Confounding of Cerebral Blood Flow Velocity by Blood Pressure During Breath Holding or Hyperventilation in Transient Ischemic Attack or Stroke

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Background and Purpose—Breath holding (BH) and hyperventilation are used to assess abnormal cerebrovascular reactivity, often in relation to severity of small vessel disease and risk of stroke with carotid stenosis, but responses may be confounded by blood pressure (BP) changes. We compared effects of BP and end-tidal carbon dioxide (etCO₂) on middle cerebral artery mean flow velocity (MFV) in consecutive transient ischemic attack and minor stroke patients.

Methods—In the population-based, prospective OXVASC (Oxford Vascular Study) phenotyped cohort, change in MFV on transcranial Doppler ultrasound (ΔMFV, DWL-DopplerBox), beat-to-beat BP (Finometer), and etCO₂ was measured during 30 seconds of BH or hyperventilation. Two blinded reviewers independently assessed recording quality. Dependence of ΔMFV on ΔBP and ΔetCO₂ was determined by general linear models, stratified by quartiles.

Results—Four hundred eighty-eight of 602 (81%) patients with adequate bone windows had high-quality recordings, more often in younger participants (64.6 versus 68.7 years; P<0.01), whereas 426 had hyperventilation tests (70.7%). During BH, ΔMFV was correlated with a rise in mean blood pressure (MBP; r²=0.15, P<0.001) but not ΔCO₂ (r²=0.002, P=0.32), except in patients with ΔMBP <10% (r²=0.13, P<0.001). In contrast during hyperventilation, the fall in MFV was similarly correlated with reduction in CO₂ and reduction in MBP (ΔCO₂: r²=0.13, P<0.001; ΔMBP: r²=0.12, P<0.001), with a slightly greater effect of ΔCO₂ when ΔMBP was <10% (r²=0.15). Stratifying by quartile, MFV increased linearly during BH across quartiles of ΔMBP, with no increase with ΔetCO₂. In contrast, during hyperventilation, MFV decreased linearly with ΔetCO₂, independent of ΔMBP.

Conclusions—In older patients with recent transient ischemic attack or minor stroke, cerebral blood flow responses to BH were confounded by BP changes but reflected etCO₂ change during hyperventilation. Correct interpretation of cerebrovascular reactivity responses to etCO₂, including in small vessel disease and carotid stenosis, requires concurrent BP measurement. (Stroke. 2020;51:468-474. DOI: 10.1161/STROKEAHA.119.027829.)

Key Words: blood pressure • humans • ischemic attack, transient • leukoaraiosis • linear models • stroke
assumed to measure cerebrovascular endothelial responses to CO₂, disregarding any contribution of changes in BP.

Therefore, we determined the feasibility, practicality, and physiological validity of BH and hyperventilation testing for assessment of CVR on transcranial Doppler ultrasound in a pragmatic, large population-based prospective cohort of patients with acute transient ischemic attack or minor stroke.

Methods

Study Population

Consecutive, consenting patients with transient ischemic attack or minor stroke were recruited between April 2011 and April 2018, as part of the phenotyped cohort of the OXVASC (Oxford Vascular Study). Participants were recruited at the OXVASC daily emergency clinic, either following a referral after attendance at the Emergency Department or after direct referral from primary care, usually within 24 hours. Patients were referred after transient neurological symptoms or symptoms consistent with a minor stroke, not requiring direct admission to hospital. The OXVASC population consists of >92 000 individuals registered with about 100 primary-care physicians in Oxfordshire, United Kingdom, with extensive clinical assessment and prospective face-to-face follow-up, as reported previously.

As part of the OXVASC cohort, a routine prospective cardiovascular physiological assessment is performed at the 1-month visit, including a BH assessment of CVR since April 2011 and hyperventilation since September 2011. Participants were excluded if they were <18 years of age, cognitively impaired (The Mini-Mental State Exam <23), pregnant, had active cancer, autonomic failure, a recent myocardial infarction, unstable angina, heart failure (New York Heart Association 3 to 4 or ejection fraction <40%), or untreated bilateral carotid stenosis (>70%). OXVASC is approved by the Oxfordshire Research Ethics Committee. Requests for access to the data and analysis tools in this article will be openly considered. Please contact the chief investigator of OXVASC for further information (peter.rothwell@ndcn.ox.ac.uk).

Assessment of CVR

CVR was assessed by BH and hyperventilation. Patients underwent continuous, noninvasive BP monitoring (Finometer MIDI; Finapres Medical Systems), end-tidal CO₂ (etCO₂) via nasal cannulae (Capnocheck Plus; Smith Medical), ECG at 200 Hz (Finometer MIDI), and bilateral middle cerebral artery blood flow velocity. The middle cerebral artery was ideally defined by the peak flow velocity identifiable at the nearest depth to 50 mm at an appropriate insonation angle, in a vessel with flow toward the probe extending for at least 0.5 cm.

