Cerebral blood flow velocity underestimates cerebral blood flow during modest hypercapnia and hypocapnia

Nicolette S. Coverdale,1 Joseph S. Gati,2 Oksana Opalevych,2 Amanda Perrotta,1 and J. Kevin Shoemaker1,3

1Neurovascular Research Laboratory, School of Kinesiology, Western University, London, Ontario, Canada; 2Robarts Research Institute, Western University, London, Ontario, Canada; and 3Department of Physiology and Pharmacology, Western University, London, Ontario, Canada

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Cerebral blood flow velocity underestimates cerebral blood flow during modest hypercapnia and hypocapnia. During HO, CSA decreased from 5.8 to 4.6 mm2 (P < 0.001). During HC, CSA increased from 5.6 to 6.0 mm2 (P < 0.001). CBFVs during baseline, HO, and HC were 18 ± 8% greater (P < 0.001) for CBF than TCD CBFV during HC, and the relative decrease of CBF during HO was 7 ± 4% greater than the change in TCD CBFV (P < 0.001). These findings challenge the assumption that the CSA of the MCA does not change over modest changes in PaCO2.

Cerebral blood flow velocity; middle cerebral artery; magnetic resonance imaging; transcranial Doppler ultrasound

The responsiveness of the cerebral circulation to altered carbon dioxide (CO2) partial pressures (PaCO2) has long been recognized (12) and is the basis of one method to assess cerebrovascular health (9, 14).1 Alterations in vessel diameter have been observed with in vitro preparations or craniotomy, with changes in inspired CO2 at the level of the smaller cerebral arteries, including the anterior cerebral artery, the M2 segment of the middle cerebral artery, and the intraparenchymal cerebral arterioles (1a, 7). Whether or not larger cerebral arteries constrict and/or dilate in conscious humans is important, because the standard tool for measuring cerebral hemodynamics, transcranial Doppler ultrasound (TCD), measures cerebral blood flow velocity (CBFV), which is used as a surrogate for cerebral blood flow (CBF). However, the change in CBFV is equivalent to the change in CBF, only if the diameter of the insonated vessel does not change. This assumption is particularly relevant to the M1 segment of the MCA, as it is the cerebral vessel that is most often studied using TCD. Early studies used magnetic resonance imaging (MRI) and reported that MCA diameter did not change in response to manipulations of end-tidal PCO2 (PetCO2) (22, 25). Serrador et al. (22) examined MCA diameter during hypercapnia (HC) at a PetCO2 of ~45 Torr and during hypocapnia (HO) at 24 Torr in six subjects and found no change in either condition. The voxel dimensions in this case were 0.47 × 0.47 × 3.0 mm. Valdueza et al. (25) had seven subjects hyperventilate to an PetCO2 of 27 Torr, and no change in MCA diameter was detected with a voxel size of 0.8 × 0.4 × 3.0 mm. Both of these investigations were performed at 1.5 T with limited spatial resolution relative to higher field systems currently available.

In contrast to MRI data (22, 25), indirect measures suggest that MCA diameter does change across levels of PetCO2. For example, combined measures of the HC-induced flow difference between CBFV in the MCA and the sphenoparietal sinus (which drains the MCA) indicated a greater increase on the venous side (26). The authors attributed this observation to MCA vasodilation (26).

Measures of CBFV as an index of CBF are challenged not only by the assumption of constant MCA diameter, but also by velocity errors. A problem inherent to the Doppler signal is overestimation of peak values due to spectral broadening, which has a greater impact as the angle of insonation increases (11). Spectral broadening occurs intrinsically due to the nature of transmitting and receiving acoustic energy (8) and has been documented with other types of Doppler ultrasound systems (11). In addition to TCD, MRI-based phase contrast (PC) imaging can estimate CBFV based on the principle that applied magnetic gradients induce a phase shift in moving protons that is proportional to fluid velocity. PC imaging has been validated against a flow phantom for estimation of total CBF through the basilar artery and internal carotid arteries and has been used to estimate MCA CBFV (2, 15, 23). Leung et al. (15) reported that PC-based estimates were lower than those measured by TCD with the error attributed to Doppler spectral broadening. Overall, the agreement between TCD and PC estimates of CBFV requires further study.

