Circulatory Response to Blood Gas Perturbations in Vasospasm

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Purpose. To investigate the response of the optic nerve head and the choroidal circulation to blood gas perturbations in otherwise healthy subjects with a history of cold hands.

Methods. Thirty-five healthy subjects were selected and grouped according to the related history of cold hands. All 12 selected male subjects, aged 21 to 38 years (mean ± SD = 28 ± 5.2 years) had a negative history of cold hands. Female subjects were almost equally divided between the groups with a negative (11 subjects, aged 18–36 years; mean, 25.7 ± 5.5) or positive (12 subjects, aged 19–45 years; mean, 25 ± 6.8) history of cold hands. Blood gas perturbations were created by having subjects breath a gas mixture consisting of 21% O_2, 74% N_2, and 5% CO_2. The partial pressures pCO_2 and pO_2 were continuously monitored transcutaneously. Choroidal and optic nerve head blood flow response was evaluated by means of laser Doppler flowmetry.

Results. Systolic and diastolic blood pressure (SBP/DBP at baseline, three-group average: 111.2/71.9 mm Hg), heart rate (HR; 70.3 bpm), and intraocular pressure (IOP; 14.7 mm Hg) increased during the blood gas perturbation phase (123.1/77.7 mm Hg, 78.5 bpm, and 15.6 mm Hg, respectively) and returned to baseline in the recovery phase (109.9/73.4 mm Hg, 69.5 bpm, and 13.5 mm Hg, respectively). There was no difference between groups (one-way ANOVA of the percentage change from baseline for SBP, $P = 0.75$; DBP, $P = 0.36$; HR, $P = 0.95$; and IOP, $P = 0.72$). pCO_2 increased from 5.52 to 6.59 kPa and returned to 5.50 kPa. pO_2 increased from 10.64 to 13.12 kPa and returned to 10.73 kPa. Again, there was no difference between groups (one-way ANOVA for the percentage change: pCO_2, $P = 0.17$; pO_2, $P = 0.78$). In the women with vasospasm, optic nerve head blood flow increased 17.1% and the choroidal blood flow decreased −3.6%, whereas in the women and men without vasospasm the optic nerve head blood flow decreased −5.8% and −4.8%, and the choroidal blood flow increased 13.3% and 18.3%, respectively (two-way ANOVA interaction; $P = 0.001$).

Conclusions. The pCO_2 increase was accompanied by a pO_2 increase. Blood pressure and HR increased comparably in all groups, indicating sympathetic arousal. The women with vasospasm demonstrated an inverse response pattern of choroidal and optic nerve head circulation to blood gas perturbation compared with the women without vasospasm and compared with the men. (*Invest Ophthalmol Vis Sci.* 2005;46:3288–3294) DOI:10.1167/iovs.05-0158

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Vascular dysregulation, or more exactly, the propensity to react by exaggerated vasospasm to various stimuli such as coldness and emotional stress has been suggested to represent a potential risk factor in various ocular conditions, including glaucoma,[1,2] retinal venous occlusion,[3] acute ischemic optic neuropathy,[4] and central serous chorioretinopathy.[5] Dysregulation-related blood flow disturbances are limited in time and, in a given patient with such a condition, blood flow measurement does not necessarily show a difference from baseline measurements obtained in normal subjects.[6] Therefore, similar to the cardiovascular stress test, which may be necessary to unmask the effects of ischemia in the heart muscle, a provocation test may be more suitable to determine vascular dysregulation in the ocular circulation. In vitro, animal and clinical studies suggest that blood flow in the retina and the optic nerve is increased by hypercapnia, that arteriolar constrictions in the anterior optic nerve microvasculature correlate with the level of arterial blood gases, that increased arterial oxygen constricts retinal vessels, and that hypoxia induces retinal vasodilation.[7–20] Although measurements of the choroidal blood flow response to changes in arterial oxygen tension have provided more erratic results, hypercapnia consistently increases choroidal blood flow.[21–28] Subjects with peripheral vasospasms are prone to ocular vascular dysregulation.[6,29,30] We sought in this study to evaluate the reactivity to blood gas perturbations in the subfoveal choroid and the neuroretinal rim of the optic nerve head (ONH) in healthy subjects and in otherwise healthy subjects with systemic vascular dysregulation, defined as a positive history of cold extremities.

