Cyclooxygenase inhibition abolishes age-related differences in cerebral vasodilator responses to hypercapnia

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Barnes JN, Schmidt JE, Nicholson WT, Joyner MJ. Cyclooxygenase inhibition abolishes age-related differences in cerebral vasodilator responses to hypercapnia. J Appl Physiol 112: 1884–1890, 2012. First published March 22, 2012; doi:10.1152/japplphysiol.01270.2011.—Blood flow and vasodilatory responses are altered by age in a number of vascular beds, including the cerebral circulation. To test the role of prostaglandins as regulators of cerebral vascular function, we examined cerebral vasodilator responses to CO₂ (cerebrovascular reactivity) in young (26 ± 5 yr; 6 males/6 females) and older (65 ± 6 yr, 5 males/5 females) healthy humans before and after cyclooxygenase inhibition (using indomethacin). Middle cerebral artery velocity (MCAv) responses to stepped hypercapnia were measured before and 90 min after indomethacin. Changes in MCAv during the recovery from hypercapnia (vasoconstrictor responses) were also evaluated before and after indomethacin. Cerebrovascular reactivity was calculated using linear regression between MCAv and end-tidal CO₂. Young adults demonstrated greater MCAv (55 ± 6 vs. 39 ± 5 cm/s; \( P < 0.05 \)) and MCAv reactivity (1.67 ± 0.20 vs. 1.09 ± 0.19 cm/s⁻¹/mmHg⁻¹; \( P < 0.05 \)) to hypercapnia compared with older adults (\( P < 0.05 \)). In both groups MCAv and MCAv reactivity decreased between control and indomethacin. Furthermore, the age-related differences in these cerebrovascular variables were abolished by indomethacin. During the recovery from hypercapnia, there were no age-related differences in MCAv reactivity; however, indomethacin significantly reduced the MCAv reactivity in both groups. Taken together, these results suggest that cerebral blood flow velocity and cerebrovascular reactivity are attenuated in aging humans, and may be due to a loss of prostaglandin-mediated vasodilatation.

BLOOD FLOW AND VASODILATOR responses decline with advancing age in many vascular beds (4), including the cerebral circulation (15). In this context, cerebral microvessels are highly sensitive to changes in the partial pressure of carbon dioxide (\( \text{PaCO}_2 \)), dilating in response to hypercapnia and constricting in response to hypcapnia. Tight control of central \( H^+ \) is facilitated by cerebrovascular reactivity to \( \text{PaCO}_2 \) and the sensitivity of respiratory chemoreceptors to central \( H^+ \). The increase in middle cerebral artery (MCA) blood flow velocity measured during stepped increases in \( \text{CO}_2 \) is often used to estimate cerebral microvascular vasodilator function (i.e., reactivity) (42). Currently, the effect of aging on cerebrovascular reactivity is controversial, with both reductions in cerebrovascular reactivity (20, 28, 30, 37) or no changes reported in older individuals (5, 7, 32). These conflicting results may be related to differences in the technique used to evaluate reactivity (transcranial Doppler, xenon-133 inhalation, or BOLD fMRI), underlying comorbidities, or the specific age, sex and medication use by the participants. For example, Kastrup et al. (13) reported no age-related change in cerebrovascular reactivity in men but declines in women after the fifth decade of life. In addition, prostaglandins play an important role in regulating cerebral blood flow and cerebrovascular responses to hypercapnia in young adults (38, 39), and the contribution of prostaglandins to the regulation of vascular tone may be altered with advancing age (14, 21, 35). Therefore, a change in prostaglandin synthesis and/or production is one potential mechanism underlying any age-related changes in cerebrovascular reactivity (3, 14, 21, 36, 42). However, this mechanism has not been systematically examined in healthy older adults.

