

# Acetazolamide-augmented dynamic BOLD (aczBOLD) imaging for assessing cerebrovascular reactivity in chronic steno-occlusive disease of the anterior circulation: An initial experience



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## ABSTRACT

The purpose of this study was to measure cerebrovascular reactivity (CVR) in chronic steno-occlusive disease using a novel approach that couples BOLD imaging with acetazolamide (ACZ) vasoreactivity (aczBOLD), to evaluate dynamic effects of ACZ on BOLD and to establish the relationship between aczBOLD and dynamic susceptibility contrast (DSC) perfusion MRI. Eighteen patients with unilateral chronic steno-occlusive disease of the anterior circulation underwent a 20-min aczBOLD imaging protocol, with ACZ infusion starting at 5 min of scan initiation. AczBOLD reactivity was calculated on a voxel-by-voxel basis to generate CVR maps for subsequent quantitative analyses. Reduced CVR was observed in the diseased vs. the normal hemisphere both by qualitative and quantitative assessment (gray matter (GM):  $4.13\% \pm 1.16\%$  vs.  $4.90\% \pm 0.98\%$ ,  $P = 0.002$ ; white matter (WM):  $2.83\% \pm 1.23\%$  vs.  $3.50\% \pm 0.94\%$ ,  $P = 0.005$ ). In all cases BOLD signal began increasing immediately following ACZ infusion, approaching a plateau at ~8.5 min after infusion, with the tissue volume of reduced augmentation increasing progressively with time, peaking at 2.60 min (time range above 95% of the maximum value: 0–4.43 min) for the GM and 1.80 min (time range above 95% of the maximum value: 1.40–3.53 min) for the WM. In the diseased hemisphere, aczBOLD CVR significantly correlated with baseline DSC time-to-maximum of the residue function ( $T_{max}$ ) ( $P = 0.008$  for the WM) and normalized cerebral blood flow ( $P = 0.003$  for the GM, and  $P = 0.001$  for the WM). AczBOLD provides a novel, safe, easily implementable approach to CVR measurement in the routine clinical environments. Further studies can establish quantitative thresholds from aczBOLD towards identification of patients at heightened risk of recurrent ischemia and cognitive decline.

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## 1. Introduction

Chronic steno-occlusive disease of the cerebrovascular system is associated with increased risk of ischemic stroke, a leading cause of long-term disability (Adams et al., 1993; Hankey and Warlow, 1991b; Mozaffarian et al., 2016). Cerebrovascular reactivity (CVR) has proved useful in predicting the risk of subsequent ischemic events among these patients (Bouvier et al., 2015; Gupta et al., 2012; Markus and Cullinane, 2001). The measurement of CVR is performed by quantifying the cerebrovascular response to vasodilatory stimuli, such as inhalation of air with increased CO<sub>2</sub> concentration (e.g. 5%) or intravenous administration of acetazolamide (ACZ) (Gupta et al., 2012; Siero et al., 2015; Vagal et al., 2009). Cerebral perfusion has historically been measured using single-photon emission computed tomography (SPECT) (Lee et

al., 2004; Sugawara et al., 2002), ultrasound (Dahl et al., 1995; Kimiagar et al., 2010), xenon-CT (Yonas et al., 1993), and in some cases positron emission tomography (PET) (Isozaki et al., 2010) in the setting of using acetazolamide challenge for measuring CVR. However, these modalities are limited by the use of radioactive materials, technical challenges with complex set-up, availability and/or high costs. A new approach for CVR measurement that is easily accessible, easy to implement, and free of ionizing radiation would provide a valuable replacement or addition to the diagnostic armamentarium in the clinical setting.

Recent development of MRI methods, including arterial spin labeling (ASL) and blood oxygenation level-dependent (BOLD) imaging, has allowed non-invasive measurements of cerebral blood flow (CBF) and related hemodynamic parameters using standard MRI hardware system. Despite growing motivation for the use of ASL in the setting of cerebrovascular disease, its use in CVR studies may be challenged by inherent limitations of the technique, including quantitative errors due to the long tag arrival time in regions with hemodynamic compromise (Bokkers et al., 2012; Petersen et al., 2006). BOLD imaging was more recently reported as a viable alternative (Ovadia-Caro et al.,

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2014), offering relative immunity to such errors, and thus represents a promising imaging method for interrogation of CVR.

