The Effect of Systemic Hyperoxia on Optic Nerve Head Blood Flow in Primary Open-Angle Glaucoma Patients

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Purpose. To assess the optic nerve head blood flow (ONH BF) response to hyperoxia in glaucoma patients using laser speckle flowgraphy (LSFG), and determine factors influencing vasoreactivity within the ONH.

Methods. We performed oxygen provocation testing in 15 eyes of 15 primary open-angle glaucoma (POAG) patients and 15 eyes of 15 age-matched control subjects. During the test, LSFG-derived tissue mean blur rate (MBR_T) and clinical variables, including blood pressure, were recorded. We evaluated differences in MBR_T alteration during systemic hyperoxia between the groups. Additionally, we calculated the mean % change in MBR_T against baseline and determined contributing factors.

Results. Despite similar clinical variables during systemic hyperoxia in both groups, the mean % change in MBR_T against baseline was significantly lower in the POAG than control subjects ($P < 0.0001$). Multiple regression analysis revealed that baseline MBR_T and systolic blood pressure (SBP) were contributing factors to mean % change in MBR_T ($\beta = 0.44$, $\beta = -0.32$, respectively). Additionally, baseline MBR_T and SBP were strongly correlated to mean % change in MBR_T only in the POAG group ($r = 0.83$, $P < 0.0001$; $r = -0.60$, $P = 0.02$, respectively).

Conclusions. POAG patients had a weaker vasoreactive response to hyperoxia than controls, and this impaired response was associated with lower basal ONH BF and higher SBP. These findings suggest that pre-existing vasoconstriction in the ONH of eyes with glaucoma might reduce the capacity of the vasoconstrictive response to hyperoxia. Alternatively, the pathways that mediate hyperoxia-induced vasoconstriction could be altered in POAG.

Keywords: ocular blood flow, laser speckle flowgraphy, autoregulation, hyperoxia

Primary open-angle glaucoma (POAG), the second most common cause of blindness worldwide,[1] is characterized by progressive retinal ganglion cell death and associated visual field loss.[2] High intraocular pressure (IOP) is the only evidence-based risk factor for glaucoma progression,[3] but glaucoma can progress even with normal IOP. Many non-IOP risk factors have been investigated,[4] but glaucoma pathogenesis remains imperfectly understood.

One suspected non-IOP risk factor for progression is damage to the optic nerve head (ONH) and associated low blood flow (BF). Low BF contributes to glaucoma by interrupting vascular autoregulation, or vasoreactivity,[5] the intrinsic ability of vascular beds to maintain constant BF despite fluctuating perfusion pressure and varying metabolic demand.[6] Vasoreactivity is usually assessed with oxygen, CO_2, or light flicker provocation tests, which measure the capacity of the vessels to constrict during systemic hyperoxia and to dilate during hypercapnia or flicker stimulation. Provocation testing in glaucoma patients shows impaired vasoreactivity in the middle cerebral artery,[7] retrobulbar vessels,[8–11] retinal arteries and veins[12–14] and superficial ONH.[15,16]

Assessing vasoreactivity is an important part of research into glaucoma pathophysiology, but previous reports could not measure hemodynamics in the deep layers of the ONH. Moreover, laser Doppler flowmetry, used in many reports to measure ONH microcirculation, is slow, has limited reproducibility, and has a limited ability to measure BF within the deep ONH, due to the relatively short wavelength of its laser.[17] There is, therefore, a need for a new, convenient, and reproducible way to assess ocular hemodynamics during provocation tests. Recently, optical coherence tomography angiography (OCTA) has been used in glaucoma research to reliably visualize the capillary response to provocation outside the ONH in healthy subjects.[18–20] However, OCTA shares the same weaknesses as laser Doppler flowmetry in visualizing deep ONH capillaries, due to image artifacts.[21]

Laser speckle flowgraphy (LSFG) promises to overcome previous limitations. LSFG is a convenient, reproducible way to assess ocular hemodynamics during provocation tests, and can evaluate BF within the deep ONH, from the posterior tissue to the lamina cribrosa, with high reproducibility.[22] LSFG is already gaining popularity in Japan,[23–25] and its clinical usability for estimating ocular perfusion has been validated in Caucasian

subjects.[26] Previously, we successfully used LSFG to measure ONH hemodynamics during oxygen inhalation provocation in healthy volunteers, revealing new details on dynamic changes in ONH BF and confirming the reproducibility of our LSFG-based protocol.[27] Here, we extend this protocol to include POAG patients to investigate the effect of POAG on vasoreactivity.

Other background factors may influence the baseline/provocation vasoreactivity ratio. However, only factors influencing vasodilation during flicker stimulation have been investigated,[14,15] and factors influencing vasoconstriction remain unexplored. Clarifying factors influencing vasoreactivity during provocation illuminated the mechanisms underlying vasoreactivity and glaucoma pathophysiology. Thus, this study carried out oxygen inhalation provocation in POAG patients and age-matched control subjects, investigated differences in vasoreactivity in the deep ONH of these subjects, and determined factors influencing the vasoconstrictive response ratio.

