Measuring Cerebrovascular Reactivity: Sixteen Avoidable Pitfalls

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Abstract: An increase in arterial PCO$_2$ is the most common stressor used to increase cerebral blood flow for assessing cerebral vascular reactivity (CVR). That CO$_2$ is readily obtained, inexpensive, easy to administer, and safe to inhale belies the difficulties in extracting scientifically and clinically relevant information from the resulting flow responses. Over the past two decades, we have studied more than 2,000 individuals, most with cervical and cerebral vascular pathology using CO$_2$ as the vasoactive agent and blood oxygen-level-dependent magnetic resonance imaging signal as the flow surrogate. The ability to deliver different forms of precise hypercapnic stimuli enabled systematic exploration of the blood flow-related signal changes. We learned the effect on CVR of particular aspects of the stimulus such as the arterial partial pressure of oxygen, the baseline PCO$_2$, and the magnitude, rate, and pattern of its change. Similarly, we learned to interpret aspects of the flow response such as its magnitude, and the speed and direction of change. Finally, we were able to test whether the response falls into a normal range. Here, we present a review of our accumulated insight as 16 "lessons learned." We hope many of these insights are sufficiently general to apply to a range of types of CO$_2$-based vasoactive stimuli and perfusion metrics used for CVR.

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Measuring Cerebrovascular Reactivity: Sixteen Avoidable Pitfalls

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An increase in arterial PCO\textsubscript{2} is the most common stressor used to increase cerebral blood flow for assessing cerebral vascular reactivity (CVR). That CO\textsubscript{2} is readily obtained, inexpensive, easy to administer, and safe to inhale belies the difficulties in extracting scientifically and clinically relevant information from the resulting flow responses. Over the past two decades, we have studied more than 2,000 individuals, most with cervical and cerebral vascular pathology using CO\textsubscript{2} as the vasoactive agent and blood oxygen-level-dependent magnetic resonance imaging signal as the flow surrogate. The ability to deliver different forms of precise hypercapnic stimuli enabled systematic exploration of the blood flow-related signal changes. We learned the effect on CVR of particular aspects of the stimulus such as the arterial partial pressure of oxygen, the baseline PCO\textsubscript{2}, and the magnitude, rate, and pattern of its change. Similarly, we learned to interpret aspects of the flow response such as its magnitude, and the speed and direction of change. Finally, we were able to test whether the response falls into a normal range. Here, we present a review of our accumulated insight as 16 “lessons learned.” We hope many of these insights are sufficiently general to apply to a range of types of CO\textsubscript{2}-based vasoactive stimuli and perfusion metrics used for CVR.

Keywords: carbon dioxide, cerebral blood flow, vascular responses, cerebrovascular reactivity, cerebrovascular reactivity to carbon dioxide

INTRODUCTION

For the last two decades, our laboratory has been engaged in interrogating cerebral vascular function. The overarching approach has been to observe changes in regional brain flow in response to a vasoactive stimulus. This is referred to as cerebrovascular reactivity, or cerebral vascular reactivity (CVR). There are a variety of well-described vasoactive stimuli and outcome measures. Our laboratory employs precise targeting of end-tidal PCO\textsubscript{2} (P\textsubscript{ET}CO\textsubscript{2}) and blood oxygen-level-dependent (BOLD) magnetic resonance imaging (MRI) as the surrogate measure of cerebral blood flow (CBF). The ability to precisely duplicate a stimulus has enabled us to, retrospectively and prospectively, re-examine the way we generate and interpret our data. Indeed, over time we have identified unwarranted assumptions—including some in which we had high levels of confidence that led to weak methodology and misguided data analysis, and conclusions, regrettably, some after they appeared in our own published work. We have
published most of these insights in multiple separate papers. Nevertheless, we thought that it would be useful to the CVR community for us to review these insights in summary format in one paper. We believe the principles can selectively inform on the strengths and limitations of other CVR studies performed under a range of stimuli such as infusion of acetazolamide, breath hold, inspiration of fixed inspired PCO$_2$ (FiCO$_2$), and with the use of outcome measures such as transcranial Doppler (TCD) and various MRI methods.

