

Hypoxic, Hypercapnic, and Hyperoxic Responses of the Optic Nerve Head and Subfoveal Choroid Blood Flow in Healthy Humans

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PURPOSE. To investigate the impact of different gas mixtures (hyperoxia, hypoxia, and hypercapnia) on the optic nerve head (ONH) and choroidal (Ch) hemodynamics.

METHODS. Twenty-three healthy subjects (28 ± 6 years) took part in the study. Variations in inspired oxygen and carbon dioxide fraction were produced by a gas mixing device. Arterial oxygen saturation (SpO₂) was measured continuously using a transcutaneous sensor and end-tidal carbon dioxide partial pressure by capnography. The experiment comprised three successive periods: 3-minute baseline (room air breathing), 15-minute gas mixture inhalation (normocapnic hypoxia, hypercapnia, or hyperoxia), and 15-minute recovery (room air breathing). Laser Doppler flowmeter parameters—velocity (VEL), volume (VOL), and flow (BF) of red blood cells—were measured. Two-way ANOVAs were performed for statistical analysis.

RESULTS. In response to hyperoxia, ONHBF significantly decreased by $-18\% \pm 6\%$ ($P = 0.04$) from baseline, due to significant changes in VEL ($-12\% \pm 3\%$ $P = 0.0002$). During hypoxia at 85% SpO₂, ONH VEL increased by $+12\% \pm 3\%$ ($P = 0.0009$), whereas VOL and BF did not change significantly. ChBF significantly increased by $+7\% \pm 2\%$ ($P = 0.004$) in response to hypoxia, due to significant changes in VEL $+5\% \pm 2\%$ ($P = 0.03$). Both Ch and ONHBFs did not vary significantly in response to hypercapnia.

CONCLUSIONS. The magnitude of the blood flow response is the most significant during hyperoxia for ONH and hypoxia for ChBF. For ONHBF, a 37% difference between hyperoxia and hypoxia can be useful when vasoreactivity to O₂ will be tested in patients.

Keywords: ocular blood flow, optic nerve head, choroid, gas mixture

The regulation of blood flow in the human eye fundus is complex due to the presence of two vascular systems, which differ anatomically and physiologically: the retinal vessels, which supply the neural region of the retina and the prelaminar region of the optic nerve head (ONH) and the choroidal blood vessels, which lie between the retina and the sclera.^{1,2} Modulation of the diameter of the resistance vessels (vasoreactivity) by myogenic, metabolic, neurogenic, and/or humoral processes allows a fine regulation of the blood flow (BF) in these vascular systems.³ In addition, regulation of choroidal BF (ChBF) also occurs through the autonomic parasympathetic and sympathetic nervous systems.⁴

Changes in the arterial blood partial pressures of oxygen (PaO₂) and carbon dioxide (PaCO₂) are efficient stimuli to activate ocular BF regulatory mechanisms. Elevated PaO₂ (hyperoxia) results in retinal vasoconstriction and a significant decrease in BF within the retina (RBF) and the ONH (ONHBF).⁵⁻⁷ In contrast, this stimulus has little influence on ChBF.^{8,9} Whereas studies have suggested that decreased PaO₂ (hypoxia) results in retinal vessels' vasodilation and an increase in RBF,^{5,6,10} the effect of this stimulus on ONHBF and ChBF is still unknown.

Hypoxia has been involved in the pathophysiology of eye diseases,¹¹ such as nonarteritic anterior ischemic optic neuropathy,¹² diabetic retinopathy, or retinal vein occlusion.¹³ Ocular complications can also occur in systemic hypoxic diseases such as obstructive sleep apnea¹⁴ or scleroderma.¹⁵

Using noninvasive laser Doppler flowmetry (LDF) in healthy subjects,^{16,17} the present prospective study determined vasomotor responses of ONHBF to isocapnic hypoxia, isocapnic hyperoxia, and hypercapnia and the responses of ChBF to isocapnic hypoxia and hypercapnia. Its aim was to obtain for the first time the magnitude and subject-to-subject variability of these responses in the normal human eye and assess the potential applicability of the method in patients with various ocular pathologies.