Methods

Subjects

This prospective study comprised 15 eyes of 15 POAG patients (mean deviation: −4.0 ± 3.8 dB) and 15 eyes of 15 control subjects who visited Tohoku University Hospital, located in Miyagi, Japan, between December 2014 and October 2015. Informed consent was obtained from the subjects before the oxygen inhalation test was performed. This study followed the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the Tohoku University School of Medicine (Protocol number: 2015-2-240-1). Reliable results were obtained from all POAG subjects in visual field testing (fixation errors <20%, false positives <33%, and false negatives <33%) and OCT examination (signal strength >60[28]). When both eyes satisfied the inclusion criteria, one eye was randomly selected for the analysis. POAG was diagnosed by a glaucoma specialist (TN).

The inclusion criteria were (1) age between 30 and 75 years old, (2) the presence of glaucomatous optic disc changes, with corresponding visual field defects, matching the Anderson-Patella criteria, (3) abnormally reduced circumpapillary retinal nerve fiber layer thickness (cpRNFLT), and (4) a normal, open angle in a gonioscopic examination. Subjects were excluded if they had a history of ocular or systemic disease causing optic nerve damage. The control group comprised the healthy fellow eyes of patients with dry eye, cataract, macular hole, or epiretinal membrane. The inclusion criteria were (1) normal findings in a slit lamp or funduscopic examination (2) and no history of elevated IOP. Because of the systemic side effects of inhaling pure oxygen,[29,30] subjects with heart disease or respiratory disorders were excluded from this study.

Additionally, subjects with best-corrected visual acuity (BCVA) less than 20/20, high IOP (higher than 21 mm Hg as measured with Goldmann applanation tonometry) on the test day, high myopia (axial length longer than 26.5 mm or refractive error worse than −8 diopters), or clinically evident cataract were excluded. Subjects with a history of smoking within the previous 4 years were also excluded, because of the effect of a smoking habit on the vasoreaction to hyperoxia.[31] All POAG patients continued their currents courses of antiglaucoma drugs. All 15 patients used prostaglandin analogues (PGs) (100%), five used beta-blockers (33.3%), five used carbonate anhydrase inhibitors (33.3%), and four used α_2 stimulators (26.7%).

Measurement of Baseline Clinical Characteristics

Baseline clinical variables were measured before testing. Logarithmic minimum angle of resolution (logMAR) was used to represent BCVA. IOP was measured with Goldmann applanation tonometry, axial length was measured with the IOL Master (Carl Zeiss Meditec, Dublin, CA, USA), cpRNFLT was measured with spectral-domain OCT (3D OCT-2000 v. 8.11; Topcon, Inc., Tokyo, Japan), and the visual field was measured with the 24-2 program of the Humphrey Field Analyzer (Carl Zeiss Meditec). Pupil dilation was performed with 0.4% tropicamide (Mydrin M; Santen Pharmaceutical Co., Ltd, Osaka, Japan). Mean blood pressure (MBP) and mean ocular perfusion pressure (MOPP) were calculated as follows: MBP = diastolic blood pressure (DBP) + 1/3 (systolic blood pressure [SBP] − DBP); MOPP = 2/3 MBP − IOP.

ONH BF Assessment With LSFG

The principles of LSFG have previously been described in detail.[32] Briefly, this instrument consists of a fundus camera equipped with a diode laser (wavelength: 830 nm) and an ordinary charge-coupled device camera (resolution: 750 × 360 pixels). The device measures the pattern of speckle contrast produced by the interference of a laser scattered by blood cells moving in the blood vessels. This study used the LSFG-NAVI device (Softcare Co., Ltd., Fukutsu, Japan). Mean blur rate (MBR), which indicates relative BF, is the main LSFG variable and is expressed in arbitrary units. MBR images of the fundus are acquired continuously at the rate of 30 frames per second over a 4-second period and then averaged to produce a composite map of ocular BF. The accompanying analysis software can automatically divide the MBR map into the large vessel and tissue (capillary) areas of the ONH and determine specific values for each. The focus of this study was on tissue-area MBR (MBR_T), because MBR_T has been reported to represent BF in the deeper regions of the ONH, in contrast to vessel-area MBR, which represents BF in the surface retinal vessels, and because MBR_T has been found to be usable for interindividual comparisons.[22,33]

