A new two-breath technique for extracting the cerebrovascular response to arterial carbon dioxide

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Edwards, Michael R., Zigniew L. Topor, and Richard L. Hughson. A new two-breath technique for extracting the cerebrovascular response to arterial carbon dioxide. Am J Physiol Regul Integr Comp Physiol 284: R853–R859, 2003; 10.1152/ajpregu.00601.2002.—Cerebrovascular autoregulation is evaluated from spontaneous fluctuations in mean flow velocity (MFV) by transcranial Doppler ultrasound of the middle cerebral artery (MCA) with respect to changes in arterial blood pressure (BPMCA), but the effects of spontaneous fluctuations in arterial PCO2 on MFV have been largely ignored. Autoregressive moving average analysis (ARMA), a closed-loop system identification technique, was applied to data from nine healthy subjects during spontaneous breathing, during inspiration of 10% CO2 for two breaths once per minute for 4 min, and during sustained breathing of 7% CO2. Cerebrovascular resistance index (CVRi) was calculated (CVRi = BPMCA/MFV). Reliable estimates of gain for BPMCA → MFV were obtained for spontaneous breathing and the two-breath method. In contrast, reliable gain estimates for PCO2 → MFV or PCO2 → CVRi were achieved only under the two-breath method. PCO2 → MFV gain was smaller with the two-breath method than during sustained 7% CO2 (P < 0.05). BPMCA was elevated by 7% CO2 but not by the two-breath method. The closed-loop model provides insight into interactions between BPMCA and PCO2 on cerebrovascular control, but reliable solutions for PCO2 effects with ARMA analysis require perturbation by the two-breath method.

autoregressive moving average analysis modeling; arterial carbon dioxide partial pressure; Doppler ultrasound; cerebral blood flow; autoregression

CEREBROVASCULAR AUTOREGULATION maintains a relatively constant cerebral blood flow across a wide range of arterial blood pressure [BPMCA, corrected at the level of the middle cerebral artery (MCA)], and its impairment can be associated with cerebrovascular disease (1). Frequency domain analysis has become popular to characterize dynamic cerebrovascular autoregulation from spontaneous fluctuations in BPMCA and mean flow velocity (MFV) (2, 3, 8, 15, 22, 34). However, these analyses fail to consider a possible confounding influence of PCO2 on the cerebrovasculature. Indeed, under resting conditions, PCO2 shows spontaneous variability (17), and recent observations suggest that the dynamic relationship between BPMCA and MFV is altered under different background levels of PCO2 (11, 21).

Autoregressive moving average analysis with exogenous inputs (ARMA), a closed-loop system identification technique (24), allows for a simultaneous solution of a model with two inputs (BPMCA and PCO2) and one output [either MFV or an index of cerebrovascular resistance (CVRi)], where CVRi = BPMCA/MFV. The model parameters can be used to generate theoretical ideal impulse and step responses. The impulse represents the change in MFV or CVRi that would result if a 1-mmHg increase in BPMCA or PCO2 occurred as a brief pulse at time 0, while the step response represents the change after a sustained 1-mmHg increase in BPMCA or PCO2. ARMA offers a number of advantages over transfer function analysis; the most important is that it can discriminate between multiple inputs, thus allowing for causal relationships. The role of PCO2 in the cerebral circulation, independent of and in combination with BPMCA, can thereby be determined.

Evidence from ARMA (10) and similar models (23, 30) indicated that the BPMCA → MFV step response obtained under spontaneous resting conditions was similar to results obtained by altering MFV and BPMCA with the release of cuffs placed around the upper thigh (1). Furthermore, the PCO2 → MFV response from the multiple input model (10, 23) was similar to results acquired with step changes in end-tidal CO2 (26) although this latter approach often causes a significant increase in BPMCA (12). A major limitation of ARMA analysis when applied to resting conditions was that

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the PCO2 signal often lacked sufficient bandwidth and amplitude (23) to yield a valid and consistent solution (10). We hypothesized that this limitation could be overcome, while at the same time avoiding elevations in BP_MCA, by introducing a short series of two inspired breaths of 10% CO2 during spontaneous breathing. ARMA solutions were compared with measured values determined during sustained breathing of 7% CO2. We also determined the reproducibility of the method by examining the responses with a total of four series of the two-breath method collected on 2 separate days.