Previous studies (Bouvier et al., 2015; Duffin et al., 2015; Mandell et al., 2008; Thomas et al., 2014) using BOLD for CVR measurement have largely utilized induced hypercapnia through inhalation of gas with increased CO<sub>2</sub> concentration; however, this approach requires specialized hardware for gas delivery and sampling, as well as good subject cooperation (Ringelstein et al., 1992), limiting its easy translation to clinical settings. In contrast, ACZ administration is safe and highly tolerated among patients from vast SPECT studies (Vagal et al., 2009), requiring only slow intravenous infusion achievable in the majority of subjects. While direct comparisons between competing approaches to inducing cerebrovascular reactivity are sparse, ACZ challenge has been shown to better detect compromised CVR than CO<sub>2</sub> gas challenge (Kazumata et al., 1996).

In this study, we proposed an MRI based approach for measuring CVR employing the combination of dynamic BOLD imaging with acetazolamide vasoreactivity (aczbOLD). We aimed to (i) examine the feasibility of this approach for evaluating hemodynamic compromise in patients with chronic cerebral steno-occlusive disease; (ii) characterize the dynamic changes in BOLD signal following ACZ infusion; (iii) study the relationship between CVR and baseline values of commonly used MRI dynamic susceptibility contrast (DSC) perfusion parameters, including CBF and the time-to-maximum of the residue function ( $T_{\max}$ ).

## 2. Material and methods

### 2.1. Participants

Eighteen patients with unilateral chronic steno-occlusive disease of the anterior circulation (age,  $48.22 \pm 13.04$  years; range, 29–70 years; 4 males, 14 females) underwent acetazolamide-challenge MRI scans, and included 4 patients with idiopathic Moyamoya disease, 10 patients with atherosclerotic occlusion of the middle cerebral artery (MCA) or internal carotid artery (ICA), and 4 patients with atherosclerotic high-grade (>75%) stenosis of the ICA. A subset of 9 patients had MRI examination the subsequent day without the administration of ACZ. This study was approved by the Institutional Review Board at Emory University School of Medicine.

### 2.2. Data acquisition

MRI data were acquired using a Siemens Tim Trio 3.0-Tesla clinical scanner (Siemens Medical Solutions, Erlangen, Germany) with a 32-channel head array coil. The protocol included a T<sub>1</sub>-weighted magnetization-prepared rapid acquisition with gradient echo (MPRAGE) anatomical imaging (TR/TE = 1900/3.52 ms, FA = 9°, voxel size =  $1 \times 1 \times 1$  mm<sup>3</sup>, 176 slices). A 20-min continuous BOLD imaging acquisition was performed with a gradient-echo echo-planar imaging (EPI) sequence (TR/TE = 2000/30 ms, FA = 78°, voxel size =  $3.44 \times 3.44 \times 4$  mm<sup>3</sup>, 30 slices). At 5 min following initiation of the BOLD scan, a standard dose of ACZ (1 g dissolved in 10 mL normal saline) (Vagal et al., 2009) was infused intravenously over 3–5 min in aliquots of approximately 2 mL/min, followed by normal saline flush.

A subset of 9 patients returned the next day, undergoing a second MRI examination, which included DSC perfusion imaging without the infusion of ACZ to obtain baseline DSC perfusion status. DSC perfusion was performed using a gradient-echo EPI sequence (TR/TE = 1500/40 ms, FA = 60°, voxel size =  $1.88 \times 1.88 \times 5$  mm<sup>3</sup>, 19 slices) with antecubital intravenous injection of 0.1 mmol/kg of gadobenate dimeglumine (MultiHance; Bracco Diagnostics, Princeton, New Jersey, USA) at 4 mL/s, and followed by normal saline flush at the same rate.

### 2.3. Data analysis

BOLD images and T<sub>1</sub>-weighted MPRAGE images were processed using SPM8 (Wellcome Trust Center for Neuroimaging, University College London, London, UK) and in-house MATLAB (MathWorks, Natick, Massachusetts, USA) scripts. Preprocessing of BOLD data comprised removal of the first 10 volumes (which can be affected by the establishment of magnetization equilibrium), motion correction, normalization to Montreal Neurological Institute (MNI) stereotaxic space, and spatial smoothing with a Gaussian kernel of 4 mm full-width-at-half-maximum. MPRAGE images were co-registered with the realigned BOLD data, and segmented to generate gray matter (GM) and white matter (WM) masks using an SPM segmentation routine.

