The CO₂ stimulus for cerebrovascular reactivity: Fixing inspired concentrations vs. targeting end-tidal partial pressures

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Abstract
Cerebrovascular reactivity (CVR) studies have elucidated the physiology and pathophysiology of cerebral blood flow regulation. A non-invasive, high spatial resolution approach uses carbon dioxide (CO₂) as the vasoactive stimulus and magnetic resonance techniques to estimate the cerebral blood flow response. CVR is assessed as the ratio response change to stimulus change. Precise control of the stimulus is sought to minimize CVR variability between tests, and show functional differences. Computerized methods targeting end-tidal CO₂ partial pressures are precise, but expensive. Simpler, improvised methods that fix the inspired CO₂ concentrations have been recommended as less expensive, and so more widely accessible. However, these methods have drawbacks that have not been previously presented by those that advocate their use, or those that employ them in their studies. As one of the developers of a computerized method, I provide my perspective on the trade-offs between these two methods. The main concern is that declaring the precision of fixed inspired concentration of CO₂ is misleading: it does not, as implied, translate to precise control of the actual vasoactive stimulus – the arterial partial pressure of CO₂. The inherent test-to-test, and therefore subject-to-subject variability, precludes clinical application of findings. Moreover, improvised methods imply widespread duplication of development, assembly time and costs, yet lack uniformity and quality control. A tabular comparison between approaches is provided.

Keywords
Cerebrovascular reactivity, carbon dioxide, cerebral blood flow, carbogen, end-tidal forcing, end-tidal targeting

Introduction
The use of cerebral vascular reactivity (CVR), the change in blood flow normalized for a change in stimulus, has long been used to elucidate underlying brain vascular physiology and pathophysiology in the presence of neurovascular disease. Changing the arterial carbon dioxide partial pressure (PaCO₂), the actual vasoactive stimulus in a controllable fashion, and magnetic resonance imaging (MRI), to provide a high spatial and time resolved measure of cerebral blood flow, is progressively more popular in non-invasive CVR studies. However, translation from physiological to clinical investigations will require precise PaCO₂ changes in order to minimize test–retest variability.

Methods to implement hypercapnic (increased PaCO₂) stimuli have long depended heavily on techniques adopted from the field of respiratory physiology. In recent decades, these have included the development of complex and expensive computerized systems targeting end-tidal (end-exhalation) PCO₂ (PETCO₂) such as dynamic end-tidal forcing, and prospective targeting. CVR investigators have recently attempted to circumvent the complexity and expense of these systems by reverting to simpler respiratory circuits that administer fixed concentrations of inspired gases. Although it is readily acknowledged that the performances of these ‘simpler’ circuits do not match those of the computer-
controlled systems, a comprehensive presentation of their limitations has not been presented. It has been argued that their simplicity, low cost, MRI compatibility, and ready availability of components are sufficient to commend them as alternative means to generate stimuli for the measure of CVR.

In this opinion essay, I provide (a) a comprehensive list of the limitations of circuits using fixed inspired concentrations of gases to target arterial gas concentrations; (b) general descriptions of the computer-controlled systems of dynamic end-tidal forcing and prospective targeting, and (c) comparisons of the performance, convenience, and cost of a computerized system (prospective targeting, which is still available for research) to those providing fixed inspired concentrations of gases. I conclude that a requirement of an accurately targetable and repeatable stimulus cannot be met using fixed inspired gas concentration circuits.

**Fixed inspired gas concentration circuits**

Two recent examples of respiratory circuits advocated for the control inspired gases for CVR using MRI are shown in Figure 1. Both methods, adopted from early respiratory experiments, use non-rebreathing valves, a fixed flow of a gas with fixed CO2 and O2 concentrations, and an inspired gas reservoir – one a flexible bag and the other, a rigid tube – to provide a constant inspired fractional concentrations of CO2 (FiCO2) and O2 (FiO2).

With these circuits it might be assumed that a fixed FiCO2 provides a standardized, predictable PETCO2.
However, that is not the case as is evident from a consideration of the alveolar gas equation

\[ F_{ETCO_2} = F_{ICO_2} + VCO_2 / VA \]

\( VCO_2 \) is minute CO₂ production, characteristic of a subject’s metabolism; \( VA \) is alveolar ventilation, the volume of gas per minute entering and exiting the alveoli for gas exchange with the blood.

Note that \( F_{ETCO_2} \) is \( P_{ETCO_2} / \) barometric pressure; similarly \( F_{ICO_2} = \) inspired \( PCO_2 / \) barometric pressure.

