Transcranial Doppler estimation of cerebral blood flow and cerebrovascular conductance during modified rebreathing.

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Abstract

Clinical transcranial Doppler assessment of cerebral vasomotor reactivity (CVMR) uses linear regression of cerebral blood flow velocity (CBFV) versus end-tidal CO$_2$ (P$_{ETCO2}$) under steady-state conditions. However, the CBF-P$_{ETCO2}$ relationship is nonlinear, even for moderate changes in CO$_2$. Moreover, CBF is increased by increases in arterial blood pressure (ABP) during hypercapnia. We used a modified rebreathing protocol to estimate CVMR during breath-by-breath changes in CBFV and P$_{ETCO2}$. Ten healthy subjects (6 men) performed 15 s of hyperventilation followed by 5 min of rebreathing, with supplemental O$_2$ to maintain arterial oxygen saturation (SaO$_2$) constant. To minimize effects of changes in ABP on the estimation of CVMR, cerebrovascular conductance index (CVCi) was calculated. The CBF-P$_{ETCO2}$ and CVCi- P$_{ETCO2}$ relationship were quantified using both linear and nonlinear logistic regression. In three subjects, muscle nerve sympathetic activity (MSNA) was recorded. From hyperventilation to rebreathing, robust changes occurred in P$_{ETCO2}$ (20 to 61 mmHg), CBFV (-44 to +104 % of baseline), CVCi (-39 to +64 %) and ABP (-19 to +23 %), (all p < 0.01). MSNA increased by 446 % during hypercapnia. The linear regression slope of CVCi vs. P$_{ETCO2}$ was less steep than that of CBFV (3 vs. 5 %·mmHg$^{-1}$, p = 0.01). Logistic regression of CBF-P$_{ETCO2}$ ($r^2$, 0.97) and CVCi-P$_{ETCO2}$ ($r^2$, 0.93) was superior to linear regression ($r^2$, 0.91; $r^2$, 0.85; p = 0.01). CVMR was maximal (6-8 % mmHg$^{-1}$) in the range of 40-50 mmHg P$_{ETCO2}$. In conclusion, CBFV and CVCi responses to transient changes in P$_{ETCO2}$ can be described by a nonlinear logistic function, indicating that estimation of CVMR varies within the range from hypocapnia to hypercapnia. Furthermore, quantification of the CVCi-P$_{ETCO2}$ relationship may minimize the effects of changes in ABP on the estimation of CVMR.
The method developed provides insight into CVMR under transient breath-by-breath changes in CO$_2$.

Key words: cerebral blood flow; Doppler; blood pressure; carbon dioxide
The prominent cerebral blood flow (CBF) responses to changes in arterial CO₂ are a unique characteristic of the cerebral vasculature (2; 27). These responses have been quantified to reflect cerebral vasomotor reactivity (CVMR) (44), even though the underlying mechanisms are still not completely understood (23). With the advent of transcranial Doppler (TCD) for measurement of CBF velocity (CBFV) with high temporal resolution, the Doppler-CO₂ test of CVMR has become a widely adopted method in research and clinical practice. Research exploring the physiological characteristics of CVMR has demonstrated that the relationship between arterial CO₂ and CBF is nonlinear and that this relationship is affected by CO₂ induced changes in arterial blood pressure (ABP) (11; 13; 14; 18; 31; 38; 39; 42; 44). In addition, the control mechanisms that govern the responses of CBF and ABP to CO₂ have dynamic properties (11; 31; 39). Despite these complexities associated with the CBF-CO₂ relationship, measurement of CVMR has been widely applied in clinical practice to evaluate cerebral vascular function, e.g. in patients with carotid artery stenosis or hypertension (5; 28; 44; 53). These clinical studies have quantified CVMR using linear regression of steady-state responses of CBF to changes in CO₂, without incorporation of the effects of ABP. The likely reason for this simplicity is that well-controlled experiments and complex modeling methods may not be practical for clinical use (31, 32). In this regard, development of a new method that is clinically applicable and may assess CVMR reliably under conditions of breath-by-breath changes in end-tidal CO₂ (P_{ETCO₂}) is warranted.

We used a modified rebreathing protocol, consisting of a period of voluntary hyperventilation, followed by rebreathing, to obtain a wide range of changes in P_{ETCO₂} to
assess CVMR under breath-by-breath conditions (10). We hypothesized that the CBF-P\textsubscript{ETCO2} relationship is nonlinear during transient changes in P\textsubscript{ETCO2}. In addition, we speculate that measurement of cerebrovascular conductance index (CVCi) during this process may reveal direct effects of changes in ABP on CBF leading to a more precise estimation of CVMR based on the CVCi- P\textsubscript{ETCO2} relationship. Finally, to explore the underlying mechanisms of increases in ABP during hypercapnia, we recorded muscle sympathetic nerve activity (MSNA) in three subjects in this study.