Methods

Subjects

Thirty-five healthy subjects were recruited. The protocol was approved by the local ethics committee, the tenets of the Declaration of Helsinki were observed, and each subject signed an informed consent form before any examination, according to the International Conference on Harmonization's tripartite guidelines for Good Clinical Practice. A notification in the University Eye Clinic Basel informed potential volunteers (collaborators, students, parents and friends of patients) of the opportunity to participate in a research project. A detailed medical and ophthalmic history was recorded for each subject, and a history of cold hands and feet was taken. Only candidates with no history of ocular or systemic disease, chronic or current systemic or topical medication, and drug or alcohol abuse were selected. Smoking was not an exclusion criterion. Systolic blood pressure (SBP) had to be in the range of 95 to 140 mm Hg; diastolic blood pressure (DBP) 55 to 90 mm Hg; best corrected visual acuity above 20/25; ametropia no greater than −5 or +3 of spherical equivalent and <1 D astigmatism in the experimental eye; intraocular pressure (IOP) lower than 20 mm Hg; and no pathologic finding on slit-lamp examination and indirect fundoscopy. A clear classification into either a "vasospastic" or "nonvasospastic" group was mandatory. Subjects were classified as having vasospasm if they related an unambiguous history of frequently cold hands (answering yes to the questions: "do you always have cold hands, even during the summer?" and "do other people tell you that you have cold hands?") and as normal subjects if they reported no such history. Subjects describing "sometimes having cold hands" were excluded from the present anal-

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ysis. No objective assertion of acral vasospasm was determined, because it has been demonstrated that a simple history of cold hands can distinguish ocular features related to vascular dysregulation[6,30] and sometimes even superior to complex methods such as finger laser Doppler flowmetry.[31]

Blood Flow Measurements in the Neuroretinal Rim of the ONH

Blood flow in the neuroretinal rim of the ONH was determined by a method based on the laser Doppler flowmetry (LDF) technique.[32–34] In brief, a continuous laser light is projected on the neuroretinal rim of the ONH, and the backscattered light is then analyzed. The LDF parameters obtained are velocity, volume, and flow. The instrument used was a laser Doppler flowmeter produced by Oculix SA (Arbaz, Switzerland), mounted on a fundus camera (Model TRC; Topcon, Tokyo, Japan). After pupil dilation, the probing laser beam was positioned to a distinct spot ~160 μm in diameter on the neuroretinal rim, distant from large-caliber vessels. The examined area was chosen so that anatomic landmarks ensured high placement–replacement reproducibility. Blood flow parameters were obtained during 20 to 30 seconds' recording time, and a mean value was computed for each parameter for each experimental phase.

Subfoveal Choroidal Blood Flow Measurements

Subfoveal choroidal blood flow was also determined by using a method based on the LDF technique. A device that measures subfoveal choroidal blood flow in the fovea has been developed (Geiser MH, et al. *IOVS* 1998;39:ARVO Abstract S995).[35–37] The optical system is based on a confocal arrangement. A polarized laser source (wavelength, 810 nm and 95 μW at the cornea) is focused with a microscope objective lens to a point source that is then relayed by a lens on the image plane of an ocular lens. When the subject is looking at this image, the latter is projected on the fovea. The light of the laser beam is focused on a small spot 10 to 20 μm in diameter. The light scattered by the moving particles in the choriocapillaris, in this case red blood cells, is collected by the optic system. The detection sites are organized with six fibers arranged circularly around a central fiber pointing at the fixation point, all within the avascular area of the fovea, so that they do not touch any retinal blood vessels. According to Bonner and Nossal,[32] this disposition favors measurements of subfoveal choroidal blood flow and minimizes the contribution of retinal blood flow. Based on their model, it is hypothesized that the indirect mode of detection senses scattering alteration that occurs as deep within the tissue as 100 to 300 μm, corresponding to the choriocapillaris. The photocurrent from the photodetector is Fourier transformed, and the hemodynamic LDF parameters flow, volume, and velocity are processed. The subjects were seated and asked to fixate the laser beam. One measurement of 20 to 30 seconds was obtained for each phase of the experiment.

Systemic Blood Pressure, Heart Rate, and IOP

During the experimental procedures, SBP and DBP and heart rate (HR), were monitored with an oscillometric device. IOP was measured with a handheld tonometer (Tonopen; Mentor, Norwell, MA).