Lower cerebrovascular reactivity has been observed in patients with cognitive impairment, thus providing an association linking cerebral microvascular dysfunction to cognitive decline (7, 29, 34). These findings are consistent with the epidemiological results suggesting a connection between so-called “vascular risk factors” and cognitive function (22). Importantly, it remains unclear if cerebral blood flow velocity responses to hypercapnia are altered by aging in cognitively normal individuals free of confounding risk factors. In this context, we tested the hypothesis that cerebral vasodilator responses to stepped hypercapnia, but not cerebral vasoconstrictor responses during recovery from hypercapnia, are reduced in healthy older adults. Because a loss of prostaglandins (vasodilating prostaglandins in particular) may explain this age-related reduction, we examined the cerebral vasodilator responses to \( \text{CO}_2 \) before and after cyclooxygenase (COX) inhibition with indomethacin.

METHODS

Subjects. Twenty-two volunteers, including 12 young (aged 18–34 yr) and 10 older (aged 59–76 yr) healthy adults, participated in the study. Subjects were nonsmoking, nonobese [body mass index (BMI) < 30 kg/m²], normotensive, and did not have any underlying cardiovascular, metabolic, or other chronic pathologies (determined by a health questionnaire and a brief clinical assessment). Blood samples were obtained after a 12-h fast and analyzed for hemoglobin, glucose, cholesterol, and triglyceride concentrations. Prescription and over-the-counter medications were reviewed by a physician prior to the study day. Subjects were not taking antihypertensive medication or any other vasoactive medications other than statins (2 older male subjects). In addition, the use of any nonsteroidal anti-inflammatory drugs (NSAIDs) and vitamin and antioxidant supplements was restricted for 10 days prior to the blood draw and 10 days prior to the experimental study day. All subjects were sedentary or recreationally active (no structured training in the previous 3 mo). Older female subjects were postmenopausal and were not on hormone replacement therapy. Young female subjects were studied in early follicular phase of the menstrual cycle. Informed consent was obtained and subjects were familiarized with experimental conditions during an initial screening visit. All older subjects underwent cognitive testing to rule
out any undetected cognitive abnormalities. All procedures had ethical approval from the Institutional Review Board at the Mayo Clinic and were performed according to the Declaration of Helsinki including written informed consent.

**Experimental protocol.** The cerebral blood flow velocity experiments were conducted in the Clinical Research Unit in the morning, and subjects arrived to the laboratory after an overnight fast. In addition, subjects were asked to abstain from alcohol, caffeine, and chocolate for at least 24 h prior to the study. Figure 1 is a schematic diagram of the experimental protocol. Arterial blood pressure (BP) was monitored noninvasively using finger photoplethysmography (Finometer, TPD Biomedical Instrumentation). Heart rate (HR) from a standard three-lead ECG and oxygen (O2) saturation using pulse oximetry were monitored continuously throughout the study (Datex-Ohmeda, Datex-Ohmeda, Louisville, CO). During the stepped hypercapnia trials, breath-by-breath end-tidal CO2 (ETCO2; Datex-Ohmeda) and tidal volume via a turbine (model VMM-2a, Face Associates, Laguna Nigel, CA) were recorded.

**Cerebral blood flow velocity.** During the initial screening, subjects were imaged using a 2-MHz Doppler probe (Transcranial Doppler, Neurovision System, Multigon, Yonkers, NY) to estimate middle cerebral artery (MCA) blood flow velocity and determine the optimal settings for each subject. The basal portion of the right MCA was insonated by placing the probe over the temporal bone just above the zygomatic arch between the frontal process and the front of the ear. The Doppler signal was optimized by varying the sample volume depth in incremental steps and varying the angle of insonance to obtain the best-quality signal. On the experimental study day, the process was repeated and once the optimal signal was determined, the probe was secured with a headband device to maintain the proper position and angle throughout the protocol.

**Stepped hypercapnia trials.** The hypercapnic responses were assessed using a steady-state, open-circuit technique (1). Subjects were in a semi-reclined position on a hospital bed with a mask covering the nose and mouth attached to one-way Hans Rudolph valves to prevent rebreathing. After breathing room air, three stepwise ETCO2 elevations were applied to each subject by adding 2%, 4%, and 6% rebreathing. After breathing room air, three stepwise ETCO2 elevations were applied to each subject by adding 2%, 4%, and 6% fractional concentration of inspired CO2 (FICO2), while the oxygen saturations were applied to each subject by adding 2%, 4%, and 6% fractional concentration of inspired CO2 (FICO2) and minimum ETCO2 (during the recovery from hypercapnia). Between the maximum and minimum ETCO2, MCAv and CVCi were averaged for each breath.