METHODS

Study Population

Twenty-three healthy young adult volunteers were recruited after full medical and ophthalmologic examinations. Exclusion



criteria included local or systemic medication, ocular or systemic disease, and pregnancy. The initial ophthalmologic examination comprised visual acuity and intraocular pressure (IOP) measurement, slit-lamp examination, gonioscopy, and funduscopy. All volunteers included had a normal optic disc and refractive errors varying between -1 and $+1$ diopters. The study was conducted in accordance with the Declaration of Helsinki for research involving human subjects and adhered to Good Clinical Practice guidelines. Informed consent was obtained from the subjects after explanation of the study. The study protocol was approved by the local institutional review board (IRB #6705) and was registered on ClinicalTrials.gov (NCT00874913).

Laser Doppler Flowmetry

Two previously described LDF instruments were used, one for the measurement of ONHBF¹⁷ and the other for the measurement of ChBF.^{16,18} Each delivers a coherent near-infrared laser probing beam (wavelength of 785 nm; power of 90 μ W at the cornea). This power value complies with American National Standards Institute (ANSI) standard Z 136.1 for laser safety. The light scattered by the tissue at the ONH or the subfoveal choroid is focused onto an avalanche photodiode detector, the output photocurrent of which is sampled at a frequency of 240 kHz with a 16-bit resolution and processed with software developed on Labview (National Instruments, Austin, TX, USA) to determine the LDF parameters based on the theory of Bonner and Nossal.¹⁹ These parameters, which were sampled at a rate of 17 Hz, are velocity (VEL, in kHz), the relative average velocity; volume (VOL, in arbitrary units, au), the relative number of red blood cells; and blood flow (BF = VEL \times VOL, in au), the relative flux of the red blood cells. Area of the probed volume was approximately 280 mm in diameter for the choroid¹⁸ and 300 mm for the ONH.¹⁷ The parameters were further processed to obtain mean values over the heart cycle. It can be legitimately assumed that the hematocrit remains constant during the experiment.^{16,17} In this case, BF is proportional to the flux of red blood cells.

Each flowmeter was mounted on a table equipped with a chin rest and head holder. Subjects were seated and the operator aligned the instrument with the eye under test. Positioning of the laser beam at the fundus occurred under video monitoring by having subjects fixate a target consisting of a point-like light from a red diode laser that could be precisely moved within the field of view.^{17,20} For measurements of ONHBF, the probing beam was placed at a temporal site of the optic disc. For ChBF measurements, subjects were asked to fixate directly at the probing beam, thus ensuring foveal location of the probed tissue volume. Entrance of the beam at the pupil was chosen to obtain a Doppler signal DC (direct current) output suitable for further processing. This DC is proportional to the intensity of the light reaching the detector. Furthermore, care was taken to keep this DC as constant as possible, as assessed visually on the monitor of the LDF flow processor.

Study Protocol

Out of the 23 volunteers, 13 (8 males, 5 females; mean age, 28 ± 6 years) participated in the study on the effect of hyperoxia and hypoxia on ONHBF and ChBF, and 13 subjects (6 males, 7 females; mean age, 26 ± 7 years) were assessed in the study on the effect of hypercapnia on these flows. Three subjects participated in both experiments, with a delay of 3 weeks.

This study was single-masked; the subject did not know which gas mixture was used at each experiment. The order of the gas mixture was randomized for each subject using a

random list. Randomization was stratified for location (ONH or Ch) and gas experiment (hypoxia, hypercapnia, air).

Subjects were asked to abstain from alcohol and caffeine for at least 12 hours before undergoing measurements. An Opticlude patch (Opticlude, OrthopticEye Patch; Nexcare First Aid, 3M Health Care, St. Paul, MN, USA) was placed over the nondominant eye of the subject, whereas the measured eye was dilated with one drop tropicamide 0.5% (Mydriaticum; Thea, Clermont-Ferrand, France). Dominant eye was determined by the pointing test repeated four times: The subject was asked to point the examiner's nose. The eye with which the finger was aligned was considered the dominant eye.

Before the start of the LDF measurements, subjects were seated for at least 20 minutes. Only one eye was measured in each subject.