After establishing reliable monitoring, patients remained at supine rest for 10 minutes. They were instructed how to perform the BH procedure and then asked to hold their breath at the end of a normal volume inspiration for 30 seconds. At the end of 30 seconds, or if the patient was unable to maintain apnea, they were asked to exhale via their nose to end expiration. If an inadequate BH was achieved, or if there was a significant Valsalva effect (mean blood pressure [MBP] decrease of >10 mm Hg in the first half of the BH), the patient was instructed again on how to perform the maneuver, concentrating on taking a normal tidal volume inspiration, and the procedure was repeated. After normalization of cerebral blood flow and a minimum of 1 minute of rest, the patient was instructed as to how to hyperventilate. They were then asked to hyperventilate via their nose for 30 seconds at a normal tidal volume and a rate of 30 to 60 breaths per minute. If the fall in etCO₂ was inadequate, the patient was asked to increase their respiratory rate or tidal volume to target a minimum 10% fall in etCO₂. The test could be repeated once if inadequate.

Analysis

All data were acquired in Labchart v8 and analyzed with dedicated in-house software (Matlab). Systolic BP, diastolic BP, MBP, etCO₂, and peak, trough, and mean flow velocity (MFV) on transcranial Doppler (TCD) were extracted, with automated detection of baseline values (mean over 5 seconds before apnea or hyperventilation) and minimum or maximum values at end of respiratory manoeuvres and during the first 30 seconds of recovery. All records were visually reviewed and manually corrected by 2 independent assessors blind to clinical information and scored for quality of recording (unsuitable, severe artifact and likely inaccurate, adequate quality, high quality, optimal quality). Only records with adequate to optimal quality of BP, capnography, and at least 1 TCD side were included. For TCD analysis, the side with the better recording quality on blinded assessment was used; if both sides were considered of equal quality, the right side was used.

Change in BP, flow velocity, or etCO₂ was calculated as the absolute increase (BH) or decrease (hyperventilation) from baseline to the greatest absolute change, and as the change in MBP per mm Hg change in etCO₂, expressed as absolute values or percentage change from baseline. Associations between change in each variable and age or demographics were determined by linear regression for continuous variables or t tests for binary variables. Agreement between left and right MCA was visualized by Bland-Altman plots.

Associations between demographics, etCO₂, and BP with change in MFV, peak systolic velocity (PSV), or end-diastolic velocity (EDV) were determined by general linear models for change in etCO₂ and change in systolic BP, diastolic BP, or MBP, unadjusted and adjusted for age and sex. A nonlinear interaction between ΔBP and ΔetCO₂ was empirically determined by simulation with sequential inclusion of individuals by ΔBP for a threshold above and below which there was an independent association between ΔetCO₂ and ΔMFV. General linear models were repeated with stratification of independent variables (ΔBP, ΔetCO₂) at this empirically determined threshold and across quartiles. P<0.05 was taken as significant. Analyses were performed in IBM SPSS v25, Matlab r2015, or Windows Excel.

Results

Four hundred eighty-eight of 602 patients with adequate bone windows (81%) had good-quality recordings during BH, of whom 426 had good-quality hyperventilation tests. Good-quality recordings were more common in younger participants (64.6 versus 68.7 years; P<0.005), with no significant differences in demographics or baseline TCD measures between patients with only BH, only hyperventilation, or both tests (Table 1). For patients with bilateral recordings, there was good agreement between sides, with no significant evidence of bias on regression of Bland-Altman plots (BH: r=0.129, R²=0.17, P=0.087; hyperventilation: r=0.039, R²=0.002, P=0.606; Figure 1 in the online-only Data Supplement).

Baseline BP, MFV, and etCO₂ were negatively correlated with age, while women had lower etCO₂ and MFV. Patients with hypertension had lower baseline MFV, whereas current smokers had higher baseline MFV (Table 2). However, there was no correlation between baseline etCO₂ and MFV (r=0.089, P=0.051) and between baseline MBP and MFV (r=-0.027, P=0.559).

Age was associated with a smaller increase in MFV and MBP but a larger increase in etCO₂ during BH but during hyperventilation was only associated with a greater fall in MBP, with no association with change in MFV or etCO₂ (Table 2). In contrast, MBP and MFV increased more in women during BH, whereas there was no significant difference between sexes in response to hyperventilation. Use of antihypertensives was associated with a lesser increase in MFV during BH, despite only a nonsignificant relationship with decrease in MBP, with no significant change during hyperventilation (Table 2). In contrast, although atrial fibrillation and smoking were associated with significant differences in change in etCO₂, they were not associated with any difference in change in MFV.
Table 1. Clinical Characteristics of Patients With Adequate Quality Assessments of Cerebrovascular Reactivity