Oxygen Inhalation Test

Clinical characteristics were measured before testing. The oxygen inhalation test itself was performed in the same way as our previous investigation.[27] Briefly, the protocol had three phases (shown in Fig. 1): baseline, hyperoxia, and recovery. In the baseline phase, the subjects inhaled room air for no less than 5 minutes; baseline measurements of blood pressure (BP), pulse rate (PR), and saturation of pulse-oximetry oxygen (SpO_2) were made once, while MBR_T represented the average of five measurements. In the hyperoxia phase, the subjects inhaled pure oxygen (6 L/min) for 12 minutes; MBR_T was measured at 1, 2, 3, 4, 8, and 12 minutes after the start of oxygen inhalation. In the recovery phase, the subjects inhaled room air for 4 minutes; MBR_T was measured again. Our previous report showed that MBR_T already decreased significantly 2 minutes after the start of oxygen inhalation in normal subjects, so we shortened the total oxygen inhalation and recovery time and widened the measurement intervals to minimize the burden on the patients. BP, PR, and SpO_2 were monitored every 5 minutes during the protocol. SpO_2 was measured with an oximeter (Onyx II Model 9580 Finger Pulse Oximeter, Nonin Medical, Inc., Plymouth, MN, USA). MBP and MOPP were calculated as follows: MBP (t) = DBP (t) + 1/3 (SBP (t) − DBP (t)). MOPP (t) = 2/3 MBP (t) − baseline IOP; "t" indicates minutes after the start of oxygen inhalation (t = 0, 5, 10, and 15). The IOP values used to calculate MOPP(t)

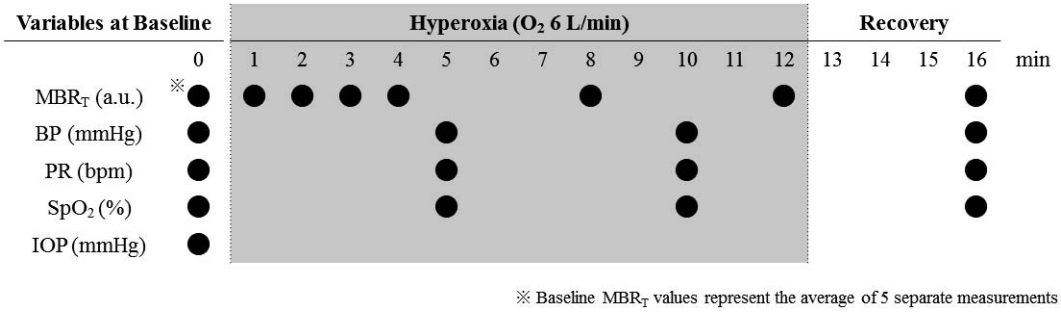

Figure 1. Oxygen inhalation test protocol. The protocol had three phases: baseline, hyperoxia, and recovery. The hyperoxia phase is shown in *gray*; recovery comprised the 4 minutes after pure oxygen inhalation ended. During hyperoxia, pure oxygen (6 L/min) was inhaled for 12 minutes. The *circles* indicate the measured time points for each variable. During baseline, BP, PR, SpO_2, IOP, and MBR_T were measured. MBR_T was measured five times while the subjects inhaled room air for no less than 5 minutes, and the average was used as the baseline MBR_T.

represented only a single baseline measurement, because our previous work showed that IOP is not affected by oxygen inhalation time.[27]

Statistical Analysis

All data are shown as mean ± standard deviation. Dynamic changes in MBR_T (%MBR_T) were defined as the percentage of baseline (100%). Repeated analyses of variance (ANOVA) were used to analyze the significance of differences in baseline variables in the POAG patients and control subjects. Two-way ANOVA and a post hoc Dunnett's test were used to analyze the significance of differences in systemic variables in each phase, as well as the %MBR_T alteration in the hyperoxic and recovery phases. We performed an a priori power analysis to determine the necessary total sample sizes for the 2-way ANOVA, with the values a = 0.05, power = 0.80, and effect size = 0.40. The calculated minimum total sample size was 30 (these calculations were made with G * Power, Version 3.1.9.2; program written by Franz Faul, University of Kiel, Kiel, Germany). Pearson's correlation coefficient was used to determine the correlation between the mean % change in MBR_T and other ophthalmic and systemic variables. The independent variable in the multiple regression analysis was mean % change in MBR_T, and the dependent variables were the variables that the univariate analysis showed were correlated with mean % change in MBR_T. The value of mean % change in MBR_T was defined as follows:

$$\text{mean \% change in MBR}_T(\%) = 100 - \frac{1}{6}\sum_{t=1}^{12} \%\text{MBR}_T(t)$$
$$(t = 1, 2, 3, 4, 8, 12)$$

All statistical analyses were performed with JMP software (Pro version 11.2.0; SAS Institute Japan, Inc., Tokyo, Japan). The significance level was set at $P < 0.05$.

