

**Retinal Arteriolar and Middle Cerebral Artery Responses to Combined Hypercarbic/Hyperoxic Stimuli**

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**PURPOSE.** The relative effect of simultaneously administered oxygen and carbon dioxide on the retinal and cerebral vessels is still controversial. The purpose of this study was to quantify and compare the superior-temporal retinal arteriole (RA) and middle cerebral artery (MCA) responses to hypercarbic and combined hypercarbic/hyperoxic stimuli.

**METHODS.** Twelve young, healthy volunteers participated in the study. End-tidal pressure of carbon dioxide was raised and maintained at 22% from baseline (hypercarbia), while end-tidal pressures of oxygen (PETO2) of 100 (normoxia), 500, and 300 mm Hg (hyperoxia) were instituted. RA diameter and blood velocity were measured with laser Doppler velocimetry and simultaneous vessel densitometry; MCA blood velocity was measured with transcranial Doppler ultrasound.

**RESULTS.** Normoxic hypercarbia increased RA blood velocity by +17% and calculated flow by +21%. Hypercarbia/hyperoxia-500 mm Hg decreased RA diameter by ~8%, velocity by ~16% and calculated flow by ~29%. MCA blood velocity increased by +45% in response to normoxic hypercarbia, significantly greater than RA blood velocity (P < 0.001). Increase in PETO2 did not affect the hypercarbia-induced increase in MCA blood velocity.

**CONCLUSIONS.** Hyperoxia reversed hypercarbia-induced vasodilation in RA in a concentration-dependent manner. Hypercarbia induced greater vasodilation in the MCA than in the RA but MCA blood velocity was unaffected by increases in PETO2. *(Invest Ophthalmol Vis Sci. 2008;49:5503-5509)* DOI:10.1167/iovs.08-1854

Dysregulation of ocular blood flow plays a prominent role in the pathogenesis of many ocular diseases.1,2 Alterations of blood flow in intraocular, retrolubar, and cerebral vessels have been reported in patients with diabetes,5–6 glaucoma,3 and Behçet’s disease with ocular involvement.8 Moreover, it is generally accepted that the status of the retinal vessels not only serves as a predictor of retinal disease development, but also is an indicator of cerebrovascular health. Abnormal retinal vessel calibers are associated with cerebrovascular disease, an increased risk of stroke, and lower brain oxygenation.9–12 Correlation between decreased cerebral and retinal vascular reactivity were found in patients with cerebral small vessel disease.13 Innovative imaging techniques to assess ocular hemodynamics have contributed to the understanding of the role of the vasculature in the pathophysiology of ocular vascular diseases (see Ref. 14 for review). Homeostatic ocular blood flow measurements exhibit large intersubject variability. Consequently, provocative stimuli (vasoconstrictor or vasodilator) have been used to quantify vascular reactivity, which shows greater consistency.15 In the eye, oxygen (O2) is a potent vasoconstrictor,16 whereas carbon dioxide (CO2) causes vasodilation.17,18 Retinal vessels show greater response to O2, whereas choroidal and cerebral vascular beds respond more to CO2.17,19 Controversy still exists, however, in regard to the combined effect of O2 and CO2 on these vascular beds (see Ref. 16 for review). The conflicting conclusions can be partly explained by the interpretation of data from different techniques used to assess blood flow as well as differing methodologies used to provoke vascular reactivity. We have shown that concentration-dependent hyperoxia-induced vasoconstriction predominates over hypercarbia-induced vasodilation in the retinal arterioles.20 The purpose of this study was to quantify and compare the vascular responses of retinal arterioles and the middle cerebral artery to a series of standardized vasoactive stimuli.