The primary purpose of this study was to determine whether the MCA constricts during HO and/or dilates during HC and to quantify the impact of such changes on CBF. A secondary purpose was to compare velocity measures from TCD to velocity measures collected with PC MRI over a range of PetCO2 values.

1This article is the topic of an Invited Editorial by Philip N. Ainslie and Ryan L. Hoiland (1).

Address for reprint requests and other correspondence: J. K. Shoemaker, Neurovascular Research Laboratory, Western Univ., Rm. 3110 Thames Hall, London, ON, Canada N6A 3K7 (e-mail: kshoemak@uwo.ca).
MATERIALS AND METHODS

In total, 19 subjects (24 ± 2 yr, 8 men) participated in this study. Subjects were not on any medications and were nonsmokers with no history of cardiovascular disease. All subjects gave informed consent, and the protocol was approved by the Health Sciences Research Ethics Board at Western University.

Experimental Protocols

Subjects participated in two testing sessions, including an MRI day and a physiological data collection (Lab) day that were matched for time of day. Subjects were asked to refrain from alcohol, caffeine, and physical activity for 12 h before testing. The protocol consisted of 5 min of baseline measures followed by 5 min of HC or HO (assigned randomly), then a 3- to 4-min recovery period. The CO2 manipulation that had not yet been performed (HC or HO) was then completed for 5 min after another 5-min baseline. To induce HC, the subjects breathed air that consisted of 6% CO2, 21% oxygen, and balanced nitrogen. For HO, participants breathed at a rate of 30 breaths/min guided by a metronome. The protocols were designed to increase PetCO2 by ~10 Torr during HC and decrease PetCO2 by ~15 Torr during HO.

Measurements

Lab session. Heart rate was acquired from standard ECG. Finger arterial blood pressure (BP) was measured continuously, and the brachial waveform (Finometer, Finapres Medical Systems BV, Amsterdam, The Netherlands) was corrected to brachial sphygmomanometric values. PetCO2 (CO2100C analyzer, Biopac Systems Canada Inc., Montreal, QB, Canada) and breathing frequency (respiratory strain gauge) were measured continuously. CBFV of the MCA was measured in a supine position using a 2-MHz pulsed TCD probe (Neurovision system, Multigon Industries, Elmsford, CA). The average depth of the ultrasound beam was 5.0 ± 0.4 cm.

MRI session. A 3T MRI (Magnetom TIM TRIO, Siemens Medical Solutions, Erlangen, Germany) was used for data collection. A three-dimensional time-of-flight sequence was used to select the location on the M1 segment of the right MCA to apply a T2 fast spin echo sequence and a PC sequence that were used to determine the vessel cross-sectional area (CSA) and CBFV, respectively. Both T2 and PC sequences were applied during baseline conditions (pre-HC and pre-HO) and each experimental condition (HC and HO) for 9 subjects (25 ± 2 yr, 5 men). In each condition, two T2 image acquisitions were performed (~1 min per acquisition), followed by one PC acquisition (~2 min) followed by two more T2 acquisitions. In the MRI session for the remaining subjects, two to five T2 images were taken at baseline and during HC and HO for assessment of MCA CSA. For T2 images used to calculate MCA CSA [8 slices, repetition time (TR) = 3,000 ms, echo time (TE) = 100 ms, flip angle = 120°, voxel dimensions 0.4 × 0.4 × 2.0 mm3], the pulse sequence was gated to the peak of the pulse wave from the continuous signal derived from an MRI-compatible pulse oximeter (8600FO MRI, Nonin Medical, Plymouth, MN) as measured at the right third finger.

For the PC acquisition, which was used to determine CBFV, (TR = 24.75 ms, TE = 6.01 ms, flip angle = 15°, voxel dimensions 0.7 × 0.7 × 3.0 mm3), 25 phases were retroactively gated to the signal from the pulse oximeter. The velocity-encoding factor (Venc), which is the maximum measurable velocity, was individually determined since the Venc too low can result in aliasing (18). The Venc for pre-HC and pre-HO conditions was, on average, 111 ± 14 and 145 ± 13 m/s for HC, and 98 ± 14 m/s for HO. Respiration was collected with a strain gauge around the upper abdomen.