To create CVR maps, both the first (after removal of the first 10 volumes) and the last 30 volumes (60 s) of BOLD data were averaged, representing the mean images before (BOLD<sub>pre-ACZ</sub>) and after (BOLD<sub>post-ACZ</sub>) ACZ administration, respectively. CVR was then calculated as  $\text{CVR} = (\text{BOLD}_{\text{post-ACZ}} - \text{BOLD}_{\text{pre-ACZ}}) / \text{BOLD}_{\text{pre-ACZ}} \times 100\%$ . Since only patients with predominantly unilateral steno-occlusive cerebrovascular disease were included in the study, the more severely affected hemisphere was treated as the diseased hemisphere, and the contralateral side as the “normal” hemisphere, hereafter. After determining the mean CVR values of the normal hemisphere for the segmented GM and WM respectively for each subject, normalized CVR (nCVR) was calculated by dividing the CVR value of each voxel by the mean value of the respective tissue classification (i.e. GM or WM) derived from the normal hemisphere. A voxel-wise nCVR of <70% was defined as reduced augmentation, and the total tissue volume with reduced augmentation was calculated for each subject. The temporal dynamics of BOLD signal changes following the administration of ACZ were then evaluated by estimating both the absolute mean CVR values, and the volumes of reduced augmentation, as a function of time to characterize any temporal dependency of the aczbOLD response. A sliding window of 30 volumes was used to calculate BOLD<sub>post-ACZ</sub> at different time ranges.

Baseline DSC were post-processed to produce parametric maps of relative CBF (rCBF) and  $T_{\max}$  using an in-house developed software pipeline with automated detection of arterial input and venous output functions, followed by a delay-insensitive deconvolution operation with a regularization threshold of 15% of the maximum singular value (Straka et al., 2010). While CBF measures the amount of blood delivered to a voxel per unit tissue volume per unit time,  $T_{\max}$  measures the difference in blood arrival time between a voxel and a reference location where arterial input function is sampled, typically in the MCA. Stenosis or occlusion of arteries usually results in lengthening of  $T_{\max}$  in downstream tissues. Calculated rCBF value was normalized, voxel-by-voxel, to the mean value of a region-of-interest (ROI) manually placed in the occipital WM of the normal hemisphere, and is reported as normalized CBF (nCBF). All perfusion maps were further spatially transformed to MNI space.

### 2.4. Statistical analysis

Statistical comparisons between the mean CVR values of the normal and the diseased hemispheres were performed using paired *t*-test. When assessing the temporal dynamics of the total tissue volume with reduced augmentation (nCVR < 70%), the maximum points were identified for both the GM and the WM, and the values were compared with the mean of the last 30 volumes (60 s) of BOLD data using paired *t*-tests.

A mixed-effects model was used to evaluate the correlation between the CVR and baseline  $T_{\max}$  values, as well as the correlation between the CVR and baseline nCBF values. For the correlation between the CVR and baseline  $T_{\max}$ , the mean values of both measures were calculated in areas with different  $T_{\max}$  bins (0–1 s, 1–2 s, 2–3 s, 3–4 s, 4–5 s, 5–6 s, and 6–10 s). For the correlation between the CVR and baseline nCBF, the mean values of both measures were calculated in areas with

different nCBF bins (0–1.2, 1.2–1.6, 1.6–2, 2–2.4, 2.4–2.8, 2.8–3.2, 3.2–3.6, 3.6–6). The linear relationships were estimated by modeling subject-specific slope and intercept as random effects.

### 3. Results

Fig. 1 shows the BOLD image, the CVR map, and the time course of BOLD signal changes in a patient with high-grade (>75%) stenosis of the left internal carotid artery. Compromised CVR was identified in the left anterior cerebral artery and middle cerebral artery territories. BOLD signals of both the left- and the right-sided ROIs increased after ACZ administration and approached maxima approximately 1110 s after the initiation of BOLD acquisition, i.e., 8.5 min after ACZ administration. In all cases, qualitative inspection by a neuroradiologist and a vascular neurologist experienced in cerebrovascular imaging revealed a detectable region of hemodynamic compromise corresponding to the territory of the middle cerebral artery, anterior cerebral artery, or entirety of the anterior circulation. Among the 18 study patients, paired *t*-test showed that CVR was significantly lowered in the diseased hemisphere compared to the normal hemisphere for both the GM ( $P = 0.002$ ) and the WM ( $P = 0.005$ ) (Fig. 2).

Fig. 3 shows nCVR maps at different times after the initiation of ACZ infusion in a representative patient. Across all the time points, the nCVR was reduced in the diseased hemisphere compared to the normal hemisphere. The nCVR showed more severely reduced relative reactivity 6–7 min after the initiation of ACZ infusion, and progressively recovered partially thereafter while approaching an asymptotic value at 10–11 min.