In every subject, the LDF measurements were scheduled as follows: day 1, choroidal LDF during ambient air followed by hypoxia; day 2, ONH LDF during ambient air followed by hypoxia or hyperoxia; day 3, choroidal and ONH LDF during ambient air followed by hypercapnia. Hyperoxia has been previously reported to have no effect on the human choroid.^{8,9} Subjects were masked with regard to the inspired gas mixture. They breathed through a mouthpiece connected to a bidirectional valve: The inspiratory side was connected to a gas mixing device (Altitrainer 200; SMTEC, Nyon, Switzerland) allowing inhalation of normoxic, hypoxic, hyperoxic, and hypercapnic gas mixtures, and the expiratory side was used for determining end-tidal CO₂ partial pressure (PetCO₂).

During the hypoxic condition, inspiratory oxygen fraction was reduced (usually at 10%–11%) to reach arterial oxygen saturation (SpO₂) levels of 90% after approximately 10 minutes of gas breathing and 85% after 15 minutes of gas breathing. Hyperoxia was defined by a 100% O₂ inhalation. Only during the hypoxia experiment, PetCO₂ was maintained at the value measured during the baseline period (i.e., normocapnia) by continuously adjusting inspiratory CO₂ fraction. Hypercapnia was achieved by increasing inspiratory CO₂ fraction to reach a target PetCO₂ of 10 mm Hg above baseline value.

An experiment comprised three successive periods (Fig. 1): a baseline, consisting of ≈ 3 -minute room air breathing; a ≈ 15 -minute gas mixture inhalation, and a ≈ 15 -minute recovery (room air breathing). Systolic and diastolic blood pressures, heart rate, SpO₂, and PetCO₂ were measured at the end of the baseline, every minute during gas mixture inhalation, and every 5 minutes during the recovery (IPM-9800; MINDRAY Biomedical Electronic, Shenzhen, China). Mean blood pressure was calculated as diastolic blood pressure plus one-third of the pulse pressure.

LDF parameters were recorded for 30 seconds. As indicated in Figure 1, three recordings were obtained during baseline; five during the gas mixture inhalation, which were initiated as soon as the subject's SpO₂ or PetCO₂ targets were reached; and at least four during the recovery period.

Statistical Analysis

During each recording, the LDF signal underwent a digital filtering procedure, by which only the parameter data corresponding to DC values lying within $\pm 20\%$ of the DC mean value of the recording were kept. This was done in order to minimize the deleterious effect of the microsaccades and blinks.²¹ Furthermore, for each experiment (i.e., baseline, gas inhalation, and recovery) we eliminated each value for which the corresponding DC was not within $\pm 12\%$ of the mean DC, calculated based on all remaining data of the experiment. These final data were analyzed using a 2-way ANOVA test performed with the R-commander (Rcmdr) software (<https://cran.r-project.org>; in the public domain). Differences reaching

