Evidence for Shear Stress–Mediated Dilation of the Internal Carotid Artery in Humans


Abstract—Increases in arterial carbon dioxide tension (hypercapnia) elicit potent vasodilation of cerebral arterioles. Recent studies have also reported vasodilation of the internal carotid artery during hypercapnia, but the mechanism(s) mediating this extracranial vasoreactivity are unknown. Hypercapnia increases carotid shear stress, a known stimulus to vasodilation in other conduit arteries. To explore the hypothesis that shear stress contributes to hypercapnic internal carotid dilation in humans, temporal changes in internal and common carotid shear rate and diameter, along with changes in middle cerebral artery velocity, were simultaneously assessed in 18 subjects at rest and during hypercapnia (6% carbon dioxide). Middle cerebral artery velocity increased significantly (69±10–103±17 cm/s; P<0.01) along with shear in both the internal (316±52–518±105 1/s; P<0.01) and common (188±40–275±61 1/s; P<0.01) carotids. Diameter also increased (P<0.01) in both carotid arteries (internal: +6.3±2.9%; common: +5.8±3.0%). Following hypercapnia onset, there was a significant delay between the onset of internal carotid shear (22±12 seconds) and diameter change (85±51 seconds). This time course is associated with shear-mediated dilation of larger conduit arteries in humans. There was a strong association between change in shear and diameter of the internal carotid (r=0.68; P<0.01). These data indicate, for the first time in humans, that shear stress is an important stimulus for hypercapnic vasodilation of the internal carotid artery. The combination of a hypercapnic stimulus and continuous noninvasive, high-resolution assessment of internal carotid shear and dilation may provide novel insights into the function and health of the clinically important extracranial arteries in humans. (Hypertension. 2016;68:1217-1224. DOI: 10.1161/HYPERTENSIONAHA.116.07698.)

Key Words: carbon dioxide ■ carotid arteries ■ cerebral arteries ■ shear stress ■ vasodilation

Small cerebral arteries and arterioles (eg, pial vessels) respond rapidly to changes in their metabolic milieu and are highly sensitive to the partial pressure of arterial carbon dioxide (PaCO₂). Vasomotor responsiveness to PaCO₂, termed CO₂ reactivity, is integral to stabilizing blood pH levels, and previous studies have associated lower CO₂ reactivity to increased cardiovascular and all-cause mortality. Recent magnetic resonance imaging studies in humans have revealed that vasomotor changes also occur in the middle cerebral artery (MCA) and basilar artery across the hypo- and hypercapnic range. These studies demonstrate the involvement of larger cerebral arteries in the PaCO₂ reactivity response, findings consistent with well-controlled, animal studies. Other recent studies using Duplex ultrasound have investigated extracranial artery responses during hypo- and hypercapnia. These studies provide direct evidence that changes in end-tidal CO₂ (PETCO₂) are associated with directionally similar, and dose-dependent, changes in internal carotid artery (ICA) diameter. The mechanism(s), however, mediating these changes in extracranial ICA diameter remain unclear.

Significant and rapid changes in extracranial artery blood flow occur across the hypo- and hypercapnic range. In peripheral conduits such as the radial and brachial arteries, such changes in flow and attendant arterial shear stress represent potent vasoactive stimuli. Although Pohl et al were the first to identify that flow-mediated dilation (FMD) is endothelium dependent, it is now well established that this phenomenon occurs in humans and that NO plays a significant role. The widely used FMD test relies on dilation of small arteries and arterioles in the limbs, as a consequence of cuff-induced ischemia, to induce an increase in upstream conduit artery shear stress and dilation. In the context of these studies, it is conceivable that rapid and profound dilation of intracranial vessels in response to hypercapnia induces extracranial (ICA) dilation as a consequence of increased shear stress.