Fig. 4 shows the mean CVR values in the diseased and the normal hemispheres, and the tissue volumes with reduced augmentation (nCVR < 70%) in the diseased hemisphere, as a function of time following initiation of ACZ infusion, for all 18 patients. CVR of both the GM and the WM increased gradually, approaching a plateau at ~8.5 min after ACZ infusion. The mean tissue volume of reduced augmentation increased progressively with time after ACZ administration, peaking at 2.60 min for the GM, and 1.80 min for the WM, followed by a relatively slow decrease. Paired *t*-test showed that the peak values of the volumes of reduced augmentation of both the GM ( $P = 0.013$ ) and the WM ( $P = 0.006$ ) were significantly higher compared to the averages values of the last 30 volumes (60 s) of BOLD data. The time ranges above 95% of the maximum values were 0–4.43 min for the GM, and 1.40–3.53 min for the WM.

Mixed-effects model showed statistically significant correlation between baseline voxel  $T_{\max}$  values and CVR in the WM of the diseased

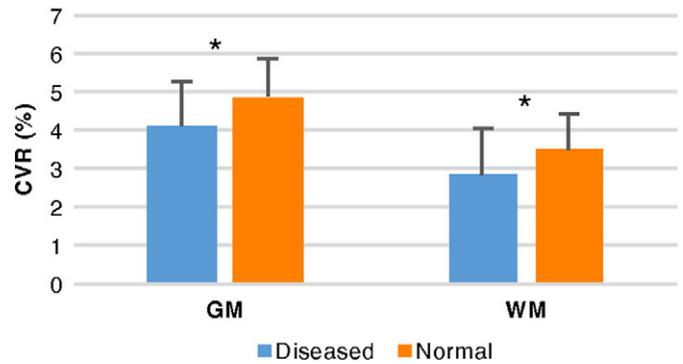


Fig. 2. Mean CVR values of the gray matter (GM) and the white matter (WM) in the diseased and the normal hemispheres. Paired *t*-test showed significantly lowered CVR in the diseased hemisphere compared to the normal hemisphere for both the GM ( $P = 0.002$ ) and the WM ( $P = 0.005$ ). Error bars represent standard deviation of the mean. Asterisks indicate statistical significance of the differences ( $P < 0.05$ ).

hemisphere ( $P = 0.008$ ), while no significance was observed in the GM ( $P = 0.344$ ) (Fig. 5). The correlations between baseline nCBF and CVR in the diseased hemisphere were significant for both the GM ( $P = 0.003$ ) and the WM ( $P = 0.001$ ). For the correlations between  $T_{\max}$  and CVR, one of the patients demonstrated a trend distinctly deviating from the rest of the cohort (arrows in Fig. 5). To evaluate the effects of this potential outlier, the same analyses were repeated after the exclusion of this subject. Similar results were obtained (correlations derived from mixed-effects model between  $T_{\max}$  and CVR:  $P = 0.139$  for the GM and  $P = 0.004$  for the WM; correlations derived from mixed-effects model between nCBF and CVR:  $P = 0.005$  for the GM and  $P = 0.002$  for the WM). The tissue volumes within different  $T_{\max}$  and nCBF bins in the diseased hemisphere are shown in supplementary Fig. S1.

### 4. Discussion

This represents, to our knowledge, the first study reporting the combined use of dynamic BOLD with ACZ challenge (aczBOLD), for characterizing CVR in patients with anterior circulation steno-occlusive disease. Our results suggest that aczBOLD for CVR measurement is feasible in this patient population, and can identify regions with compromised vasodilatory response. Compared to the normal hemisphere, reduced CVR was observed in the diseased hemisphere both visually and quantitatively, and in all subjects a dynamic response of BOLD signal in tissues was also observed and quantified. CVR furthermore