Data Analysis

Lab data. Values of heart rate, BP, breathing frequency, and mean CBFV were averaged for the 5-min baseline periods. These measures were averaged every minute during HC and HO, and the value reported corresponds to when the maximal and minimal CSA measurements from the MRI were recorded.

MRI data. Figure 1 shows a representative baseline T2 image. Using Osirix imaging software (Pixmeo, Geneva Switzerland), the CSA of each MCA image was measured in triplicate by two blinded observers. The MCA was assumed to be circular, but, rather than fitting a circle to the vessel, we chose to outline the lumen manually point by point for the best fit, and this was done at each operator’s discretion after training by the same investigator. An intraclass correlation coefficient (ICC) was calculated to examine the agreement between the two observers. The ICC between the two observers was 0.92; thus the average CSA between the two observers is reported. The HC and HO CSAs are reported as the maximal CSA for HC and the minimal CSA for HO, as well as the average of all CSAs during HC and HO. The diameter was then determined by dividing the square root of the CSA by π and multiplying by 2.

An index of vascular reactivity was assessed relative to the corresponding baseline as the change (Δ) in CSA/PetCO2 during each of HC and HO. CBF was calculated (CSA × CBFV) using TCD CBFV as the basis for CBF calculations unless otherwise noted. Percent change (%Δ) from baseline was calculated for CBF and CBFV. Cerebrovascular reactivity (CVR) for CBF and CBFV was calculated as both absolute and relative change (ΔCBF/ΔPetCO2, ΔCBFV/ΔPetCO2, %ΔCBF/ΔPetCO2, %ΔCBFV/ΔPetCO2).

PC data were assessed using cvi42 (Circle Cardiovascular Imaging, Calgary, AB, Canada). Phases were quantified into velocities based on the Venc. The lumen of the MCA was identified, and the mean velocity for each voxel was determined over the 25 phases. To coincide with the TCD analysis, a peak velocity for each of the 25 images was determined from the voxel with the highest velocity within the MCA, and these values were averaged over the cardiac cycle. The analysis was performed by two blinded observers, and values were averaged. The ICC between the two observers was 1.0 (P < 0.001).

Statistical Analysis

Data are presented as means ± SD, unless otherwise indicated. SigmaStat 12.0 was used for statistical analysis. All comparisons were made with a paired t-test. Average and maximal/minimum data are reported for HC and HO. The maximum/minimum CSA were the main outcome of interest because this represents the maximum to
and HO in the MRI and Lab sessions (P unchanged. During HO, systolic BP increased above baseline (systolic BP and mean arterial pressure (MAP) increased above baseline to a maximal CSA of 6.5 ± 1.0 mm² (P < 0.001; Fig. 2A) and an average CSA of 6.3 ± 0.9 mm² (P < 0.001). During HO, the CSA decreased from 5.8 ± 0.9 mm² at baseline to a minimum of 5.3 ± 0.9 mm² (P < 0.001; Fig. 2B) and an average CSA of 5.5 ± 0.9 mm² (P = 0.01). For HC, Cohen’s d was 0.94, and the achieved power was 0.87. For HO, Cohen’s d was 0.50, and achieved power was 0.44. Figure 2 illustrates the individual patterns of response showing a homogeneous dilatory response for all subjects during HC and