Stimulus Response
Cerebral vascular reactivity is a provocative cerebral vascular test analogous to a cardiac stress test. For both, provocation is required to elicit a flow response exceeding baseline perfusion to ascertain the flow reserve. In the case of the cardiac stress testing, treadmill exercise or vasoactive agent stressors indicate the presence of flow deficits in the form of chest pain, ECG changes, or flow reductions in cardiac perfusion imaging. The results are then interpreted using angiographic findings of the vascular patho-anatomy.

Stressors
For studies of the heart, a standard stimulus may consist of aerobic exercise pushed to the threshold where anaerobic metabolism becomes active. When using pharmacological vasoactive agents such as adenosine, regadenoson, and dipyridamole as standard stimuli, uniformity of the vasodilatory stimulus is achieved by supramaximal dosing, that is, the dose beyond which no further flow response occurs.

In the brain, activation of neurovascular coupling throughout the brain is not an option as there is no safe stimulus that can activate all neurons. However, global vasoactive stimulation can be affected pharmacologically by the intravenous injection of hypotensive agents or using carbonic anhydrase blockers such as acetazolamide. The former is not considered safe for this indication. Acetazolamide can be injected at supramaximal response doses, but its time course is not predictable (Dahl et al., 1995; Grossmann and Köberle, 2000) and results in frequent uncomfortable side effects (Ringelstein et al., 1992; Dahl et al., 1995; Grossmann and Köberle, 2000; Saito et al., 2011).

Hypercapnia, defined an increased arterial partial pressure of CO$_2$ (PaCO$_2$), is easily implemented, safe, well-tolerated, and is therefore, the most used stressor. Each mmHg increase in PaCO$_2$ increases CBF by ~6–8% (Kety and Schmidt, 1948; Willie et al., 2012; Al-Khazraji et al., 2019). Supramaximal levels of PaCO$_2$ (greater than 90 mmHg) (Reivich, 1964) cannot be used to obtain a standard stimulus as levels that exceed 50–60 mmHg are very uncomfortable (Willie et al., 2012) and levels acutely exceeding ~80 mmHg begin to cause confusion and unconsciousness. However, implementation of a known stimulus requires the ability to precisely target PaCO$_2$. This is not a trivial task. First, while it may seem that PaCO$_2$ should simply be a function of the inspired fractional concentration of CO$_2$ (FiCO$_2$), it is in fact also a function of the minute ventilation, which itself changes when inhaling CO$_2$ (Fisher, 2016). Since the change in minute ventilation in response to a change in FiCO$_2$ cannot be predicted, a particular PaCO$_2$ cannot be targeted by designating the FiCO$_2$ (Peebles et al., 2007).

Measuring PaCO$_2$, the independent variable of CBF, is problematic. The only non-invasive measure of PaCO$_2$ available is the P$_{ET}$CO$_2$, but, unfortunately, without rebreathing, it is not a reliable surrogate for PaCO$_2$ (Jones et al., 1972). The P$_{ET}$CO$_2$ does approach PaCO$_2$ only with complete rebreathing (Duffin and McAvoy, 1988), and prospective targeting using sequential gas delivery (SGD; Ito et al., 2008; Fisher et al., 2016). End-tidal forcing (Robbins et al., 1982) is able to target P$_{ET}$CO$_2$, but its equivalence to PaCO$_2$ has not been demonstrated (Jones et al., 1972; McDonald et al., 2002; Tymko et al., 2016). When using breath hold as the vasoactive stimulus, the P$_{ET}$CO$_2$ is related to breath hold duration, but PaCO$_2$ is unknown (Totaro et al., 1999).

Stress Indicators
The stress indicator, CBF, is measured indirectly by surrogate measures. All surrogates can be characterized by fidelity to flow and their limitations in this regard. TCD measures velocity with high temporal resolution. The output, however, reflects velocity only in a single arterial segment rather than the brain parenchyma. Even so, its relation to flow assumes no particular corresponding change in vessel diameter with the stimulus, which may not hold (Willie et al., 2012; Verbee et al., 2014; Al-Khazraji et al., 2019). In our laboratory, we use BOLD signal changes as surrogates for changes in flow. These signals have high temporal resolution and high spatial resolution, providing whole brain maps of tissue perfusion. Although BOLD reflects flow-induced deoxyhemoglobin dilution, to its credit the signal has a reasonably linear relation to flow at moderate levels of hypercapnia (Hoge et al., 1999). Both TCD and BOLD are internally consistent but are poorly correlated (Burley et al., 2020). Arterial spin labeling uses magnetic labeling of water protons as a flow tracer and is a very good measure of flow. However, its limitations for CVR include imperfect labeling efficiency that can change from patient to patient based on difference in anatomy, and the magnetic label decays as a function of T1-relaxation over time. Delays in arrival time caused by steno-occlusive disease can result in problems with labeled proton localization and increasing image noise.