The aim of this study was to identify whether hypercapnia induces shear-mediated dilation in the carotid arteries. Using high-resolution Duplex ultrasound combined with novel, edge-detection software, we assessed simultaneous common
carotid artery (CCA) and ICA dilator responses evoked by hypercapnia. We tested the hypotheses that: (1) hypercapnia would induce significant increases in CCA and ICA shear rate, (2) vasodilation of the vessels would occur secondary to this increase in shear rate, and (3) there would be a strong association between the magnitude of shear and subsequent diameter change in carotid arteries.

Methods

Subject Characteristics
Eighteen participants were recruited (9 women, 9 men; age, 26±4 years; height 1.75±0.12 m; weight 69±12 kg, and body mass index 22±2 kg/m²). Participants who had a history of cardiovascular, musculoskeletal, or metabolic disease; were smokers (or <6 months cessation); or taking medication of any kind were excluded. To control for the effect of changes in circulating hormones on vascular function, all women were assessed within the first 7 days of the follicular phase of the menstrual cycle. The study was approved by the University of Western Australia’s Human Research Ethics Committee and conformed to the standards outlined in the Declaration of Helsinki. Participants were informed of all experimental procedures and any potential associated risks. Written informed consent was obtained from all participants before commencement of the study.

Study Design
Subjects attended the laboratory having fasted for a minimum of 8 hours and abstained from alcohol, caffeine, and vigorous exercise for at least 24 hours. Once instrumented, participants lay supine for 10 minutes. All data were continuously recorded, beginning with a 2-minute baseline period before inhalation of 6% CO₂, 21% O₂, and balance nitrogen, for 4 minutes, followed by a 1-minute recovery period. During this time, simultaneous assessment of intracranial velocity (measured by transcranial Doppler of the left MCA) and beat-by-beat extracranial blood flow (measurement by Duplex ultrasonography of the left ICA and CCA) was assessed along with beat-to-beat arterial pressure (Finapres Medical Systems, Amsterdam, The Netherlands). To minimize the influence of turbulent flow on vascular responsiveness, the CCA and ICA were imaged at least 2 cm below and above the point of bifurcation, respectively. All studies were performed in a quiet, temperature-controlled laboratory.

Experimental Measures

Transcranial Doppler and Systemic Hemodynamics
Middle cerebral artery velocity was measured using a 2-MHz, pulsed, ST3 transcranial ultrasound system (Spencer Technologies, Seattle, WA). The MCA was identified using search techniques described in detail elsewhere. A Marc 600 headframe (Spencer Technologies) was secured around the participant’s head to allow for adjustments to the insonation angle until an optimal M-mode image was found. Raw analogue MCA cerebral velocity was exported from PowerLab to LabChart 7 for post hoc analysis. The PETCO₂ was measured in all subjects via a sampling tube connected to a Hans Rudolph mask via an online and calibrated gas analyzer (ML206; ADInstruments, Sydney, Australia). Beat-to-beat reconstructed brachial blood pressure, mean arterial pressure, and heart rate were measured using a Finometer Pro (Finapres Medical Systems, Amsterdam, Netherlands).

Vascular Ultrasonography
The CCA and ICA were assessed on the left side by 2 expert vascular sonographers. The diameter and blood flow velocity of each artery were simultaneously recorded throughout the hypercapnic protocol using 2 identical 10-MHz, multifrequency, linear array probes attached to a high-resolution ultrasound machine (T3200; Terason, Burlington, MA). The CCA and ICA were identified, and the images were optimized in accordance with recent methodological guidelines. Simultaneous recordings began after optimization of the longitudinal B-mode image of the lumen-arterial walls. Concurrently, Doppler velocity assessments were collected using the lowest possible insonation angle (always <60°). Our group have previously reported high reproducibility for the assessment of ICA diameter, with a within-day coefficient of variation of 1.5%.²⁰