|                        | BH (n=488) | Hyperventilation (n=426) | P Value |
|------------------------|------------|--------------------------|---------|
| Age, y; mean (SD)     | 64.9 (13)  | 64.8 (15)                | 0.90    |
| Sex—male; n (%)        | 298 (61.1%)| 262 (61.5%)              | 0.89    |
| Hypertension, n (%)    | 202 (41.4%)| 179 (42.0%)              | 0.84    |
| Diabetes mellitus, n (%)| 44 (9.02%)| 41 (9.62%)               | 0.75    |
| AF, n (%)              | 37 (7.58%) | 32 (7.51%)               | 0.97    |
| Hyperlipidemia, n (%)  | 135 (27.7%)| 115 (27.0%)              | 0.82    |
| Migraine, n (%)        | 146 (29.9%)| 127 (29.8%)              | 0.93    |
| Smoke current, n (%)   | 85 (17.4%) | 71 (16.7%)               | 0.72    |
| Carotid stenosis—any degree, n (%) | 26 (5.32%) | 22 (5.16%) | 0.84 |
| PWV mean, mean (SD)   | 9.16 (2.5) | 9.12 (2.4)               | 0.85    |
| SBP base, mean (SD)   | 127 (22)   | 130 (23)                 | 0.05    |
| DBP base, mean (SD)   | 70.6 (11)  | 71.2 (12)                | 0.38    |
| MBP base, mean (SD)   | 89.2 (13)  | 90.7 (15)                | 0.11    |
| CO2 base, mean (SD)   | 42.3 (6.7) | 42.6 (6.0)               | 0.49    |
| PSV base, mean (SD)   | 78.5 (20)  | 78.0 (20)                | 0.69    |
| EDV base, mean (SD)   | 37.9 (10)  | 37.0 (10)                | 0.20    |
| MFV base, mean (SD)   | 51.4 (13)  | 50.7 (13)                | 0.38    |

Comparisons are by t tests. AF indicates atrial fibrillation; BH, breath holding; CO2, carbon dioxide; DBP, diastolic blood pressure; EDV, end-diastolic velocity; MBP, mean blood pressure; MFV, mean flow velocity; PWV, pulse wave velocity; and SBP, systolic blood pressure.

Relationship Between BP, CO2, and Cerebral Blood Flow

During BH, there was a significant increase in MFV, which was correlated with a concurrent increase in MBP (r2=0.15, P<0.001; Table 3) but was not correlated with the increase in CO2 (r2=0.002, P=0.32). However, during hyperventilation, the fall in MFV was correlated with both reduction in CO2 and reduction in MBP (Figure 1), with a similar strength of association between both CO2 and MBP with MFV. These associations persisted after adjustment for age and sex (Table 3) and when assessing percentage change from baseline (Table 3) or absolute change (Table I in the online-only Data Supplement), with an average 0.3% increase in MFV per 1% increase in etCO2 during hyperventilation, consistent before and after adjustment, and across strata of change in BP.

Change in MFV was significantly associated with both changes in PSV and EDV for both respiratory tests. Although with both tests there were similar associations between systolic BP with PSV and diastolic BP with EDV (Tables II and III in the online-only Data Supplement), there was no relationship during BH between change in etCO2 and either PSV or EDV. In contrast, during hyperventilation, etCO2 was associated with change in both PSV and EDV.

Although there was no significant linear interaction between change in CO2 and change in MBP with change in MFV during BH, there was a nonlinear interaction on empirical modeling (Figure II in the online-only Data Supplement); conversely, there was a significant linear interaction between change in CO2 and change in MBP during hyperventilation.

The association between change in etCO2 with change in MFV with sequentially increasing levels of change in MBP demonstrated a threshold effect for a significant association at an ≈10% change in MBP during both respiratory tests (Figure III in the online-only Data Supplement). In the subgroup of patients with a change in MBP of <10% during BH, there was a significant association between change in etCO2 and change in MFV, with no association in patients with a change in MBP >10% (Table 3). Similarly, there was a stronger association between change in MBP and change in MFV in patients with change in CO2 <10%.

During hyperventilation, the relationship between change in etCO2 and change in MFV was slightly greater in patients with a change in MBP <10%, but it remained significant in patients with a change in MBP >10%, while there was a stronger relationship between MBP and MFV in patients with a change in CO2 <10% (Table 3).