CVR MEASURES

Basic CVR Measures
Failing precise targeting of PaCO$_2$, a reasonable fallback position is to assume that the ΔP$_{ET}$CO$_2$ is close to ΔPaCO$_2$ and therefore can be used to index the change in flow for the stimulus by dividing it by ΔP$_{ET}$CO$_2$ and defining the

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**Abbreviations:** BOLD, blood oxygen-level dependent; CBF, cerebral blood flow; CVR, cerebrovascular reactivity; FiCO$_2$, fractional concentration of inspired carbon dioxide; P$_{ET}$CO$_2$, end-tidal partial pressure of carbon dioxide; MAP, mean arterial pressure; MCA, middle cerebral artery; MCAw, middle cerebral artery velocity; MRI, magnetic resonance imaging; PaCO$_2$, arterial partial pressure of carbon dioxide; ROI, region of interest; SGD, sequential gas delivery.
stress indicator as the slope of line of best fit, CVR (Veselý et al., 2001; Sobczyk et al., 2014).

We initially considered the magnitude of the CVR as reflecting the vasodilatory vigor of the underlying vasculature (Bhogal et al., 2014). In patients with known vascular pathology, we identified specific regions of interest (ROI) in some patients where, rather than flow increasing in response to a stimulus, it declined. Such areas of “steal” in response to hypercapnia had been described more than 40 years ago (Brawley, 1968; Symon, 1969) and have been reviewed recently (Fisher et al., 2018).

Enhanced CVR Model and Physiological Interpretations

We also developed a more comprehensive model to explain additional behaviors of the vasculature during hypercapnia. This model consisted of blood vessels organized in a series of hierarchical vascular beds where each downstream bed has a greater flow potential than its supply vessels (Duffin et al., 2017, 2018). Consequently, on the application of a vasoactive stimulus, vascular territories perfused in parallel from a common source must compete for inflow such that increased inflow to beds capable of more robust vasodilation is at the expense of those with less robust vasodilation, i.e., with steal (Sobczyk et al., 2014). The presence of steal is known to be a strong marker for risk of stroke (Reinhard et al., 2014) and is therefore important to identify. Importantly, the absence of steal indicates sufficient collateral blood flow to meet the flow requirements downstream from the stenosis and seems protective for stroke (Bang et al., 2008; Sheth et al., 2016; Tan et al., 2016).

This view is the basic model we followed in studying CVR in the first 434 patients examined (Spano et al., 2013). While maintaining consistency in the methodology of our studies, we nevertheless explored alternatives to our initial underlying assumptions. The aim of the remainder of this paper assembles some of the subtle, but retrospectively obvious lessons we learned over the last two decades about optimizing the stimulus, developing new stress indicators, and furthering the understanding of cerebral vascular physiology.

The Two-Point Stimulus

As defined above, the vasoactive stimulus is the PaCO$_2$. Note that when the hypercapnic stressor is implemented by breath hold or fixing the FiCO$_2$, the magnitude of the stressor, that is, the change in the independent variable PaCO$_2$, is unknown. With breath hold, $\Delta$CBF is a function of time and can only be indexed by breath hold duration, which correlates poorly with $\Delta$PaCO$_2$. For FiCO$_2$ inhalation, $\Delta$P$_{ET}$CO$_2$ is known, but not $\Delta$PaCO$_2$. As such, CVR can only have a binary outcome: either positive or negative (steal absent or present). This information is retained despite the uncertainty in $\Delta$PaCO$_2$ because the denominator $\Delta$P$_{ET}$CO$_2$ is always positive, leaving the sign of the slope to depend only on the numerator, $\Delta$CBF.

