Precision of Cerebrovascular Reactivity Assessment with Use of Different Quantification Methods for Hypercapnia Functional MR Imaging

S.D. Goode, S. Krishan, C. Alexakis, R. Mahajan and D.P. Auer

AJNR Am J Neuroradiol 2009, 30 (5) 972-977
doi: https://doi.org/10.3174/ajnr.A1496
http://www.ajnr.org/content/30/5/972
Cerebral blood flow (CBF) is tightly controlled to meet metabolic demands according to neural activity levels and to counterbalance systemic variation in blood pressure. Several control mechanisms are integrated to maintain adequate cerebral tissue perfusion. Metabolic, myogenic, and local mechanisms interact to maintain constant CBF despite wide variations in arterial blood pressure, referred to as cerebral autoregulation. Cerebrovascular reserve (CVR) reflects the spare capacity of the cerebral circulation to increase CBF in response to regulatory vasodilatory stimuli. The main cerebral vasodilatory stimulus is carbon dioxide (CO₂); hence, exogenous CO₂ and acetazolamide have been used to assess CVR. Alterations in these cerebral hemodynamic control parameters are present in various disease states with increasingly recognized pathophysiologic relevance. Tools for noninvasive mapping of cerebrovascular parameters including CVR would thus be desirable to complement emerging physiologic imaging techniques to predict tissue at hemodynamic risk.

Many techniques have been developed for noninvasive assessment of CVR. In a clinical setting, measurements of CVR are performed with use of transcranial Doppler (TCD), by quantifying change in flow velocity in the middle cerebral artery (MCA) on a vasodilatory challenge such as hypercapnia, apnea, or acetazolamide. Positron-emission tomography and single-photon emission CT (SPECT) have also been used to detect and locate quantitative brain perfusion changes with similar stimuli. However, these latter 2 techniques are not widely available, are expensive, and involve significant radiation, therefore limiting their clinical usability. More recently, the blood oxygen level–dependent (BOLD) functional MR imaging (fMRI) technique has been applied to assess CVR in healthy volunteers and in patients, but the quantitative reliability is uncertain. Recent publications have also shown the value of hypercapnia BOLD fMRI to predict normal and abnormal hemispheric CVR.

BOLD fMRI is sensitive to changes in the local deoxygenation concentration, which varies with perfusion and blood volume changes in a complex but qualitatively predictable manner. BOLD contrast is based on the fact that deoxygenation acts as an endogenous contrast agent. BOLD hypercapnia fMRI detects increase in tissue oxygenation resulting from increases in CBF induced by CO₂ inhalation while demands in oxidative metabolism remain constant. This mechanism leads to an observed BOLD signal intensity increase related to measurable T₂* prolongation. Over a reasonable range of increases in CO₂, there is a linear increase in CBF and cerebral blood volume (CBV) with an exponential plateauing close to maximal dilation. It has previously been assumed that BOLD signal intensity increases also linearly with CO₂, but this is speculative and more recent theoretic models of hemodynamic BOLD response suggest a nonlinear relationship.
Previous studies have used simple ratios to normalize fMRI response to varying levels of hypercapnia expressed as % BOLD signal intensity or %R2* change/mm Hg end-tidal (ET)CO2 change. The validity of this approach has not been tested.

There are potential advantages of this technique versus TCD because BOLD fMRI assesses the hemodynamic effects on tissue oxygenation rather than blood flow velocity. It is noteworthy that BOLD hypercapnia fMRI 1) affords whole-brain mapping and, hence, assessment of regional heterogeneity and 2) is sensitive to vasodilatory effects mediated by collateral arteries, inaccessible to TCD techniques. The disadvantage of hypercapnia fMRI is its semiquantitative nature and complex dependence on blood flow and volume changes and on the ratio between arterial and venous blood volume. Arterial spin-labeling (ASL) would be a quantitative method to directly assess CBF and, hence, CVR; however ASL has poor signal-to-noise intensity at 1.5T, which is still currently the main clinical platform for MR imaging.

Current clinical practice is to increasingly offer brain MR imaging to patients with cerebral ischemia (stroke and transient ischemic attack) that would allow for uncomplicated routine use of an integrated CVR assessment. Application of such a technique to map CVR would be extremely useful in patients with a hemodynamically significant brain lesion such as an arteriovenous malformation whereby the normal autoregulatory mechanisms have been altered, or in patients with intracranial or extracranial steno-occlusive disease to evaluate future stroke risk, perhaps even as a risk stratification tool for carotid intervention. However, the clinical usefulness of such a test is dependent on sufficient reliability and reproducibility of the diagnostic test.