METHODS

Ten healthy subjects (4 women) with a mean age (± SD) of 37 ± 8 yrs, height 177 ± 10 cm, and weight 84 ± 18 kg voluntarily participated in the study. Participants were nonsmokers, were not on any medication, and all were normotensive. They were free of known cardiovascular, cerebrovascular, or respiratory disease. Each subject was informed of the experimental procedures and signed a consent form, approved by the Institutional Review Boards of the University of Texas Southwestern Medical Center and Presbyterian Hospital of Dallas.

Protocol

Each participant visited the laboratory twice, three to four weeks apart (in three participants the time interval was three to four months). All experiments took place in a quiet laboratory with a room temperature of 22\textdegree C. The subjects refrained from heavy exercise and caffeinated or alcoholic beverages at least 12 h before the tests. The study
was conducted with the subjects in the supine position. Therefore, assessment of CVMR would be comparable with those obtained with other imaging technology (32). They then wore a nose clip and breathed through a mouthpiece with a Y-valve, with one end connected to a 5 L rubber bag and the other end open to room air. This system was held in place by an adjustable cable, rendering it practically weightless to avoid any discomfort for the subject. After at least 20 min of stabilization, 2 min of baseline data during spontaneous breathing were recorded on room air. A modified Read rebreathing protocol was used (10; 33; 51). The procedure contains a period of voluntary hyperventilation preceding rebreathing to obtain a wider range of CO$_2$ changes. Specifically, a short period of 15 s of hyperventilation with a duty cycle of 1 second and maximal voluntary deep breaths was used. This short period of hyperventilation reduced P$_{ETCO2}$ to ~20 mmHg, but did not cause respiratory muscle fatigue or central hypoxia possibly associated with a prolonged hyperventilation as employed in the other rebreathing methods (33; 51). At the end of a deep inspiration, the respiratory valve was switched to the empty bag and then the bag was filled with the subjects’ own breathing gas during expiration (with a P$_{ETCO2}$ of ~20 mmHg, comparable to a gas mixture with 2.6 % CO$_2$). Rebreathing was continued for 5 min, before returning to room air for recovery (4 min). During rebreathing, a small amount of oxygen was bled into the rebreathing bag at the subject’s basal metabolic rate (estimated using the Harris-Benedict formula) to maintain arterial oxygen saturation (SaO$_2$) constant (19).
**Instrumentation and Data Acquisition**

The middle cerebral artery (MCA) was insonated using a 2-MHz pulsed Doppler ultrasound system (Multi-Dope X2, DWL). The probe was securely attached to the cranium using a mold that was cast individually to fit the facial bone structure (16). In this way, the exact position and angle of the probe were preserved during the repeat tests.

Throughout the test, ABP was monitored non-invasively using a Finapres (Ohmeda 2300), and was corroborated by concurrent electrosphygmomanometry (SunTech Medical) with measurements at intervals of one minute. $P_{ETCO_2}$ was recorded using a capnograph (POET TE, Criticare Systems), $SaO_2$ was measured using a pulse oximeter (Biox 3700, Ohmeda), and heart rate was monitored using a 3-lead ECG connected to a cardiotachometer (Quinton Instruments).

Muscle sympathetic nerve activity (MSNA) was recorded in three participants. MSNA signals were obtained using the microneurographic technique (50). Sympathetic bursts were identified by a computer program and then were confirmed by an experienced microneurographer. The number of bursts per min (burst frequency), and the sum of the integrated burst area per min (total activity) were used as quantitative indices. Specifically, total activity was normalized to the baseline value (set at 100 %) to reflect changes in sympathetic activity during CO$_2$ stimuli.

**Data Analysis**

Off-line data analysis was performed using commercially available software (Acknowledge, BIOPAC systems). For hyperventilation, the time delay between the onset of hyperventilation and the onset of changes in CBFV and ABP was measured.
Similarly, at the end of rebreathing, the time delay for recovery between $P_{ETCO2}$, CBFV, and ABP was measured. Values for CBFV, ABP and $P_{ETCO2}$ at the last min of recovery were compared to their pre-test baseline. Breathing cycles were identified from the respiratory CO2 signal. For each cycle, the corresponding value for $P_{ETCO2}$, mean value for CBFV, ABP and HR were measured. Averaging a number of cardiac cycles (usually 6) has been applied previously in attempt to reduce respiratory hemodynamic variability (17). We preferred to identify each respiratory cycle (22) to derive the breath-to-breath CBFV-$P_{ETCO2}$ relationship. During the short breathing cycles in hyperventilation, an average of data from two cycles was used. Since CBFV is affected directly by changes in ABP (11; 13; 27; 38), an index of cerebrovascular conductance (CVCi) was calculated by dividing mean CBFV by mean ABP within each breath cycle to reveal intrinsic vascular responses to changes in CO2. Furthermore, percentage changes from baseline CBFV and CVCi values were calculated for data analysis. Data ranging from the nadir of $P_{ETCO2}$ during hyperventilation to the end of rebreathing were utilized for curve fitting to quantify the CBFV-$P_{ETCO2}$ and CVCi-$P_{ETCO2}$ relationship.