Blood Gas Perturbations and Monitoring

Blood gas perturbations were created by having subjects breath room air and then a combination of 5% CO_2, 21% O_2, and 74% N_2. A partly closed mask system covering both mouth and nose and connected to the gas monitor (Capnomac Ultima; AVL Medical Systems AG, Schaffhausen, Switzerland) was used for gas delivery. Variations in systemic partial pressures of oxygen and carbon dioxide (pO_2 and pCO_2) were monitored transcutaneously in the periphery on the upper arm (Microgas 7650; Acutronic, Hirzel, Switzerland). Transcutaneous blood gas measurement is a well-known, simple, reliable, and noninvasive method of monitoring systemic pO_2 and pCO_2.[38–41] We used it as a reliability criterion, which provided direct evidence that pulmonary gas changes were well reflected in the extrapulmonary circulation and peripheral tissues and that the blood flow measurements were performed in a timely manner. However, sensor positioning, taping, and warming for the purpose of vasodilation make handling the device somewhat difficult. In an attempt to validate the measurements with this device, we explored the concordance of end-tidal, transcutaneous radial artery blood and ear-lobe blood CO_2 concentrations in three healthy subjects at baseline. The mask system allows an inspiration either of the gas mixture from the reservoir bag or room air when the bag is empty. The excess dead gas was evacuated through a one-way valve on the side of the face mask. As stated earlier, the gas mixture contained 5% CO_2, 21% O_2, and 74% N_2. The rate of delivery was adjusted to the respiration rate and volume of each subject by observing the inflation and deflation of the reservoir bag. No inspiration of the ambient air was allowed during the second phase of the experiment. During the data collection, the subjects were not asked to control their respiration rate. At the end of the experiment, subjects were asked to quantify their comfort during the gas-mixture phase. An arbitrarily chosen reporting scale from 1 (could not tolerate) to 10 (noticed no difference from ambient air) was offered.

Experimental Design

During the assessment of ocular blood flow parameters, the operator was masked to the vasospastic status of the subject. Systemic pCO_2 and pO_2 levels were monitored continuously as just outlined, and one value for each phase of the experiment was recorded. Blood flow in the neuroretinal rim of the optic nerve and of the subfoveal choroid was recorded at the end of each of three phases in the duration of 20 to 30 seconds. One eye per subject was randomly chosen, and the pupil was dilated with 0.5% tropicamide. The signal gain (magnification) level was set to obtain a direct current (DC) between 1 and 2 mV. DC is, after correcting for gain level, a measure of captured light intensity.[37] If there was a frequent loss of fixation with that eye, with DC falling below 0.5 mV in more than 20% to 25% of the recordings, the other eye was dilated and measured. In the *phase one* (baseline), subjects breathed ambient air. Blood pressure, IOP, and blood flow measurements were conducted, first in the ONH and then, after repositioning the subject, in the subfoveal choroid. In *phase two* (gas perturbations), the gas-mixture delivery followed. It lasted, on average, 10 minutes until systemic pCO_2 and pO_2 reached a steady state. Measurements were repeated, and subjects returned to ambient air breathing. In *phase three* (recovery), all the measurements were repeated after a stabilization period lasting ~10 minutes. These phase durations were in agreement with the literature pertaining to cerebral blood flow[42,43] and with our own experience,[44] according to which an average period of 5 to 15 minutes was anticipated to reach a stable plateau.

Data Analysis

Systemic cardiovascular (SBP/DBP and HR) and IOP response, as well as blood gas levels (transcutaneous and end-tidal pCO_2 and pO_2), are reported. To perform a direct comparison between groups, a percentage change from baseline for individual subjects was first calculated for each parameter in the second, gas-mixture breathing, phase, to account for heteroscedasticity.[45] Differences in response between groups were tested with one-way ANOVA.

Similarly, all ocular circulatory parameters in the three phases of the experiment are reported. Again, a percentage change from baseline was calculated for individual subjects for the LDF parameter flow in the ONH and in the choroid in the second, gas-mixture breathing, phase, to account for heteroscedasticity. To perform an analysis of the difference between groups and between vascular beds, we applied the two-way ANOVA model. One independent factor was the grouping variable (pertinent to the three study groups), and the other was the vascular bed (either ONH or the choroid). Using model contrasts, we performed planned comparisons between the individual groups and groups pooled in various combinations.

TABLE 1. Blood Gas Parameters in Three Subjects

Subjects	End-Tidal pCO_2	Transcutaneous pCO_2	Arterial pCO_2	Capillary pCO_2 (Ear Lobe)
1	5.5	5.5	5.6	—
	5.5	5.6	—	5.5
	6.5	6.3	6.5	—
2	—	4.3	—	4.4
3	5.0	5.2	—	5.2
	6.5	6.4	—	6.4

The end-tidal partial pressure of carbon-dioxide pCO_2 (in %), transcutaneous pCO_2 (on the upper arm, in kPa), arterial (in the radial artery, in kPa) and capillary (ear lobe, in kPa) blood pCO_2 at baseline. Results show good agreement.

Pearson's linear correlation and Spearman's rank test were used to explore relationships between the subjective comfort scale and parameters at baseline and during gas perturbation. In all tests, $P < 0.05$ was considered significant.

RESULTS

Table 1 shows good agreement between radial artery blood and earlobe blood CO_2 concentrations and end-tidal and transcutaneous CO_2 levels in the small prestudy experiment with three healthy subjects at baseline, confirming the validity of the transcutaneously measured levels of pCO_2 and pO_2.