**METHODS**

The study was approved by the Research Ethics Board of the University Health Network, University of Toronto and adhered to the guidelines of the Declaration of Helsinki. Informed consent was obtained from each subject. Twelve healthy nonsmokers (1 woman) of mean age 25 years (SD = 5) were recruited into the study. All subjects breathed via a commercial sequential gas delivery breathing circuit (HiOx-80; VIASYS Healthcare Inc., Dublin, OH) modified by adding a rebreathing bag to the expiratory port.18,21 Adhesive tape (Tegaderm; 3M Health Care, St. Paul, MN) was used to ensure an airtight seal of the mask to the face. Gas was sampled continuously from inside the mask and analyzed for end-tidal partial pressures of CO2 (PETCO2) and O2 (PETO2). Noninvasive systolic and diastolic arterial blood pressures (at 1 minute intervals), and blood oxygen saturation and heart rate were monitored continuously by pulse oximetry (Cardiocap/5; Datex-Ohmeda, Tewksbury, MA). The method of gas administration and its advantages have been published.22,23 Briefly, targeted end-tidal gas concentrations (partial pressures), as described in the protocol were preprogrammed and implemented with a custom-built computer-controlled automated gas blender and sequencer (RespirAct; Thornhill Research Inc., Toronto, ON, Canada).

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Procedures

The breathing protocol is illustrated in Figure 1. Each stage of the experiment was performed when PETCO2 and PETO2 stabilized (i.e., <2 mm Hg change over a 2-minute period). After stabilization at baseline values of PETCO2 and PETO2 (normocarbia/normoxia I), PETCO2 was then targeted to achieve a 20% increase from the baseline at a PETO2 of 100 mm Hg (hypercarbia/normoxia I). PETO2 was then increased to target 500 mm Hg (hypercarbia/hyperoxia-500) and subsequently decreased to normoxia, while maintaining PETCO2 constant at 20% above baseline (hypercarbia/normoxia II). Next, PETCO2 was decreased to baseline (normocarbia/normoxia II). Finally, both PETCO2 and PETO2 were increased simultaneously to achieve 20% increase in PETCO2 and 300 mm Hg PETO2 (hypercarbia/hyperoxia-300). Each condition was maintained for 5 minutes after targeted levels of PETCO2 and PETO2 were achieved and had stabilized. Our approach differed cardinally from previously published studies in that, by implementing continuous end-tidal gas monitoring, we ensured that all blood flow measurements were taken when similar respiratory parameters were achieved and confirmed across subjects. The overall time of each experimental condition for every subject varied from 8 to 15 minutes, depending on the length of transition phase when no blood flow measurements were taken.

Quantitative Assessment of Retinal Blood Flow. The pupil of the study eye was dilated with 1 drop of tropicamide 1%. Retinal blood flow was assessed with laser Doppler velocimetry and simultaneous vessel densitometry (Laser Blood Flowmeter, CLBF, model 100; Canon, Tokyo, Japan) in the superior–temporal arteriole, approximately 1 disc diameter from the optic nerve head, in a straight vessel segment distant from bifurcations. The instrument and measurement site selection details have been described previously.29 With this technique, retinal blood flow was calculated from measured diameter and velocity values. All measurements were made by a single experienced observer (MK). The subjects were masked to the breathing gas mixture composition. Retinal blood flow measurements were made for each condition only when end-tidal gas concentrations were stable. The quality of velocity waveforms was assessed against agreed-upon standards but the observer was masked to the quantitative results of the reading. Readings with loss of fixation and/or aberrant velocity waveforms were deleted at the time of measurement. The measurement of diameter was saved only if the coefficient of variation was less than 2%. At least 10 measurements were taken for each experimental condition.

Quantitative Assessment of Cerebral Blood Flow. Cerebral blood velocity was measured in the MCA with a 2-MHz pulsed Doppler ultrasound system (Multidop X4; DWL Elektronische System GmbH, Sipplingen, Germany). The MCA was identified by using an insonation pathway through the right or left temporal window just above the zygomatic arch. The Doppler signal was optimized by varying the depth (45–55 mm) and angle of insonation.24 When the optimal signal was obtained, the probe was fixed in place with a headband. Velocity (time averaged maximum or V̇̇max) was monitored continuously.

Statistical Analysis

Statistical analysis was performed with commercial software (Statistica ver. 6; StatSoft, Tulsa, OK). All data are presented as the mean ± SD for each experimental condition. Repeated measures ANOVA was performed to test for any change in respiratory, systemic hemodynamic, retinal or cerebral hemodynamic parameters across experimental conditions and study visits. When appropriate, Tukey HSD post hoc tests were undertaken to determine the significance of any change relative to baseline. Tests of average correlations between arterial pressure parameters and retinal and cerebral hemodynamic parameters were used to assess any influence of systemic blood pressure on retinal and cerebral blood flows.