**Drug administration.** Indomethacin, a COX inhibitor, was given orally at 1.2 mg/kg along with 10 ml of simethicone to reduce possible stomach irritation. Subjects then rested for 90 min before repeat measurements of cerebrovascular reactivity were made. To account for potential differences in absorption time and metabolism between groups, cerebrovascular reactivity was again assessed at 120 min postindomethacin in 16 subjects; however, there were no statistical differences between the 90- and 120-min measurements so only data from 90 min are presented.

**Data analysis and statistics.** Data were collected at 250 Hz, stored on a laboratory computer, and analyzed off-line with signal processing software (WinDaq, DATAQ Instruments, Akron, OH). All variables of interest (heart rate, blood pressure, ETCO2, and MCAv) were continuously monitored throughout the hypercapnia trials. Beat-by-beat hemodynamic measurements were averaged over the final minute of room air breathing and at each level of hypercapnia. The linear slopes of the relationship between ETCO2 and MCAv or cerebrovascular conductance index (CVCi = MCAv/MAP) were calculated to estimate cerebrovascular reactivity. Subject demographics and baseline characteristics were compared using a one-way ANOVA. Primary variables of interest during the hypercapnia trials were compared between groups (young vs. old) and conditions (control vs. indomethacin) using a two-way repeated-measures ANOVA followed by a Tukey’s post hoc analysis. Statistical significance was set a priori at P < 0.05.

**RESULTS**

Subject characteristics at baseline are listed in Table 1. We attempted to match young and older adults for BMI, blood pressure, and cardiovascular risk factors. Older subjects had a maximum (during 6% FICO2) and minimum ETCO2 (during the recovery from hypercapnia). Between the maximum and minimum ETCO2, MCAv and CVCi were averaged for each breath.
higher fasting blood glucose compared with younger subjects \((P < 0.05)\), although this was still within the normal range.

**Effects of aging on hemodynamic and cerebrovascular variables.** There were no differences between young and older subjects in mean arterial pressure (MAP) measured using a sphygmomanometer at baseline (Table 1) or using a Finometer while breathing room air, or at any stage of hypercapnia (Table 2). At baseline, older subjects had significantly lower MCAv (Table 2, Figure 2) and CVCi (Table 2) during the room air condition compared with younger subjects (Fig. 2). In addition, older subjects also demonstrated significantly lower MCAv while breathing 2%, 4%, and 6% \(\text{FiCO}_2\) and lower CVCi at 2% and 6% \(\text{FiCO}_2\). Indomethacin decreased MCAv reactivity by 10.2 ± 0.3 cm/s; 1 to 6 cm/s (Fig. 3) and percent change in CVCi reactivity slope (Fig. 4) during hypercapnia was significantly lower in older adults compared with young adults. ETCO\(_2\) at all stages of hypercapnia was similar between groups. During the recovery after hypercapnia, the change in MCAv was greater in young compared with older adults (36 ± 4 vs. 23 ± 3 cm/s; \(P < 0.01\)); however, there were no age-related differences in MCAv reactivity or CVCi reactivity (Fig. 5).

**Effects of indomethacin on cerebral blood flow velocity and reactivity.** All subjects completed both control and indomethacin hypercapnia trials. Indomethacin reduced cerebral blood flow velocity of the MCA in both the young (42%) and older subjects (30%) while breathing room air (Fig. 2) and at all levels of hypercapnia (Table 2). To further examine the effect of indomethacin, we calculated the absolute change in cerebrovascular variables before and after indomethacin. Delta MCAv was higher in young subjects compared with older subjects (36 ± 4 vs. 23 ± 3 cm/s, respectively; \(P < 0.05\)). CVCi was also decreased after indomethacin in young subjects at each time point \((P < 0.05)\) while the reduction in CVCi in the older subjects only reached significance during room air and 6% \(\text{FiCO}_2\). Indomethacin decreased MCAv reactivity by 65% in the young and 56% in the older subjects, thereby abolishing the age-related differences in MCAv reactivity (Figs. 3 and 4). Indomethacin did not change ETCO\(_2\) values during any stage of hypercapnia.