METHODS

Subjects. Nine healthy subjects (4 men and 5 women, mean age 22.1 yr, range 18–24 yr) voluntarily participated in this study approved by the Office of Research Ethics of the University of Waterloo. Informed consent was obtained in all cases.

Experimental protocol. Subjects reported to the laboratory on 2 separate days in a rested state, at least 2 h after food and caffeine ingestion. Subjects were instrumented and remained in a seated position for the duration of the tests. On each day, collection included 10 min of spontaneous resting baseline data followed by two series of a two-breath method. Subjects were switched by a four-way valve from breathing room air to inspired 10% CO2 (21% O2, balance N2) for two-breaths, every minute, for 4 consecutive minutes. The two-breath series were separated by 5 min of spontaneous collection where the valve was switched from one room air source to another as a form of control experiment. Subjects were not informed of the exact design of this protocol. After a 20-min break, 5 min of resting spontaneous data were collected before the valves were switched so that the subjects inspired from a large bag containing 7% CO2 (21% O2, balance N2) for 5 min. Continuous measurements of MFV, BP_MCA, heart rate and expired CO2 were collected.

Experimental measures. Heart rate was determined from a standard three-lead electrocardiogram. MFV was measured by transtracranial Doppler ultrasonography of the MCA (1). Briefly, a 2-MHz Doppler probe (Multigon, Mt. Vernon, NY) was placed over the right temporal window and fixed at a constant angle by a head-gear apparatus (Marc 600, Spencer Technologies, Seattle, WA) for the duration of the collection period. Arterial blood pressure was measured by noninvasive arterial tonometry (Colin, Pilot, San Antonio, TX) in which the sensor was placed over the radial artery. Calibration of the tonometry system was automatic against an oscillometric cuff placed on the upper arm. This measured pressure was then corrected to estimate BP_MCA by measurement of the distance between the tonometry sensor and the transtracranial Doppler probe (27). Cuff calibration of the Colin was turned off during data collection. PCO2 was collected continuously using a face mask connected to a mass spectrometer (MGA-1100, Perkin-Elmer, Pomona, CA). The face mask allowed breathing through both the nose and mouth.

Data analysis. All data were recorded on digital audiotape using an eight-channel recorder (TEAC, Montebello, CA) and were then transferred for analysis by a computer-based system to yield a dataset sampled at 100 Hz. The Doppler signal was processed using a fast Fourier transform to yield an outer envelope of MFV. Alveolar PCO2, which was taken as a surrogate for arterial PCO2, was calculated from the expired PCO2 profile as described by Whipp et al. (33). Each cardiac cycle was marked allowing beat-by-beat averaging of MFV, BP_MCA, and PCO2 calibrated waveforms. CVRi was calculated from the average values of each cardiac cycle as BP_MCA/MFV.

After removal of artifact, autospectral analysis using a Welch periodogram method (Matlab, Math Works, Natick, MA) was performed on the last 5-min of the baseline time series as well as on each of the two-breath series for the variables BP_MCA, MFV, CVRi, and PCO2. A two-input (BP_MCA and PCO2) and one output (MFV or CVRi) ARMA modeling procedure was also applied to baseline and each of the four, two-breath series as previously described in detail by Perrott and Cohen (24). Briefly, ARMA represents a linear, time-invariant system where the parameters of the model were estimated using a modified autoregressive parameter reduction algorithm (20). An appropriate model was selected based on two criteria: 1) minimal residuals, where the residuals represent the difference between the measured response and the modeled response, and 2) residuals with a Gaussian distribution that did not correlate with the inputs. The model parameters were used to generate the output responses to ideal impulse and step transitions of the input variables to estimate system gain.

Statistical analysis. Total autospectral power, ARMA impulse gain, and step responses were compared across the six trials (day 1 baseline, day 1 first 2-breath series, day 1 second 2-breath series, day 2 first 2-breath series, and day 2 second 2-breath series) with a one-way ANOVA with repeated measures. If significance was obtained (P < 0.05), a Student-Newman-Keuls post hoc test was used to isolate the differences. The same statistical model was used to compare the change per millimeter Hg PCO2 induced in MFV and CVRi from sustained breathing of 7% CO2 with the theoretical step gain obtained from the ARMA parameters for the PCO2 → MFV and PCO2 → CVRi relationships. The differences between baseline MFV, CVRi, and BP_MCA and steady-state changes in these variables from breathing 7% CO2 were compared with one-tailed, paired t-tests. An intra-class correlation coefficient was calculated and used to assess the reliability among the four, two-breath series (29). By convention, <0.4 indicates poor reliability (reproducibility), between 0.4 and 0.75 indicates fair to good reliability, and >0.75 indicates excellent reliability.