Data Analysis

The reactivity of MCA velocity (MCAv), ICA blood flow, and CCA blood flow with elevations in PETCO₂, was analyzed by averaging 30 seconds of baseline data to that of the peak velocity/blood flow responses and PETCO₂ obtained during the last 30 seconds of hypercapnia. Linear regression analysis was then performed to calculate reactivity slopes. All data from LabChart (MCAv, raw brachial and mean arterial pressure, raw PETCO₂, O₂, and PETCO₂max and O₂max) were exported as 1-second averaged bins into Excel. Analysis of CCA and ICA diameter and flow were performed using custom-designed, edge-detection, and wall-tracking software (BloodFlow Analysis, version 5.1)—an approach that is independent of investigator bias and has previously been comprehensively described and validated.²³,²⁴ From synchronized diameter and velocity data, blood flow (the product of lumen cross-sectional area and Doppler velocity) and shear rate (4xmean blood velocity/vessel diameter) were calculated at 30 Hz. Vascular data were then interpolated from 30 Hz to 1 Hz and exported into an excel spreadsheet. The LabChart and vascular data were time aligned in Excel for subsequent analysis using in-house, carotid shear-mediated dilatation software (General Purpose Data Graphing Software, version 1).

Carotid Shear–Mediated Dilatation Software
All data were passed through a 2-stage filtering process; a median filter (with a rank of 7) was applied to the parameter data, followed by passage through a Savitzky–Golay finite impulse response Smoothing Filter with a window size of 13 data points and a polynomial order of 1. These filters removed high-frequency noise to reveal the underlying lower frequency physiological response profiles. All subsequent analyses were performed using this graphed, filtered data. The following variables were automatically detected and calculated by the software: (1) baseline: the median value of the 2-minute baseline period preceding hypercapnia; (2) peak response: the autodetected maximum value of the filtered data identified after the onset of CO₂; and (3) relative (%) change: change from baseline to peak, calculated as ((peak−baseline)/baseline)×100. In addition, a thresholding algorithm was applied to each data array (eg, ICA shear, ICA diameter, CCA shear, and CCA diameter), which identified threshold points. These thresholds were defined as the point at which each variable began to systematically increase, above the baseline level, after the application of the CO₂ stimulus. The threshold point was calculated as follows:

\[
\text{Threshold point} = \left[ \text{max value} - \text{min value} \right] \times \left( \frac{\text{variation factor} \times \text{baseline median value}}{\text{max value} - \text{min value}} \right)
\]

[max value−min value] was across the whole study and took into account the maximum variation in each study. Two researchers analyzed the variables using a standard variation factor of 10% in the equation across all studies. This variation factor was chosen to ensure that the variable had increased to a point that represented a definitive deviation from baseline, which also exceeded fluctuations associated with cardiac and respiratory cycles. Once the software had automatically detected the threshold points, they were depicted on the raw data array and visually inspected to ensure they met the following criteria: (1) the algorithm-detected threshold point occurred before the peak value and (2) the variable did not decrease below the algorithm-detected threshold point before the peak value occurring. Of the 90 responses analyzed, 78 met the agreed criteria, and their automatically detected points were accepted and not modified. In the remaining 12 cases (13%), the threshold points were manually adjusted independently by the 2 analyzers to a point where each deemed there was a...
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Results

In response to 6% CO2 inhalation, PETCO2 increased from 41±5 at baseline to a peak of 54±3 mmHg (P<0.01), whereas PETO2 also increased from 120±5 to 135±2 mmHg (P<0.01). Heart rate (58±9–73±11 bpm; P<0.01) and mean arterial pressure (92±9–104±11 mmHg; P<0.01) were also elevated with hypercapnia. Average MCA cerebrovascular reactivity was 2.44±0.58 cm/s/mm PETCO2, whereas ICA and CCA reactivity were 14.0±7.4 and 16.1±5.8 mL/min/mmHg PETCO2, respectively.

Arterial Blood Flow, Shear Rate, and Diameter Responses

There was an increase in MCAv by 49±19% from baseline to a peak value of 69±17 cm/s (both P<0.01; Figure 1), with a greater relative increase observed in the ICA when compared with the CCA (P<0.01; Table). Finally, with no differences between the arteries (P=0.53; Figure 2), the CCA dilated by 5.8±3.0% and the ICA by 6.3±2.9% (both P<0.01).