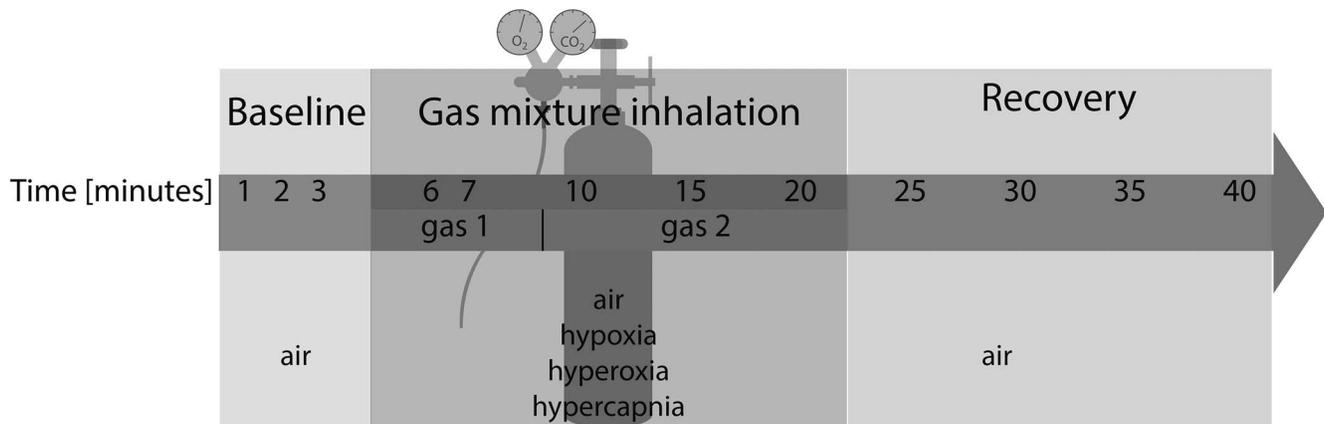


FIGURE 1. Schema of experimental design. Diagrammatic representation of experimental timeline. The *gray area* shows the duration of gas breathing for a subject. There was a four-part gas inhalation sequence: hypoxia (inspiratory oxygen fraction, $FiO_2 \sim 10\%$ -11%), hyperoxia (FiO_2 100%), hypercapnia (end-tidal CO_2 partial pressure, $PetCO_2 + 10$ mm Hg), air. The 30 seconds of laser Doppler flowmetry (LDF) measurements were recorded at minutes 1, 2, and 3 during baseline period; at minutes 6, 7, 10, 15, 20 during gas breathing; at minutes 25, 30, 35, 40 during recovery period. During gas breathing, gas1 is obtained from averaged measures at minutes 6, 7, and 10; gas2 is obtained from averaged measures at minutes 15 and 20.

a P value less than 0.05 were considered statistically significant.

The detection sensitivity (Se) of a given LDF parameter, that is, the minimum change in this parameter that is detectable for a given group of subjects, was calculated based on the data obtained during the air inhalation experiment. Sensitivity was calculated according to the formula:

$$Se = \frac{SD\Delta_{bl}}{\text{mean}(x)} \cdot \frac{t[N-1]}{\sqrt{N}},$$

where $SD\Delta_{bl}$ is the standard deviation of the difference between the mean of baseline and inhalation LDF measurements, N the number of eyes measured, $\text{mean}(x)$ represents the mean value of these measurements, and $t_{[N-1]}$ the 2-tailed critical value of the t -distribution for $N-1$ degrees of freedom at the 0.05 level of significance.²² With this definition, the higher the Se, the bigger change in a LDF parameter is needed to be statistically significant at a 0.05 significance level P value. Our choice of including in each recording only data lying within $\pm 12\%$ of the mean DC resulted in a Se of 3.4% for choroidal BF and 11.7% for ONHBF.

RESULTS

ONH Vasomotor Responses

In response to hyperoxia (Fig. 2), BF was significantly reduced by $18\% \pm 4\%$ ($P = 0.02$) from baseline, mainly due to a significant decrease in VEL ($12\% \pm 3\%$ $P = 0.001$). Changes in VOL were not significant ($-0.1\% \pm 7.6\%$ $P = 0.99$). SpO_2 increased by $1.4\% \pm 0.7\%$ ($P < 0.001$) while $PetCO_2$ did not change significantly (-0.3 ± 1.5 mm Hg $P = 0.4$). SpO_2 decreased to the baseline level during the recovery period ($P = 0.9$).

In response to normocapnic hypoxia (Fig. 2), the 90% SpO_2 target significantly increased VEL by $12\% \pm 3\%$ ($P < 0.005$) while changes in VOL ($+5\% \pm 11\%$; $P = 0.6$) and BF ($+11\% \pm 9\%$ $P = 0.2$) were not significant compared to baseline. At the 85% SpO_2 target, VEL increased significantly by $19\% \pm 3\%$ ($P < 0.001$) while the changes in VOL ($+9\% \pm 11\%$; $P = 0.8$) and BF by $20\% \pm 8\%$ ($P = 0.12$) were not significant. During the recovery period, the LDF parameters returned to values not significantly different from baseline for VOL ($-0.4\% \pm 7\%$ $P =$

0.97) and BF ($+8\% \pm 7\%$ $P = 0.8$), whereas VEL was still significantly above baseline ($+10\% \pm 3\%$ $P = 0.03$). SpO_2 returned to baseline values during the recovery period ($P = 0.3$).

In response to hypercapnia, all LDF parameters did not change significantly (VEL $5.6\% \pm 4.5\%$ $P = 0.79$; VOL $-2.2\% \pm 6.7\%$ $P = 0.99$; BF $-0.1\% \pm 4.8\%$ $P = 1.0$). $PetCO_2$ increased by 10 ± 1 mm Hg ($P < 0.001$). No significant change in SpO_2 was observed ($+0.4\% \pm 1\%$ $P = 0.2$).