Table 1. Physiological results during HC and HO in the Lab and MRI

<table>
<thead>
<tr>
<th></th>
<th>Pre-HC</th>
<th>HC</th>
<th>Pre-HO</th>
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<tr>
<td><strong>MCA</strong></td>
<td></td>
<td></td>
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<tr>
<td>End-tidal CO₂</td>
<td>37 ± 3</td>
<td>46 ± 5*</td>
<td>36 ± 4</td>
<td>23 ± 3†</td>
</tr>
<tr>
<td>Heart rate</td>
<td>13 ± 3</td>
<td>15 ± 3</td>
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<td>28 ± 1†</td>
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<tr>
<td>Lab</td>
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<tr>
<td>End-tidal CO₂</td>
<td>39 ± 3</td>
<td>49 ± 5*</td>
<td>36 ± 4</td>
<td>25 ± 9†</td>
</tr>
<tr>
<td>Heart rate</td>
<td>14 ± 2</td>
<td>16 ± 3</td>
<td>14 ± 3</td>
<td>29 ± 1†</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>107 ± 11</td>
<td>112 ± 13*</td>
<td>110 ± 8</td>
<td>114 ± 9†</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>69 ± 8</td>
<td>70 ± 10</td>
<td>69 ± 8</td>
<td>70 ± 8</td>
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<tr>
<td>Mean arterial BP</td>
<td>82 ± 9</td>
<td>86 ± 10*</td>
<td>84 ± 7</td>
<td>85 ± 7</td>
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Values are means ± SD; n = 13 (MRI HC), 15 (MRI HO), and 7 subjects (Lab HC and HO). HC, hypercapnia; HO, hypocapnia; Lab, physiological data collection day. *P < 0.05 for Pre-HC vs. HC; †P < 0.05 for Pre-HO vs. HO.

which CSA changes may impact TCD estimates of CBFV. Therefore, all calculations relating to the change in CSA relate to the maximal CSA during HC and the minimum during HO. Bland-Altman analysis was performed to examine the spread of differences between PC and TCD for estimates of CBFV and CVR. The differences between the two estimates were plotted against the corresponding means, and fixed and/or proportional biases were assessed. A one-sample t-test was performed between PC and TCD difference values, to test for fixed bias, since the differences should be equal to zero if no fixed bias exists (3). The regression between the differences and corresponding means was plotted, and a slope different from zero was used to indicate the presence of proportional bias (3). The ICC was also calculated to examine the agreement between PC and TCD, and a value > 0.8 was considered to indicate good agreement.

Cohen’s d was calculated for the change in CSA during HC and HO to provide an estimate of the effect size. Additionally, a post hoc power analysis was performed for the change in CSA during each of the HO and HC conditions using GPower 3.1 (6), specifying two tails, an α-value of 0.05, the sample size, and the effect size of Cohen’s d.

RESULTS

T2 images were collected for 19 subjects, and analysis of CSA was performed on images from 15 subjects for HO and on 13 subjects for HC for whom image quality provided clear MCA edges. PC images of sufficient quality to determine CBFV were obtained in seven of nine individuals for both the HC and HO trials, and TCD CBFV was collected in these same subjects. The average time between the Lab and MRI session was 53 ± 58 days. This estimate is variable because 13 subjects had their Lab and MRI sessions within 29 days of one another, while the other six had 3–4 mo between test dates due to the reduced availability of the MRI.

Table 1 illustrates the physiological responses to HC and HO in both the laboratory and MRI sessions. PreCO₂ increased during HC and decreased during HO in each session (P < 0.01 for all cases). Breathing rate was not different during HC in the MRI or in the Lab session. By design, breathing rate was elevated compared with baseline during HO in each session (P < 0.001). Additionally, heart rate increased during both HC and HO in the MRI and Lab sessions (P < 0.05 for all cases). BP was measured only in the Lab session and during HC, systolic BP and mean arterial pressure (MAP) increased above baseline (P < 0.05 for both cases), while diastolic BP remained unchanged. During HO, systolic BP increased above baseline (P < 0.01) while MAP and diastolic BP remained unchanged.

During HC, CSA of the MCA increased from 5.6 ± 0.8 mm² at baseline to a maximal CSA of 6.5 ± 1.0 mm² (P < 0.001; Fig. 2A) and an average CSA of 6.3 ± 0.9 mm² (P < 0.001). During HO, the CSA decreased from 5.8 ± 0.9 mm² at baseline to a minimum of 5.3 ± 0.9 mm² (P < 0.001; Fig. 2B) and an average CSA of 5.5 ± 0.9 mm² (P = 0.01). For HC, Cohen’s d was 0.94, and the achieved power was 0.87. For HO, Cohen’s d was 0.50, and achieved power was 0.44. Figure 2 illustrates the individual patterns of response showing a homogeneous dilatory response for all subjects during HC and

Fig. 2. Individual and mean changes in the cross-sectional area (CSA) of the MCA. A: hypercapnia (HC; n = 13). B: hypocapnia (HO; n = 15). *P < 0.05.
of the regression line was different from zero \((r = 0.57; P = 0.032)\), indicating proportional bias was present. The ICC for CVR was 0.86 \((P < 0.001)\).