Results

Twelve subjects completed retinal hemodynamic assessment, and their results were included in the analysis of retinal vascular reactivity. Ten subjects completed cerebral hemodynamic assessment, whereas two subjects did not have a temporal bone window that provided a good-quality Doppler signal. The data of these two subjects were excluded from the comparison of retinal and cerebral vascular reactivity.

Respiratory Parameters

A stable increase in PETCO2 was achieved throughout the experiment (+8.9 mm Hg (SD ±1) during assessment of retinal vascular reactivity and +9.8 mm Hg (SD ±1) during assessment of cerebral vascular reactivity. Attained PETCO2 and PETO2 values for each condition did not differ between the two study days (Table 1).

Systemic Hemodynamic Parameters

Systemic hemodynamic changes are shown in Table 2. The differences in systolic (SP), diastolic (DP), and mean arterial blood pressure (MAP) between the first baseline and hyperoxia-500 were small and reached statistical significance only for SP (P < 0.001) and DP (P < 0.05). The differences in SP, DP, and MAP between the first baseline and hyperoxia-300 were larger than those between the first baseline and hyperoxia-500 and in the former reached statistical significance for SP, DP, and MAP (P < 0.05). This effect was consistent across the two visits. Systolic blood pressure was slightly higher on the second study day (P < 0.05). Mean arterial pressure was not different on the two study days.

Superior–Temporal Retinal Arteriolar Vascular Reactivity

RA diameter did not change in response to hypercarbia/normoxia I (115 ± 14 μm vs. 113 ± 13 μm, P = 0.99). RA diameter decreased to 105 ± 14 μm (P < 0.001) in response to hypercarbia/hyperoxia-500. RA diameter returned to baseline during hypercarbia/normoxia II and during normocarbia/normoxia II (112 ± 12 and 112 ± 13 μm, respectively) and then decreased to 107 ± 12 μm (P < 0.05) during hypercarbia/hyperoxia-500 (Fig. 2).

RA blood velocity increased in response to hypercarbia/normoxia I and decreased during hypercarbia/hyperoxia-500 (from baseline 30 ± 6 to 35 ± 6 mm/s, P < 0.01, and 25 ± 7
mm/s, \( P < 0.05 \), respectively). Velocity returned to baseline levels during hypercarbia/normoxia II, normocarbia/normoxia II, and hypercarbia/hyperoxia-300 (Fig. 3).

Calculated RA blood flow increased in response to hypercarbia/normoxia I and then decreased during hypercarbia/hyperoxia-500 (from baseline 9.2 ± 2.6 µL/min to 11.1 ± 3.5 µL/min, \( P < 0.05 \) and 6.5 ± 2.2 µL/min, \( P < 0.001 \), respectively). Calculated RA flow returned to baseline during hypercarbia/normoxia II and normocarbia/normoxia II. During hypercarbia/hyperoxia-300, calculated RA blood decreased significantly compared with normoxia/normocarbia II (\( P < 0.01 \)) but not compared with normoxia/normocarbia I (Fig. 4).

Hypercarbia/normoxia I increased RA blood velocity by +17% ± 14% and calculated flow by +21% ± 19% from baseline. Hypercarbia/hyperoxia-500 reduced diameter by −8% ± 5%, velocity by −16% ± 17%, and calculated flow by −29% ± 14% from baseline values. There were strong correlations between \( P_{ETCO2} \) and RA diameter, blood velocity, and calculated flow (\( r = -0.762, r = -0.675 \) and \( r = -0.807 \), respectively, \( P < 0.0001 \)).

**MCA Vascular Reactivity**

During hypercarbia/normoxia I, MCA blood velocity (MCAV) increased to 76 ± 15 cm/s from baseline 54 ± 13 cm/s (\( P < 0.001 \)) and then remained unchanged during hypercarbia/hyperoxia-500 and hypercarbia/normoxia II (82 ± 21 and 79 ± 19 cm/s, respectively, \( P < 0.001 \)). During normocarbia/normoxia II, MCAV returned to baseline and then increased to 81 ± 19 cm/s during hypercarbia/hyperoxia-300 (\( P < 0.001 \); Fig. 5).