Although 53.1% (259 of 488) of patients performed an involuntary Valsalva manoeuvre during BH, despite repeated instruction, there was no difference in the strength of associations with change in MFV in patients performing a Valsalva for either CO2 (P=0.151 and P=0.631, respectively) or MBP (r2=0.147, P<0.0005, versus r2=0.128, P<0.0005), despite a lower average change in MFV (Table 2). Baseline MBP was associated with ΔMFV during both BH and hyperventilation, but baseline etCO2 was only associated with ΔMFV during hyperventilation. This reflected the association between baseline values and ΔMBP and ΔetCO2, respectively (Table 2). Across quartiles of baseline MBP during hyperventilation, ΔMBP decreased with a corresponding smaller decrease in MFV (Figure IV in the online-only Data Supplement). There were no significant associations between the cerebrovascular responses to hyperventilation versus BH, either for percentage change in MFV (r2=0.006, P=0.09) or percentage change in MFV per percentage change in CO2 (r2=0.0006, P=0.61), although there was a weak association between absolute changes in MFV on the 2 respiratory manoeuvres (r2=0.05, P=0.0003), reflecting the overall magnitude of MFV.

On stratifying change in MBP or etCO2 into quartiles, ΔMFV increased linearly across all quartiles of ΔMBP during BH, with no difference in ΔMFV across quartiles of ΔetCO2. However, there was a linear decrease in ΔMFV across quartiles of ΔetCO2 during hyperventilation (Figure 1). Similarly, the magnitude of the relationship between ΔetCO2 and ΔMFV varied by quartile of ΔMBP during BH, before and after adjustment for confounders, with the only significant association in the lowest quartile of ΔMBP (Figure 2). However, during hyperventilation, the magnitude of association (the CVR) between ΔetCO2 and ΔMFV was consistent across quartiles of ΔMBP, before and after adjustment for baseline MFV and age and sex.

Discussion

In the prospective OXVASC phenotyped cohort of patients with recent transient ischemic attack or minor stroke, cerebral blood flow responses to apnea were principally determined by increases in systemic BP, while etCO2 only induced changes...
when BP change was <10%. In contrast, change in MFV during hyperventilation was determined by both change in systemic BP and change in etCO₂, with a consistent relationship with etCO₂ across different levels of change in BP and demographic characteristics. Findings were consistent for change in systolic or diastolic measures and for absolute and percentage changes.

Previous studies identified a reduction in CVR in patients with lacunar stroke, white matter hyperintensities, and cognitive impairment, but a direct causative role for endothelial dysfunction is unproven. Yet, with evidence of blood-brain barrier breakdown in small vessel disease, a number of studies are targeting CVR to prevention of progression of small vessel disease. TREAT-SVDs [Effects of Amlodipine and Other Blood Pressure Lowering Agents on Microvascular Function in Small Vessel Diseases], OXHARP [Oxford Haemodynamic Adaptation to Reduce Pulsatility Trial], Valsalva and occurred despite efforts to train the participant, likely reflecting endogenous sympathetically driven BP rises to the stress of apnea. However, during hyperventilation, the response was consistently dependent on both BP change and CO₂. Although this affects the interpretation of the result for an individual, this allows for reliable interpretation of the importance of BP and CO₂-driven responses across a population where both indices are measured. Even in an individual, CO₂-dependent CVR could be estimated by adjusting for the expected change in MFV because of BP change. The greater validity of hyperventilation is supported by its reduced association with age, consistent with studies reporting greater reproducibility and preserved cerebrovascular function.

### Table 2. Associations Between Change in CO₂, MBP, and MFV With Clinical Characteristics

| Characteristic                  | Index | Baseline (n=488) | BH (n=488) | Hyperventilation (n=426) |
|--------------------------------|-------|-----------------|------------|------------------------|
|                                |       | CO₂ % | MBP % | MFV % | ΔCO₂ % | ΔMBP % | ΔMFV % | ΔCO₂ % | ΔMBP % | ΔMFV % |
| Age, y                         | R     | -0.125* | 0.169† | -0.292† | 0.114† | -0.192† | -0.252† | -0.026 | -0.137* | 0.015  |
| Below vs above median age      | MD    | 1.27†  | -3.10* | 6.45†  | -1.94 | 2.88*  | 6.32†  | -0.29  | 1.34    | -1.09  |
| Sex (M vs F)                   | MD    | -1.38‡ | 2.07   | -5.16† | 0.88  | -3.24* | -3.84* | -0.02  | 1.37    | 1.26   |
| Hypertension (N vs Y)          | MD    | 0.18   | -3.41* | 2.75†  | 0.13  | -0.36  | 2.45   | -1.30  | 0.23    | 0.70   |
| Treated for hypertension (N vs Y) | MD  | -2.33‡ | 0.06   | 1.99   | -0.27 | 2.15   | 4.52†  | 1.23   | 0.88    | 1.47   |
| Diabetes mellitus (N vs Y)     | MD    | -1.16  | -0.50  | 0.78   | -0.75 | 2.56   | 0.18   | 2.75   | -0.40   | 0.29   |
| Atrial fibrillation (N vs Y)   | MD    | 1.68   | 1.34   | 2.81   | -4.37‡| 2.53   | 3.69   | -1.56  | -0.32   | -0.31  |
| Dyslipidemia (N vs Y)          | MD    | 0.31   | 0.47   | 2.29   | -2.42‡| 1.82   | 1.93   | -0.07  | 0.77    | 0.26   |
| Smoking—current (N vs Y)       | MD    | 1.73‡ | -1.93   | -3.31‡ | -2.04 | -2.71‡ | -0.64  | -5.34† | 0.55    | -1.84  |
| Smoking—ever (N vs Y)          | MD    | 0.86   | -2.03  | 1.24   | -2.43‡| 0.55   | 1.36   | -1.31  | -0.53   | -1.32  |
| Migraine (N vs Y)              | MD    | -1.22  | 1.95   | -2.89† | 1.00  | -0.32  | -2.97† | 1.62   | 0.73    | 1.43   |
| Carotid stenosis—>50% (N vs Y) | MD    | 0.67   | -4.50  | -4.20  | 2.24  | 0.70   | 4.09   | -0.91  | -1.19   | -1.46  |
| Valsalva (N vs Y)              | MD    | -0.03  | -2.67‡ | -0.08  | -1.16 | 3.32†  | 6.41†  | ...    | ...     | ...    |
| Basal MBP                      | R     | 0.42   | ...    | -0.027 | 0.018 | -0.232†| -0.136*| -0.106†| -0.143*  | -0.131*|
| Basal etCO₂                    | R     | ...   | 0.042  | 0.089  | -0.421 | -0.030 | -0.037 | -0.290†| -0.066  | -0.201†|