Therefore, the aim of our study was to assess the reliability of a standardized quantitative hypercapnia fMRI protocol with use of a clinical 1.5T scanner. Specifically, we aimed to assess intrasubject and intersubject reproducibility for different analysis techniques and CO2 normalization. For the different analysis techniques, we determined BOLD amplitude changes by using a visually defined block design or automated regression to ETCO2.

Materials and Methods

Study Sample
Sixteen healthy volunteers (7 women and 9 men with a mean age of 27.7 years (age range, 19–34 years)) without neurologic signs and symptoms and no medical or drug history were included in the study after giving informed written consent. All subjects were medication free and were asked not to consume caffeine for the 4 hours before the scanning session. Eight subjects underwent subsequent scans on the same day after a 30-minute break outside the scanner. The hospital research and development department and medical school research ethics committee approved the study.

MR Imaging
MR imaging studies were performed on a clinical 1.5T scanner (Intra; Philips, Best, the Netherlands). A standard 8-channel head coil was used for imaging, with use of a gradient-echo echo-planar sequence with the following parameters: TR, 3500 ms; TE, 60 ms; flip angle, 90°; matrix size, 64 × 64; FOV, 192 mm; sections, 33; thickness, 3 mm; and no gap. A total of 160 volumes (approximately 9 minutes acquisition time) were acquired for each experiment.

CO2 Stimulation
We controlled ETCO2 by using a standard nonrebreathing anesthetic circuit with 2 one-way valves to prevent rebreathing. The circuit incorporated a reservoir bag of 2 L to compensate for any large tidal volume breaths. Subjects breathed through a comfortably secure standard anesthetic mask to ensure a closed circuit. ETCO2 was continuously monitored via a sampling tube at the mouthpiece level. ETCO2 recordings were made during the entire experiment, and a reading was taken every 3.5 s to correlate with the length of time of each volume acquisition or 1 dynamic (ie, 1 TR).

We used free gas flow of 1-L/min O2 and 8-L/min air and then provided a fixed amount of CO2 (1 L/min), therefore providing 10% CO2. Control of CO2 gas flow was via an external source outside of the MR imaging scanner. We used high 10% CO2 to achieve maximal vasodilation, and we monitored the ETCO2 level aiming for an increase in ETCO2 between a 7- and 8-mm Hg change. This was performed by 2 experienced and medically qualified practitioners (S.D.G., S.K.) who were familiar with the experiment setup. During the 9-minute long paradigm, all of the subjects underwent 2 periods of hypercapnia and 3 periods of normocapnia. We also continuously monitored the subjects’ blood pressure, pulse, and arterial oxygen saturations continuously by using a MR compatible device (In vivo; Siemens, Malvern, Pa).

Data Analysis
We analyzed all data using FSL software (http://www.fmrib.ox.ac.uk/fsl/), FMRI Expert Analysis Tool (FEAT) deploying standard preprocessing with high-pass filtering, and smoothing and motion correction using MCFLIRT. During the FSL analysis, the ETCO2 data acquired during the scanning session are incorporated into the design matrix of the general linear model by the 2 methods described below. A Gaussian model was used for modeling the hemodynamic response function. A limitation of our CO2 setup included a sampling delay in ETCO2 from patient to capnometer outside the scanner room. There was also a second time lag of BOLD signal intensity change after increase in ETCO2 level. The FSL analysis method compensated for these time lags by allowing for a temporal derivative of the design matrix waveform. This essentially shifts the waveform slightly in time during the analysis to enable a better fit of the data and model.

Fig 1. Example of boxcar analysis method showing BOLD signal intensity timecourse and corresponding ETCO2 model.
We found a significant linear correlation between PSC and ETCO2 change for both methods of BOLD amplitude estimation ($R^2 = 0.244$ ($P = .0001$) for the boxcar method and $R^2 = 0.18$ ($P = .003$) for the automated method (see Fig 4 for boxcar method). To further explore the possibility of a nonlinear relationship between PSC and $\Delta_{ET}CO_2$ change, we tested the data using a quadratic term and found no significant relationship.

**Reliability of Boxcar vs Automated Determination of Absolute PSC**

There was a small but significant bias toward higher absolute PSC when using the boxcar compared with the regression model that failed to reach significance for whole-brain gray matter (Table 1). A good test-retest short-term reproducibility was found for absolute PSC for both the boxcar and regression analysis methods (range, 4.8% to 10% COV; Table 2).