Subfoveal Choroidal Vasomotor Responses

In response to normocapnic hypoxia, LDF parameters did not change significantly (VEL $3.2\% \pm 2.3\%$ $P = 0.64$; VOL $-3.7\% \pm 2.7\%$ $P = 0.77$; BF $2\% \pm 1.7\%$ $P = 0.85$ at SpO_2 85%, Fig. 3). During the gas inhalation, SpO_2 decreased during hypoxia by $12\% \pm 5\%$ on average ($P < 0.001$).

In response to hypercapnia, LDF parameters did not change significantly (VEL $5.8\% \pm 3.1\%$ $P = 0.54$; VOL $-3.3\% \pm 3.4\%$ $P = 0.91$; BF $1.5\% \pm 1.6\%$ $P = 0.97$, Fig. 3). $PetCO_2$ increased during hypercapnia by 12 ± 5 mm Hg ($P < 0.001$). No change in SpO_2 was observed ($+1\% \pm 1\%$ $P = 0.3$).

No significant changes in respiratory rate and blood pressure were measured during all the experiments (Table).

DISCUSSION

This study demonstrates for the first time that normocapnic hypoxia induces an increase in ONHBF and has no significant effect on ChBF. In contrast, hyperoxia reduces ONHBF significantly. Hypercapnia did not produce detectable changes in ONHBF and ChBF. The magnitude of ONHBF response is maximum between hyperoxia and hypoxia in healthy subjects.

BF Response to Hypoxia

The LDF data reveal a significant increase of ONHBF associated with a simultaneous increase in VEL during acute isocapnic hypoxia, which was observable in all volunteers. In both tissues VOL did not change significantly. The response of ONHBF, close to that reported for the retina (increase of retinal BF from 15% to 38%),^{6,10} contributes to the maintenance of a stable PO_2 within the ONH,²³ as it does for the inner retina.²⁴⁻²⁶ This effect could be due to the release of retinal lactate, mediated by