A four parameter logistic function was used for curve fitting (26):

$$f(x) = y_0 - \frac{a}{1 + e^{(b(x-x_0))}}$$

(1)

where $f(x)$ represents percentage changes in CBFV or CVCi; $x$ represents $P_{ETCO2}$. The model parameter $a$ represents the range of change in CBFV or CVCi; $y_0$ is the maximum value of CBFV or CVCi during hypercapnia; $x_0$ is the level of $P_{ETCO2}$ where the first-order derivative of the logistic function (the slope of the curve) is maximal; and $b$ is related to the overall curvilinear properties of the sigmoid curve (Fig. 1). Nonlinear curve fitting was performed using a Marquardt-Levenberg algorithm for model parameter
identification (SigmaPlot 8.02, SPSS Inc.). The selection of this model was based on the feature of curvilinear distribution of breath-by-breath changes in CBFV or CVCi versus $P_{ETCO2}$ (Fig. 2) and the fact that model parameters of the selected logistic function have clear physiological implications as shown in figure 1 (26).

The first-order derivative of the logistic function was calculated using the following equation:

$$f'(x) = \frac{a \cdot b \cdot e^{b(x-x_0)}}{(1 + e^{b(x-x_0)})^2}$$  \hspace{1cm} (2)

This derivative function yields the specific CO$_2$-sensitivity (or CVMR) for each $P_{ETCO2}$. At $x = x_0$, CVMR becomes maximal (CVMR$_{max}$), and equation (2) becomes: $(a \cdot b)/4$. Of note, CVMR$_{max}$ derived from the CBFV-$P_{ETCO2}$ relationship was denoted as CBFV-CVMR$_{max}$ and CVMR$_{max}$ derived from the CVCi-$P_{ETCO2}$ relationship was denoted as CVCi-CVMR$_{max}$.

For comparison, linear regression of changes in CBFV and CVCi versus $P_{ETCO2}$ was conducted for the entire range of changes in $P_{ETCO2}$ from the nadir of hyperventilation to the end of rebreathing. The slopes of these linear regressions were defined as CBF-CVMR$_0$ and CVC-CVMR$_0$, respectively. In addition, to complement the assessment of CVMR$_{max}$ with the derivative method, CVMR was estimated by the linear regressions in the steep part of the sigmoid curves and these results were defined as CBF-CVMR$_1$ and CVC-CVMR$_1$, respectively.

**Statistics**

The results of curve fitting were examined by the correlation coefficients ($r^2$) and by model residual analysis of individual data (SigmaPlot 8.02, SPSS Inc.). Thus,
individual data for $r^2$ as well as mean sum of squares were available for both linear and logistic curve fits, allowing comparisons of both methods on using paired t-tests (Sigma Stat 3.11, Systat Software Inc.). Comparisons of cerebral hemodynamics at baseline, during hyperventilation and during rebreathing were made using one-way repeated measurements ANOVA. Comparisons between the estimates of CVMR using different methods were made with paired-t tests. Test-retest reproducibility for curve-fitting parameters and for CVMR estimation was examined by the analysis of typical error (the standard deviation of the differences between the two tests, divided by square root of 2), which was expressed as a coefficient of variation (21). Data are presented as mean ± SD and a $P < 0.05$ was considered to be statistically significant.