Indomethacin reduced the magnitude of change in MCAv during the recovery from hypercapnia in both young (from 36 ± 4 to 16 ± 2 cm/s; \(P < 0.01\)) and older adults (from 23 ± 3 to

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**Table 2. Hemodynamic and gas exchange variables during stepped hypercapnia**

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\(\ast P < 0.05\) vs. young; †\(P < 0.05\) vs. control.

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**Fig. 2. MCAv and cerebrovascular conductance index (CVCi) before (control trial) and 90 min after indomethacin (indomethacin trial). Measurements were taken while subjects were breathing room air. \(*P < 0.05\) vs. young; †\(P < 0.05\) vs. control.**
Furthermore, indomethacin attenuated the MCAv reactivity and CVCi reactivity slope in both groups during the recovery period (Fig. 5).

DISCUSSION

The present study shows, for the first time, that COX inhibition abolishes the age-related differences for both cerebral blood flow velocity at rest and cerebrovascular reactivity to stepped increases in CO2. Thus our results suggest that the age-related decline in cerebrovascular reactivity to hypercapnia may be mediated by a loss of vasodilating prostaglandins. In addition, the present study found that there were no age-related differences in cerebral vasoconstrictor responses to a decrease in CO2 and that COX inhibition reduces cerebral vasoconstrictor responses in both young and old adults.

Methodological limitations. There are several methodological considerations with our experimental approach. First, cerebral blood flow and cerebrovascular reactivity measurements in this study used transcranial Doppler measurements and therefore use blood velocity to estimate blood flow of the MCA. Changes in MCA blood velocity correspond to changes in cerebral blood flow velocity measured using other techniques (2, 26, 33). For our protocol, measuring MCA velocity using Doppler is an ideal approach to determine the beat-by-beat changes during hypercapnia and recovery. Blood velocity is a reliable indicator of flow when diameter or cross-sectional area of the insonated vessel is constant. Accordingly, there is little to no change in MCA cross-sectional area during hypercapnia using MRI or angiography (3, 8, 9, 26). Furthermore, altered PaCO2 induces changes in microvasculature downstream from the MCA allowing the use of MCA velocity as an indicator of cerebral blood flow (3, 26).

Second, we evaluated cerebral responses using a stepped hypercapnia (steady state) technique and measured ETCO2 to calculate the cerebrovascular reactivity slopes. In this study, we did not have an arterial catheter and did not measure PaCO2 directly. Other studies have used a similar approach (11, 42) and reported similar cerebrovascular reactivity values in a young healthy population. There were no differences in ETCO2 between young and old subjects during any stage of hypercapnia. Thus we believe the stimulus for cerebral vasodilator responses was similar between young and old subjects.

Effect of aging on cerebrovascular responses to hypercapnia. The effect of normal aging on cerebrovascular responses to hypercapnia, independent of pathological conditions, is controversial. Reductions in cerebrovascular reactivity with age have been reported using xenon-133 inhalation technique (28, 30, 37), imaging techniques (10, 29), and transcranial Doppler (17, 20). One study using the xenon-133 inhalation technique reported that lower cerebrovascular reactivity was associated with advancing age (44). In a prospective 4-yr longitudinal study, gradual reductions in reactivity for each year interval were reported (30). The xenon-133 method has been used for decades; however, transcranial Doppler allows for continuous measurement of hemodynamic variables (18). In the present study, we chose the transcranial Doppler method to enable continuous hemodynamic monitoring to compare two distinct subject populations. Several other studies have used transcranial Doppler to assess reactivity using 5% inhalation of CO2 (combined with 95% O2) (17, 20), where we administered three levels of CO2 to construct dose-response curves similar to the human vascular biology studies that use the forearm model. Because hyperoxia causes cerebral vasoconstriction (6), balancing with 95% O2 may obscure the vasodilatory effect of CO2 on the cerebral vessels (7).