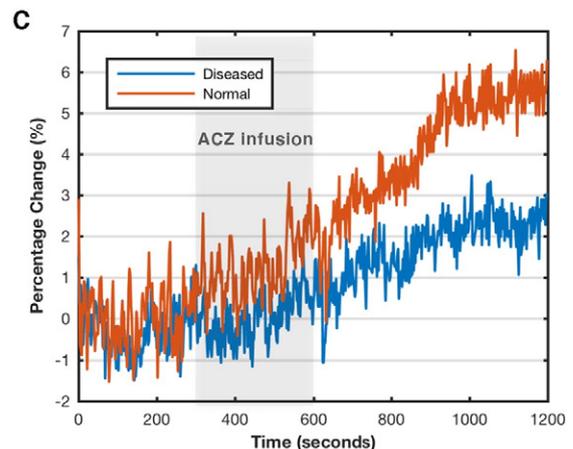
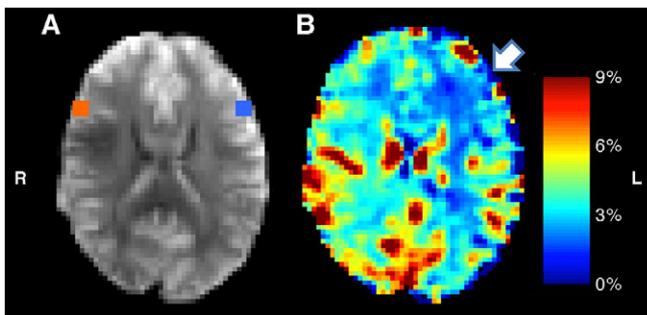
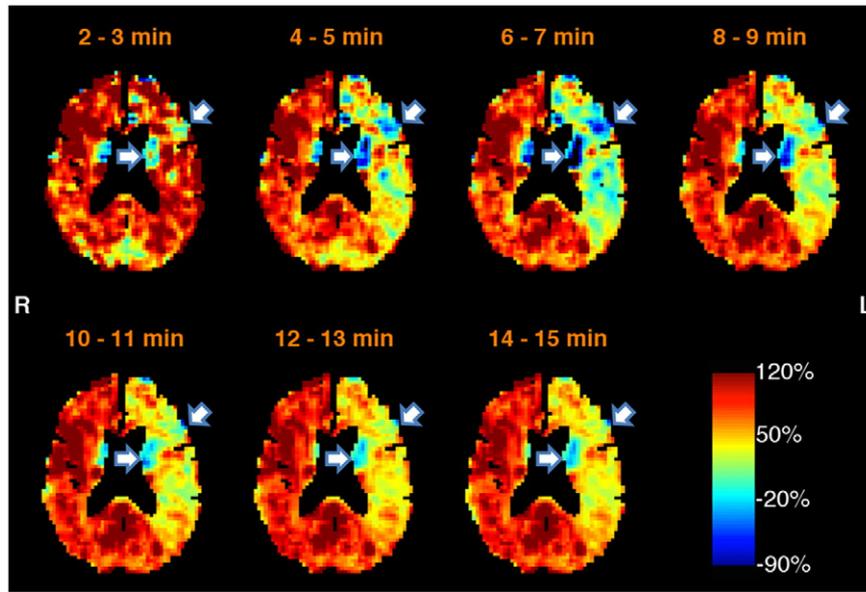


Fig. 1. Example results from a patient with high-grade stenosis of the left internal carotid artery. (A) Mean BOLD image with two ROIs indicated by blue and orange boxes, (B) CVR map showing reduced CVR in the left anterior cerebral and middle cerebral artery territories (arrow), compared to the right hemisphere, and (C) time series of BOLD signal changes of the ROIs in (A) within the diseased and the normal hemispheres. Both ROIs demonstrated increasing signal intensity following the administration of acetazolamide (ACZ), with detectable dampening of signal augmentation in the diseased hemisphere. Gray shadow indicates time period of ACZ infusion.



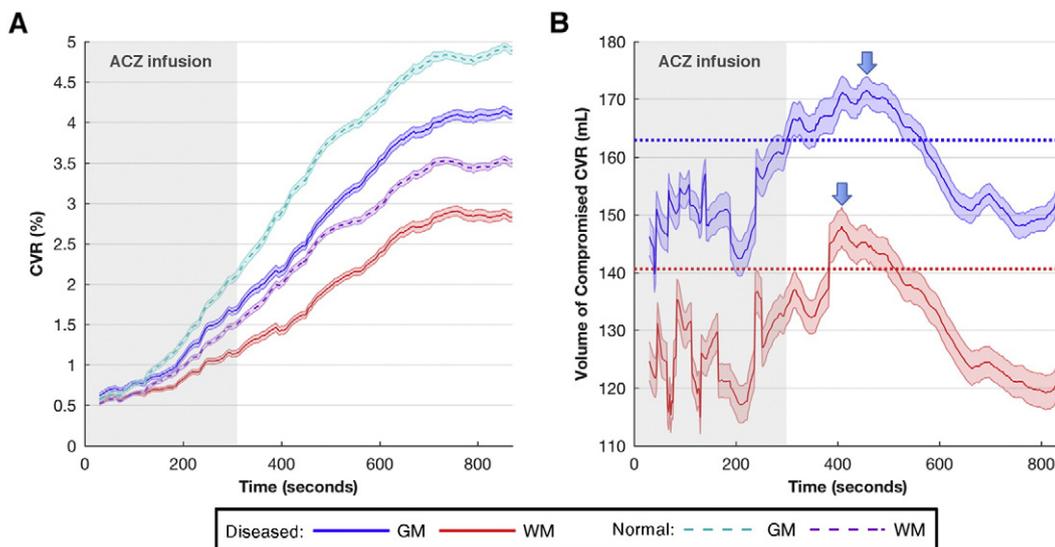
**Fig. 3.** Normalized CVR (nCVR) maps at different times after the initiation of acetazolamide (ACZ) infusion in a representative patient. Across all time points, the nCVR was reduced in the diseased (left) hemisphere. The nCVR showed more severely reduced relative reactivity 6–7 min after the initiation of ACZ infusion, and progressively recovered partially towards an asymptotic value at 10–11 min (arrows indicated).