RESULTS

A time series dataset for one representative subject is shown in Fig. 1. Ten minutes of resting spontaneous baseline data followed by two series of the two-breath CO2 method are observed in the PCO2 plot. MFV and CVRi clearly responded to the P CO2, while BP_MCA remained relatively unaffected by the changes in PCO2.

Autospectral analysis. Total autospectral power for BP_MCA was unchanged across all trials (P > 0.05, Fig. 2), while there was a significant increase in total autospectral power for MFV (Fig. 2), CVRi, and PCO2 (P < 0.05). Post hoc analysis indicated that baseline trials and double-breath series differed significantly (P < 0.05). The two baseline trials did not differ from each other nor did the four two-breath series (P > 0.05).

ARMA solution for BP_MCA + PCO2 → MFV. The impulse-response function represents the change in MFV that would occur in response to a unit area impulse (1-mmHg increase) in the input variable at time 0, while the step response represents the change after a sustained 1-mmHg increase. As anticipated based on measurements of MFV in response to sustained changes in blood pressure at the level of the MCA, for example with head-up tilt (11), the calculated MFV increased rapidly in response to the increase in BP_MCA but returned close to
baseline in <10 s (Fig. 3A). Averaged over subjects, there was no significant difference across all trials for the gain of \( \text{BPMCA} \rightarrow \text{MFV} \) determined from the peak impulse or step \((P > 0.05, \text{Table 1})\).

The impulse-response for the \( \text{PCO}_2 \rightarrow \text{MFV} \) relationship was positive but was much slower (Fig. 3B) compared with the response to \( \text{BPMCA} \). Averaged over all six trials, the time at which the step response reached 95% of the plateau value was 17.9 ± 5.4 s (mean ± SD). The step values were not different across trials \((P > 0.05)\). The peak impulse for \text{day 2 baseline} was greater than all other trials \((P < 0.05, \text{Table 1})\), but three subjects were not included because no solution was found for the ARMA model under the spontaneous breathing condition. ARMA solutions were found in all of the two-breath series.

\text{ARMA solution for BP}_{\text{MCA}} + \text{PCO}_2 \rightarrow \text{CVRi}.\) The impulse-response for the relationship between \( \text{BP}_{\text{MCA}} \rightarrow \text{CVRi} \) was positive, and the step response showed the anticipated increase in \( \text{CVRi} \) in an attempt to maintain \( \text{MFV} \) in the face of sustained increase in \( \text{BP}_{\text{MCA}} \) (Fig. 3C). Mean values for the peak impulse and step response were not significantly different across trials \((P > 0.05, \text{Table 1})\).

The \( \text{PCO}_2 \rightarrow \text{CVRi} \) impulse and step responses were negative, indicating that an increase in \( \text{PCO}_2 \) caused a decrease in \( \text{CVRi} \) (Fig. 3D). There were no significant differences across trials for the peak impulse and step values as there was large variability between trials, especially in the baseline trials. A solution for \( \text{PCO}_2 \) could not be determined in the baseline trials for two subjects on \text{day 1} and three subjects on \text{day 2}. Solutions were determined for all two-breath series, and there was less variation in the mean values (Table 1). Averaged over all trials, the step reached 95% of the plateau value within 16.3 ± 6.6 s. This was consistent with the \( \text{PCO}_2 \rightarrow \text{MFV} \) response.

\text{Sustained breathing of 7% CO}_2.\) MFV and \( \text{BP}_{\text{MCA}} \) were significantly increased whereas \( \text{CVRi} \) was significantly decreased under sustained breathing of 7% \( \text{CO}_2 \) \((P < 0.05, \text{Table 2})\). The absolute change in \( \text{MFV} \) per millimeter Hg change in \( \text{PCO}_2 \) (Table 2) was significantly greater than the change determined for the step
response from the ARMA model for all conditions except day 2 baseline (Table 1). The relative change in MFV per millimeter Hg PCO2 during the sustained 7% CO2 trial was 3.4 ± 0.4% (18). Contrary to the differences between ARMA and sustained CO2 models for the MFV response to CO2, the CVRi response to the 7% CO2 (Table 2) was not significantly different from the ARMA PCO2 → CVRi step responses across all six trials (Table 1, P > 0.05). Reliability. Based on the convention for evaluating intraclass correlation with <0.4 indicating poor, 0.4–0.75 as fair to good, and >0.75 as excellent reliability (29), most solutions for the two-breath series ARMA models provided fair to excellent reliability (Table 3). The impulse responses for BPmca as the input proved to be more reliable than the step responses, whereas the step responses for PCO2 as the input were more reliable than the impulse responses (Table 3).