Table 1. Clinical Characteristics and BF Variables in Both Groups at Baseline

Variable	POAG, $n = 15$	Control, $n = 15$	P Value
BCVA, logMAR	−0.13 ± 0.06	0.12 ± 0.05	0.94
Refractive error, diopter	−2.1 ± 2.6	−2.2 ± 2.9	0.92
Axial length, mm	24.5 ± 1.1	24.8 ± 1.7	0.54
IOP, mm Hg	12.6 ± 1.5	14 ± 3.0	0.12
CpRNFLT, μm	88.8 ± 12.6	111.8 ± 9.6	<0.001
MBR_T, a.u.	11.3 ± 3.1	13.5 ± 2.7	0.048
Age, y	54.7 ± 12.9	55.6 ± 7.6	0.82
Gender, male, female	7 : 8	8 : 7	1.0*
Diabetes mellitus, *n*	2	0	0.48*
Dyslipidemia, *n*	4	2	0.65*
Hypertension, *n*	3	1	0.60*
SBP, mm Hg	138.4 ± 5.9	131.1 ± 5.9	0.39
DBP, mm Hg	86.2 ± 3.3	87.4 ± 3.3	0.81
MBP, mm Hg	103.6 ± 15.4	101.9 ± 14.7	0.77
MOPP, mm Hg	56.6 ± 9.6	54.6 ± 10.2	0.60
PR, bpm	68.5 ± 7.7	71.0 ± 9.0	0.44
SpO_2, %	97.6 ± 1.3	97.4 ± 0.9	0.63

Unmarked *P* values, ANOVA test.
* χ^2 test.

Results

Clinical Characteristics and BF Variables in Both Groups at Baseline

As shown in Table 1, clinical characteristics did not significantly differ in the two groups (P = 0.12–0.94), although cpRNFLT ($P < 0.001$) and baseline MBR_T ($P = 0.048$) were significantly lower in the POAG patients.

Changes in BF Variables During Oxygen Inhalation Test

As shown in Table 2, SpO_2 rose significantly in both groups during oxygen inhalation ($P < 0.0001$). During systemic hyperoxia, no systemic variables, including SBP, DBP, MBP, MOPP, and PR, changed significantly in either group (P = 0.51–0.99).

Differences in the Vascular Response to Hyperoxia

As shown in Figure 2, MBR_T decreased significantly in the control group 2 minutes after the start of oxygen inhalation (%MBR_T = 87.7 ± 6.5%, P = 0.0006) and after 3 minutes in the POAG patients (%MBR_T = 90.5 ± 9.3%, P = 0.02). The response to hyperoxia was significantly lower in the POAG patients (2-way ANOVA: $P < 0.0001$).

Relationship Between Mean % Change in MBR_T and Other Variables

In the total group of POAG and control subjects ($n = 30$), a single regression analysis showed that mean % change in MBR_T was significantly correlated with cpRNFLT ($r = 0.47$, P = 0.007), baseline MBR_T (r = 0.68, $P < 0.0001$), baseline SBP (r = –0.52, $P = 0.005$), and baseline MBP (r = –0.37, $P = 0.033$),

Table 2. Changes in BF Variables During Oxygen Inhalation

Variable	Baseline	Hyperoxia		Recovery	*P* Value
		5 min	10 min	16 min	
POAG, $n = 15$					
SBP, mm Hg	138.4 ± 7.7	140.4 ± 7.7	140.2 ± 7.7	139.4 ± 7.7	0.99
DBP, mm Hg	86.2 ± 4.1	89.7 ± 4.1	86.5 ± 4.1	89.0 ± 4.1	0.91
MBP, mm Hg	103.6 ± 15.4	104.4 ± 20.0	105.8 ± 19.8	106.6 ± 20.8	0.98
MOPP, mm Hg	56.6 ± 9.6	58.6 ± 13.1	57.2 ± 12.7	58.1 ± 12.5	0.97
PR, bpm	68.5 ± 7.7	66.8 ± 7.5	64.8 ± 7.2	69 ± 9.4	0.51
SpO_2, %	97.6 ± 1.3	99.1 ± 0.9	99.1 ± 0.6	97.1 ± 1.4	<0.0001
Control, $n = 15$					
SBP, mm Hg	131.1 ± 4.8	128.2 ± 4.8	125.4 ± 4.8	127.4 ± 4.8	0.86
DBP, mm Hg	87.4 ± 2.9	84.7 ± 2.9	85.0 ± 2.9	86.6 ± 2.9	0.90
MBP, mm Hg	101.9 ± 14.7	98.5 ± 10.8	100.2 ± 12.3	99.2 ± 12.2	0.90
MOPP, mm Hg	54.6 ± 10.2	52.6 ± 8.6	51.9 ± 7.3	53.4 ± 7.7	0.87
PR, bpm	71.0 ± 9.0	69.3 ± 11.3	68.7 ± 10.0	71.8 ± 12.4	0.86
SpO_2, %	97.4 ± 0.9	98.9 ± 0.5	99 ± 0.6	97.6 ± 0.9	<0.0001

Unmarked *P* values, ANOVA test.

while mean % change in MBR_T was not significantly correlated with age, axial length, IOP, baseline DBP, or baseline MOPP ($P = 0.08$–0.47).