RESULTS

Changes in Cerebral and Systemic Hemodynamics

All 10 participants completed the experimental protocol, so that 20 data sets were collected. Data from one test could not be used due to poor signal quality; hence, reproducibility results were available only for 9 subjects. Representative changes in CBFV, ABP, $P_{ETCO2}$, HR and MSNA are shown in figure 2. Individual values for SaO$_2$ varied within the range of 96-100% during baseline breathing of room air, and remained unchanged during rebreathing owing to the supplementation of oxygen. There was a delayed response of CBFV (mean: 7 s, range 6-10) and ABP (mean: 14 s, range 12-16) to changes in $P_{ETCO2}$ during hyperventilation. Correction for the time delay did not significantly alter the result of data analysis. Since the physiological validity of applying
a time correction for CVMR estimation was uncertain, analysis of the raw (uncorrected) data is presented. Following the rapid return of $P_{ETCO2}$ to the pre-test baseline level at the onset of recovery, a considerable time delay was observed for the ensuing recovery of CBFV (mean: 21 s, range 14-35) and of ABP (mean: 22 s, range 12-30). However, this delay did not influence the data analysis because the recovery period was not included in the models used to assess CVMR in this study. Whereas ABP gradually returned to the pre-test baseline after rebreathing, CBFV displayed an undershoot and then gradually returned to a sustained level which was below the pre-test baseline level (mean: 81 % of baseline, range 65-90), even at the end of the 4 min of recovery.

On average, $P_{ETCO2}$ was reduced by 22 mmHg during hyperventilation, and increased by 20 mmHg during rebreathing from the pre-test baseline ($p < 0.001$). These changes in $P_{ETCO2}$ resulted in a respective reduction of CBFV by 46 % and an increase by 104 % relative to the baseline ($p < 0.001$). CVCi fell by 39 % during hyperventilation and increased by 64 % during rebreathing ($p < 0.001$). Of note, ABP was reduced by 19 % during hyperventilation, and augmented by 23 % during rebreathing ($p < 0.01$). During rebreathing, increases in ABP appeared to be proportional to increases in $P_{ETCO2}$, but leveled off at high levels of $P_{ETCO2}$ (Fig. 2). HR increased by 29 % during hyperventilation, and by 14 % during rebreathing ($p < 0.01$) (Fig 2, Table 1).

In the 3 subjects that underwent recording of MSNA, averaged burst frequency increased from 24 at baseline to 30 bursts/min during hyperventilation, and total nerve activity increased by 166 % ($p = 0.01$). MSNA returned rapidly to baseline after cessation of hyperventilation, and subsequently increased to 41 bursts/min and 446 % for total
nerve activity during the last min of rebreathing ($p = 0.01$, Fig 2). Changes in MSNA were similar in all 3 subjects.

Curve Fitting and Model Parameters

Figure 3 depicts the representative results of sigmoid curve fitting of the CBFV/CVCi –$P_{ETCO2}$ relationship in one subject. The specific CVMR derived from the first-order derivative of the logistic function is also plotted. The group-averaged data of changes in CBFV and CVCi as well as logistic regression of pooled data for all subjects are shown in figure 4. Since there were no significant differences between results from the first and second test, all data were used in the pooled data analysis. Logistic regression of individual data demonstrated an excellent curve-fitting result (mean $r^2 0.95$, SD 0.04; average of mean sum of squares (MSS) 37, SD 19), superior to the use of linear regression (mean $r^2 0.88$, SD 0.06; average MSS 85, SD 51, $p < 0.01$) for both the CBFV- $P_{ETCO2}$ and the CVCi- $P_{ETCO2}$ relationship. Specifically, logistic regression was performed well in all 18 tests for changes in CBFV ($r^2 0.97$, SD 0.02) and in 16 of 18 tests for CVCi ($r^2 0.93$, SD 0.06). In those two cases of CVCi where logistic regressions could not be performed reliably, linear regressions yielded an $r^2$ of 0.71 and 0.76 for CBFV and CVCi respectively.

Table 2 shows the group averaged model parameters and the calculated CVMR indices derived respectively from the logistic and linear regressions. Over the entire range of changes in $P_{ETCO2}$, the linear regression slope of changes in CVCi (CVCi-CVMR0) was significantly smaller than that of CBFV (CBFV-CVMR0). The steep ranges of the sigmoid curves were found to lie between 40 to 50 mmHg $P_{ETCO2}$ for most subjects (Figs.
3, 4). As expected, in the steep ranges of the sigmoid curves, linear regression slopes of changes in CBFV (CBFV-CVMR1) and CVCi (CVCi-CVMR1) were similar to those obtained from the first-order derivatives of the identified logistic function (CBF-CVMRmax and CVC-CVMRmax respectively). Of note, both CVMRmax and CVMR1 were higher than the estimates of CVMR from the linear regressions over the entire range of changes in $P_{ETCO2}$ (CVMR0). Finally, for test-retest reproducibility, coefficients of variation for curve-fitting parameters and for estimates of CVMR are presented in Table 2.

**DISCUSSION**

We have developed a clinically applicable method to assess CVMR that addresses the nonlinear relationship and interactions between transient changes in CO$_2$, CBFV and ABP. Based on a modified rebreathing method, we have observed a wide range of breath-by-breath changes in $P_{ETCO2}$ (20 ~ 61 mmHg) and corresponding changes in CBFV, CVCi and ABP.