CVR Beyond the Two-Point Stimulus

To obtain information beyond this binary labeling, we set out to address one major unknown by precisely targeting the PaCO$_2$. For this purpose, we developed a system involving SGD (Slessarev et al., 2007; Fisher et al., 2016). SGD enables the establishment of P$_{ET}$CO$_2$ within 2 mmHg of a target, independent of the level and pattern of breathing. A further benefit of SGD is that the difference between P$_{ET}$CO$_2$ and PaCO$_2$ falls within the range of error of the blood gas analyzer, making the equivalent value to PaCO$_2$ accessible non-invasively (Ito et al., 2008; Fierstra et al., 2011, 2013). Also employing SGD, Willie et al. (2012) reported a regression of PaCO$_2$ vs. P$_{ET}$CO$_2$ over a range of 15–65 mmHg had slope 0.88 with a $r^2$ of 0.98 and Bland–Altman analysis showing a bias where P$_{ET}$CO$_2$ exceeded PaCO$_2$ by about 5 mmHg at the highest PCO$_2$ levels.

Note that henceforth in this paper, when referring to a stressor administered via SGD, we will cite PaCO$_2$ when referring to the stimulus and P$_{ET}$CO$_2$ when referring to the measured parameter.

CVR in Health

In healthy people the relationship between PaCO$_2$ and CBF is sigmoidal with a midpoint close to baseline PaCO$_2$ showing vasodilatory and vasoconstrictor reserves (Battisti-Charbonney et al., 2011; Bhogal et al., 2014; Duffin et al., 2017). The sigmoid response characteristics vary between white matter and gray matter (Bhogal et al., 2015) and indeed between more specific anatomical locations (Sobczyk et al., 2015; van Niftrik et al., 2017; Duffin et al., 2018). Nevertheless, in health, the slope of a voxel-wise line of best fit between P$_{ET}$CO$_2$ and CBF provides a reasonably accurate, simplifying, linear representation of the sigmoidal CO$_2$-CBF response (Bhogal et al., 2014; Sobczyk et al., 2014). Sobczyk et al. (2015) and McKetton et al. (2018)
have provided an atlas mapping normative CVR with voxel-wise mean ± SD for CVR in health.

CVR in Neurovascular Disease

The Shape of the PCO$_2$-Flow Relationship

In the presence of neurovascular disease, the sigmoidal relationship of flow to PaCO$_2$ is degraded such that curves become flattened (Harper and Glass, 1965; Ringelstein et al., 1988; Sobczyk et al., 2014). Figure 1 represents flow in selected voxels over a range of PaCO$_2$ in a patient with cerebrovascular disease.

Note that a suitably strong stressor is required to stimulate a sufficiently robust vasodilation in healthier vascular territories such that blood flow is preferentially directed to those territories and away from those with a weaker vasodilatory response, resulting in reduced flow or steal. The greater the vasodilation in the healthy vasculature, the greater the sensitivity for exposure of vasodilatory reserve (see also Figure 3).

From these considerations, we have the first four lessons:

1. When CBF responses to changes in P$_{ET}$CO$_2$ are curved, a two-point stressor will result in a reduced CVR.
2. The less sigmoidal the flow vs. PaCO$_2$ curve, the more tenuous the connection between the CVR calculation and the vascular reactivity and the more the CVR is affected by the initial PaCO$_2$ and ΔPaCO$_2$ (see Figure 2).
3. Small differences in the ΔPaCO$_2$ (~2 mmHg) result in measurable changes in CVR throughout the hypocapnic and hypercapnic ranges (Figure 2).
4. The CVR depends on whether the ΔPaCO$_2$ is applied in the hypocapnic or hypercapnic range and the direction of change (see also Ide et al., 2003; Coverdale et al., 2014; Figure 3).

ASPECTS OF THE STIMULUS AFFECTING CVR

Baseline PCO$_2$ and CVR

Following from (2): What baseline PCO$_2$ should be used for measuring CVR? In the first decade, we assumed that the normal PCO$_2$ for humans was nominally 40 mmHg and would provide a universal starting point. However, over the years we found that resting P$_{ET}$CO$_2$ varied between 30 mmHg and over 47 mmHg, but each baseline P$_{ET}$CO$_2$ remained mostly constant over time, in some people over the many years in which we followed them.