The relative increases in MCAV were +45% ± 20% and +48% ± 18% during hypercarbia/normoxia I and II, respectively, and +54% ± 15% and +54% ± 20% during hypercarbia/hyperoxia-500 and hypercarbia/hyperoxia-300, respectively (Fig. 6). Hypercarbia increased MCAV by 4.6% per mm Hg \( P_{ETCO2} \). The increase in MCAV to normoxic hypercarbia was greater than that of the RA (\( P < 0.001 \)). There was strong correlation between \( P_{ETCO2} \) and MCAV (\( r = 0.994, P < 0.0001 \)).

**DISCUSSION**

There were two major findings in the study. Hyperoxia reversed the hypercarbia-induced increase in RA blood flow in a concentration-dependent manner. Identical hyperoxic-hypercarbic stimuli caused profoundly different responses of MCA and RA blood velocities.

First, we confirmed our previous findings that hyperoxia is a stronger vasoactive stimulus than hypercarbia in the retinal circulation and that with combined hyperoxia/hypercarbia, the constrictor effect of \( O_2 \) on RAs predominates over the vasodilator effect of \( CO_2 \). A limitation of our previous study was that the hyperoxic stimuli followed consecutively and progressively without a return to baseline. In the present study, we reversed the order of the application of the \( O_2 \) stimuli, giving the higher \( PO_2 \) first and returning conditions to baseline between stimuli to minimize any persistent effects of arterial oxygen saturation on retinal and cerebral blood flow. Previously, we found that a 23% increase in \( P_{ETCO2} \) at a \( P_{ETCO2} \) of 556 mm Hg caused a 36% decrease in retinal blood flow.

<table>
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<tr>
<th>Table 2. Effect of Inhalation of Air, Hypercarbic, and Combined Hypercarbic/Hyperoxic Gas Mixtures on Systemic Hemodynamic Parameters</th>
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<tr>
<td>RA Vascular Reactivity Study</td>
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<td>SP (mm Hg)</td>
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All data are expressed as the mean (SD). Level of significance of change from baseline: *\( P < 0.05 \); †\( P < 0.001 \). Data in bold, level of significance of change between the two study days: \( P < 0.05 \).
in this study, a 22% increase in P<sub>ET</sub>CO<sub>2</sub> at a P<sub>ET</sub>O<sub>2</sub> of 483 mm Hg decreased retinal blood flow by 29%. These results support the reproducibility of our retinal hemodynamic assessment technique. Of interest, we found less hypercarbia-induced increase in blood flow after exposure to the hypercarbia/hyperoxia-500 stimulus, suggesting a persistent vasoconstrictive effect of O<sub>2</sub> on retinal arterioles. The underlying mechanisms of cerebral and ocular vascular regulation in response to hyperoxia and hypercarbia are not completely understood. In both vascular beds, the primary involvement of endothelium-derived nitric oxide and endothelin (ET)-1 has been demonstrated. Endothelin-1 is a potent vasoconstrictor with lasting effect, whereas nitric oxide has a much shorter half life, which may explain why hyperoxia causes a concentration-dependent cumulative response in the retinal circulation. Moderate hyperoxia (P<sub>ET</sub>O<sub>2</sub> 300 mm Hg) combined with hypercarbia induced mild vasoconstriction in the retina relative to the preceding normocarbic/normoxic II condition; however, there was no change in calculated RA blood flow between the hypercarbia/hyperoxia-300 stimulus and baseline. Of note, there was no difference in flow between normocarbic/normoxia I and normocarbic/normoxia II, suggesting that the retinal blood flow response to hypercarbia/hyperoxia-300 was not uninfluenced by possible persistent ET-1 effects.

The second major finding is the discrepancy in responses of two organs of close embryologic origin, whose vascular beds are still widely assumed to behave congruently, even though conflicting evidence was available as early as 1964. The present study is the first in which two identical provocative protocols were performed and the vascular reactivity of the RA and MCA compared in the same subjects. Both the superior–temporal RA and MCA velocities increased substantially in response to hypercarbia. However, hypercarbia induced greater increase in blood velocity in MCA than RA. The degree of MCA vascular reactivity agreed with results from previous studies. Although adding hyperoxia to the hypercarbia constricted the RA, it had no discernible effect on MCA velocity.