The main findings of this study are twofold. First, consistent with our hypothesis, transient, breath-by-breath responses of both CBFV and CVCi to changes in $P_{ETCO2}$ demonstrate nonlinear properties that can be quantified by a logistic function. Second, the magnitude of increases in CBFV during hypercapnic rebreathing exceeded CVCi (104 % versus 64%) because of the presence of significant increases in ABP by 23 %. Consistently, during hypocapnic hyperventilation, the reduction in CBFV (46 %) surpassed CVCi (39 %), while ABP fell by 19 %. These observations suggest that changes in ABP during CO$_2$ stimuli have direct effects on CBF.
Study limitations

The reproducibility of model parameters and estimates of CVMR are comparable with other Doppler-CO₂ tests (44; 45; 48). Nonetheless, the coefficient of variation of the model parameter b was relatively large as compared with the other parameters (Table 2). Parameter b reflects the overall curvilinear properties of the sigmoid curve. Therefore, estimation of parameter b may have been affected by the lack of precise control of levels of $P_\text{ETCO}_2$ reached during voluntary hyperventilation between the tests. The technique of dynamic end-tidal forcing may provide much better control of $P_\text{ETCO}_2$ to improve the reproducibility of model parameter’s estimation (40).

An additional limitation, inherent to the use of TCD, is that CBFV was measured to reflect changes in CBF. The TCD method has been well validated against other modalities used to measure CBF (4; 9; 30). However, changes in CBFV reflect changes in CBF only if the diameter of the insonated middle cerebral artery remains constant. Direct and indirect measurements of the MCA diameters as well as comparison of changes in CBFV with changes in CBF measured using different modalities during either hypo- or hypercapnia suggest the validity of using TCD (15; 46). We cannot exclude the possibility that a vasodilatation in the MCA may occur at high levels of CO₂ (49). If this was the case, the magnitude of maximal changes in CBF may have been underestimated by the measurement of CBFV during hypercapnia. However, the maximum changes in CBFV at a $P_\text{ETCO}_2$ level of 60 mmHg (~100 % above baseline), are consistent with results of direct measurements of CBF in other studies with a similar level of changes in CO₂ (14; 27).


Magnitude of changes in CBFV and ABP

We found marked changes in CBFV with a range of nearly 150% from hyperventilation to rebreathing, with rebreathing accounting for an estimated doubling of baseline CBF. This range of changes in CBF is comparable with those found in other studies using TCD or other techniques to measure CBF (14; 24; 27; 35; 40; 51).

Prominent CBF responses to arterial CO2 are well-established (23; 27; 42; 44). These changes in CBF have been attributed mainly to cerebral vasodilatation during hypercapnia and vasoconstriction during hypocapnia (3; 52).

However, changes in arterial CO2 elicit complicated autonomic reflexes and exert vascular effects not only in the cerebral but in the systemic circulation (29; 43). ABP increases substantially even during moderate increases in arterial CO2, due largely to sympathetic activation (6; 11; 38; 47), which clearly occurred in our study as well. This chemoreflex mediated sympathetic activation may have confounding and opposing effects for the cerebral circulation, especially in the setting of CO2 induced cerebral vasodilatation. For example, systemic hypertension may increase cerebral blood flow prominently when normal autoregulatory mechanisms are impaired by CO2 (13; 18; 37). Conversely, sympathetic activation in the brain may restrain CO2 induced vasodilatation (12); (55); (7; 52) though it is less certain whether such effects occur consistently in humans (25). Both of these opposing effects will influence the measurement of CVMR during rebreathing.

In the present study, we found that estimates of CVMR based on CBFV (during either hyper- or hypocapnia) exceeded those based on CVCi. For example, model parameters a and y0 (which reflect the range and maximal changes in CBFV and CVCi
respectively) derived from the CBF-P_{ETCO2} relationship were significantly higher than those obtained from the CVCi-P_{ETCO2} relationship. Likewise, linear regressions resulted in higher values for CVMR when derived from CBFV instead of CVCi.

According to Ohm’s law, the much higher increases in CBFV (104 %) during hypercapnia relative to the increases in CVCi (64 %) could be attributed to the 23% increases in ABP. Consequently, increases in ABP led to an overestimation of CVMR based on the linear regression of changes in CBFV vs. P_{ETCO2} (CBFV-CVMR0) (Table 2) (13). Taken together, these findings suggest that the net CBF responses to acute hypercapnia in humans are dominated by an overwhelming vasodilatory effect of CO₂, which impairs cerebral autoregulation and thereby enhances the effects of ABP on CBFV.