correlated with baseline voxel  $T_{max}$  in the WM and baseline normalized CBF in both the GM and the WM from DSC perfusion.

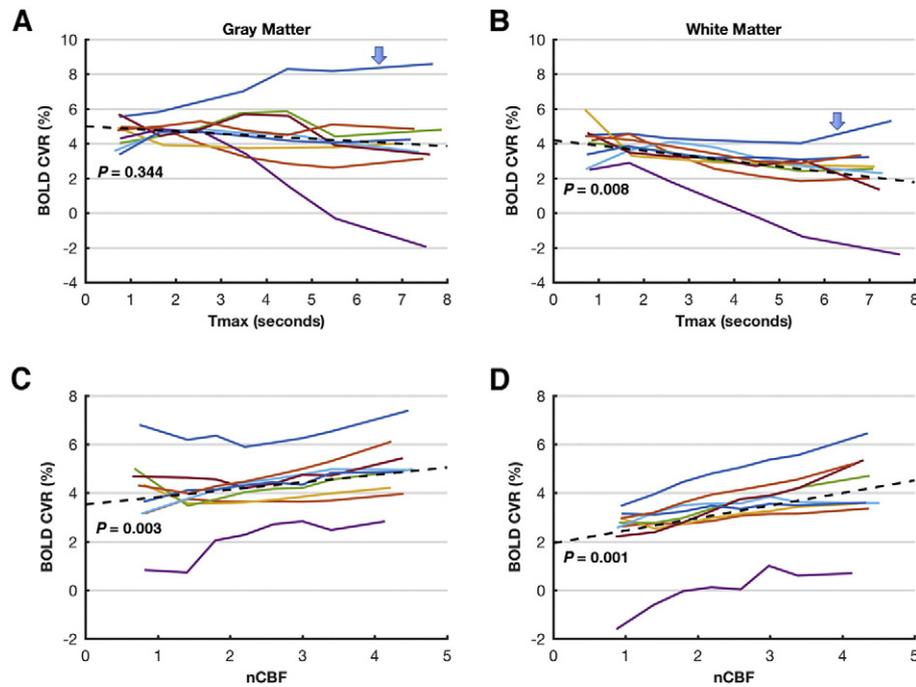
Patients with symptomatic carotid occlusion carry increased risk for the development of recurrent stroke compared to the general population, with an annual stroke rate of 2% to 6% ipsilateral to the occluded carotid artery (Hankey and Warlow, 1991a; Klijn et al., 1997); the use of OEF imaging with PET allows for identification of patients at significantly higher risk of stroke, among whom so-called stage II hemodynamic failure portends a particularly tenuous hemodynamic state of *misery perfusion*, at even greater risk for infarction with an odds ratio of 6.0 compared to those without misery perfusion (Grubb et al., 1998). In clinical practice, PET OEF imaging proves challenging, due to requisite need for an onsite cyclotron due to the short half-life of  $^{15}O$  radiotracer, which limits its availability to a small number of academic centers, and

carries the attendant risk of ionizing radiation. As an alternative to the identification of the misery perfusion state, a meta-analysis of 13 studies showed that CVR measurement as commonly employed using non-PET radionuclides and SPECT, allow for stratification of stroke risk with an odds ratio of 3.96 (Gupta et al., 2012).