Table 1. ARMA maximum impulse and step responses for the models BPmca + PCO2 → MFV and BPmca + PCO2 → CVRi

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
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<th>Day 2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>1</td>
<td>2</td>
<td>Baseline</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>BPmca impulse, cm/s mmHg BPmca⁻¹</td>
<td>0.55(0.07)</td>
<td>0.58(0.08)</td>
<td>0.58(0.06)</td>
<td>0.60(0.09)</td>
<td>0.61(0.07)</td>
<td>0.58(0.07)</td>
</tr>
<tr>
<td>BPmca step, cm/s mmHg BPmca⁻¹</td>
<td>0.12(0.05)</td>
<td>0.24(0.10)</td>
<td>0.22(0.16)</td>
<td>0.06(0.14)</td>
<td>0.12(0.26)</td>
<td>0.31(0.10)</td>
</tr>
<tr>
<td>PCO2 impulse, cm/s mmHg PCO2⁻¹</td>
<td>0.17(0.03)</td>
<td>0.15(0.03)</td>
<td>0.15(0.02)</td>
<td>0.28(0.05)</td>
<td>0.13(0.02)</td>
<td>0.13(0.02)</td>
</tr>
<tr>
<td>PCO2 step, cm/s mmHg PCO2⁻¹</td>
<td>1.26(0.20)</td>
<td>1.16(0.21)</td>
<td>1.12(0.22)</td>
<td>1.65(0.58)</td>
<td>1.23(0.25)</td>
<td>1.05(0.18)</td>
</tr>
<tr>
<td>BPmca impulse, CVRi U/mmHg BPmca</td>
<td>0.0039(0.0008)</td>
<td>0.0036(0.0008)</td>
<td>0.0041(0.0006)</td>
<td>0.0058(0.0016)</td>
<td>0.0039(0.0005)</td>
<td>0.0042(0.0006)</td>
</tr>
<tr>
<td>BPmca step, CVRi U/mmHg BPmca</td>
<td>0.0132(0.0033)</td>
<td>0.0108(0.0028)</td>
<td>0.0080(0.0039)</td>
<td>0.0149(0.0033)</td>
<td>0.0159(0.0034)</td>
<td>0.0086(0.0028)</td>
</tr>
<tr>
<td>PCO2 impulse, CVRi U/mmHg PCO2</td>
<td>-0.0036(0.0008)</td>
<td>-0.0030(0.0006)</td>
<td>-0.0034(0.0006)</td>
<td>-0.0052(0.0022)</td>
<td>-0.0027(0.0006)</td>
<td>-0.0029(0.0004)</td>
</tr>
<tr>
<td>PCO2 step, CVRi U/mmHg PCO2</td>
<td>-0.0260(0.0078)</td>
<td>-0.0217(0.0038)</td>
<td>-0.0241(0.0038)</td>
<td>-0.0477(0.0347)</td>
<td>-0.0252(0.0059)</td>
<td>-0.0222(0.0039)</td>
</tr>
</tbody>
</table>

Values are means; SEs are in parentheses. ARMA, autoregressive moving average analysis; CVRi, cerebrovascular resistance index; U, units; BPmca, arterial blood pressure corrected at level of middle cerebral artery; MFV, mean flow velocity. For both day 1 and day 2, 1 and 2 refer to 1st and 2nd 2-breath series. *Significant difference across trials. No solution was found in 1 subject (†) and 3 subjects (‡).
Table 2. Responses to sustained breathing of 7% CO₂

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>7% CO₂</th>
<th>∆U/mmHg PCO₂</th>
<th>%∆/mmHg PCO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFV, cm/s</td>
<td>60.6(4.8)</td>
<td>87.1*(6.8)</td>
<td>2.03*(0.26)</td>
<td>3.4(0.4)</td>
</tr>
<tr>
<td>BPMCA, mmHg</td>
<td>78.4(5.8)</td>
<td>87.1*(6.1)</td>
<td>0.51(0.32)</td>
<td>0.70(0.4)</td>
</tr>
<tr>
<td>CVRi, mmHg·cm⁻¹·s⁻¹</td>
<td>1.4(0.2)</td>
<td>1.0*(0.1)</td>
<td>-0.0267(0.0049)</td>
<td>-1.9(0.26)</td>
</tr>
</tbody>
</table>

Values are means; SEs are in parentheses. *Significant difference between baseline and 7% sustained CO₂; †significant difference between change in measured values during 7% CO₂ per mmHg PCO₂ and PCO₂ → MFV ARMA step responses in Table 1 (except day 2 baseline). 7% CO₂ = 12.7 ± 1.4 mmHg PCO₂.