BH indicates breath holding; CO₂, carbon dioxide; etCO₂, end-tidal carbon dioxide; F, female; M, male; MBP, mean blood pressure; MD, mean difference between groups; MFV, mean flow velocity; N, no; R, Pearson correlation coefficient; and Y, yes. *P<0.01, †P<0.001, ‡P<0.05.
We did not study change in BP during inhaled CO₂, but these tests are innately stressful with ≤5% of patients not tolerating CO₂ inhalation during magnetic resonance imaging and may well be associated with sympathetically driven rises in BP that would confound interpretation of CVR in the absence of BP measurement. This

Table 3. Middle Cerebral Artery Reactivity to Changes in Blood Pressure or CO₂

|                      | BH (n=488) |                      | Hyperventilation (n=426) |
|----------------------|------------|----------------------|--------------------------|
|                      | Per Percentage Change in MBP | Per Percentage Change in etCO₂ | Per Percentage Change in MBP | Per Percentage Change in etCO₂ |
|                      | CVR  | P Value | R²  | CVR  | P Value | R²  | CVR  | P Value | R²  | CVR  | P Value | R²  |
| Unadjusted           | 0.522 | <0.001 | 0.152 | 0.058 | 0.327 | 0.002 | 0.396 | <0.001 | 0.121 | 0.301 | <0.001 | 0.134 |
| Univariate (n)       |                      |                      |                          |
| <10% change etCO₂ (43, 88) | 0.761 | <0.001 | 0.333 | -1.075 | 0.004 | 0.183 | 0.484 | <0.001 | 0.182 | 0.233 | 0.035 | 0.051 |
| >10% change etCO₂ (445, 338) | 0.495 | <0.001 | 0.136 | 0.131 | 0.061 | 0.008 | 0.310 | <0.001 | 0.076 | 0.302 | <0.001 | 0.069 |
| <10% change MBP (188, 179) | 0.481 | 0.008 | 0.037 | 0.226 | 0.009 | 0.036 | 0.777 | <0.001 | 0.130 | 0.318 | <0.001 | 0.152 |
| >10% change MBP (300, 247) | 0.342 | <0.001 | 0.055 | -0.030 | 0.683 | 0.001 | 0.117 | 0.174 | 0.008 | 0.171 | 0.001 | 0.041 |
| Adjusted: Baseline MFV | 0.522 | <0.001 | 0.152 | 0.057 | 0.336 | 0.002 | 0.391 | <0.001 | 0.127 | 0.310 | <0.001 | 0.154 |
| Adjusted: ∆MBP+∆etCO₂ | 0.525 | <0.001 | 0.154 | 0.083 | 0.130 | 0.005 | 0.296 | <0.001 | 0.070 | 0.236 | <0.001 | 0.085 |
| Interaction MBP×etCO₂ | -0.012 | 0.024 | 0.011 | -0.012 | 0.024 | 0.011 | 0.016 | 0.001 | 0.026 | 0.016 | 0.001 | 0.026 |
| Baseline MFV         | 0.526 | <0.001 | 0.154 | 0.081 | 0.141 | 0.004 | 0.286 | <0.001 | 0.073 | 0.247 | <0.001 | 0.101 |
| Adjusted: ∆MBP, ∆etCO₂, age, sex | 0.462 | <0.001 | 0.123 | 0.113 | 0.037 | 0.009 | 0.300 | <0.001 | 0.071 | 0.236 | <0.001 | 0.086 |
| Interaction MBP×etCO₂ | -0.010 | 0.057 | 0.008 | -0.010 | 0.057 | 0.008 | 0.016 | 0.001 | 0.025 | 0.016 | 0.001 | 0.025 |
| Baseline MFV         | 0.454 | <0.001 | 0.120 | 0.106 | 0.049 | 0.008 | 0.285 | <0.001 | 0.071 | 0.247 | <0.001 | 0.101 |