Previous reports have proposed that cerebrovascular reactivity to vasodilatory stimuli can be monitored by ASL and/or BOLD in normal subjects and patients with cerebrovascular disease (Mandell et al., 2008). While traditional ASL methods suffer from errors in regions with long arterial transit time of blood, recently developed ASL technique with multiple post-labeling delays could mitigate some of these errors and quantify changes in bolus arrival time in response to vasodilatory stimuli in regions with mildly increased arterial transit time (Donahue et al., 2014). The vascular challenge in previous studies



**Fig. 4.** (A) Mean CVR in the diseased (solid curves) and the normal (dashed curves) hemispheres, and (B) mean volumes of reduced augmentation (normalized CVR < 70%) in the diseased hemisphere for the gray matter (GM) and the white matter (WM) as a function of time with a sliding window of 30 volumes (60 s of acquisition) after the initiation of acetazolamide (ACZ) infusion in all 18 patients. The CVR increased gradually and approached plateau at approximately 8.5 min after ACZ infusion. The tissue volumes of reduced augmentation also increased after ACZ administration, peaking at 2.60 min for the GM, and at 1.80 min for the WM (indicated by arrows), followed by a relatively slow decrease. Dotted lines denote 95% of the maximum values. Error bars represent standard error of the mean. Gray shadows indicate time period of ACZ infusion.



**Fig. 5.** Correlations between baseline  $T_{max}$  and CVR (A and B), as well as between baseline nCBF and CVR (C and D) of the gray matter (GM) (A and C) and the white matter (WM) (B and D) in the diseased hemisphere. Mixed-effects model showed statistically significant correlation between baseline voxel  $T_{max}$  values and CVR, in the diseased hemisphere for the WM ( $P = 0.008$ ), while no significance was observed for the GM ( $P = 0.344$ ). The correlations between baseline nCBF and CVR in diseased hemisphere were significant for both the GM ( $P = 0.003$ ) and the WM ( $P = 0.001$ ). Each colored solid line represents a patient. The fitting lines (black dash lines) are also shown to indicate trends. Arrows indicate a single patient with a different trend in the correlations from the others.

is typically achieved with induced hypercapnia, using complex gas delivery and sampling apparatus. Such set-ups are well suited for normal subjects and patients with mild diseases who are cooperative. However, such implementations can be laborious and technically challenging in translating to the clinical setting, including the need to consider possible dilution of delivered gas, potential inaccuracy in the measurement of end-tidal  $CO_2$  concentration and subject tolerance among other factors. In contrast, ACZ can be easily administered through standard intravenous access, by slow infusion of a highly tolerated agent that is in longstanding and widespread use for CVR measurement in this patient population. The use of  $CO_2$ -based hypercapnia for CVR measurement may be further challenged as the calculation of CVR is commonly based on a linear regression of BOLD signals against end-tidal partial pressures of  $CO_2$ . This approach can give rise to artificially low CVR values in regions that have normal vasodilatory reserve but a relatively slower cerebrovascular regulatory response (Regan et al., 2014). Such challenges can be mitigated with ACZ administration, where CVR estimation is insensitive to the response speed when coupled to continuous imaging interrogation as proposed using dynamic BOLD in our population. Moreover, the regions with slow response can be identified by investigating the temporal dynamic effects of ACZ on BOLD (see Figs. 3 and 4B for more details), permitting an additional element of tissue contrast which may inform the tissue state. It is noteworthy that for  $CO_2$ -based hypercapnia, new analysis methods, such as time delay processing, were shown to provide some information on blood flow augmentation delays and reduce errors (Donahue et al., 2016).

Although ACZ has several advantages over  $CO_2$ , it should be noted that the mechanism of vasodilation induced by ACZ is similar to that by  $CO_2$ .  $CO_2$  challenge is suitable for cooperative participants, less invasive compared to venous infusion of ACZ, more informative on the vasoconstrictor capabilities of the cerebral vasculature (Ringelstein et al., 1992), and makes on/off switching and the control of carbonic acidosis easier. The side effects of ACZ include paresthesia and headache (Burt et al., 1992; Dahl et al., 1995; Sullivan et al., 1987), most of which are transient and acute. Furthermore, no long-term side effects were reported in over

1000 cases with ACZ challenge (Piepgras et al., 1990), indicating the safety of ACZ. A potential criticism might be that the CVR assessment would be associated with the dose of ACZ. A dose of 15 to 18 mg/kg is recommended to produce maximal vasodilatory response and reduce the risk of side effects (Dahl et al., 1995). However, 1 g of ACZ was given in the present study, which is a standard dose for ACZ challenge test (Vagal et al., 2009). Future studies could establish whether a weight-based dosing strategy might provide more accurate CVR assessment.