Until now, age-related changes in cerebrovascular reactivity have not been investigated using stepped increases in CO2 combined with transcranial Doppler. We have demonstrated

![Fig. 3. MCAv vs. ETCO2 before (control trial) and 90 min after indomethacin (indomethacin trial) in young subjects (left panel) and older subjects (middle panel). Calculated reactivity slope for MCAv is on the right. *P < 0.05 vs. young. †P < 0.05 vs. control.](http://jap.physiology.org/)

![Fig. 4. The slope of the relationship between percent change from baseline in CVCi (cm·s⁻¹·mmHg⁻¹) and percent change from baseline in ETCO2 (mmHg). *P < 0.05 vs. young. †P < 0.05 vs. control.](http://jap.physiology.org/)
reduced cerebral vasodilator responses to CO2 in older adults. However, our results conflict with several studies showing no change in cerebrovascular reactivity with advancing age (5, 7, 32). In the study by Schieve et al. (32), the young healthy subjects also had psychiatric conditions or were diagnosed with cancer, complicating the interpretation of their data. A recent transcranial Doppler study by Galvin et al. and an older study by Davis et al. reported no change in CO2 reactivity but a significant elevation in MAP (Δ5 and Δ15 mmHg, respectively), which was inversely correlated with cerebral blood flow (5, 7). Because MAP is an important determinant of blood flow, and cerebral autoregulation may be altered by age, it is important to calculate cerebrovascular conductance to determine age-related differences. Our results demonstrate that healthy older adults have ~35% lower cerebrovascular reactivity and 27% lower cerebrovascular conductance index (taking into account MAP) compared with young adults. When we examined the slope of the relationship between the percent change in CVCi and the percent change in ETCO2 (to account for differences in baseline MCAv and MAP), the difference between young and old adults remained. In this context, our results are especially robust because we studied healthy, normotensive older subjects and used several concentrations of CO2 (all containing 21% O2), allowing us to construct dose-response curves of CO2.

During the recovery from the hypercapnic stimulus, we found no age-related differences in cerebrovascular reactivity. This suggests that cerebral vasodilator responses to stepped CO2 are reduced, and cerebral vasoconstrictor responses (to decreasing CO2) are preserved, in aging humans. Our approach is different from previous studies where cerebral vasoconstrictor responses were examined using hypocapnia induced by hyperventilation. However, the drop in PaCO2 after removal of the 6% FICO2 still produces a rapid lowering of CO2. Yamaguchi et al. (43) reported a reduction in cerebral vasoconstrictor responses to hypocapnia was associated with advancing age. The authors attribute the reduced responses to hypocapnia to potential atherosclerotic changes within the vessel. However, Galvin et al. (7) report an increase in hypocapnic vasoconstrictor responses in older adults and in older patients with coronary artery disease. The conflicting results could be due to the method of inducing hypocapnia, methodology of cortical blood flow measurement, or the health of the subjects participating in these studies. For example, our approach measured breath-by-breath averages of MCAv during passive recovery from hypercapnia whereas previous studies rely on the subjects to voluntarily hyperventilate to lower CO2 to 15–25 mmHg. Few studies have investigated the effect of aging on cerebral vasoconstrictor responses to decreasing CO2, and more research is necessary.

Potential role of prostaglandins in cerebrovascular responses to hypercapnia. The present study tested the hypothesis that prostaglandins contribute to cerebral blood flow regulation and may be an underlying mechanism in the age-related reduction in cerebral responsiveness to hypercapnia. We found that cyclooxygenase inhibition using indomethacin reduces baseline cerebral blood flow velocity and reactivity to hypercapnia in young adults, similar to other reports (12, 13, 42). However, indomethacin blunted cerebral blood flow velocity and reactivity less in older adults compared with young adults. Importantly, after cyclooxygenase inhibition, cerebrovascular reactivity was similar in young and old adults. These results
suggest that potential age-related reductions in prostaglandin synthesis and/or vascular sensitivity to vasodilating prostaglandins explain, at least in part, why both baseline cerebral blood flow velocity and the vasodilator response to \(\text{CO}_2\) are reduced with aging. In addition, indomethacin blunted cerebral vasoconstrictor responses to decreasing \(\text{CO}_2\) in both groups, again highlighting the importance of prostaglandins in the regulation of cerebral vascular tone.