Estimates are shown as change in MFV from baseline (%) per percentage change from baseline in MBP or etCO₂ for BH and hyperventilation tests. Estimates are presented for the whole population, stratified by percentage change in MBP or etCO₂, and adjusted for MBP, etCO₂, age, sex, and the interaction between MBP and etCO₂. R² presented is partial R². BH indicates breath holding; etCO₂ carbon dioxide; CVR cerebrovascular reactivity; etCO₂ end-tidal carbon dioxide; MBP mean blood pressure; and MFV mean flow velocity.

Figure 1. Percentage change in mean flow velocity (MFV) during breath holding or hyperventilation by quartile of change in mean blood pressure (MBP) or end-tidal carbon dioxide (etCO₂). A and B show change in MFV during breath holding by quartile of change in MBP (A) and etCO₂ (B), C and D show ∆MBP% during hyperventilation by ∆etCO₂ (C) or ∆etCO₂ (D), with magnitude of change increasing from Q1 to Q4. Data show mean change with 95% CIs as error bars.
Figure 2. Magnitude of cerebrovascular reactivity (CVR) during breath holding and hyperventilation by quartile of change in mean blood pressure (MBP). CVR (percentage change in mean flow velocity [MFV] per percentage change in MBP or carbon dioxide [CO2]) is derived from general linear models, unadjusted and adjusted for age, sex, and baseline MFV. *P<0.05.

does not undermine the interpretation that CVR impairment assessed by BH in carotid stenosis is prognostically significant but undermines the interpretation that abnormalities in CVR in small vessel disease reflect cerebrovascular endothelial dysfunction and that CVR as opposed to autoregulation to BP is the optimal treatment target.

There are limitations to our study. First, the study was performed in consecutive, unselected patients with transient ischemic attack or minor stroke, resulting in a high proportion of elderly and frail patients who may have had greater difficulties in performing the manoeuvres, and a relatively high proportion of patients with poor TCD windows or poor quality recordings. However, the analysis was restricted to good-quality recordings, and this population reflects the population of interest for assessment of CVR. Second, we did not assess the effects of BP during CO2 inhalation or during magnetic resonance imaging–based methods of cerebral blood flow assessment. This limits extrapolation of these results to these tests, which require further study to determine whether they are similarly confounded by concurrent BP changes. Third, although the OXVASC study and the physiological tests were prespecified in the protocol of the study, the details of the analysis were not. Fourth, the time constraints of performing a complex series of physiological tests in a pragmatic clinical population resulted in a maximum of 2 repeats of each test. However, this study, therefore, reflects an assessment of the validity of these tests used within a busy clinical setting. Finally, because of practical limitations in an elderly, population-based cohort, the respiratory manoeuvres did not include techniques to control the magnitude of etCO2 change, such as paced breathing, end-tidal forcing, or biofeedback. However, this was not practical without limiting inclusivity, and etCO2 change was linearly associated with MFV during hyperventilation and sufficiently large in most patients to allow for reliable estimation of the effect of etCO2 change.

Overall, this study identified that hyperventilation is a reliable and practical method to assess CVR in a large population with acute cerebrovascular disease, but concurrent measurement of continuous BP response is critical for validly interpreting the results. However, BH principally reflects the cerebrovascular response to BP change in this population. Although this may still have prognostic value in a large population and may reflect autoregulation of cerebral blood flow responses to BP, concurrent BP measurement is critical and the measured outcome cannot be interpreted as reflecting endothelium-dependent CO2 responses. Further research is required to assess the role of BP changes in CVR assessment during inhaled CO2, to assess the prognostic significance of BH and hyperventilation, to determine the CVR-dependent effects of antihypertensive and statin-based treatments, and for stroke prevention, and to address which determinants of the cerebrovascular response are associated with cerebral small vessel disease.

Acknowledgments
We are grateful to the staff in the general practices that collaborated in the OXVASC (Oxford Vascular Study): Abingdon Surgery, Stert St, Abingdon; Malthouse Surgery, Abingdon; Marcham Road Family Health Centre, Abingdon; The Health Centre, Berinsfield; Key Medical Practice; Kidlington; 19 Beaumont St, Oxford; East Oxford Health Centre, Oxford; Church Street Practice, Wantage. This work uses data provided by patients and collected by the National Health Service as part of their care and support and would not have been possible without access to these data. A.J.S. Webb devised, acquired, supervised, and analyzed physiological assessments and statistical analysis and drafted, edited, and submitted the manuscript. Dr Paolucci acquired and analyzed physiological data, performed statistical analyses, and had equal responsibility for the manuscript. Dr Mazzucco and L. Li acquired physiological assessments. P.M. Rothwell established and supervised the OXVASC study and devised, initiated, and supervised the physiological studies, analyses, and manuscript.