CBF, calculated from TCD CBFV and MCA CSA, increased from baseline during HC and decreased during HO \((P < 0.01\) for each; Fig. 6A). The \(\%\Delta\) was greater for CBF than for CBFV during HC and HO \((P < 0.001\) for each comparison; Fig. 6B).

There were no differences in CVR between CBFV estimates obtained by TCD and PC for HC or HO \(\text{TCD: } 2.42 \pm 3.98 \text{ cm}^2\text{s}^{-1}\text{mmHg}^{-1}\) and \(\text{PC: } 2.87 \pm 1.63 \text{ cm}^2\text{s}^{-1}\text{mmHg}^{-1}\) for HC, \(P = 0.33\); and TCD: \(-1.67 \pm 1.37 \text{ cm}^2\text{s}^{-1}\text{mmHg}^{-1}\) and \(\text{PC: } -0.92 \pm 1.17 \text{ cm}^2\text{s}^{-1}\text{mmHg}^{-1}\) for HO, \(P = 0.14\). There was a strong linear association between TCD and PC CVR \(r = 0.81, P < 0.001\); Fig. 5B). Bland-Altman analysis for CVR (Fig. 5A) revealed one point that fell outside the limits of agreement of \(-3.6\) and 4.8. No fixed bias (mean difference 0.6 \text{cm}^2\text{s}^{-1}\text{mmHg}^{-1}; \(P = 0.10\) ) was observed; however, the slope

Table 2. CBFV during HC and HO obtained from TCD and PC MRI

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<thead>
<tr>
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<th>Pre-HC</th>
<th>HC</th>
<th>Pre-HO</th>
<th>HO</th>
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<tbody>
<tr>
<td>TCD CBFV</td>
<td>68 ± 9</td>
<td>85 ± 15*</td>
<td>69 ± 8</td>
<td>55 ± 16†</td>
</tr>
<tr>
<td>PC CBFV</td>
<td>64 ± 10</td>
<td>85 ± 15*</td>
<td>61 ± 8</td>
<td>51 ± 8†</td>
</tr>
</tbody>
</table>

Values are means ± SD in cm/s; \(n = 7\) subjects for TCD and PC HC and HO. TCD, transcranial Doppler ultrasound CBFV, cerebral blood flow velocity; PC, phase contrast. *\(P < 0.05\) for Pre-HC vs. HC. †\(P < 0.05\) for Pre-HO vs. HO.

Fig. 3. Relationship between the change in end-tidal PETCO2 (PETCO2) and the change in MCA CSA from HO to HC. Solid squares represent mean data with SD bars, and open squares show individual data \(n = 19; n = 9\) with data points for HO and HC, \(n = 6\) for HO alone, and \(n = 4\) for HC alone.

heterogeneous vasoconstriction during HO, where six participants showed a \(<5\%\) change in MCA CSA. Figure 3 shows the mean and individual data for the relationship between PETCO2 and CSA. The average increase during HC was \(0.11 \pm 0.07 \text{mm}^2/\text{Torr}\), while the average decrease during HO was \(0.04 \pm 0.03 \text{mm}^2/\text{Torr}\).

Both PC and TCD methods produced similar significant changes in CBFV during both HC and HO stimuli (Table 2). Overall, there was a strong linear association between TCD and PC estimates of CBFV \(r = 0.71, P < 0.001\); Fig. 4B). The limits of agreement with Bland-Altman analysis were at \(-27\) and 20 on the y-axis (Fig. 4A), and one value fell outside this range at approximately \(y = 23\). Bland-Altman analysis revealed no significant fixed (mean difference \(-3.56\) cm/s; \(P = 0.13\)) or proportional biases \((r = 0.01; P = 0.98\)). However, the spread of the differences between PC and TCD produced limits of agreement with a wide range. The ICC for CBFV was 0.83 \((P < 0.001)\).