Time Course of Arterial Hemodynamic Responses

MCAv increased 18±9 seconds after the onset of hypercapnia (Figure 3), followed by ICA (22±12 seconds) and CCA shear (32±44 seconds); however, there were no significant differences between the above variables. Dilation of the CCA occurred at 53±30 seconds, significantly earlier than the ICA (85±51 seconds; P<0.05). Finally, a difference was evident between the onset of change in shear and the onset of change in diameter of the ICA (P<0.01), with an average latency period of 64±53 seconds. No difference was evident between the onset of shear and diameter in the CCA (P=0.12).

Influence of Sex on Responses

Although no difference was evident between baseline MCAv values (P=0.07), peak MCAv responses were higher in women compared with men (114±17 versus 92±7 cm/s; P=0.002). A 2-way ANOVA between CCA and ICA baseline and peak shear rate data revealed a significant difference for sex (P<0.001). Post hoc tests revealed that while there was no difference in CCA shear rate at baseline between the groups, peak CCA shear rates were higher in the female group (P=0.008). Baseline ICA shear was higher in women, along with peak shear responses (P=0.009 and P<0.001, respectively). No difference was evident between CCA and ICA baseline or peak diameters between the groups (Table). No difference was evident between groups for the threshold point data. Finally, women displayed greater MCA cerebrovascular reactivity compared with men (2.84±0.50 versus 2.04±0.32 cm/s/mmHg PETCO2; P=0.001); however, there was no difference between sexes in ICA or CCA reactivity.

Relationships Between Selective Variables of Interest

The relative increase in shear between the CCA and ICA were significantly correlated (r=0.68; P<0.01). A significant correlation was also evident between the percentage changes in shear for the CCA and ICA respectively.
change in pooled CCA and ICA diameter and shear rate ($r=0.43; P<0.01$; Figure 4A). Interestingly, further analysis revealed a significant association between ICA shear and diameter ($r=0.68; P<0.01$; Figure 4B). The correlation between percentage change in CCA diameter and shear rate was not significant ($r=0.15; P=0.55$; Figure 4C). Mean arterial pressure increased 51±73 seconds after the onset of hypercapnia, and there was no association between threshold time points for increase in MAP and ICA diameter or MAP and CCA diameter ($r=-0.21$ and $r=17$, respectively). However, a positive correlation was evident between change in MAP (mm Hg) and ICA ($r=0.56; P<0.01$) and CCA diameter (mm) ($r=0.53; P<0.01$). Finally, no association was evident between time to peak for MAP and CCA dilation ($r=0.31; P=0.22$) or dilation of the ICA ($r=0.26; P=0.30$).

**Discussion**

The primary aim of this study was to examine the shear rate and diameter responses of the extracranial cerebral arteries, the ICA and CCA, during transient hypercapnia. Our principle novel findings are (1) both the ICA and CCA dilate significantly in response to hypercapnia, (2) changes in artery velocity and shear in response to hypercapnia occurred first in the MCA, followed by the ICA and then the CCA, (3) there was a significant time delay between the hypercapnia-induced onset of increase in shear and the onset of increase in diameter in both the ICA and CCA, characteristic of shear-mediated dilation (but not direct CO$_2$-mediated diameter change), and (4) there was a strong association between changes in shear and diameter in the ICA, but not in the CCA. These findings are suggestive that increases in shear play an important role in ICA dilation during hypercapnia and also suggest distinct regulatory differences between the ICA and CCA.