Sources of Funding
OXVASC (Oxford Vascular Study) is funded by the National Institute for Health Research (NIHR) Oxford Biomedical Research Centre, Wellcome Trust, Wolfson Foundation, British Heart Foundation, and the European Union’s Horizon 2020 programme (grant 666881, SVDs@target). P.M. Rothwell is in receipt of an NIHR Senior Investigator award. A.J.S. Webb is funded by a Wellcome Trust clinical research career development Fellowship (206589/Z/17/Z) and British Heart Foundation Project Grant (PG/16/38/32080).

Disclosures
None.

References
1. Lassen NA. Cerebral blood flow and oxygen consumption in man. *Physiol Rev*. 1959;39:183–238. doi: 10.1152/physrev.1959.39.2.183
2. Ainslie PN, Duffin J. Integration of cerebrovascular CO2 reactivity and chemoreflex control of breathing: mechanisms of regulation, measurement, and interpretation. Am J Physiol Regul Integr Comp Physiol. 2009;296:R1473–R1495. doi: 10.1152/ajpregu.91008.2008.

3. Powers WJ. Cerebral blood flow and metabolism: regulation and pathophysiology in cerebrovascular disease. Stroke. Pathophysiology, Diagnosis, and Management. 2016;28:46–62.

4. Wardlaw JM, Smith C, Dichgans M. Small vessel disease: mechanisms and clinical implications. Lancet Neurol. 2019;18:684–696. doi: 10.1016/S1474-4422(19)30079-1.

5. Sam K, Crawley AP, Conklin J, Poublanc J, Sobczyk O, Mandell DM, et al. Development of white matter hyperintensity is preceded by reduced cerebrovascular reactivity. Ann Neurol. 2016;80:277–285. doi: 10.1002/ana.24711.

6. Tsvigoulis G, Alexandrov AV. Cerebral hemodynamics in acute stroke: pathophysiology and clinical implications. J Vasc Interv Neurol. 2008;1:65–69.

7. Jokinen H, Kaksha H, Ylikoski R, Madureira S, Verdelho A, van der Flier WM, et al. LADIS Group. Longitudinal cognitive decline in subcortical ischemic vascular disease—the LADIS Study. Cerebrovasc Dis. 2009;27:384–391. doi: 10.1159/000020744.

8. Smolifiski L, Członkowska A. Cerebral vasomotor reactivity in neurodegenerative diseases. Neurol Neurochir Pol. 2016;50:455–462. doi: 10.1016/j.pjnn.2016.07.011.

9. Matteis M, Vernieri F, Caltagirone C, Troisi E, Rossini PM, Silvestrini M. Patterns of cerebrovascular reactivity in patients with carotid artery occlusion and severe contralateral stenosis. J Neurol. 1999;168:47–51. doi: 10.1002/000015943.

10. Vernieri F, Pasqualetti P, Passarelli P, Rossini PM, Silvestrini M. Outcome of carotid artery occlusion is predicted by cerebrovascular reactivity. Stroke. 1999;30:593–598. doi: 10.1161/01.str.30.3.593.

11. Silvestrini M, Vernieri F, Pasqualetti P, Matteis M, Passarelli P, Troisi E, et al. Impaired cerebral vasoreactivity and risk of stroke in patients with asymptomatic carotid artery stenosis. JAMA. 2000;283:2122–2127. doi: 10.1001/jama.283.16.2122.

12. Yoshida J, Ogasawara K, Chida K, Oikawa K, Matsumoto Y, Nomura J, et al. Preoperative prediction of cerebral hyperperfusion after carotid endarterectomy using middle cerebral artery signal intensity in 1.5-tesla magnetic resonance angiography followed by cerebrovascular reactivity to acetazolamide using brain perfusion single-photon emission computed tomography. Neurol Res. 2016;38:1–9. doi: 10.1080/01616412.2015.1114291.

13. Gupta A, Chazen JL, Hartman M, Delgado D, Anunula N, Shao H, et al. Cerebrovascular reserve and stroke risk in patients with carotid stenosis or occlusion: a systematic review and meta-analysis. Stroke. 2012;43:2884–2891. doi: 10.1161/STROKEAHA.112.663716.

14. Reinhard M, Schwarzer G, Briel M, Altamura C, Palazzo P, King A, et al. Cerebrovascular reactivity predicts stroke in high-grade carotid artery disease. Neurology. 2014;83:1424–1431. doi: 10.1212/WNL.0000000000000888.