In a subject at rest, \( VCO_2 \) is relatively constant, but \( VA \) varies from person to person in an unknown manner because the respiratory chemoreflex ventilatory response to CO₂ varies from person to person. As a result, a constant \( F_{ICO_2} \) does not result in a constant, or predictable, \( PETCO_2 \). This can be appreciated intuitively by considering that we all breathe a constant \( F_{ICO_2} (0.039 \text{ CO}_2) \) in breathing ambient air, yet the \( PETCO_2 \) varies throughout the population. The same holds true for any fixed \( F_{ICO_2} \), as Figure 2 illustrates. More to the point, a fixed \( F_{ICO_2} \) does not produce a consistent \( PaCO_2 \) (see Figure 2). The gradient between \( PETCO_2 \) and \( PaCO_2 \) varies between people and varies at different \( PETCO_2 \). Therefore, \( F_{ICO_2} \) cannot be applied to target any particular \( PETCO_2 \) or \( PaCO_2 \).

There are additional confounding aspects to using fixed \( F_{ICO_2} \) as a repeatable stimulus. First, step changes in \( PETCO_2 \) or \( PaCO_2 \) (i.e., to a plateau value) cannot be achieved because the volume of the bag-lung system buffers the rate of change in lung gas concentrations, producing instead an exponential rise. This is illustrated in Figure 4 in Tancredi et al., using the circuit shown in Figure 1(B) and 5% CO₂ as the inspired gas mixture. Second, despite maintenance of a constant \( F_{ICO_2} \), a steady stimulus is not achieved, because \( PETCO_2 \) varies from breath to breath due to normal changes in ventilation and breathing pattern affects \( PETCO_2 \). Figure 2 in Lu et al., using the circuit shown in Figure 1(A) and Figure 4 in Tancredi et al., using the circuit shown in Figure 1(B) show the breath-by-breath variability of \( PETCO_2 \), which is apparent even after averaging is applied. Indeed, using an open-ended tube as a reservoir (Figure 1(B)) may not even maintain a constant \( F_{ICO_2} \) as Figure 4 in Tancredi et al. shows. This variability is caused by the diffusion and mixing of the gas in the reservoir tube with room air during ventilatory excursions, which themselves vary breath to breath. Finally, it should also be apparent that

**Figure 3.** The dynamic end-tidal forcing system adapted from Wise et al. O₂, CO₂, and N₂ are blended breath-by-breath to provide the respective inspired gas concentrations. Inspired gas concentrations are calculated from the respective exhaled gas concentrations of the preceding breath and the target gas concentrations. High inspiratory flows are required to meet peak inspiratory flows. Gases are dry and require efficient humidification.
changes in PETCO₂ in turn alter minute ventilation, and thereby also PETO₂. Isoxia therefore cannot be maintained with fixed inspired gases (e.g., see Bulte et al.¹⁶).

Implications of unknown, variable and inconsistent stimuli:

a. Effect of accuracy of vasoactive stimulus on CVR. In considering the accuracy and reproducibility of CVR, we need to consider both the accuracy of the stimulus and that of the blood flow measurement. The inaccuracy of each component is additive in the calculation of CVR. Making one component more precise markedly increases the precision of CVR even if the other is unchanged.

b. Cost of research. The greater the variability of the stimulus, the greater the number of subjects required in a clinical investigation.

c. Clinical implications. Few conclusions can be drawn from a single test in a single subject if there is high variability in the test. This precludes any clinical application.

Computer-based gas control systems

Both computer-based gas control systems, dynamic end-tidal forcing and prospective targeting, are capable of providing precise and repeatable sequences of PETCO₂ and PETO₂, with low breath-to-breath variability. The prospective targeting method employs sequential rebreathing, which has been shown to equalize PETCO₂ and PaCO₂.¹⁷-¹⁹ These systems can produce a variety of stimulus patterns, including rapid changes in end-tidal gases, as well as targeting PETCO₂ and PETO₂ independently. The latter ability allows maintenance of isocapnia with changes of PETO₂ and isoxia with changes of PETCO₂. Figures 3 and 4 show the dynamic end-tidal forcing, and prospective targeting systems, respectively. These systems are generally capable of providing repeatable, complex, stimulus patterns as Figures 5 and 6 demonstrate for the prospective targeting system. However, some operator expertise and experience with the particular computerized system, and some minimal patient cooperation (i.e., continuing to breathe at their resting