Our results suggest that BOLD signal increases immediately after ACZ infusion and approaches plateau at approximately 8.5 min, which is in agreement with previous studies using ASL (Inoue et al., 2014), phase-contrast MR angiography (Hartkamp et al., 2012), and Doppler ultrasonography (Dahl et al., 1995). In the previous studies, post-ACZ imaging has typically been performed at 10–15 min after intravenous administration (Bokkers et al., 2010; Ni et al., 2016; Yun et al., 2016) by presumption that peak CBF response occurs within this time range (Vagal et al., 2009). Our findings support the use of this time window for single-point post-ACZ measurement. Importantly, by comparison to most previous approaches, aczBOLD allows for dynamic examination of vascular reactivity. Specifically, in addition to the measurement of response at the delayed equilibrium stage, aczBOLD also provides information on vascular reactivity immediately following ACZ administration. We found that within this initial time frame, some regions showed more pronounced reduction in relative reactivity when compared to the delayed equilibrium phase. Interestingly, our results showed that the volumes of reduced augmentation (nCVR < 70%) peaked at 2.60 min for the GM and 1.80 min for the WM, probably due to slower vascular regulatory response in some diseased areas. Moreover, the time ranges above 95% of the maximum values were similar between the GM (0–4.43 min) and the WM (1.40–3.53 min). Whether this provides further prognostic information towards stroke risk stratification or cognitive decline remains to be demonstrated in future study with longitudinal design and larger cohorts.

The findings from previous studies on the relationship between CVR and baseline perfusion parameters are not consistent (Bouvier et al.,

2015; Kawano et al., 2016), probably due to different imaging methods and correlation measures used. In the present study, we applied a mixed-effects model to account for the intra-subject repeated-measure dependency. The results showed that CVR correlated with baseline  $T_{\max}$  in the WM and baseline CBF in both the GM and the WM, suggesting that baseline hemodynamic state may be predictive of cerebrovascular reserve within a certain regime. This is in line with an earlier study that showed lower CBF and CVR in the hemisphere ipsilateral to cerebrovascular stenosis or occlusion (Okazawa et al., 2007). It is noteworthy that perfusion deficits can also be identified using the BOLD data by performing time delay analysis, and that this information can be used to correct for the analysis of resting-state functional brain networks (Wu et al., 2016). Also the use of ACZ reduces the effectiveness of time delay and functional connectivity analyses. Whether DSC perfusion, time delay analysis of BOLD, and aczBOLD provide complementary value in stroke risk stratification and the prediction of cognitive impairment in patients with chronic steno-occlusive diseases remains to be established.

There are several issues remaining to be addressed in the future. First, BOLD signal depends on not only CBF, but also cerebral blood volume (CBV), cerebral metabolic rate of oxygen (CMRO<sub>2</sub>), arterial partial pressure of oxygen, and hematocrit (Ogawa et al., 1993). ASL can provide non-invasive quantitative measurement of CBF despite its limitation in regions with long arterial transit time. Further studies are required to investigate whether BOLD and ASL could provide complementary information about ACZ-induced CVR. Simultaneous evaluation of ASL and BOLD, such as with dual-echo ASL (Smeeing et al., 2016), may prove valuable as a synergistic approach for further interrogation. Combining dual-echo ASL imaging and ACZ to characterize CVR is currently underway in our group. Second, both stroke and cognitive decline are well-recognized consequences of chronic cerebrovascular disease (Pendlebury, 2012; Weinstein et al., 2014). Existing approaches to risk stratification using <sup>15</sup>O PET prove difficult to implement in large cohort examination of this patient population, and the development of non- and minimally-invasive MR biomarkers in this context would greatly facilitate study of the natural progression of such patients. The capacity of CVR as approximated by aczBOLD herein to identify patients at heightened risk is however not established, and remains the subject of ongoing and further studies. Thirdly, identification of CVR onset time is at present challenging, owing to relatively long infusion time (3–5 min in this study) and noise in the acquired signals (see Fig. 1C for example). Such perfusion timing might be monitored using CO<sub>2</sub> challenge with step stimuli (Donahue et al., 2016). Evaluating differences in onset time between the GM and the WM is an interesting topic for future studies. Fourthly, although nCVR < 70% was used as a criterion for identifying regions with reduced augmentation in the present study, systematic investigations should be performed to establish the threshold for quantifying pathological volumes. Finally, this initial experience applied aczBOLD on patients with cerebrovascular disease. Comparisons with healthy controls are needed to deepen our understanding of the underlying mechanism.

In conclusion, we have proposed a new approach using dynamic BOLD with acetazolamide challenge (aczBOLD) for CVR measurement in chronic cerebrovascular disease. AczBOLD can be easily implemented on most MRI systems. AczBOLD can be used to detect compromised CVR in patients with cerebrovascular disease, in whom characterization of the dynamic effects of ACZ on BOLD may allow for the detection of regions with slow or impaired vascular regulatory response, and may allow for identification of patients at higher risk of recurrent ischemic events and cognitive decline.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.nicl.2016.11.018>.

#### Declaration of interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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