**Estimation of CVCi**

This study applies changes in cerebrovascular conductance to assess CVMR during transient changes in P_{ETCO2}. The use of CVCi, in contrast to changes in CBFV, may better reflect the physiological process of cerebral vasodilatation during hypercapnia and cerebral vasoconstriction during hypocapnia. When an index of cerebrovascular resistance (the inverse of conductance) is used rather than CBFV to assess CVMR, the outcome was less influenced by changes in ABP (13). However, using resistance, rather then its inverse conductance, to assess CVMR may lead to difficulties in data interpretation, especially under conditions with increased CBF (34). Because of their reciprocal relationship, in a condition where resistance is low and blood flow is high, a large increase in conductance (vasodilatation) leads to only a small (further) decrease in resistance. Therefore, if vasomotor responses are estimated from changes in resistance,
such a small decrease in resistance could be falsely interpreted to represent a small increase in vasodilatation. Because CVMR testing generates high blood flow, conductance rather than resistance was used in this study.

Alternatively, studies have analyzed the ABP-CBFV relationship within each cardiac cycle, and used linear extrapolations to calculate the zero-flow pressure (also known as critical closing pressure) or resistance-area product indices (1; 22; 36). Hypocapnia increases and hypercapnia reduces zero-flow pressure; therefore, changes in these indices may serve as alternative measures to assess cerebrovascular responses to CO₂ (17; 22). Whereas the clinical relevance of assessment of CVMR has been demonstrated in follow-up studies in patients at risk for cerebrovascular events (28; 53), similar findings have not been reported for zero-flow pressure or resistance-area product parameters.

**Temporal characteristics of changes in CBFV and ABP**

The CBF response to transient changes in CO₂ has a time delay of about 5-7 s (31; 41). It is therefore of interest to consider whether this factor could affect the estimation of CVMR using the proposed modeling. Identification of the time delay between transient changes in CBF and PETCO₂ as performed in these studies, requires complex and stringent control of respiration and CO₂ levels. However, in the present study we are able to approximate the time delay between changes in PETCO₂, CBFV and ABP based on the individual data from each subject. The average time delay of the reduction in CBFV in response to the reduction in PETCO₂ during hyperventilation was ~ 7 s, consistent with the reported time delay after a negative step change in PETCO₂ (6.8 s) (41), and with a time
delay of ~5 s found when spontaneous CO₂ oscillations were analyzed (39). This time delay is possibly explained by CO₂ transportation time (54). The identification of a similar time delay during hypercapnic rebreathing is less evident, because, as can be seen in figure 2, rebreathing caused a ramp-like increase in P_{ETCO₂}. Despite the time delay between transient changes in CBFV and CO₂, it had little effect on the estimation of CVMR. With a total duration of hyperventilation of 15 s, a (short) plateau phase with minimum and stable CO₂ as well as CBFV values was reached in all individuals. Values at the end of hyperventilation (i.e., in this plateau phase) were used as a starting point for calculation of CVMR. Therefore, “correction” for the time delay did not affect the modeling and data outcome. Similarly, the observed reduction in ABP during hyperventilation also showed a time delay, lagging behind the changes in CBFV by an additional 7 s. This delayed response in ABP may partly explain why there was a smaller difference between changes in CVCi and CBFV during hyperventilation when compared with hypercapnic rebreathing.

During rebreathing, CBFV increased steadily associated with a continuous increase in P_{ETCO₂} and then appeared to level off at high levels of P_{ETCO₂}. Two mechanisms may have contributed to the saturation of CBFV. First, cerebral vasodilatation may have reached its maximal levels at or greater than a P_{ETCO₂} of 60 mmHg (42; 44). Second, substantial sympathetic activation may constrain the magnitude of the CBFV responses (7; 25; 52).

With the onset of recovery, P_{ETCO₂} returned rapidly to the pre-test baseline level within about 1-2 breaths of room air (< 10 s). However, there was a substantial time delay of about 20 s for CBFV to reach a new sustained level, which was lower than the pre-test
baseline value. Of note, ABP returned to the pre-test baseline level without a similar undershoot. These data suggest a sustained cerebral vasoconstriction during recovery from prolonged acute hypercapnia. In addition, these data confirm that adaptive processes modify the response in both ABP and CBF to CO₂, leading to limitations in using steady-state data to estimate CVMR (14; 41). However, the time delay in the recovery of CBFV appears to be much longer than that reported using a step change in CO₂ (6 s) (40). These discrepancies might be related to the differences in the duration as well as the intensities of CO₂ stimuli used.