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Figure 4. The prospective targeting system (RespirAct™). The system consists of a gas blender and a breathing circuit. Gas A is a blend of O₂ and N₂. Gas B is a blend of O₂ and CO₂ with balance N₂. Gas C is O₂. All gases contain O₂ as a safety measure. A computer pre-calculates the breath-by-breath inspired gas concentrations and flow to attain end-tidal gas concentration targets and controls the gas blender delivery to a breathing circuit. The breathing circuit provides sequential gas delivery as follows. The subject exhales to the expiratory reservoir. During exhalation, the inspiratory reservoir fills with blended gas. On inspiration, the inspiratory reservoir is emptied and any additional gas is inhaled from the expiratory reservoir. Gas inhaled from the expiratory reservoir has already equilibrated with the blood and does not affect gas exchange, so that the gas inspired from the blender constitutes entirely of VA (see text). Thus, considering the alveolar gas equation (FETCO₂ = FiCO₂ + VCO₂/VA), VCO₂ is a user input function, and VA is imposed by the gas blender; control of these variables enables the targeting of PETCO₂ and PETO₂ independent of minute ventilation and breathing pattern. For further details see literatures.⁴,⁵,¹⁵
Figure 5. Screen capture of sinusoidal changes in \( \text{PETCO}_2 \) and \( \text{PETO}_2 \) produced using the RespirAct™. (a) compressed time course; (b) expanded time course. Note the precise breath-to-breath changes and lack of breath-to-breath variability during the steady segments. Note also that changes in \( \text{PETCO}_2 \) and \( \text{PETO}_2 \) are independent of each other. Red tracing is tidal PCO\(_2\); blue dots are end-tidal values. Green tracing is tidal PO\(_2\); red dots are end-tidal values.
**Figure 6.** Screen capture of end-tidal targeting using RespirAct\textsuperscript{TM}. Simultaneous tracing from a step algorithm targeting sharp step changes in PCO\textsubscript{2} and PO\textsubscript{2}. Typically (but not always) PCO\textsubscript{2} transitions occur within 1–3 breaths. Red tracing is tidal PCO\textsubscript{2}; blue dots are end-tidal values. Green tracing is tidal PO\textsubscript{2}; red dots are end-tidal values.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Fixed inspired CO\textsubscript{2} methods</th>
<th>Computerized: e.g. prospective targeting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attain a target PaCO\textsubscript{2}?</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>Designate a target PetO\textsubscript{2}</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>Breath-to-breath PetCO\textsubscript{2} variability in the steady state</td>
<td>~4 mmHg</td>
<td>&lt;1 mmHg</td>
</tr>
<tr>
<td>Time to achieve a steady state PCO\textsubscript{2} (ramp protocol)</td>
<td>Many minutes\textsuperscript{1,20}</td>
<td>1–3 breaths (see Figure 6)</td>
</tr>
<tr>
<td>Maintain steady change in PO\textsubscript{2}</td>
<td>NO</td>
<td>YES</td>
</tr>
</tbody>
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(continued)
rate or greater) are required to obtain consistent, optimal control of gas targeting.

**Concluding comments**

There are four factors which affect \( \text{PETCO}_2 \): (1) the metabolic \( \text{CO}_2 \) production rate (\( \text{VCO}_2 \)); (2) the \( \text{FiCO}_2 \); (3) the time constant of gas exchange in the lungs; and (4) the alveolar ventilation (\( \text{VA} \)). While (1) and (2) are, or can be made, constant, (3) prevents the possibility of an abrupt change \( \text{PETCO}_2 \) or \( \text{PETO}_2 \) resulting from an abrupt change in inspired gas concentrations. As the extent or pattern of ventilation (and therefore \( \text{VA} \)) cannot be controlled in spontaneously breathing subjects, so \( \text{PETCO}_2 \), \( \text{PaCO}_2 \), cannot be targeted with any chosen \( \text{FiCO}_2 \) and \( \text{FiO}_2 \). Finally, the gradient between \( \text{PETCO}_2 \) (which is measured) and \( \text{PaCO}_2 \) (which determines the hemodynamic response) is not known. Thus, standardized and repeatable stimuli unfortunately require more complex and expensive computer-controlled systems. A detailed comparison of issues such as complexity/simplicity,
effectiveness and cost between the fixed inspired concentration methods and computer-controlled prospective targeting is provided in Table 1.

**Declaration of conflicting interests**
The author declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: JAF is Chief Scientist at Thornhill Research Inc. (TRI), a for profit spin-off company from the University Health Network that developed the RespirAct™. RespirAct™ has been a non-commercial research tool assembled, and made available by TRI to research institutions to enable CVR studies.

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**References**