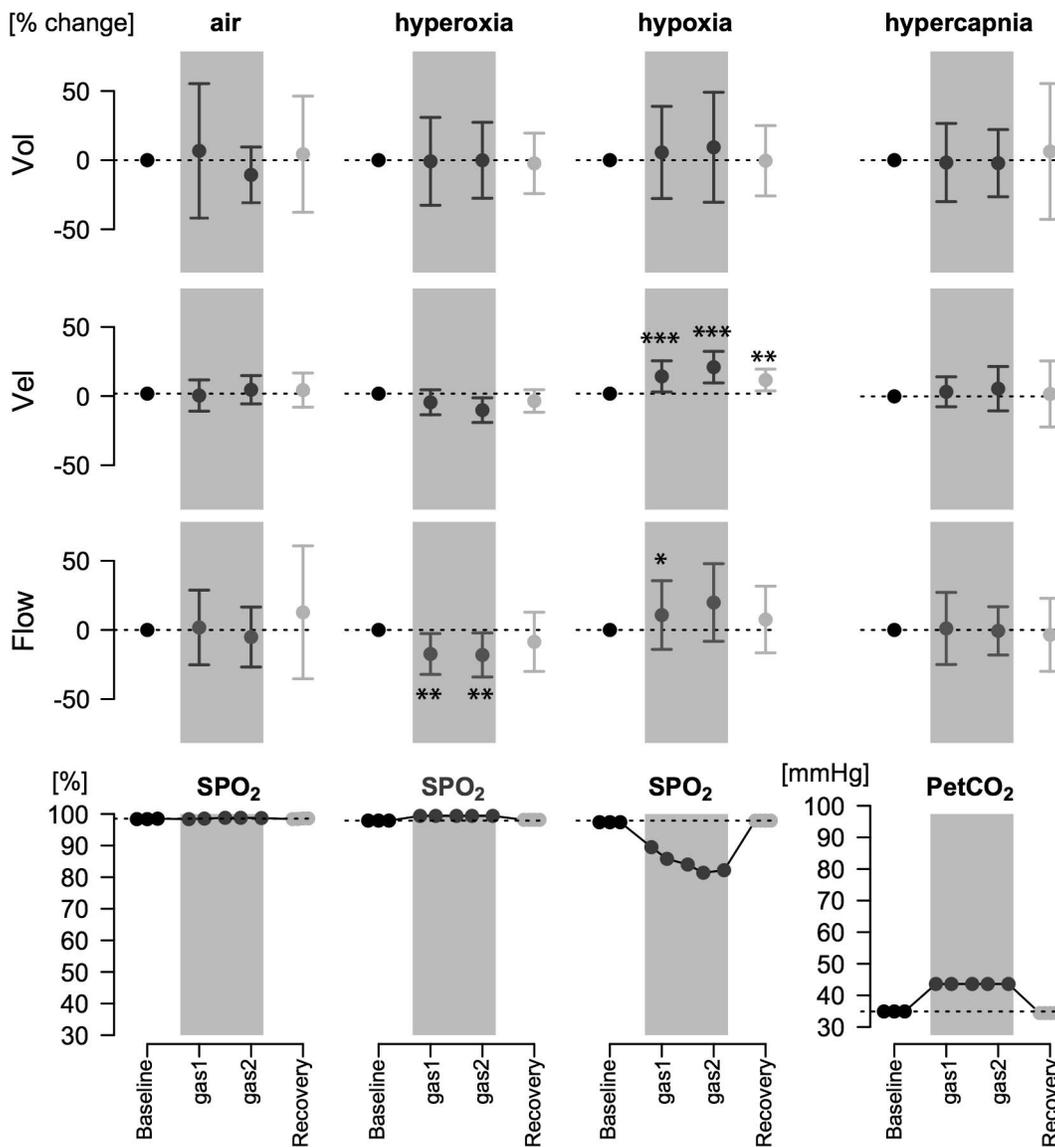


FIGURE 2. Group average time course of ONH LDF parameters (VEL, VOL, BF) in response to four conditions of gas breathing (room air breathing, hyperoxia, normocapnic hypoxia, hypercapnia) in healthy subjects. Mean arterial oxygen saturation (SpO₂), mean end-tidal CO₂ partial pressure (PetCO₂) are represented. Error bars are mean ± standard error. Significance was tested while comparing to baseline. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

endothelium-derived NO,²⁷ prostaglandin PGI₂,^{28,29} and the retinal-derived relaxing factor.^{30,31}

Subfoveal ChBF is not significantly responsive to acute hypoxia. A previous study using the Pulsatile Ocular Blood Flow system³² reported that mild hypoxia (corresponding to SpO₂ values of 89%) did not alter the pulsatile component of the overall choroidal BF. This technique, however, does not provide information on the nonpulsatile component and consequently on the total ChBF (pulsatile and nonpulsatile components).^{33,34} Subfoveal LDF provides a measurement of choriocapillaris BF beneath the fovea and did not represent the response of the entire choroid. LDF measures the blood perfusion locally, whereas interferometry estimates the overall BF.

The reduced vasoreactivity of the choroid compared to that described in the retina and the ONH is consistent with the linear decrease of the PO₂ close to the choroid and in the outer retina when the blood PO₂ decreases during hypoxia as described in the cat.^{24,35} The absence of ChBF changes could be explained in humans by the opposite effect of the choroidal

vasoconstriction secondary to activation of the orthosympathetic system during acute hypoxia^{36,37} and the vasodilation effect of the choroidal hypoxia.

ONHBF Response to Hyperoxia

Hyperoxia induced an average ONHBF reduction by 18%. This is less than the 25% to 37%^{38,39} responses obtained using scanning LDF. One of the reasons for this discrepancy between the two techniques may be due to instrumental differences. While the depth of the scattering volume is the same (300 μm), the area of the illuminated tissue is 130 μm for LDF versus 400 μm for the scanning LDF. Investigations on a model eye using ONH histologic sections of different thickness⁴⁰ suggest that, when the light-collecting aperture coincides with the tissue volume illuminated by the probing laser, layers of the ONH tissue as deep as 300 μm contribute to the signal. However, in both instruments used for this study, the light-collecting aperture is an annulus, which collects multiple scattered light