The alternate approach we considered was that of identifying a participant's resting PCO$_2$ and applying a stimulus from resting P$_{ET}$CO$_2$ to resting + 10 mmHg. It was counterintuitive that a P$_{ET}$CO$_2$ stressor of 34 mmHg to 44 mmHg in one person with resting P$_{ET}$CO$_2$ of 34 mmHg would be equivalent to one of 44 mmHg to 54 mmHg in another with resting P$_{ET}$CO$_2$ of 44 mmHg. However, over time we were convinced that this was indeed the case and changed to a stressor P$_{ET}$CO$_2$ spanning the range from baseline to baseline + 10 mmHg. Figure 4 is a vivid illustration of the wisdom of this approach. These findings are consistent with the idea that people normalize their resting

![Figure 2](image-url)
The Effect of Blood Pressure on CVR

The brain is protected from hypoperfusion resulting from reductions in perfusion pressure by reflex vasodilation, mostly of small arterioles (50–200 μm) downstream from pial vessels (~300 μm; Kontos, 1989), a response termed “autoregulation” (Kulik et al., 2008). Hypercapnia is distressing for some subjects, and a few may respond with increases in blood pressure. Hypercapnia dilates both the pial and penetrating arterioles, blunting the autoregulatory vasoconstriction restraining increases in CBF due to increases in blood pressure. In the presence of hypercapnia, an apparent “autoregulatory break-through” may occur, as depicted in Figure 3.

From these considerations, we have the sixth lesson:

6. Monitor blood pressure during assessment of CVR. Interpret flow results in the presence of hypercapnia and hypertension with caution.

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FIGURE 3 | The effect of direction of change of PaCO\textsubscript{2} on CVR. The patient is the same individual whose data are shown in Figures 1, 2. The experimental paradigm of changes in P\textsubscript{ET}CO\textsubscript{2} is shown in red in the graph; BOLD signal changes are shown in blue. CVR maps shown in the lower figure correspond to the periods depicted on the graph, with arrows showing the direction of change in P\textsubscript{ET}CO\textsubscript{2}. (A) As P\textsubscript{ET}CO\textsubscript{2} is reduced from 40 mmHg to 30 mmHg, there is a symmetrical reduction in BOLD signal in the white and gray matter in both hemispheres. (B) The mirror image change in P\textsubscript{ET}CO\textsubscript{2} from 30 mmHg to 40 mmHg results in an asymmetrical change where flow increases in the left hemisphere while decreasing in the right (steal). The blood vessels in right hemisphere paradoxically increase their flow, a feature attributed to the improved perfusion pressure as left hemisphere vessels constrict. Modified from Sobczyk et al. (2014).

The Effect of PO\textsubscript{2} on CVR

When administering a fixed FrCO\textsubscript{2} such as “carbogen” (5% CO\textsubscript{2}), whether the balance is composed of air or O\textsubscript{2} is consequential. If the balance is O\textsubscript{2}, then compared to the balance being air, there will be a higher ventilatory response and, as a result, a smaller ΔPaCO\textsubscript{2} (Prisman et al., 2007; Fisher, 2016) and therefore a smaller ΔCBF. Concurrent hypoxia to arterial saturations below 70% may also increase the CBF response to hypercapnia (Poulin et al., 1996; Ainslie and Burgess, 2008; Mardimae et al., 2012; Willie et al., 2012).

Accordingly, seventh and eighth lessons are:

7. With fixed FrCO\textsubscript{2}, the minute ventilation, and therefore the PaCO\textsubscript{2}, depends on the inspired PO\textsubscript{2} and ventilatory response, neither of which can be predicted or corrected post hoc. This confounder should be considered for all fixed FrCO\textsubscript{2} protocols.

8. A fixed inspired PO\textsubscript{2} does not result in a fixed arterial PO\textsubscript{2}; the latter varies depending on the level of ventilation in a mechanism analogous to that of breathing a fixed FrCO\textsubscript{2}. Such small changes in PO\textsubscript{2} due to differences in ventilation have consequential changes for the CVR.

Non-sigmoidal Flow Responses and Calculation of CVR

In our early investigations of the way in which CBF responds to PaCO\textsubscript{2}, we applied a gradual “ramp” increase in PaCO\textsubscript{2} from resting baseline to baseline + 15 mmHg over 4 min (Sobczyk et al., 2014). The results made it clear that, in the presence of cerebrovascular disease, many vascular beds, some as large as an entire hemisphere, have complex flow responses that are decidedly not sigmoidal, nor could they be accurately summarized by a linear regression. We studied the voxel-by-voxel changes in signal over the range of P\textsubscript{ET}CO\textsubscript{2} (Fisher et al., 2017) and were able to classify the P\textsubscript{ET}CO\textsubscript{2} response patterns...
into four basic types: proportional to PaCO₂, biphasic rise-fall, negatively proportional to PaCO₂, and biphasic fall-rise (Figure 6).