Intrasession, interhemispheric reproducibility of absolute PSC was excellent (range, 1.2% to 2.2% COV) depending on tissue and analysis method. Correlation between hemispheric absolute PSC was very high for both analysis methods (0.963–
Mean hemispheric asymmetry indices were low for all regions of interest (Table 3).

**Effect of ETCO2 Normalization**

Linear correction for ETCO2 unexpectedly led to substantial degradation of reliability. Short-term reproducibility of normalized data yielded an unacceptably high COV of more than 23% (Table 2). Deterioration was significant for all the regions/tissue types except for whole-brain and gray matter data analyzed with the automated method that still showed a similar trend.

**Between-Subject Variation**

Intersubject variability. Similar to intrasubject reproducibility, the between-subject variation was lower for the non-normalized compared with CO2 normalized data (Table 4). Of note, the automated regression analysis method revealed lower COV, suggesting improved correction for between-subject variability of physiologic factors with lowest values for the MCA territory (14.2%) and white matter (11.3%).

---

**Table 1: Summary data of normalized and nonnormalized mean PSC and SD for both analysis methods**

<table>
<thead>
<tr>
<th>N</th>
<th>Nonnormalized Data</th>
<th></th>
<th>Normalized Data</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PSC Boxcar</td>
<td>Mean SD</td>
<td>PSC Automated</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole brain (GM+WM)</td>
<td>2.86</td>
<td>0.60</td>
<td>2.70*</td>
<td>0.43</td>
</tr>
<tr>
<td>Whole-brain GM</td>
<td>2.93</td>
<td>0.68</td>
<td>2.73</td>
<td>0.48</td>
</tr>
<tr>
<td>Whole-brain WM</td>
<td>2.65</td>
<td>0.43</td>
<td>2.54*</td>
<td>0.29</td>
</tr>
<tr>
<td>GM MCA territory</td>
<td>2.88</td>
<td>0.61</td>
<td>2.68*</td>
<td>0.39</td>
</tr>
</tbody>
</table>

**Table 2: Test retest reliability given as COV for nonnormalized and normalized data for both analysis methods**

<table>
<thead>
<tr>
<th>N</th>
<th>COV/%</th>
<th>PSC Boxcar</th>
<th>PSC Automated</th>
<th>PSC/mm Hg Boxcar</th>
<th>PSC/mm Hg Automated</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole brain (GM+WM)</td>
<td>6</td>
<td>8.5</td>
<td>25.8*</td>
<td>23.9</td>
<td></td>
</tr>
<tr>
<td>Whole-brain GM</td>
<td>6.6</td>
<td>9</td>
<td>25.6*</td>
<td>23.8</td>
<td></td>
</tr>
<tr>
<td>Whole-brain WM</td>
<td>4.8</td>
<td>7.4</td>
<td>25.7</td>
<td>24.7</td>
<td></td>
</tr>
<tr>
<td>GM MCA territory</td>
<td>6.7</td>
<td>10</td>
<td>27.2</td>
<td>26.1</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3: Hemispheric asymmetry index values given for boxcar and automated analysis methods**

<table>
<thead>
<tr>
<th>hAI</th>
<th>PSC Boxcar</th>
<th>95% CI</th>
<th>PSC Automated</th>
<th>95% CI</th>
<th>t test</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB Hemi</td>
<td>−0.72</td>
<td>3.38</td>
<td>0.27</td>
<td>7.59</td>
<td>0.09</td>
</tr>
<tr>
<td>GM Hemi</td>
<td>−0.68</td>
<td>4.54</td>
<td>0.40</td>
<td>7.93</td>
<td>0.10</td>
</tr>
<tr>
<td>WM Hemi</td>
<td>−0.13</td>
<td>4.82</td>
<td>0.84</td>
<td>8.75</td>
<td>0.04</td>
</tr>
<tr>
<td>GM MCA</td>
<td>−1.92</td>
<td>3.55</td>
<td>−0.34</td>
<td>8.48</td>
<td>0.05</td>
</tr>
</tbody>
</table>

**Note:** CI indicates confidence interval; WB, whole brain; Hemi, hemisphere. *P values are given for differences between the methods, and 95% confidence intervals are given for each method.
Table 4: Intersubject nonnormalized and normalized COV given for both analysis methods