Indomethacin may alter basal cerebrovascular tone; however, it has been argued that indomethacin does not affect the vasodilatory response seen with hypercapnia (25). The study by Pickles et al. (25) examined cerebrovascular reactivity in six subjects after 3 days of oral indomethacin instead of a single dose. A single dose of oral indomethacin reduces basal cerebral blood flow within 60–90 min (42) and has a half-life of \(~4.5\) h. In addition, the subjects consumed 50 mg of indomethacin prior to arriving in the laboratory, but it is not reported how long it was between the final dose of indomethacin and the cerebral blood flow measurement (25); therefore part of the experiment may have fallen outside the window of 90–120 min typically used to study the acute effect of indomethacin. Furthermore, because 3 days of indomethacin was given, other hemodynamic changes (41) may have occurred to “balance” the vasoconstrictors and vasodilators back to normal. In this context, the study by Pickles et al. is difficult to compare to the present study where we found both a reduction in basal cerebral blood flow velocity and attenuation of the vasodilatory response to hypercapnia in young subjects after one dose of indomethacin [in accordance with previous work (42)]. We have also shown that older adults have a similar directional response to indomethacin, albeit lower in magnitude, compared with young adults. However, the question remains whether the inhibition of cyclooxygenase metabolites accounts for the majority of the change seen in this study. Other cyclooxygenase inhibitors like ibuprofen and aspirin are not associated with a reduction in cerebral blood flow during normocapnia (19, 38), and prostacyclin partially restores the reduction in cerebral blood flow (23). This suggests that indomethacin may have additional effects on the cerebral vasculature, beyond arachidonic acid metabolism. Cerebral metabolic rate is not altered by indomethacin in young adults (31) and therefore is unlikely to be a factor in older adults. Other potential mechanisms of action of indomethacin include blocking of calcium channels, altering extracellular pH, or influencing reactive oxygen species (ROS) produced by cyclooxygenase (16, 27, 39, 40) which may be dependent on prostaglandin synthesis (23). Animal studies strongly indicate that prostaglandins are involved in cerebral blood flow regulation (24), combined with the data showing a reduction in cerebral blood flow responses after indomethacin is partially restored by prostacyclin (23), suggesting that prostaglandin-independent effects of indomethacin cannot explain the findings of the present study. Therefore, based on our data, we speculate that an age-related reduction in vasodilating prostaglandins is a primary mechanism underlying the blunted cerebrovascular reactivity with advancing age.

**Perspectives.** We have shown that cerebrovascular reactivity to \(\text{CO}_2\) is reduced in healthy older subjects and that this is likely related to a loss of prostaglandin-mediated vasodilator function in the cerebral circulation. Our findings may be especially relevant to cognitive function and aging because many of the risk factors for cognitive decline (e.g., hypertension, diabetes, dyslipidemia, and physical inactivity) are known to affect the microcirculation in many vascular beds. Future studies are thus needed to (1) explore the potential link between cognitive function and cerebral vasodilator function; and (2) determine if interventions that increase cerebral blood flow velocity and cerebrovascular reactivity improve cognitive function in normal subjects, those with risk factors for cognitive impairment, and patients with documented cognitive dysfunction.

**Conclusions.** In summary, human aging is associated with attenuated cerebrovascular reactivity to hypercapnia and lower cerebrovascular conductance index. These age-related differences were abolished after cyclooxygenase inhibition using indomethacin, suggesting that a loss of prostaglandin-mediated dilation contributes to the altered regulation of cerebral blood flow velocity and vasodilator responses to \(\text{CO}_2\) with aging.

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**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**

Author contributions: J.N.B., J.E.S., and W.T.N. performed experiments; J.N.B. analyzed data; J.N.B. and M.J.J. interpreted results of experiments; J.N.B. prepared figures; J.N.B. drafted manuscript; J.N.B., J.E.S., W.T.N., and M.J.J. edited and revised manuscript; J.E.S. and M.J.J. conception and design of research; J.E.S., W.T.N., and M.J.J. approved final version of manuscript.

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