By investigating the frequency and distribution of the various response patterns in healthy subjects and patients with cerebrovascular steno-occlusive disease, we found that the response types were not dispersed throughout the brain, but tended to cluster in large contiguous regions, strongly suggesting shared physiologic causes. Analyzing the biphasic curves as a linear regression can obscure important physiological information from the subsequently derived CVR maps (Fisher et al., 2017). Rather, multimodal analysis (transfer function analysis (Duffin et al., 2015) or vascular resistance analysis (Duffin et al., 2017, 2018) can be used to extract abundant additional nuanced information regarding vascular response functions.

From these investigations, we derived the additional lessons:

9. A ramp stimulus reveals the pattern of response to a range of PaCO₂ values.
10. A ramp stimulus to a PaCO₂ >10 mmHg above resting explores a higher range of vasodilatory reserve. This range of reserve would not be interrogated with a smaller stressor such as 5 mmHg.
11. A ramp stimulus generates the data in a form that can be analyzed for intrinsic vascular resistance (Duffin et al., 2017, 2018; McKetton et al., 2019).

A NEW CVR METRIC: SPEED OF RESPONSE

The calculation of CVR as the slope of a regression between PaCO₂ and flow becomes progressively less representative of vascular reactivity as the correlation diminishes. However, another, less apparent factor also affects the quality of representation, and likely a reliable indicator of vascular health in its own right—duration of full evolution of the CVR response after stimulus onset, i.e., the “speed of response.”

The effect of a slow response on the calculation of CVR is shown in Figure 7. The slow vascular response to a step change in P_{ET}CO₂ (Figure 7A1, green line) graphs a large range of delayed changes in BOLD signal at the 50-mmHg position on the abscissa (Figure 7A3, black dots) reducing the slope of the line of regression, compared to that of a rapid response (Figure 5 row B, red line, black dots). Clearly, a simple regression of all data points is not an accurate summary of the vascular response when the vascular response time to the stimulus is prolonged; a correction is therefore required.

Mathematical Correction of CVR for the Speed of Response

In Figure 7 we show the mathematical approach of Poublanc et al. (2015) for correcting CVR for a slow response. This process entails assuming the response is a first-order exponential and obtaining an index of that delay in the form of τ, the time constant required to match the response to the exponential function (Figures 7A2,B2). Caveat: Heretofore, we have used the input function containing both the rising and declining PaCO₂ in the calculation of τ in response to the step change in PaCO₂. This method assumes τ is the same in both directions. Figures 2, 3 provide reason to challenge this assumption in future analysis. Further mathematical treatments are needed. Transfer function analysis can be used to identify phase, gain, and coherence metrics (Duffin et al., 2015). Sinusoidal stimuli may be used (Blockley et al., 2011), and the linear fit between the observed BOLD signal and the arrival-time adjusted P_{ET}CO₂ convolved with the hemodynamic response function (Yao et al., 2021).

Physical Correction of CVR for the Speed of Response

The amplitude of CVR can also be corrected for speed of response by changing the P_{ET}CO₂ vs. time profile of the stressor from a step to a ramp (Figure 8). In the case of a slowly rising stressor, the effect of slowed response times on the CVR is reduced, resulting in an accurate calculation of amplitude of CVR (Poublanc et al., 2015).

Speed of Response as a Metric of Vascular Health

How quickly the vascular resistance in a voxel changes may be a metric of neurovascular health. We frequently observed a strong correlation between speed of response, CVR, and known vascular pathology. Our current impression is that of these three, the speed of response is the most sensitive to mapping the extent and grading the severity of pathology (Poublanc et al., 2015; Holmes et al., 2020), a conclusion also reached by others (van Niftrik et al., 2017). It is currently debated as to whether the τ slowing effect of vascular risk factors on parenchymal brain arteries can be separated from those of extra-parenchymal large vessel arterial steno-occlusive disease.
Measurement of the speed of response may be limited by the time taken for the stimulus to evolve to a steady state. Figures 7A2, B2 illustrates a range of the slowing of response to a square wave stimulus. If rather than a square wave, the PaCO\textsubscript{2} rises to a maximum with a time constant of \(\tau\)\textsubscript{stimulus}, the calculated \(\tau\)\textsubscript{response} cannot be less. With stressors such as fixed FiCO\textsubscript{2}, it is not possible to measure any \(\tau\)\textsubscript{response} less than the time constant of the lung wash-in to a new PCO\textsubscript{2}. In healthy young people, this is 20–30 s but is longer in older people and those with expiratory flow restrictions. Consequently, measuring the intrinsic speed of the vascular response requires a stressor with a very short \(\tau\)\textsubscript{stimulus} approaching a square wave, substantially changing from baseline to target PaCO\textsubscript{2} within one breath, as is possible with SGD (Fisher et al., 2016).