Fig. 4. Bland-Altman and scatterplots comparing transcranial Doppler ultrasound (TCD) and phase contrast (PC) cerebral blood flow velocity (CBFV) over the range of PETCO2 values. Pre-HC, HC, pre-HO, and HO are represented for \(7\) subjects for a total of 28 data points. A: Bland-Altman plot of CBFV. B: scatterplot with the regression line (solid line) and 95% confidence intervals (dashed lines) for CBFV.
There was also a significant change from baseline during HC (from 219 ± 38 to 333 ± 68 ml/min; *P* = 0.001) and HO (from 211 ± 34 to 156 ± 13 ml/min; *P* = 0.007) when CBF was calculated from PC CBFV and MCA CSA. CVR calculated from CBF was 19 ± 23 ml·min⁻¹·mmHg⁻¹ for HC and −8 ± 6 ml·min⁻¹·mmHg⁻¹ for HO. Comparing %ΔCBFV CVR to %ΔCBF CVR revealed that the reactivity to HC and HO was greater for %ΔCBFV CVR than %ΔCBF CVR (*P* < 0.05 for both cases; Fig. 7).

**DISCUSSION**

This study was the first to show that MCA CSA increases by 16 ± 7% and decreases by 8 ± 6% during HC and HO, respectively. Thus using CBFV as an estimate of flow underestimated true flow changes in the MCA. CVR was also lower when using CBFV compared with CBF. Additionally, there was some variability between TCD and PC estimates of CBFV during both HC and HO conditions, which was evident by the wide limits of agreement produced from Bland Altman analysis, despite the strong ICC value.

Overall, MCA diameters calculated from measures of CSA in the present study (2.66 ± 0.21 to 2.87 ± 0.21 mm for HC, and 2.69 ± 0.20 to 2.59 ± 0.21 with HO) are comparable to...
values of 2.23 mm (24) to 3.4 mm (10, 21, 22, 25, 28) from other studies that have employed MRI. In the present analysis, the percent change in diameter with HC was 8 ± 3% over an average change in PetCO2 of 9 ± 4 Torr, whereas diameter decreased 4 ± 4% during an PetCO2 decrease of 13 ± 5 Torr. Willie et al. (27) reported a change in the diameter of the internal carotid artery of ∼20% over a PaCO2 range of 50 Torr (27). Thus, the present data produced a rate of diameter change (∼0.4%/Torr) over a physiologically relevant range of PetCO2 that is consistent with that of the internal carotid artery (∼0.6%/Torr) observed over a much wider range of PetCO2.

Previously, MCA diameter was observed not to change across a similar range of PetCO2 examined in the present study (22, 25). However, Serrador et al. (22) used 1.5 T MRI with a voxel size of 0.47 × 0.47 × 5.00 mm, giving a total voxel volume of 1.11 mm3. In the present study, the resolution of the 3T system was greater with a voxel size of 0.4 × 0.4 × 2.0 mm for a voxel volume of 0.32 mm3. The voxel volume affects not only the resolution, but also the impact of partial volume effects. In particular, the smaller voxel volume of the 3T system used here will reduce the presence of two or more types of tissue in one voxel, where signal from one would “water down” signal from the second. With the larger voxel volume in the previous study, partial volume effects may have minimized any detectable change (22). In addition, a higher signal-to-noise ratio was achieved with the 32-channel head coil, and the signal-to-noise ratio is inherently two times greater at 3 T compared with 1.5 T (4). Some variability in MCA CSA was observed across subjects in the present study, especially during HO. The observation of a greater dilatory response to HC vs. constrictor response to HO is consistent with previous observations of the internal carotid artery (27). We observed high between-subject variability in the constrictor response to HO, which suggests either varying sensitivity to CO2 or that the MCA exists in a constricted state at baseline. Giller et al. (7) also observed variability in the response to HO on direct observation of the MCA during craniotomy. We speculate that the brain could be protected from inappropriate vasoconstric-

tion, which could lead to damaging ischemia, particularly if it involves the major arteries at the base of the brain. In this context, baseline flow may be the regulated variable in the brain, as opposed to the PaCO2.