The MCA, one of the larger basilar vessels, receives 80% of the internal carotid artery blood flow. It functions as a con-
ductive vessel (as opposed to a resistance vessel), and thus, without exception, changes in diameter are reported to be considerably less than 5% under conditions that affect resistance vessels such as hypercapnia, hypoxia, hypertension, and pharmacologic vasodilator and vasoconstrictor provocations. As the MCA in adults is a rather large vessel (1.5–3 mm in diameter), such diameter changes would result in less than 4% to 6% change in MCAV reading for the same flow. The MCA diameter under these conditions has been monitored by ultrasound power analy- 
sis, MRI scanning, and even by direct observation under a microscope. Furthermore, in subjects undergoing provocations to alter cerebral blood flow, there is a strong correlation between resultant changes in MCAV and changes in cerebral blood flow as measured by “reference standard” techniques such as 133Xe SPECT and electromagnetic flow probes on ipsilateral carotid artery, with good correlations of \( r = 0.85, P < 0.001 \) and \( r = 0.898 \). Although historically debat- ed, the overwhelming balance of experimental evidence leaves little doubt that in most conditions, changes in transcranial Doppler velocity signal are directly related to changes in cerebral blood flow.

Retinal and cerebral vascular reactivity was assessed on two separate visits because simultaneous measurements of retinal and cerebral hemodynamics were ergonomically difficult. Nevertheless, the considerable similarity of achieved gas provoca- tion parameters when making measurements at the two vascular sites minimizes the effect of this potential limitation. Another limitation of the present study is the use of a non-blinded observer. Even though the observer was not blinded to the composition of the inspired gas, she was blinded to the quantitative measurements of retinal blood velocity, thus diminish- ing the possible effect of observer bias. The study may also be more comprehensive if the retinal capillary, choroidal, and ophthalmic artery vascular reactivity could be assessed along with that of the retinal arterioles. Unfortunately the comfort level of subjects and the time needed to obtain good-quality recordings limited the number of vascular sites that could be examined.

Part of the underlying motivation for this work was a desire to reveal the effect of carbogen (a mixture of approximately 1–5% \( \text{CO}_2 \) in \( \text{O}_2 \)) on the vasculature when administered for therapeutic benefit. In addition to its direct action of vasoconstriction, \( \text{O}_2 \) causes hyperventilation and a reduction in \( \text{P}_{\text{ET}}\text{CO}_2 \) with a consequent additive vasoconstrictor effect of hypoxia. This has prompted the suggestion to maintain isocapnia with \( \text{O}_2 \) administration. Moreover, since hypercapnia promotes vasodilation, \( \text{CO}_2 \) is often administered in conjunc- tion with \( \text{O}_2 \) in the form of carbogen in an attempt to optimize tissue oxygenation. First, Prisman et al. have shown that carbogen does not reliably change \( \text{P}_{\text{ET}}\text{CO}_2 \) or arterial \( \text{PCO}_2 \) when administered to otherwise healthy subjects. Thus, vasodila- tion cannot be presumed on the basis of carbogen adminis- tration without measuring \( \text{P}_{\text{ET}}\text{CO}_2 \) or arterial \( \text{PCO}_2 \). Second, we demonstrated that with respect to the eye, during combi- 

FIGURE 6. Relative responses of RA and MCA blood velocities. A, normocarbia/normoxia III; B, hypercarbia/normoxia I; C, hypercarbia/hyperoxia-500; D, hypercarbia/normoxia II; E, normocarbia/normoxia II; F, hypercarbia/hyperoxia-300.

The results of the present study imply that, with respect to the eye, one must choose between increasing perfusion and increasing \( \text{PO}_2 \). Hemoglobin is almost fully saturated at a \( \text{PO}_2 \) of approximately 100 mm Hg in patients without significant lung disease. As such, increasing arterial \( \text{PCO}_2 \) will increase perfusion and thus \( \text{O}_2 \) delivery. In the presence of lung disease, it is rational to increase the inspired \( \text{PO}_2 \) to maintain arterial \( \text{PO}_2 \) near 100 mm Hg only, to maintain the efficacy of hyper- 

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may have a more effective alternative to carboxgen for optimizing ocular blood flow.

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