Although not a universal finding,$^{5,11}$ our ICA findings are broadly consistent with recent studies,$^{9,10}$ adding robustness to the notion of ICA dilation during hypercapnia. Differences in measurement approaches (manual versus automated) may explain these between-study differences.$^{10}$ Our findings are the first to show that the CCA also dilates to a similar extent to the ICA during hypercapnia. By characterizing the time course of change in MCA velocity and ICA and CCA shear and diameter after application of hypercapnia, this study...
provides insight into the mechanisms responsible for extracranial hypercapnic dilation in humans. Although conceivable that extracranial arteries are directly affected by changes in arterial CO₂ tension, or reflex impacts of hypercapnia (ie, MAP), it is logical that such mechanisms would be rapidly evoked after application of the CO₂/pH stimulus, with minimal delay between the hypercapnia and the onset of dilation. For example, pial vessels are intrinsically sensitive to CO₂ via changes in extracellular pH and respond by dilating instantaneously.1,25,26 We speculate that this is reflected in the present study by the rapid increase in MCA velocity.27 However, the ICA (and CCA) did not dilate with the same temporal dynamics as previously reported in the pial vessels. This dissimilar reactive behavior to hypercapnia suggests distinct regulation and provides evidence that ICA and CCA vasodilation is not solely mediated via intrinsic CO₂ sensitivity.

We hypothesized that the dilator responses of the ICA might be shear mediated, as is the case in peripheral conduit arteries when distal arteriolar beds dilate.13 The latencies we observed between the onset of increases in ICA shear and diameter in the present study are congruent with in vivo cerebral FMD in animals28 and are also consistent with shear-mediated dilation in peripheral arteries in humans.23 We also observed a correlation between percentage change in shear and diameter in the ICA. Cumulatively, these results are suggestive of a shear-mediated role in ICA dilation. Because there was a correlation between change in ICA dilation and MAP, the role of blood pressure in ICA dilation cannot be dismissed. However, no correlation was evident between either the threshold time points or the time to peak for ICA dilation and MAP, as would be expected if there was a passive effect of pressure on artery dilation. Increases in intra-arterial pressure have typically been associated with the activation of vasoconstrictive mechanisms such as the myogenic reflex in peripheral arteries.29,30 To our knowledge, there are no data investigating this in relation to the ICA in humans; however, if it occurred in the present study, it would result in an underestimation of our percentage dilation results. In addition, increases in blood pressure can directly increase blood velocity. This may have affected vasomotor tone via increased shear, consistent with our primary findings. Finally, previous reports of ICA vasoconstriction during hypocapnia occurred in the presence of slight increases in blood pressure,9,10 which argues against the role of blood pressure as a regulator of ICA vasodilation in the present study.

A novel aspect of the present study was the simultaneous assessment of both ICA and CCA diameter and shear changes within subjects in response to hypercapnia. Interestingly, the magnitude of dilation in the ICA and CCA were not highly correlated, despite close correlations between the eliciting shear rate stimuli within subjects (r=0.68). If flow and shear were principal determinants for vasomotor responses in both the CCA and ICA, similar diameter responses should have manifested. Yet, ICA dilation was strongly correlated with shear, whereas the CCA dilation was not. Furthermore, both arteries were imaged simultaneously on the same side, meaning the prevailing blood pressure and other systemic stimuli were identical, yet distinct within-subject vasomotor responses were observed between the arteries. These findings suggest regulatory differences in the vasomotor control between the CCA and ICA, with the latter demonstrating greater shear dependence. Possible reasons for this difference in regulation of the CCA and ICA may relate to structural differences between the arteries. In the current study, the CCA was significantly larger, with a lower shear rate compared with the ICA. It is known that artery size influences the shear stimuli and functional responsiveness of an artery, which was potentially evident in the current study.11 In addition, a previous magnetic
Some previous studies, but not all. In the present study, men and women. This interesting observation suggests that no difference was evident in ICA or CCA reactivity between men and women. This sex difference in CO2 reactivity is consistent with some previous studies, but not all. In the present study, we did not independently manipulate or clamp changes in CO2 or O2, and this may contribute to some of the variation between subjects. Indeed, we observed an increase in O2 during the hypercapnic period (120±5–135±2 PETO2 mm Hg; P<0.01). However, this represents a small change, and because the cerebrovasculature is relatively insensitive to such small changes in the hyperoxic range, changes the sex-related differences in MCA reactivity are a result of either (1) methodological issues relating to the determination of MCA reactivity from velocities, which does not account for potential sex differences in the magnitude of change in MCA diameter or (2) different regulation between the intracranial and extracranial vessels. The physiological significance of these sex differences remains unknown but may relate to artery size, which has impacts on functional responsiveness.