Factors Affecting the Vascular Response to Systemic Hyperoxia

We investigated background factors influencing the vascular response to systemic hyperoxia. As shown in Table 3, baseline MBR_T was the strongest dependent factor affecting mean % change in MBR_T in all subjects ($\beta = 0.44$, $P = 0.0096$). Additionally, SBP was a dependent contributing factor to mean % change in MBR_T ($\beta = -0.32$, $P = 0.03$). CpRNFLT did not reach the level of statistical significance ($P = 0.14$). Mean % change in MBR_T was significantly correlated with baseline MBR_T and SBP in the POAG group, but was not significantly correlated in the control group (Fig. 3). Figure 4 shows representative baseline fundus photographs, LSFG images, and the $\%MBR_T$ alteration during the test. The figure shows patients with high baseline MBR_T, low SBP, and high mean % change in MBR_T in the upper panel and low baseline MBR_T, high SBP, and low mean % change in MBR_T in the lower panel.

Discussion

In this study, we attempted to determine whether patients with glaucoma had an impaired vasoreactive response to hyperoxia. We used LSFG to test the vasoreactive response, which allowed us to obtain a detailed assessment of dynamic changes in ONH BF. We found that the response to hyperoxia was significantly reduced in POAG patients compared to control subjects. Additionally, we investigated factors influencing vasoreactivity within the ONH. We found that in eyes with POAG, mean % change in MBR_T was highly associated with MBR_T and SBP at baseline. These findings, which demonstrate the usefulness of LSFG in this field of research, suggest that an impaired vasoreaction to hyperoxia might be due to pre-existing vasoconstriction in the eyes of glaucoma patients.

In this study, LSFG measurements of MBR_T were significantly lower in glaucoma patients than control subjects (Table 1) confirming our previously reported results.[34,35] We also found that SpO_2 increased significantly during hyperoxia, confirming that systemic hyperoxia was successfully achieved, and that there were no significant changes in SBP, DBP, MBP, MOPP, or PR, confirming that the changes we observed in MBR_T during hyperoxia were caused specifically by breathing pure oxygen, not by coincidental changes in other clinical variables. A detailed examination of time-course changes in MBR_T showed that MBR_T decreased significantly after 2 minutes in the control subjects (Fig. 3). These results are consistent with our previous research.[27]

One of the new findings in this study was that vasoreactivity to systemic hyperoxia was impaired within the deep ONH in POAG patients (Fig. 3). The vascular response to hyperoxia in glaucoma has not been widely studied. Hosking et al.[9] used color Doppler imaging to show that hyperoxia resulted in reductions in both peak systolic velocity and end diastolic velocity in the ophthalmic arteries, and that this occurred only in normal subjects, not glaucoma patients. Harris et al.[7] used transcranial Doppler imaging to show that hyperoxia significantly decreased both mean and peak systolic velocities in the middle cerebral artery of control subjects, while it did not cause any significant change in open-angle glaucoma patients.[7] Our finding that the vascular response to systemic hyperoxia is significantly reduced in the ONH of eyes with POAG is consistent with these reports. MBR_T is considered to represent BF in the short posterior ciliary artery in the ONH,[22] suggesting that vasoreactivity within the deep ONH is reduced in POAG patients. This novel finding is particularly significant because the deep ONH supplies the lamina cribrosa, which is thought to be the primary site of lesion in glaucoma.[36]

In this study, we also investigated factors influencing the vasoreaction to systemic hyperoxia in a mixed group of subjects, comprising both healthy and glaucoma subjects. We set a novel variable, mean % change in MBR_T, as the independent variable in a multiple regression analysis, and found that baseline MBR_T was the strongest dependent factor (Table 3). Additionally, baseline MBR_T was correlated with mean % change in MBR_T only in the POAG patients. This result supports the hypothesis that pre-existing vasoconstriction limits the capacity of the glaucomatous eye to moderate blood velocity.[37] During hyperoxia, ET-1, which acts via the endothelin receptors and is considered to be the most important endothelium-derived vasoconstrictor, plays an important role in retinal vessel constriction in rats and humans.[38,39] Previous investigations have reported that endothelin receptors A and B are both expressed in the human ONH.[40] Thus, ET-1, acting via the endothelin receptors, might be the primary actor in the mechanism underlying the BF response to hyperoxia in the ONH and the retinal vessels.