DISCUSSION

Results from our new two-breath method support the hypothesis that solutions for both the BPMCA and PCO₂ effects on the cerebrovascular response could be obtained without causing a confounding effect on mean BPMCA. We determined that reliable estimates of autoregulatory gain could be determined from the impulse response functions for BPMCA → MFV and BPMCA → CVRi under both spontaneous baseline measurements as well as during the two-breath method. However, the two-breath method was required to yield accurate and reliable estimates of the PCO₂ step response. The closed-loop analysis approach had a distinct advantage over sustained breathing of a CO₂ gas mixture. We found a significantly greater gain for PCO₂ → MFV but similar gain for PCO₂ → CVRi during sustained breathing of 7% CO₂ than from the ARMA model. This suggests that part of the increase in MFV during the sustained CO₂ tests was probably a consequence of the elevated BPMCA and that it did not reflect exclusively CO₂ responsiveness.

The results of the BPMCA → MFV and BPMCA → CVRi impulse and step relationships were similar during baseline and with the two-breath method, and the results were consistent with the concept of cerebrovascular autoregulation. The rapid BPMCA → MFV impulse supports the concept that autoregulation operates as a high-pass filter (13). That is, faster oscillations in BPMCA are passed through to the cerebral circulation and are observed within the MFV signal. In contrast, the BPMCA → MFV step response shows that MFV is returned close to baseline in the face of sustained increases in BPMCA. The time courses of these responses are consistent with the proposal of Aaslid et al. (1) that an initial autoregulatory response could occur within a few seconds but may not be complete for up to 15 s. The adaptation of CVRi to the sustained increase in BPMCA as shown by the step response (Fig. 3C) accounts for the return of MFV toward baseline.

Table 3. Intraclass correlation coefficient for ARMA models

<table>
<thead>
<tr>
<th>Model</th>
<th>Impulse</th>
<th>Integrated Step</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPMCA → MFV, cm/s · mmHg⁻¹</td>
<td>0.94</td>
<td>0.83</td>
</tr>
<tr>
<td>BPMCA → CVRi, CVRi U/mmHg</td>
<td>0.75</td>
<td>0.51</td>
</tr>
<tr>
<td>PCO₂ → MFV, cm/s · mmHg PCO₂⁻¹</td>
<td>0.89</td>
<td>0.94</td>
</tr>
<tr>
<td>PCO₂ → CVRi, CVRi U/mmHg PCO₂</td>
<td>0.78</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Values >0.75 represent excellent reliability (29).

The absence of a solution for the PCO₂ effect when modeling BPMCA + PCO₂ → MFV has been attributed to the limited amplitude and bandwidth of the PCO₂ signal (29). In the present experiment, we successfully increased PCO₂ total autospectral power with the two-breath method compared with baseline measures. Insensitivity of two breaths of 10% CO₂ from a bag caused a decrease in CVRi, which, in turn, modified MFV (Fig. 1). However, BPMCA total autospectral power remained unchanged from baseline measures during the two-breath method (Fig. 2), suggesting that the short duration of the PCO₂ stimulus was not sufficient to evoke a blood pressure response. In contrast, the mean value of BPMCA increased by 0.51 ± 0.32 mmHg per millimeter Hg PCO₂ under sustained 7% CO₂ breathing, complicating the interpretation of the true CO₂ effect on MFV.

In further support of the argument that modeling the MFV response to BPMCA and PCO₂ was limited by insufficient amplitude and bandwidth of the PCO₂ signal (23), we found a solution for PCO₂ in only six of the nine subjects under resting baseline conditions. When the amplitude of the PCO₂ signal was increased with the two-breath method, a solution was found in all cases. Furthermore, the impulse solutions under baseline conditions were often of poor quality, leading to the high variability observed in Table 1.