All 12 selected male subjects, aged 21 to 38 years (mean ± SD, 28 ± 5.2) had a negative history of cold hands. The female subjects were almost equally divided between the group with a negative (11 subjects, aged 18–36 years; mean, 25.7 ± 5.5) and a positive (12 subjects, aged 19–45 years; mean, 25 ± 6.8) history of cold hands.

Systemic Cardiovascular and IOP Response

Table 2 reports the systemic parameter levels of each of the three groups individually in the three phases of the experiment. In general, blood pressure and HR increased in the gas-mixture–breathing phase and returned to baseline in the recovery phase. Table 3 reports each parameter response, expressed as the percentage increase during the second experimental phase. A direct between-groups comparison in a one-way ANOVA, revealed no significant group difference for any of the parameters (Table 3).

TABLE 2. Systemic Cardiovascular Parameters in Three Groups

	Group	Baseline	Breathing Gas-Mixture	Recovery
SBP	1	120.3 ± 12.7	132.3 ± 16.7*	122.4 ± 9.8
	2	106.5 ± 11.4	119.4 ± 11.8*	105.2 ± 10.5
	3	106.3 ± 14.8	117.0 ± 16.9*	101.8 ± 14.6*
DBP	1	72.8 ± 10.3	77.8 ± 8.8*	74.9 ± 8.2
	2	74.9 ± 6.8	83.7 ± 8.8*	77.6 ± 9.9
	3	68.3 ± 10.0	72.2 ± 11.7	67.9 ± 9.4
Heart rate	1	67.0 ± 7.7	74.2 ± 8.6*	65.6 ± 7.3*
	2	75.0 ± 8.2	84.8 ± 9.7*	74.2 ± 7.8
	3	69.4 ± 11.6	76.9 ± 12.4*	69.3 ± 9.6
IOP	1	16.3 ± 3.2	16.6 ± 2.9	13.8 ± 2.6*
	2	14.3 ± 1.8	15.5 ± 3.6	13.8 ± 2.7
	3	13.5 ± 2.7	14.7 ± 2.0	12.8 ± 2.1

Data are shown for, 1, men; 2, nonvasospastic women; and 3, vasospastic women, in three phases of the experiment (baseline with room-air breathing, gas-mixture breathing, and again room-air breathing in the recovery phase). All results are expressed as the mean ± S.D.

* Significant difference from baseline, paired *t*-test.

TABLE 3. Percentage Change from Baseline in the Second Phase of the Experiment, Gas-Mixture Breathing

	Group	% Change from Baseline	*P* (ANOVA)
SBP	1	10.1 ± 8.2	0.75
	2	12.5 ± 8.6	
	3	10.2 ± 8.5	
DBP	1	8.0 ± 12.4	0.36
	2	11.8 ± 6.6	
	3	5.8 ± 9.7	
Heart rate	1	11.4 ± 12.1	0.95
	2	10.4 ± 5.5	
	3	10.4 ± 5.2	
IOP	1	4.4 ± 22.3	0.72
	2	9.4 ± 24.5	
	3	11.1 ± 15.5	
tc pCO_2	1	15.9 ± 5.8	0.17
	2	21.7 ± 8.7	
	3	21.0 ± 9.3	
tc pO_2	1	23.6 ± 9.7	0.78
	2	22.1 ± 7.1	
	3	24.2 ± 3.4	

Data were calculated for each subject and then averaged. Systemic cardiovascular parameters and transcutaneous gas levels are shown for the three groups: 1, men; 2, nonvasospastic women; 3, vasospastic women. All results are expressed as the mean ± SD. One-way ANOVA was performed for each parameters change. tc, transcutaneous.

IOP did not significantly change during the gas-breathing phase (Table 2), whereas it significantly decreased in the men in the recovery phase compared with baseline ($P = 0.036$). However, when corrected for heteroscedasticity by calculating percentage change from baseline, there was no difference among groups, either in the gas-breathing (one-way ANOVA, $P = 0.72$, Table 3) or the recovery ($P = 0.081$) phases. There was also no significant group difference in the percentage change from the gas-breathing to the recovery phases ($P = 0.48$). The percentage change in IOP did not correlate with the change in ocular blood flow parameters in the gas-breathing phase or in the recovery phase (data not shown).