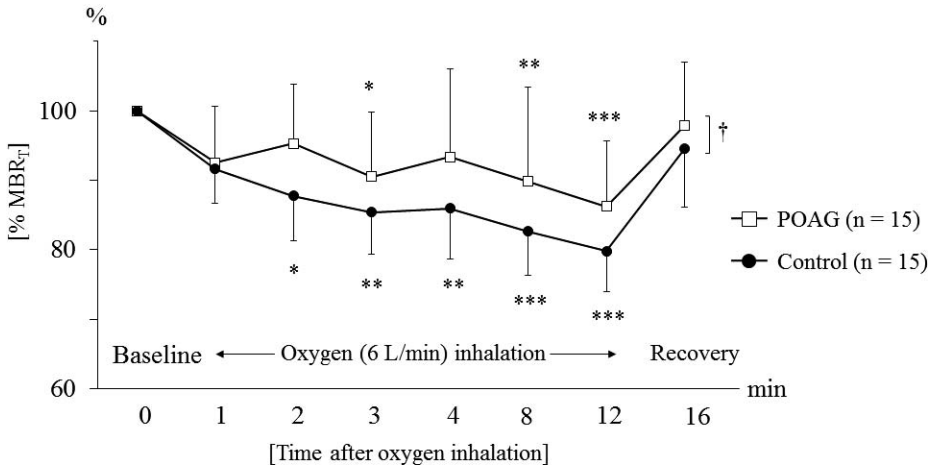

Figure 2. Differences in vascular response to hyperoxia. The *x*-axis shows the time after the start of oxygen inhalation, and the *y*-axis shows the %MBR$_T$ alteration compared to baseline. The *dagger* indicates a statistically significant difference between the POAG and control groups (2-way ANOVA: $P < 0.0001$). The *asterisks* indicate statistically significant differences from the baseline (ANOVA and post hoc Dunnett's test; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

Previously, a meta-analysis of ET-1 showed that ET-1 blood concentration is elevated in glaucoma, and another study showed that endothelin B receptor immunoreactivity was elevated in the ONH of human glaucoma patients.[41,42] ET-1 has also been shown to induce astroglial proliferation in cultured human ONH astrocytes, acting via ET (A/B) receptor activation.[43] Furthermore, in a pigmented rabbit-eye model, the intravitreous injection of ET-1 decreased ONH BF and increased optic disc cupping.[44] Taken together, these findings suggest that endothelin is associated with both decreased BF and the reduction of redundant vessel constriction capacity in eyes with glaucoma, factors that might underlie glaucomatous neurodegeneration. Further assessment will be required to clarify the relationship between abnormal vasoreactivity to hyperoxia and the endothelin pathway in glaucoma patients.

Other interesting findings in this study were that SBP independently contributed to mean % change in MBR$_T$, and that there was a significant negative correlation between SBP and mean % change in MBR$_T$, seen only in eyes with POAG. There have been various etiologic studies on the influence of BP and OPP on the prevalence, incidence, and progression of glaucoma.[45] However, these studies have disagreed, finding that both high and low BP and OPP were risk factors for glaucoma. Meanwhile, several other studies have found no association. A solution to this disagreement was suggested by the Los Angeles Latino Eye Study, which found that low DBP and high SBP were both associated with an increased prevalence of POAG. Thus, any kind of extreme change in BP, whether high or low, may cause ischemic damage to the ONH. Specifically, low DBP may cause low OPP and high SBP may cause hypertensive vasoconstriction.[45] High SBP can also cause chronic narrowing of the vessels,[46–49] creating a preexisting degree of vasoconstriction that might reduce constrictive capacity during the response to hyperoxia.

Table 3. Dependent Factors Contributing to Mean % Change in MBRT, $N = 30$, POAG = 15; Control = 15

Variable			
Independent	**Dependent**	***β***	***P* Value**
Mean % change in MBR$_T$	CpRNFLT	0.23	0.14
	Baseline MBR$_T$	0.44	0.0096*
	Baseline SBP	−0.32	0.030*

β indicates the standard partial regression coefficient.
* Indicates statistical significance.

There were several limitations to this study. First, the sample size was small. However, as described in statistical analysis section, the study included the minimum necessary sample size. We limited the sample size to this number to avoid placing an unnecessary burden on our patients. Second, although LSFG measurements in the ONH have recently been validated using the microsphere technique and the hydrogen gas clearance method in animal experiments,[22,50] an adequate understanding of the penetration depth of the laser into the ONH has not been established. Third, the use of anti-glaucoma eye drops might have contributed to altered vasoreactivity during hyperoxia in our POAG subjects. In particular, PGs have been reported to have a direct, IOP-independent effect on BF, improving it by relaxing the vasoconstriction induced by ET-1.[51,52] Considering that all POAG patients in this study were being treated with PGs, it follows that PGs might have reduced ET-1-induced vasoconstriction during hyperoxia, affecting our results. However, we found that lower vasoreactivity was closely associated with lower baseline BF, making it unlikely that any dysfunction in autoregulation was caused by PG use, which would have improved baseline BF. Other glaucoma eye drops have been reported to either protect BF or to have unclear effects.[53,54] Thus, their effect on vasoreactivity is likely similar to that of PGs. In fact, the POAG subjects showed no significant correlation between the number of antiglaucoma eye drops and the mean % change in MBR$_T$ ($P = 0.19$) and no significant difference between users and nonusers of each drug ($P = 0.15$–0.93). Fourth, our hypothesis that a chronic increase in ET-1 concentration is associated with a decrease in vasoreactivity is contradicted by several studies that found no significant difference in ET-1 concentration in eyes with glaucoma.[55,56] Nevertheless, as we mentioned in a previous report, we consider that this disagreement between existing studies as to the involvement of ET-1 concentration in the pathogenesis of glaucoma may best be resolved by a hyperoxic provocation assessment. In the future, we therefore plan to measure ET-1 concentration not just at baseline, but also after oxygen provocation. We believe that this should shed new light on blood concentration dynamics and help resolve current disagreements. Fifth, it remains unclear whether an