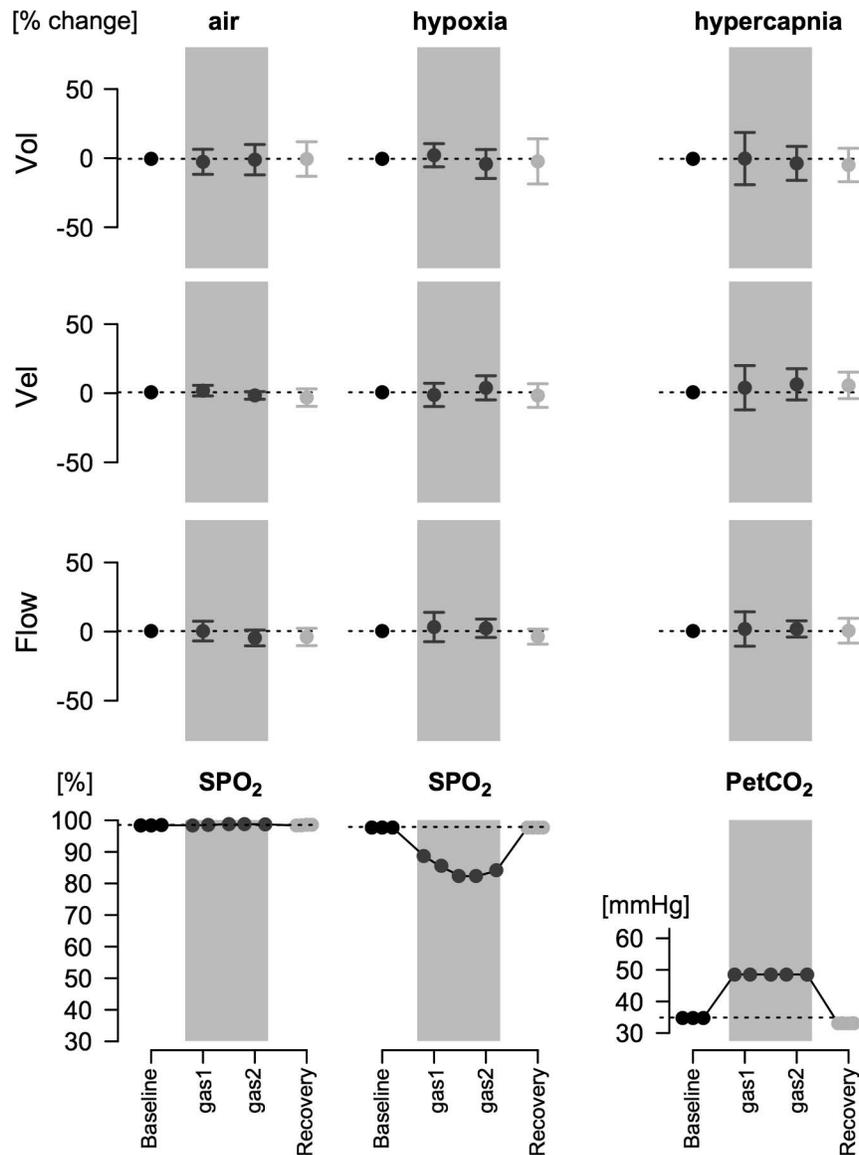


FIGURE 3. Group average time course of Ch LDF parameters (VEL, VOL, BF) in response to four conditions of gas breathing (room air breathing, hyperoxia, normocapnic hypoxia, hypercapnia) in healthy subjects. Mean arterial oxygen saturation (SpO₂), mean end-tidal CO₂ partial pressure (PetCO₂) are represented. Error bars are mean ± standard error. Significance was tested while comparing to baseline. *P < 0.05; **P < 0.01; ***P < 0.001.

only and disregards single scattered events. This suggests that the depth of probed tissue is probably larger than 300 μm. Moreover, the frequency response is 2 kHz for the scanning LDF⁴¹ versus 46 kHz for our instrument.

The response of ONHBF is smaller than that previously reported for the retinal circulation using LDF techniques (approximately -50% reduction of retinal BF).^{7,42-45} The intraretinal oxygen tension in the inner retina is stable for

inspired oxygen fractions from 20% to 100%.⁴⁶ In minipigs the ONHBF response to hyperoxia leads to a stable tissue PO₂ within the ONH.^{23,47} This effect has been reported to be associated with endothelin-1 (ET-1),⁴⁸⁻⁵⁰ via its endothelin receptor A.