Blood Gas Level Monitoring

Table 4 reports transcutaneous pCO_2 and pO_2 across experimental phases. Transcutaneous oxygen and carbon dioxide increased in the gas-mixture breathing phase, and returned to

TABLE 4. Transcutaneous Blood Gas Levels in the Three Groups

	Group	Baseline	Breathing Gas Mixture	Recovery
tc pCO_2	1	5.9 ± 0.6	6.9 ± 0.8*	5.9 ± 0.6
	2	5.3 ± 0.7	6.4 ± 1.0*	5.3 ± 0.7
	3	5.3 ± 0.7	6.4 ± 0.8*	5.3 ± 0.7
tc pO_2	1	9.9 ± 1.1	12.2 ± 1.7*	10.2 ± 1.2
	2	10.9 ± 1.0	13.4 ± 1.5*	10.9 ± 1.2
	3	11.0 ± 1.1	13.7 ± 1.4*	11.0 ± 1.2
Comfort report scale	1	—	7.3 ± 1.2	—
	2	—	6.5 ± 1.7	—
	3	—	7.0 ± 1.7	—

Data are shown for groups 1, men; 2, nonvasospastic women; and 3, vasospastic women, in three phases of the experiment (baseline with room-air breathing, gas-mixture breathing and again room-air breathing in the recovery phase). A scale from 1 (could not tolerate) to 10 (noticed no difference whatsoever) was used to quantify discomfort during the experiment. Results are expressed as the mean ± SD.

* Significant difference from baseline, paired *t*-test.

TABLE 5. Ocular Circulatory Laser Doppler Flowmetry Parameters in the Three Groups

	Group	Baseline	Breathing Gas Mixture	Recovery
Ch Vel (k H_2)	1	1.49 ± 0.33	1.52 ± 0.31	1.48 ± 0.32
	2	1.53 ± 0.26	1.62 ± 0.34*	1.54 ± 0.30
	3	1.61 ± 0.25	1.62 ± 0.23	1.62 ± 0.22
Ch Vol (AU)	1	0.67 ± 0.17	0.76 ± 0.18	0.72 ± 0.12
	2	0.71 ± 0.19	0.86 ± 0.4	0.77 ± 0.36
	3	0.88 ± 0.22	0.84 ± 0.26	0.83 ± 0.23
Ch Flow (AU)	1	22.3 ± 5.2	25.9 ± 7.6	24.6 ± 7.3
	2	25.1 ± 7.0	27.8 ± 7.7	23.9 ± 9.6
	3	32.0 ± 8.7	30.4 ± 10.1	30.6 ± 9.1
ONH Vel (kHz)	1	0.41 ± 0.07	0.38 ± 0.06	0.43 ± 0.08
	2	0.41 ± 0.06	0.41 ± 0.05	0.41 ± 0.05
	3	0.46 ± 0.07	0.43 ± 0.07	0.46 ± 0.08
ONH Vol (AU)	1	0.89 ± 0.29	0.94 ± 0.31	0.80 ± 0.30
	2	1.23 ± 0.82	1.26 ± 0.98	0.83 ± 0.32
	3	0.79 ± 0.37	1.04 ± 0.41*	0.92 ± 0.55
ONH Flow (AU)	1	28.6 ± 7.2	27.0 ± 7.7	27.6 ± 9.2
	2	32.0 ± 13.2	29.1 ± 14.1	26.2 ± 8.2
	3	28.1 ± 9.7	31.4 ± 9.4	31.2 ± 15.0

Data are shown for groups 1, men; 2, nonvasospastic women; and 3, vasospastic women, in three phases of the experiment (baseline with room-air breathing through the mask, gas-mixture breathing and again room-air breathing in the recovery phase with the mask removed). All results are expressed as the mean ± SD. LDF, laser Doppler flowmetry; AU, arbitrary units. In the choroid: Ch Vel, LDF parameter Velocity; Ch Vol, LDF parameter Volume; Ch Flow, LDF parameter Flow. In the optic nerve head: ONH Vel, LDF parameter Velocity; ONH Vol, LDF parameter Volume; ONH Flow, LDF parameter Flow.

* Significant difference to baseline, paired *t*-test.

baseline in the recovery phase. Table 3 reports parameters expressed as the percentage increase in the second experimental phase. One-way ANOVA, as shown in Table 3, revealed no significant difference in transcutaneous oxygen and carbon-dioxide responses between groups.

Table 4 also shows the subjects' self-reported comfort. One-way ANOVA was applied to test group differences. The reported grades were between 3 and 9, with no significant group differences (one-way ANOVA; $P = 0.54$).

Ocular Circulatory Response to Blood Gas Perturbation

Table 5 reports descriptive statistics for blood flow parameters in the ONH and the choroid in three groups in three phases of the experiment. To evaluate an ocular circulatory response to blood gas changes in the second experimental phase, the percentage change from baseline was calculated for individual subjects for the LDF parameter flow. In the choroid, the mean percentage change ± SD was 18.3% ± 29.1%, 13.3% ± 21.6%, and −3.6% ± 20.8% in the men, the women without vasospasm, and the women with vasospasm, respectively. In the ONH, the corresponding values were −4.8% ± 19.9%, −5.8% ± 29.1% and 17.1% ± 32.1% (Fig. 1).