15. Lim EY, Yang DW, Cho AH, Shim YS. Cerebrovascular hemodynamics on transcranial doppler ultrasonography and cognitive decline in mild cognitive impairment. J Alzheimers Dis. 2018;59:651–657. doi: 10.3233/JAD-180026.

16. Viticchi G, Falsetti L, Vernieri F, Altamura C, Bartolini M, Luzzi S, et al. Vascular predictors of cognitive decline in patients with mild cognitive impairment. Neurobiol Aging. 2012;33:1127.e1–1127.e9. doi: 10.1016/j.neurobiolaging.2011.11.027.

17. Bakker SL, de Leeuw FE, de Groot JC, Hofman A, Koudstaal PJ, Breteler MM. Cerebral vasomotor reactivity and cerebral white matter lesions in the elderly. Neurology. 1999;52:578–583. doi: 10.1212/WNL.52.3.578.

18. Blaser T, Hofmann K, Buerger T, Effenberger O, Wallesch CW, Goertler M. Risk of stroke, transient ischemic attack, and vessel occlusion before endarterectomy in patients with symptomatic severe carotid stenosis. Stroke. 2002;33:1057–1062. doi: 10.1161/01.str.0000013671.70986.39.

19. Provincialis L, Mincipieto P, Ceravolo G, Angeleri F, Sanguinetti CM. Transcranial Doppler sonography as a diagnostic tool in vascular dementia. Eur Neurol. 1999;30:98–103. doi: 10.1159/0000117320.

20. Webb AJ, Rothwell PM. Physiological correlates of beat-to-beat, ambulatory, and day-to-day home blood pressure variability after transient ischemic attack or minor stroke. Stroke. 2014;45:533–538. doi: 10.1161/STROKEAHA.113.003321.

21. Webb AJS, Mazzucco S, Li L, Rothwell PM. Prognostic significance of blood pressure variability on beat-to-beat monitoring after transient ischemic attack and stroke. Stroke. 2018;49:62–67. doi: 10.1161/STROKEAHA.117.109107.

22. Rothwell PM, Coull AJ, Silver LE, Fairhead JF, Giles MF, Lovelock CE, et al. Oxford Vascular Study. Population-based study of event-rate, incidence, case fatality, and mortality for all acute vascular events in all arterial territories (Oxford Vascular Study). Lancet. 2005;366:1773–1783. doi: 10.1016/S0140-6736(05)67702-1.

23. Stevenson SF, Doubal FN, Shuler K, Wardlaw JM. A systematic review of dynamic cerebral and peripheral endothelial function in lacunar stroke versus controls. Stroke. 2010;41:e434–e442. doi: 10.1161/STROKEAHA.109.656855.

24. Wardlaw JM, Doubal F, Armitage P, Chappell F, Carpenter T, Muñoz Maniega S, et al. Lacunar stroke is associated with diffuse blood-brain barrier dysfunction. Ann Neurol. 2009;65:194–202. doi: 10.1002/ana.21549.

25. Bruce CD, Steinback CD, Chauhan UV, Pfoh JR, Abrosimova M, Vanden Berg ER, et al. Quantifying cerebrovascular reactivity in anterior and posterior cerebral circulations during voluntary breath holding. Exp Physiol. 2016;101:1517–1527. doi: 10.1113/EP085764.

26. Oudegeest-Sander MH, van Beek AH, Abbink K, Olde Rikkert MG, Claassen JA. Incorrect perceived cerebral blood flow and cortical oxygenation. Exp Physiol. 2014;99:586–598. doi: 10.1133/exphysiol.2013.076455.

27. van De Beek AH, Van De Wit HM, Olde Rikkert MG, Claesena JA. Incorrect performance of the breath hold method in the old underestimates cerebrovascular reactivity and goes unnoticed without concomitant blood pressure and end-tidal CO2 registration. J Neuroimaging. 2011;21:340–347. doi: 10.1111/j.1552-6569.2010.00517.x.

28. Claassen JA, Zhang R, Fu Q, Witkowski S, Levine BD. Transcranial Doppler estimation of cerebral blood flow and cerebrovascular conductance during modified rebreathing. J Appl Physiol (1985). 2007;102:870–877. doi: 10.1152/japplphysiol.00906.2006.

29. Totto R, Marini C, Baldassarre M, Carolei A. Cerebrovascular reactivity evaluated by transcranial doppler: reproducibility of different methods. Cerebrovasc Dis. 1999;9:142–145. doi: 10.1159/000015943.

30. Giannopoulos S, Katsanos AH, Tsvigoulis G, Marshall RS, Statins and cerebral hemodynamics. J Cereb Blood Flow Metab. 2012;32:1973–1976. doi: 10.1038/jcbfm.2012.122.