In the present study, no significant biases were observed when comparing TCD and PC estimates of CBFV. Additionally, the ICC was strong, indicating that variation between the two methods in the same subject was minimal compared with variation between subjects. However, the variability in the differences between the two methods produced quite a spread of data, resulting in wide limits of agreement. Leung et al. (15) observed proportional bias with less agreement between the two methods at high levels of CBFV during HC. However, in this study, proportional bias was observed only with CVR due to one data point where CVR was highest. This indicates that TCD and PC may not provide similar estimates of CBFV in individuals who express a large CVR response to HC. Leung et al. (15) suggested that one way to reduce this variability between the two methods may be to compare the mean CBFV signal instead of the peak signal, which may reduce partial volume effects.

The increase in MCA CSA with HC may be influenced by the concurrent rise in BP. In this study, MAP increased during HC by ∼5 ± 3%. Under conditions of normal PetCO2, a change in BP should have minimal effect on CBF due to intact cerebral autoregulation. However, under hypocapnic conditions, dynamic cerebral autoregulation is impaired (19). Thus it is possible that the cerebral circulation may respond passively to small BP changes, leading to an effect on the CSA of the MCA during HC. Indeed, pharmacological studies indicate that larger cerebral vessels, such as the internal carotid artery, can change diameter passively during changes in BP (16). Based on these earlier data (16), the change in diameter in internal carotid artery diameter that could be expected with our change in BP is ∼3% (16), which is less than the 8% diameter change observed. However, the study of Liu et al. was performed under conditions of normocapnia, so it is unclear how HC may affect these results (16).

This study is limited by the fact that MCA CSA and TCD measurements were collected on different days that were, on average, 53 days apart. We attempted to decrease day-to-day variability by performing data collection at the same time of day in the separate sessions. Despite this limitation, studies suggest that TCD and PC estimates of CBFV are reproducible over 1 wk in the case of TCD and over 72 days for PC (5, 23). These studies are limited by the fact that they examined repeatability under conditions of normocapnia. Few studies that have assessed the reproducibility of CVR to CO2 have produced variable findings. McDonnell et al. (17) found the ICC between two estimates of TCD-based CBFV change during HC performed 1 wk apart to be fairly strong (ICC = 0.73) in one rater who had more recently been trained, but much weaker in the other rater (ICC = 0.08). Additionally, BP measurements were not taken during the MRI session.

Another limitation of the present study was that PaCO2 was not measured. Previous studies indicate that PetCO2 overestimates PaCO2 during HC but not HO (20, 27). Regardless, PetCO2 was used in all cases, so any overestimation was comparable between TCD and PC.

Lastly, it must be noted that, in a minimal number of T2 images, the vessel borders were unclear and, as a result, were

Fig. 7. CVR expressed as percent change of CBFV and CBF divided by the change in PetCO2 during HC and HO. Values are means ± SD, n = 7 for all conditions. *P < 0.05.
not analyzed. Respiratory-induced motion artifacts can be problematic and were a concern for this study. All images used in this study were carefully monitored for significant motion problems like blurring or ghosting. It is unlikely that the image quality could be significantly improved through the use of respiratory gating for the following reasons: 1) the significant increase in scan time during the T2 turbo spin echo sequence used in this study could potentially introduce patient movement artifacts other than respiration itself; and 2) the potentially large variation between TR periods in this acquisition would significantly alter the spin history and introduce other unwanted magnitude and phase variances and, therefore, image artifacts.

In summary, the present data support the conclusion that the CSA of the MCA changes in response to changes in \( \text{PetCO}_2 \). The response was more homogeneous across participants during HC than HO. These data contribute to emerging evidence that alterations in CBF due to manipulations of the \( \text{PaCO}_2 \) and/or the arterial partial pressure of oxygen are not solely due to changes in the smaller arterioles (27, 28). Therefore, caution must be applied when using CBFV as a surrogate for CBF during conditions that manipulate concentrations of arterial gases.

**GRANTS**

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: N.S.C., J.S.G., O.O., and J.K.S. conception and design of research; N.S.C., O.O., and A.P. performed experiments; N.S.C. and J.K.S. interpreted results of experiments; A.P. analyzed data; N.S.C. and J.K.S. drafted manuscript; N.S.C., J.S.G., and J.K.S. edited and revised manuscript; N.S.C., J.S.G., O.O., A.P., and J.K.S. approved final version of manuscript.

**REFERENCES**