Our findings have potential clinical relevance. FMD of the brachial and femoral arteries, introduced by Celermajer et al has been technically enhanced and formalized as a measure of in vivo endothelial function and preclinical atherosclerosis in the past 2 decades. The assessment of FMD is largely mediated by NO, strongly correlates with coronary artery function, and independently predicts cardiac events. A 1% decrease in FMD is associated with an 8% to 13% increase in cardiovascular event risk. Thus, FMD has become a useful research and clinical tool providing insight into conduit artery function and health in humans. No such in vivo test has been specifically developed to provide insight into cerebrovascular function, physiology, and risk, but it is conceivable that ICA shear-mediated dilation may provide a window on the health of the cerebrovascular endothelium. Indeed, there is a positive association between greater arterial stiffness and microvascular brain disease and cognitive impairment, and a lower ICA dilation in response to a standardized, shear-mediated stimulus may therefore provide a useful marker of cerebrovascular function and future risk. Future studies will be necessary to test this proposal.

Although our findings are suggestive of hypercapnia-induced, shear-mediated ICA dilation, we did not infuse intra-arterial antagonists of endothelium-derived substances such as nitric oxide. Future studies, particularly in animal models, involving more invasive approaches, or the modulation of shear via carotid ligation, would strengthen the notion of shear-mediated, extracranial, artery dilation. Previously, it has been demonstrated that CO2 reactivity is unaffected by prostaglandins, a known endothelium-derived relaxing factor, through utilization of multiple cyclo-oxygenase inhibitors. Although the precise role of NO remains controversial, the lack of effect of other endothelium-derived relaxing factors provides support that NO may be an important factor driving endothelium-mediated responses in the cerebral circulation of humans. It is also possible that CO2 directly influenced the extracranial arteries in the current study; however, we consider this unlikely because the time course of the vascular responses was delayed and consistent with shear-mediated adaptation, as discussed above. Future studies may also benefit from different approaches to the application of hypercapnia, for example, using a higher level of CO2 for a shorter period of time to elicit a rapid increase in arterial shear and subsequently diameter, independent of the confounding influence of longer exposure to CO2. In the present study, we did not independently manipulate or clamp changes in CO2 or O2, and this may contribute to some of the variation between subjects. Indeed, we observed an increase in O2 during the hypercapnic period (120±5–135±2 PETO2 mm Hg; P<0.01). However, this represents a small change, and because the cerebrovasculature is relatively insensitive to such small changes in the hyperoxic range, changes...
in cerebral blood flow or the function of the measured arter-ies seem unlikely. Nonetheless, future studies should consider strictly controlling PETCO2 and PETO2, for example, via end-tidal forcing systems similar to those used in the previous stud-ies by Willie et al6 and Hoiland et al.9 An additional limitation, inherent in the use of transcranial Doppler, is the assumption that the diameter of the imaged vessel does not change.7 We were unable to examine and compare the time course of velocity and diameter change between the intra- and extracranial arteries, and there are no current technologies that allow for the temporal resolution to do so accurately.

Perspectives

In summary, we report significant vasodilation in both the CCA and ICA in response to hypercapnia. By systematically determining the time latencies of cerebral blood velocity and diameter responses, we provide insight into the underlying mechanisms of extracranial vasodilation. Our findings suggest that shear stress is an important stimulus for hypercapnic ICA vasodilation in humans, secondary to the dilation of distal cerebral arteries. The combination of an easily administered and standardized hypercap-nic stimulus, with continuous high-resolution assessment of internal carotid shear and shear-mediated dilation, may provide important insights into the function and health of the clinically important extracranial arteries in humans.

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Disclosures

None.

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