From these considerations, we have drawn three further lessons:

12. CVR has two independently measurable metrics: the amplitude of response and the speed of response.
13. The measure of the speed of vascular response to a stimulus requires a stimulus shorter than the fastest vascular response time constant to be measured.
14. To measure amplitude of change, speed of change can be accounted for mathematically or physically via a slow ramp or sinusoidal stimulus paradigms.

**ASSESSING SINGLE SUBJECTS/PATIENTS: WHAT IS NORMAL?**

The repeatability of a test in one person over time (Figure 9) or between two people is the key to clinical application. Assuming two subjects receive identical stimuli and are scanned with the same scanner parameters, the CVR of a ROI, or even that of a single voxel, should be comparable after accounting for day-to-day variations in subject physiology and scanner instabilities. Administering the same stimulus and using the same scan parameters over a cohort of healthy subjects enable the generation of a map of voxel-wise normative CVR values (as mean and standard deviation) controlled for anatomical location, age, sex, or any other factor. Collectively, such normative data are referred to as a “CVR atlas” (Sobczyk et al., 2015; Figure 10).
Performing repeated CVR tests in a cohort enables the documentation of the normal range of voxel-wise test–test variability. These data can be used to assess whether repeated tests in a single subject differ over and above that due to extrinsic causes (e.g., stimulus delivery and imaging devices) of test–retest variability and therefore attributable to a disease process or surgical intervention. Such determinations are pertinent in clinical assessment and drawing conclusions from clinical data (Sobczyk et al., 2016).

From these considerations, we draw the final two lessons:

15. A standard stimulus and uniform scan parameters enable construction of an atlas of normal values and their standard deviation.

16. A merged group atlas of normative CVR metrics enables scoring a single subject/patient in terms of standard deviation differences from normal responses, thus providing clinical utility important for judging the impact of steno-occlusive disease in that individual.

CONCLUSION

Standardization and reproducibility of the CO$_2$ stressor are fundamental to advance the use of CVR for understanding cerebral vascular physiology and pathophysiology and translating the science to the bedside.
We present 16 lessons (limitations, optimizations, and interpretations) acquired from two decades using CVR as a cerebrovascular stress test in about 2,000 patients with cerebrovascular disease, as well as several healthy subjects. We investigated the method of action, and thereby the optimization and limitations of the stressor, identified suitable outcome variables, and divined the sometimes very obscure physiology at which they hinted. Many, but not all, of these have been presented in one way or another in our peer-reviewed publications over the years and various journals but we believe will benefit from collation into one document. The sum of this work may lead to the development of a quantitative repeatable standardized CVR test for both clinical and research purposes. In patients, it can be used to detect and quantify hemodynamic insufficiency, follow the natural history of the disease in individual patients, and assess the response to interventions. For research, the ability to apply standardized CVR methodology for data
acquisition and quantitation is critical in any prospective clinical trial that assesses the natural history of disease and its management.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: Anonymized data will be shared by request from any qualified investigator for purposes such as replicating procedures and results presented in the article provided that data transfer is in agreement with the University Health Network and Health Canada legislation on the general data protection regulation. Requests to access these datasets should be directed to OS, olivia.sobczyk@uhn.ca.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University Health Network, Toronto, Canada. The patients/participants provided their written informed consent to participate in the studies. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

OS, JF, LV, JD, JAF and DJM drafted the manuscript. All authors participated in the feedback and writing process following the initial drafting of the manuscript.

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Conflict of Interest: JAF and DJM contributed to the development of the automated end-tidal targeting device, RespirAct™ (Thornhill Research Inc., TRI) used in the figure studies mentioned and have equity in the company. RespirAct™ is not currently a commercial product but is made available for REB-approved research. OS and JD received salary support from TRI. TRI provided no other support for the study.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer RH declared a past collaboration with one of the authors JAF to the handling editor.

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