We previously showed no significant effect of hyperoxia on ChBF in healthy subjects using the same protocol and the same LDF device,^{8,9} which is in accordance to the linear increase in PO₂ in the choroid and outer retina in minipigs during

TABLE. Blood Pressure and Respiratory Rate During the Different Experimental Conditions With Choroidal and Optic Nerve Head Blood Flow Measurements

Measures	Choroidal Experiment			Optic Nerve Head Experiment			
	Normoxia	Hypoxia	Hypercapnia	Normoxia	Hyperoxia	Hypoxia	Hypercapnia
Mean blood pressure, mm Hg	85 ± 8	86 ± 8	79 ± 9	84 ± 11	87 ± 10	85 ± 9	89 ± 9
Respiratory rate/min	15 ± 3	15 ± 2	17 ± 3	15 ± 3	14 ± 3	15 ± 3	16 ± 3

hyperoxia.³⁵ The human ChBF response differs from that previously reported in rabbits,⁵¹ cats,⁵² and monkeys⁵³ showing a decrease during hyperoxia. However, one should be careful when comparing results obtained from animals and human studies since, firstly, anatomic and physiological differences may exist, and secondly, animal studies are conducted under systemic anesthesia, which may impair BF,⁵⁴ O₂ delivery to tissues,⁵⁴ or CO₂ response.⁵⁵

BF Response to Hypercapnia

PetCO₂ was increased by +10 mm Hg above baseline in the present study, a value identical to that used in previous studies of cerebral and ocular BF vasoreactivity.⁵⁶ The control of end-tidal PCO₂ was preferentially used in this study since that of transcutaneous PCO₂ has several limitations, including the incompatibility between the equilibration time of arterial blood gases and an instantaneous measurement.⁵⁷ To overcome the absence of evaluation of gas concentrations within the eye, spectrometry could be used to evaluate the O₂ and CO₂ saturation.

The absence of response of ONHBF to hypercapnia observed in our study is consistent with the results of previous experiments using similar LDF and gas inhalation techniques, that is, 5% inspiratory CO₂ fraction.^{38,58} The effect of carbogen has not been studied here, because it involves complex and opposite effects of O₂ and CO₂ on the ocular vasculature, systemic effect on blood pressure, and a CO₂-induced rightward shift of the oxyhemoglobin dissociation curve.⁵⁹

In contrast, our study demonstrates the significant effect of hypercapnia on ChBF. The 5% increase in ChBF is less than the reported 16% to 22% using interferometry,⁶⁰ secondary to a 10 mm Hg PCO₂ increase.^{56,61} The difference between the studies could be related to differences in the techniques to obtain information about the flow. Mechanisms underlying ocular vasodilatory capacity in hypercapnia have been essentially studied in animals. They involve metabolic intermediates such as nitric oxide in the retina,⁶² the optic disc,⁶³ and the brain,⁶⁴ prostaglandins,^{59,65} and a reduction in intracellular pH in retinal capillary pericytes.^{61,66,67} The absence of effect of hypercapnia on ONHBF and ChBF could be related to the species difference regarding tissue-related vasoreactivity (in relationship to the size of vessels), the retinal arterioles being less sensitive to hypercapnia than cerebral arteries,^{59,68} and the impact of hypercapnia on the orthosympathetic system projecting on the choroid.^{69,70}

Range of BF Responses to Gas Inhalation

Our healthy young subjects showed some heterogeneity in the magnitude of their responses to the changes in the blood gases. This variability may be secondary to the measurement technique and experimental design (sensitivity of 11% for ONHBF) and the physiological variability. Such a response variability in healthy young subjects has been recently demonstrated in the ONHBF and ChBF responses to various stimuli, such as flicker or increased ocular perfusion pressure.^{71,72} In order to reduce some variability of the ONHBF responses, we limited the ONHBF measurements in all subjects to the temporal region of the ONH rim. In healthy subjects, we were able to detect an ONHBF difference of 37% between hyperoxia and hypoxia, which can be useful if vasoreactivity to O₂ is tested in patients. In contrast, the BF change to hypercapnia is absent or small for ONH and choroid, respectively; this gas stimulus has therefore limited value for the study of ocular disease.

In conclusion, ONH capillary function test under hypoxia and hyperoxia allowed us to characterize the ONH vasoreactivity using LDF. The results confirm that ONHBF decreases

and increases under hyperoxia and hypoxia, respectively. This functional test would be useful to characterize the vascular dysregulation in patients. Considering chronic tissue hypoxia, pathologic changes might be highlighted by such a functional test, which may contribute to pathogenesis understanding. Finally, hypercapnia has a limited value for testing vasoreactivity of ONH and choroid.

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