<table>
<thead>
<tr>
<th>Intersubject COV/</th>
<th>PSC</th>
<th>PSC/mm</th>
<th>PSC/mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Boxcar</td>
<td>Automated</td>
<td>Boxcar</td>
</tr>
<tr>
<td>WB</td>
<td>21.3</td>
<td>15.9</td>
<td>32.9</td>
</tr>
<tr>
<td>GM</td>
<td>23.13</td>
<td>17.4</td>
<td>32.7</td>
</tr>
<tr>
<td>WM</td>
<td>16.1</td>
<td>11.3</td>
<td>33.4</td>
</tr>
<tr>
<td>GM MCA</td>
<td>21.3</td>
<td>14.5</td>
<td>33.7</td>
</tr>
</tbody>
</table>

Discussion

Our study investigated both the short-term reproducibility and intersubject variability of hypercapnia BOLD fMRI in a group of young healthy volunteers and compared various quantification methods. The main findings are that absolute BOLD signal intensity changes after 10% hypercapnia challenge 1) yield good short-term reproducibility (≤10%), 2) reveal very good interhemispheric reproducibility (< 4%), and 3) show better reproducibility and lower between-subject variability than BOLD ratios normalized to $ET_{CO_2}$.

BOLD fMRI techniques are becoming increasingly used in clinical studies. To be used as a diagnostic clinical test, the technique needs to be reliable (ie, precise and accurate). Accuracy of CVR assessments with hypercapnia fMRI has been assessed against SPECT,28 with favorable results. The reported accuracy of CVR assessments with hypercapnia fMRI has been deemed an important factor to consider for quantitative reproducible hypercapnia fMRI relates to variations in $ET_{CO_2}$ levels. In our data, a significant linear relationship was found between absolute PSC and $ET_{CO_2}$ levels, explaining 24% and 18% of the mutual variance according to the boxcar and regression analyses. There was no indication of a nonlinear interaction, but the linear dependence resulted mainly from the lower end of $ET_{CO_2}$ change (Fig 4). Because of the experimental setup chosen in view of safe clinical applicability, we had limited data at higher $ET_{CO_2}$ changes; hence, we are unable to exclude a nonlinear relationship.

Normalization of PSC to $ET_{CO_2}$ change substantially degraded the reliability of hypercapnia fMRI with significant worsening of short-term reproducibility and increase in intersubject variability. This was surprising against the clear linear interrelation observed and the proposed linear normalization method to present the data corrected for the $ET_{CO_2}$ change in % signal intensity change/mm Hg change format. The disadvantage of normalization with use of ratios to mm Hg and $ET_{CO_2}$ change can be explained by its oversimplification of the true interrelation that is not unity and moreover is expected to depend on the absolute baseline value with nonlinear behavior at maximal vasodilation. For strong hypercapnic challenges, BOLD normalizations to mm Hg $CO_2$ change cannot be recommended. Of note, this rationale was also applied in a previous large patient study using TCD for assessment of CVR in which no normalization to $ET_{CO_2}$ was performed.31

The main limitation of our study was the inclusion of only young healthy volunteers and a limited number of subjects. However, the study size was sufficient to demonstrate that 10% hypercapnia provides a robust BOLD signal intensity increase, allowing reliable and reproducible CVR assessment. It remains to be shown whether similar short-term reproducibility can be achieved in older subjects and patients. However, the setup was chosen for its tolerability, and we expect no difficulties for older subjects and patients.

The second limitation was the dependence of absolute BOLD PSC on the end-expiratory $CO_2$ change. Nevertheless,
we demonstrated that the standard proposed normalization was inadequate and impaired reproducibility. Until the full nature of this relationship is clarified, control of potential confounding effects from $\text{ETCO}_2$ via regression analysis is required for systematic evaluation of CVR between subjects and effects from interventions. From a practical aspect for clinical application, additional standardization of $\text{ETCO}_2$ may further mitigate the problem. Dynamic end-tidal forcing, described by Wise et al.\textsuperscript{29}, independently sets $\text{ETCO}_2$ to desired levels by rapid alteration of inspired gases on a breath-by-breath basis via computer feedback. This method targets the desired $\text{ETCO}_2$ change and may further improve reproducibility for individual CVR mapping.

Conclusions

In conclusion, assessing cerebrovascular reactivity with use of hypercapnia fMRI and absolute BOLD amplitude estimation was found to be safe and reproducible in healthy volunteers. Analysis of absolute BOLD signal intensity change yielded good short-term and very good interhemispheric stability. Larger normative data in older healthy subjects and patient studies are warranted to probe the clinical usefulness of CVR assessment by quantitatively hypercapnia fMRI.

References