**Assessment of CVMR**

A sigmoidal distribution of data was present in most subjects when breath-by-breath changes in CBFV were plotted against P_{ETCO₂}. Furthermore, similar distributions were observed for changes in CVCi, indicating the presence of threshold and saturation properties of cerebrovascular changes in response to transient changes in P_{ETCO₂} and provide further evidence for the nonlinear properties of CBF responses to changes in CO₂ (40-42; 44; 54). However, the specific physiological mechanisms underlying the nonlinear CBF-CO₂ relationship cannot be determined in the present study.

Data with a sigmoidal distribution could be fitted equally well with several nonlinear functions based on the norm of least-square curve-fitting procedures (42). A modified logistic function was employed for curve-fitting (26). As compared with other curve fitting methods, one advantage of the logistic function used in this study is that the model parameters identified have clear physiological meanings (Fig. 1). In addition, the
first-order derivative of the identified logistic function readily provides an estimation of CVMR for each level of $P_{ETCO2}$.

The linear regression slope of the CBF-$P_{ETCO2}$ relationship ($CVMR_0$, 5\%·mmHg$^{-1}$) over the entire range of changes in $P_{ETCO2}$ is consistent with previous findings (8; 14; 27; 40). However, this method of analysis, although practical, evidently underestimates the maximal cerebral vasodilatory effect of CO$_2$, as indicated by the estimates of CVMR$_{max}$ using either the first-order derivatives of the identified logistic function (8 \%·mmHg$^{-1}$) or the specific linear regressions in the steep portion of sigmoidal curves (7 \%·mmHg$^{-1}$) (Table 2).

The inflection point of the logistic function (parameter $x_0$), which corresponds to the maximal rate of changes in CBFV or CVCi to CO$_2$ (CVMR$_{max}$), was almost identical for both the CBF-$P_{ETCO2}$ and CVCi-$P_{ETCO2}$ relationship (Table 2). This demonstrates that CVMR$_{max}$ occurs during moderate hypercapnia, slightly above the baseline level of $P_{ETCO2}$ consistent with previous studies suggesting that estimation of CVMR is lower during hypocapnia than during hypercapnia (13; 24; 40; 51).

Previous studies have used similar rebreathing methods to estimate CVMR (8; 20; 35; 51). However, these studies investigated only the hypercapnic stimuli and used linear regressions to derive a CVMR from 2.8 to 5.2 \%·mmHg$^{-1}$ (8; 20; 35). Only one study included the hypocapnic range using hyperventilation prior to rebreathing, and applied a bilinear model, resulting in a smaller CVMR in the hypocapnic range than in the hypercapnic range (1.6 vs. 2.8 cm.s$^{-1}$.mmHg$^{-1}$) (51). Direct comparisons to these previous findings are difficult because hypoxic and/or hyperoxic conditions also were employed in these studies.
In conclusion, a clinically applicable rebreathing method was developed to assess CVMR during transient changes in $P_{ETCO2}$. We found that both CBFV and CVCi responses to transient changes in $P_{ETCO2}$ are nonlinear, and that these responses can be quantified well by a modified logistic function. Furthermore, we found that the magnitude of change in CBFV during CO2 rebreathing (and hyperventilation) is higher than that of CVCi, suggesting direct effects of increases (and decreases) in ABP on changes in CBFV. For assessment of CVMR, the range of changes in CO2 to be studied and the confounding influence of changes in ABP on CBFV must be considered for application of an appropriate modeling method to quantify CBFV or CVCi responses.
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DISCLOSURES

None declared.
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Figure Legends

Figure 1. Schematic representation of a logistic function with 4 parameters to be identified. “a” is the total range of changes in CVCi or CBFV, “y0” is the maximum value, “x0” is the level of PETCO2 that exhibits highest CO2 sensitivity, and “b” represents the curvilinear properties of the sigmoid curve.

Figure 2. A typical recording of baseline, hyperventilation, rebreathing, and recovery in one subject. CBFV: cerebral blood flow velocity in the middle cerebral artery, ABP: arterial blood pressure, HR: heart rate, PETCO2: end-tidal CO2, MSNA: muscle sympathetic nerve activity. Note the significant increases in ABP and MSNA, concurrent with the increase in CBFV during rebreathing. Arrows indicate start and end of hyperventilation and rebreathing periods.