A two-way ANOVA model was applied to the results. One independent factor was the grouping variable (factor A: pertinent to all three groups), and the other was the vascular bed (factor B: ONH or choroid). For factor A, $P = 0.93$; for factor B, $P = 0.15$. However, the interaction probability was 0.001, indicating an opposite behavior pattern in two ocular vascular beds. Thus, we made planned comparisons within the model: model contrasts were specified to pool the men and women without vasospasm together on one side versus the women with vasospasm on the other and found a significant response difference independently for the choroid ($P = 0.03$) and the ONH ($P = 0.029$).

Gender Analysis

An asymmetric gender distribution was the result of difficulties in recruiting healthy men with vasospasm. To investigate whether this gender factor was responsible for the observed results, the two-way ANOVA model described in the previous paragraph was repeated, only this time with the the following exclusions: (1) The women with vasospasm were excluded from the analysis, and the difference in the response pattern was tested between the men (all nonvasospastic) and the women without vasospasm. No significant difference was found, either for the choroid ($P = 0.62$) or the ONH ($P = 0.93$), separately, or as an opposite pattern behavior between the choroid and ONH ($P = 0.74$). (2) The men were excluded from the analysis, and the difference in the response pattern was tested between the women with and those without vasospasm. The corresponding probabilities were borderline ($P = 0.1$) for the choroid and ($P = 0.055$) for the ONH. The difference in behavior between the choroid and the ONH blood flow response pattern was highly significant ($P = 0.002$).

No gender differences were found in the response of systemic cardiovascular parameters and blood gas levels (Table 3).

Correlation between Systemic and Ocular Perfusion Parameters and Blood Gas Levels

Correlation analyses between blood gas levels and systemic and ocular perfusion parameters were conducted with all groups pooled together, by means of Pearson linear regression. Neither pCO_2 nor pO_2 levels correlated significantly at baseline with systemic cardiovascular parameters, ocular blood flow, or IOP (data not shown). The same held true for the percentage change from baseline of the respective parameters.

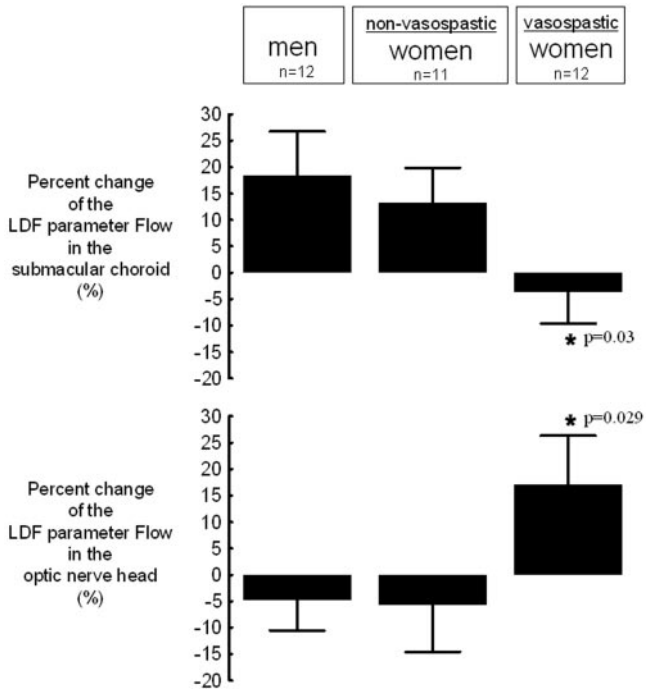

FIGURE 1. Percentage change from baseline of the LDF parameters. Flow in the choroid and in the ONH during the breathing phase, calculated for each subject individually, and then group averaged for three groups. All results are given as the mean (*box*) ± SE (*whisker*). *Top*: percentage change of the LDF parameter flow in the choroid. *Bottom*: percent change of the LDF parameter flow in the ONH. *Significant response differences of the women with vasospasm, compared with the other two groups pooled.

Subjects with smaller oxygen increase demonstrated a lower subjective tolerance for gas-mixture breathing. Self-reported comfort correlated with the percentage change of transcutaneous pO_2 (Pearson $r = 0.53$, $P = 0.001$; Spearman $R = 0.56$, $P = 0.0005$). However, it correlated significantly with the percentage change of the transcutaneous pCO_2 only in parametric testing (Pearson $r = -0.35$, $P = 0.04$), not in Spearman's rank testing ($R = -0.25$, $P = 0.14$). Subjects poorly tolerating gas-mixture breathing demonstrated a sympathetic excitation: Comfort scores and the percentage change in DBP, an indicator of sympathetic nervous system arousal, also showed significant correlation (Pearson $r = -0.36$, $P = 0.038$; Spearman $R = -0.39$, $P = 0.019$). Direct correlation between the changes in DBP or HR and blood gas changes did not reach statistical significance.