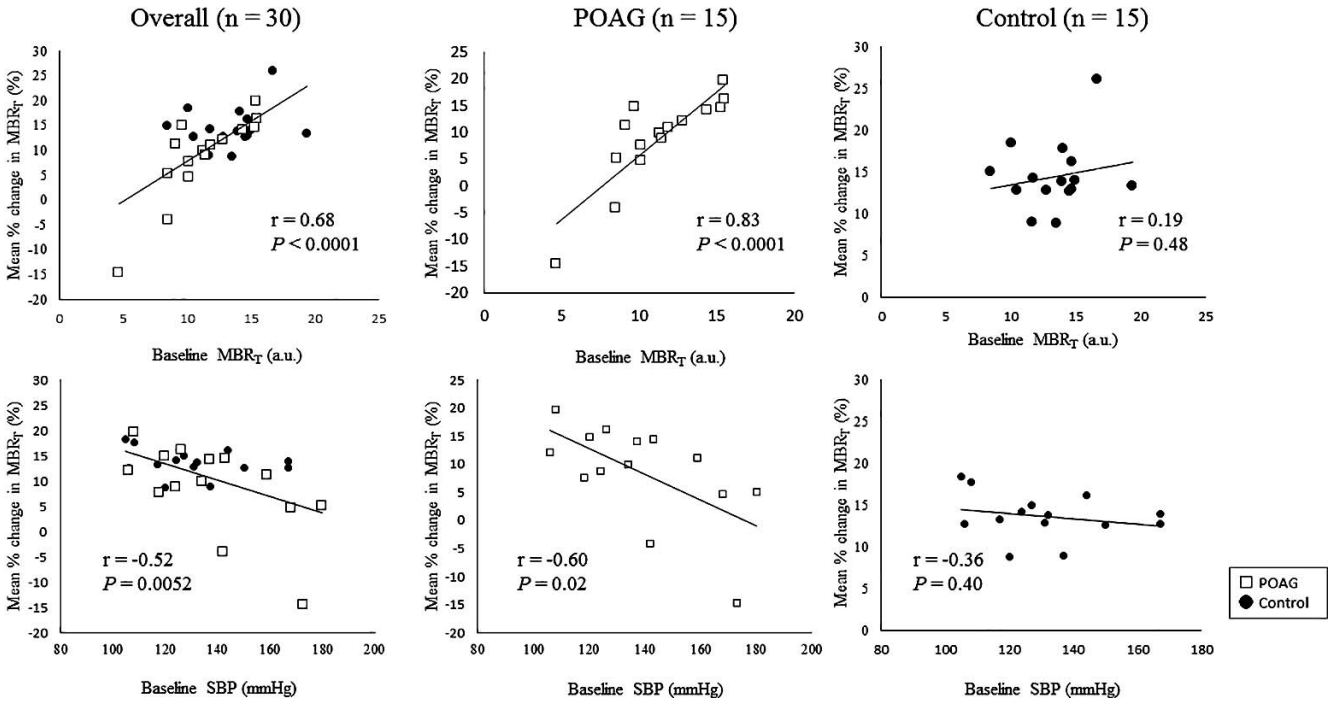

Figure 3. The relationship of mean % change in MBR_T to baseline MBR_T and SBP in the two groups. Mean % change in MBR_T was significantly correlated with baseline MBR_T in the subjects overall and in the POAG group ($r = 0.68$, $P < 0.0001$; $r = 0.83$, $P < 0.0001$, respectively). Furthermore, mean % change in MBR_T was significantly correlated with SBP in the subjects overall and in the POAG group ($r = -0.52$, $P = 0.0052$; $r = -0.60$, $P = 0.02$, respectively). However, there was no significant correlation between mean % change in MBR_T and baseline MBR_T or SBP in the control group ($r = 0.19$, $P = 0.48$; $r = -0.36$, $P = 0.40$; Pearson's correlation coefficient).

abnormal ONH BF response to hyperoxia is associated with glaucoma progression. Therefore, a prospective study should be performed in the future, including a larger number of glaucoma subjects.

In conclusion, this study used LSFG to show that POAG patients have a weaker vasoreactive response to hyperoxia than healthy controls. Furthermore, this impaired vasoreactivity was associated with lower ONH BF and higher SBP. These findings suggest that eyes with glaucoma have a pre-existing degree of vasoconstriction in the ONH, and that this might reduce the capacity of the vasoconstrictive response to hyperoxia. Alternatively, the pathways that mediate hyperoxia-induced vasoconstriction could be altered in POAG.