The relative gain for the PCO₂ → MFV relationship calculated during the sustained breathing of 7% CO₂ was similar to previous reports (5). This value was greater than that determined with the ARMA model, suggesting that some of the increase in MFV during the sustained 7% CO₂ test was due to elevation in BPMCA observed here and in other studies (12). The ability of the ARMA model to assign gain to the different inputs has also been demonstrated during investigations of the heart rate control by the arterial baroreflex where heart rate is influenced by both arterial blood pressure and the direct effect of respiration (20, 24).

The gain for the PCO₂ → MFV relationship has often been evaluated by breathing a constant level of inspired CO₂. In the current study, the relative gain determined during the sustained breathing of 7% CO₂ was similar to previous reports (5), but we observed that absolute gain was lower when we determined the ARMA solution. Consistent with previous investigations (12, 14), we observed an increase in BPMCA. Thus, for investigating the relationship with ARMA, which assigns cause between the input and output variables
(20, 24), some of the increase in MFV was attributed to the increase in BP_{MCA} as well as the increase in PCO_{2}. The gain for the PCO_{2} \rightarrow CVR_{i} was slightly but not significantly greater during sustained 7% CO_{2} breathing than with the ARMA model. Although BP_{MCA} and MFV are the measured variables, CVR_{i} is the manipulated variable that responds to change in BP_{MCA} or PCO_{2} and has been shown to provide insight into the dynamics of the cerebrovasculature, for example under conditions of altered arterial PCO_{2} (11) or with rapid release of thigh cuffs (1).

**Methodological considerations.** Transcranial Doppler ultrasound, with its high temporal resolution, combined with continuous, noninvasive blood pressure recordings have become widely used and accepted as a means of evaluating dynamic cerebrovascular autoregulation. However, Doppler ultrasound measures velocity and not flow. This issue has been extensively addressed in previous publications on dynamic autoregulation (32, 34), and recently Serrador et al. (28) reported that diameter of the MCA measured by magnetic resonance imaging under an orthostatic challenge and different levels of PCO_{2} changed at most 0.1 mm from a mean value of 2.9 \pm 0.4 mm. This further suggests that this artery remains stable under a range of cerebral blood flow and under the conditions of the current experiments.

The use of CVR_{i} as an index of cerebrovascular resistance has been discussed previously (1, 11, 15, 32). True cerebrovascular resistance depends on the pressure gradient across the vascular bed and flow through that bed. This pressure gradient was unknown as venous and intracranial pressure could not be measured in our healthy subjects. However, our subjects remained motionless in a seated position where the venous influences on resistance and flow should be fairly constant.

**Perspectives**

Our new two-breath method combined with ARMA analysis offers a number of advantages over previous approaches for characterizing the BP_{MCA} and PCO_{2} effects on the cerebrovasculature. For example, methods relying on pharmaceutical interventions or orthostatic challenge models may not be appropriate for some patient populations. Furthermore, CO_{2} sensitivity tests with constant inspired CO_{2} increase BP_{MCA}, confounding the relationship between PCO_{2} and MFV (14). Bolus injections of acetazolamide have been employed clinically to investigate the cerebrovascular reserve, for example in patients with carotid occlusive disease (25). This method does increase cerebral flow in the absence of change in arterial blood pressure (7), but there is controversy over whether it reflects the same dilatory mechanism investigated by increased PCO_{2} (7, 19). The two-breath method, in contrast to sustained increases in PCO_{2}, does not cause systematic elevation in BP_{MCA} and in addition offers the ability to clearly separate system gain for the simultaneous inputs of PCO_{2} and BP_{MCA}. This latter property is functionally important for evaluation of autoregulation. Further investigation is required to determine if the two-breath method is preferable to the acetazolamide test under conditions such as after head injury (16) given potential differences in cerebrovascular CO_{2} sensitivity and autoregulation (9, 31). The ARMA method might also prove to be valuable under conditions where there are spontaneous fluctuations in PCO_{2}, eliminating the need for a two-breath manipulation of CO_{2}, for example before the onset of orthostatic vasovagal syncope (4, 6).

We believe that the two-breath method can be applied under a range of conditions in healthy and patient populations and that it can be used with ARMA analysis to successfully extract information on cerebrovascular control. The two-breath technique is noninvasive and reproducible, and the results compare favorably with previously established reports of cerebral autoregulation in healthy persons.

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Flexible Matlab coding for the ARMA analysis is available from the authors on request.

**REFERENCES**