Discussion

In the present study, we investigated the response of two ocular vascular beds, the ONH and the choroid, to blood gas perturbations in men (all with negative cold-hands histories), and women with negative and positive histories of cold hands. A gas-mixture of 5% carbon dioxide, 21% oxygen, and 74% nitrogen was delivered through a closed-mask system. Transcutaneous pCO_2 and pO_2 were continuously monitored. Blood pressure, HR, and IOP were measured as safety criteria at baseline, during the gas-mixture breathing phase and in the recovery phase. All but IOP demonstrated an increase during the second phase of the experiment, with a subsequent decrease in the recovery phase. Hyperoxia accompanied hypercapnia in all groups. There was no difference in systemic cardiovascular or blood gas parameter response between groups. In contrast, ONH and choroidal blood flow demonstrated a different, inverse response pattern between the groups. In the women with vasospasm, the average change in the ONH blood flow was a 17.1% increase; the choroidal blood flow decreased, on average, −3.6%. In the women without vasospasm and the men, the ONH blood flow decreased, on average, −5.8% and −4.8%, and the choroidal blood flow increased 13.3% and 18.3%, respectively.

Confirming earlier results,[6,29,30] female gender had an overwhelming tendency toward a positive history of cold hands. However, the observed phenomena were not gender related. No difference in cardiovascular systemic response and in blood gas level changes was noted between the groups. In contrast, local ocular circulatory response was dependent on the vasospastic propensity, with the female vasospastic group singled out.

Although carbogen (95% O_2, 5% CO_2) was not used, but air enriched with 5% CO_2 was used, a large degree of hyperoxia (an average 23% increase of transcutaneous pO_2) constantly accompanied the desired hypercapnia (an average 19% increase of transcutaneous pCO_2). The respiration rate was spontaneous for each subject and was not controlled. Most of the studies conducting gas perturbations in pursuit of ocular blood flow response used end-tidal gas levels for monitoring. Some, however, used arterialized earlobe blood.[46–48] Our transcutaneously measured pCO_2 increase corresponded to one seen with 5% CO_2 in the inhalation mixture (15%–23% change in pCO_2 in the earlobe blood) in the mentioned studies. However, the DBP increase was rather comparable with one seen when 8% CO_2 in the inhalation mixture was used (10% DBP increase). Although blood oxygen levels at baseline were comparable (pO_2, 81–85 mm Hg) to our transcutaneously measured pO_2, no comparison of oxygen level changes was reasonable, as high oxygen concentrations were used in the studies mentioned.

Subjects in the present study experienced combined hyperoxia and hypercapnia. The superficial layer of the ONH is supplied by the retinal circulation. With regard to retinal circulatory response to gas perturbations, there are conflicting reports in the literature. The retinal circulation vasoconstricted in response to breathing 100% oxygen and in response to breathing carbogen. Luksch et al.[49] reported equivalent decreases in retinal blood flow (approximately 60%), whereas Pakola and Grunwald[15] reported that 100% oxygen-breathing reduced retinal blood flow by 56.4% ± 13.7%, whereas carbogen breathing decreased retinal blood flow by 42.2% ± 10.2%. In contrast, the initial photograph studies of the retinal circulation in humans by Hickam and Frayser[8] presented clear direct evidence that a carbogen-like gas inhalation prevents to a large degree the vasoconstriction typically associated with 100% oxygen breathing. In other supporting studies Sponsel et al.[14] reported that carbogen "can counteract the profound inhibitory effects (found with hyperoxia alone) on retinal hemodynamics in the functionally important premacular capillary bed." Haefliger et al.[50] found a similar result in the papilla of healthy subjects. Arend et al.[51] stated that a "substantial acceleration of retinal dye velocity and transit under combined hyperoxia and hypercapnia strongly suggests that (carbogen) may indeed improve oxygenation without reducing retinal perfusion." In our study, no change of ONH blood flow was observed in subjects without vasospasm. In contrast, those with vasospasm demonstrated a flow increase of 17.1%. A certain lack of autoregulatory capacity has already been described in the retinal circulation of subjects with vasospasm.[29] It is plausible that subjects with vasospasm differed from those without vasospasm and experienced a blood flow increase in the ONH due to blood pressure increase and diminished autoregulation. An alternative explanation is that subjects with vasospasm are more responsive to CO_2 in some vascular beds. This response has already been observed in migraine, a condition with obvious vascular dysregulative characteristics.[52]

It has been shown that hypercapnia provokes, acting primarily through the central chemoreceptors, hyperventilation, sympathetic drive,[53,54] and an increase in blood pressure in healthy subjects.[55] Even spontaneous hyperventilation alone causes an increase in blood pressure and HR in healthy subjects.[56] In the present study, translation of gas perturbations to a sympathetic excitation was determined by the subject's individual tolerability. Indeed, the agreement was observed between the comfort reports and the changes in DBP. The tolerability of hypercapnia was variable among subjects. There was no direct correlation between the blood gas changes and blood pressure and HR changes and only the weak association between the pCO_2 increase and the comfort reports, with a significant Pearson correlation and a nonsignificant Spearman's rank testing.