Figure 3. Typical data of logistic regression of % changes in cerebral blood flow velocity (CBFV) and cerebrovascular conductance index (CVCi) to changes in end-tidal CO2 (PETCO2) in one subject. The sigmoid curve represents the result of logistic regression; the dotted bell-shaped curve is the first-order derivative of the identified logistic function. Linear regression was performed in the steep portion of the sigmoidal curve to estimate cerebral vasomotor reactivity (CVMR), using CBFV and CVCi as the dependent variables respectively.
Figure 4. Group (n=10) averaged results for logistic regression of % changes in cerebral blood flow velocity (CBFV) and cerebrovascular conductance index (CVCi) to changes in end-tidal CO₂ (P_{ETCO₂}). The sigmoidal curve represents the result of logistic regression of pooled group data from all subjects (parameters, CBFV: a 140 %, y₀ 198 %, x₀ 47 mmHg, b 0.20 mmHg⁻¹; CVCi: a 102 %, y₀ 169 %, x₀ 47 mmHg, b 0.16 mmHg⁻¹; note that these values are similar to the parameters obtained by averaging the individual curve fitting results as listed in Table 2). The dots and error bars represent group averages and standard deviations for CBFV (%) and CVCi (%) within 5 mmHg ranges of P_{ETCO₂} (e.g. 20-25, 25-30 etc.).
Table 1. Changes in Cerebral and Systemic Hemodynamics during the Test.

<table>
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<tr>
<th>Subject</th>
<th>( P_{ETCO2} )</th>
<th>CBFV</th>
<th>MABP</th>
<th>HR</th>
<th>( P_{ETCO2} )</th>
<th>CBFV</th>
<th>MABP</th>
<th>HR</th>
<th>( P_{ETCO2} )</th>
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<th>MABP</th>
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</table>
\( P_{\text{ETCO}_2} \): end tidal CO\(_2\) (mmHg). CBFV: cerebral blood flow velocity (cm/s). MABP: mean arterial blood pressure (mmHg). HR: heart rate (bpm).

Baseline: average of 2-minute recordings before hyperventilation. Hyperventilation: lowest values following 15 seconds of hyperventilation.

Rebreathing: average of the last minute of data. Data are mean values from all available tests in n=10 subjects.

*: \( p < 0.01 \) for comparison of hyperventilation and rebreathing with baseline.
Table 2. Estimation of Logistic Function Parameters and Cerebral Vasomotor Reactivity (CVMR).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CBFV-P\textsubscript{ETCO2}</th>
<th>CVi-P\textsubscript{ETCO2}</th>
<th>CVi-P\textsubscript{ETCO2}</th>
<th>CVi-P\textsubscript{ETCO2}</th>
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<td>(a), %</td>
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<td>17</td>
<td>99 ± 29*</td>
<td>26</td>
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<tr>
<td>(y_0), %</td>
<td>202 ± 35</td>
<td>10</td>
<td>166 ± 30*</td>
<td>11</td>
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<tr>
<td>(x_0), mmHg</td>
<td>47 ± 2</td>
<td>6</td>
<td>46 ± 2</td>
<td>4</td>
</tr>
<tr>
<td>(b), mmHg(^{-1})</td>
<td>0.22 ± 0.06</td>
<td>36</td>
<td>0.28 ± 0.09</td>
<td>58</td>
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<tr>
<td>CVMR\textsubscript{max}, %·mmHg(^{-1})</td>
<td>8 ± 2†</td>
<td>23</td>
<td>6 ± 2†</td>
<td>31</td>
</tr>
<tr>
<td>CVMR\textsubscript{0}, %·mmHg(^{-1})</td>
<td>5 ± 1</td>
<td>18</td>
<td>3 ± 1*</td>
<td>18</td>
</tr>
<tr>
<td>CVMR\textsubscript{1}, %·mmHg(^{-1})</td>
<td>7 ± 1†</td>
<td>9</td>
<td>6 ± 1&quot;†</td>
<td>26</td>
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</tbody>
</table>

Values are mean ± SD from 10 subjects. CV: coefficient of variation (%). CBFV: cerebral blood flow velocity. P\textsubscript{ETCO2}: end-tidal CO\textsubscript{2}. \(a\), \(y_0\), \(x_0\), and \(b\): identified logistic function parameters (Fig 1). CVMR\textsubscript{max}: maximal cerebral vasomotor reactivity (CVMR), peak value of the first-order derivative of the identified logistic function. CVMR\textsubscript{0}: linear regression slope of percentage changes in CBFV over the entire range of changes in P\textsubscript{ETCO2}. CVMR\textsubscript{1}: linear regression slope of CBFV vs. P\textsubscript{ETCO2} in the steep portion of the sigmoidal curve.

* \(p < 0.01\) for difference in parameters between CBFV-P\textsubscript{ETCO2} and CVCi-P\textsubscript{ETCO2}. † \(p < 0.01\) for CVMR\textsubscript{0} vs. CVMR\textsubscript{max} and CVMR\textsubscript{1}. 