Vasospastic propensity influenced neither the magnitude of hypercapnia nor of sympathetic arousal, as the increase in SDP and DBP and HR was comparable in all groups. Because of autonomic innervation in the choroidal circulation, unlike in the ONH, once the sympathetic activity had been increased, it could have played an important role. Sympathetic arousal of the same magnitude as seen in the current study was shown to have a profound effect on choroidal circulation in otherwise healthy subjects with vasospasm.[6] Defined in the same way as in the present study, subjects with vasospasm demonstrated an opposite choroidal blood flow reaction to a hand-grip test compared with control subjects without vasospasm. This reaction pattern was interpreted in the mentioned study to be a disproportionate choroidal vasoconstriction in response to autonomic stimulation in subjects with vasospasm, despite the comparable sympathetic arousal in all groups. As detailed earlier, choroidal circulation is responsive to hypercapnia and

weakly reactive to oxygen level variations. In the present study, in the face of blood pressure increase and hypercapnia, choroidal blood flow increased on the average. However, subjects with vasospasm could have demonstrated an exaggerated vasoconstrictive response to sympathetic stimulation, in line with previous findings.[6]

Breathing O_2 can cause a decrease in IOP[57,58]; however, this decrease is not a universal finding.[10,28] Results of a study involving the use of a pulsatile ocular blood flowmeter and carbogen inhalation[21] explained an IOP decrease through a massaging effect. A handheld tonometer (Tonopen; Mentor) was used in the present study, which makes the massaging effect unlikely. No significant change in IOP was observed in the gas-breathing phase, and an IOP decrease in the recovery phase compared with baseline was significant only in the men. We cannot in a meaningful way interpret the variable IOPs at baseline, particularly because the baseline conditions were standardized and equal for all the participants. Nevertheless, it is not likely that the IOP influenced the observed ocular blood flow response pattern. An ANOVA of the percentage change in IOP did not reveal any significant difference between the groups in IOP behavior in the gas-breathing and the recovery phases. Furthermore, there was no correlation between the change in IOP and the change in the choroidal and ONH blood flow, either in the gas-breathing or the recovery phase.

There are several limitations to the present study. Hyperoxia and hypercapnia are conflicted stimuli; therefore, the findings do not represent a study of vascular regulation with a single variable. In addition, the cerebral vascular reactivity was not measured. Because the eyes rely mostly on blood supply from the internal carotid artery, if there were large differences in cerebral blood flow response between the groups, they may explain, through a possible steal effect by the brain, some of the observed differences in the ophthalmic vascular response. However, the main shortcomings of the present study are pertinent to the LDF technique. The exact tissue level where blood flow parameters are acquired in ONH LDF remains unclear, and this technique may be predominantly sensitive to blood flow changes in the superficial layers of the ONH and less sensitive to those in the prelaminar and deeper regions.[59,60] Such a limitation, however, does not represent a major disadvantage, since, knowing that dysregulative phenomena have been observed in the retinal vasculature of subjects with vascular dysregulation,[29] we were particularly interested in the behavior of the neuroretinal rim. Moreover, each parameter, although given in arbitrary units, varies linearly with respect to changes in blood flow,[34,61–65] and the ability of LDF to detect relative changes in human ONH hemodynamics caused by blood gas perturbations has been demonstrated.[17] In regard to choroidal LDF, the subfoveal choroidal region is not necessarily representative of the entire choroid. Furthermore, interindividual variability is very high. Nevertheless, the intraindividual reproducibility of measurements with this device is good,[37] and its ability to detect relative changes in human subfoveal choroidal hemodynamics caused by blood gas perturbations has been demonstrated.[46] The new emerging techniques, for example visualization of retinal oxygenation by means of functional magnetic resonance imaging,[66–69] may overcome the downsides of the LDF method in future gas perturbation studies.

In summary, hyperventilation-induced hyperoxia and sympathetic system excitation accompanied induced hypercapnia. Blood gas perturbations with 5% CO_2, 21% O_2, and 74% N_2 elicited a distinctive response pattern, opposite to that of the control group, in the ONH and in the choroidal circulation of subjects with a history of cold hands. An altered ocular circulatory response in subjects with cold hands may have clinical relevance in several ocular diseases.

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