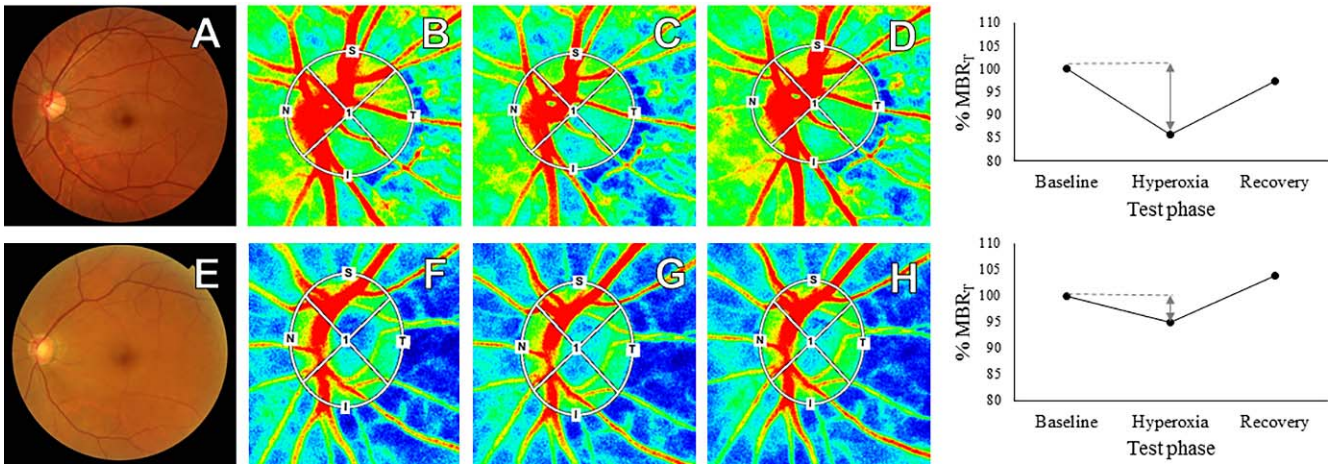

Figure 4. Representative fundus photographs, LSFG images, and $\%MBR_T$ alteration during the protocol. This figure shows representative fundus photographs, LSFG images, and $\%MBR_T$ alteration during the protocol. The *upper panel* shows a patient with high baseline MBR_T, low SBP, and high mean % change in MBR_T, while the *lower panel* shows a patient with low baseline MBR_T, high SBP, and low mean % change in MBR_T. **A**, **B**, **E**, and **F** show representative baseline fundus photographs and LSFG images for each patient. **C** and **G** show representative LSFG images during the hyperoxic phase, while **D** and **H** show the recovery phase. The average $\%MBR_T$ against baseline during hyperoxia was calculated; the *gray double arrow* indicates mean % change in MBR_T. *Upper panel*: The left eye of a 43-year-old male glaucoma patient (MD: −7.88 dB, MBR_T: 15.5 a.u., BP 126/86 mm Hg, PR 70 bpm, mean % change in MBR_T: 16.2%). *Lower panel*: The left eye of a 66-year-old male glaucoma patient (MD: −2.81 dB, MBR_T: 8.6 a.u., BP 180/103 mm Hg, PR 63 bpm, mean % change in MBR_T: 5.1%).

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References

1. Leske MC. Open-angle glaucoma—an epidemiologic overview. *Ophthalmic Epidemiol*. 2007;14:166–172.
2. Weinreb RN, Khaw PT. Primary open-angle glaucoma. *Lancet (London, England)*. 2004;363:1711–1720.
3. AGIS-Investigators. The Advanced Glaucoma Intervention Study (AGIS): 7. The relationship between control of intraocular pressure and visual field deterioration. *Am J Ophthalmol*. 2000;130:429–440.
4. Nakazawa T. Ocular blood flow and influencing factors for glaucoma. *Asia-Pacific J Ophthalmol (Philadelphia, Pa)*. 2016;5:38–44.
5. Moore D, Harris A, Wudunn D, Kheradiya N, Siesky B. Dysfunctional regulation of ocular blood flow: a risk factor for glaucoma? *Clin Ophthalmol*. 2008;2:849–861.
6. Guyton AC, Carrier O Jr, Walker JR. Evidence for tissue oxygen demand as the major factor causing autoregulation. *Circ Res*. 1964;15:60–69.
7. Harris A, Zarfati D, Zalish M, et al. Reduced cerebrovascular blood flow velocities and vasoreactivity in open-angle glaucoma. *Am J Ophthalmol*. 2003;135:144–147.
8. Zeitz O, Mayer J, Hufnagel D, et al. Neuronal activity influences hemodynamics in the paraoptic short posterior ciliary arteries: a comparison between healthy and glaucomatous subjects. *Invest Ophthalmol Vis Sci*. 2009;50:5846–5850.
9. Hosking SL, Harris A, Chung HS, et al. Ocular haemodynamic responses to induced hypercapnia and hyperoxia in glaucoma. *Br J Ophthalmol*. 2004;88:406–411.
10. Sines D, Harris A, Siesky B, et al. The response of retrobulbar vasculature to hypercapnia in primary open-angle glaucoma and ocular hypertension. *Ophthalmic Res*. 2007;39:76–80.
11. Plange N, Bienert M, Harris A, Remky A, Arend KO. Color Doppler sonography of retrobulbar vessels and hypercapnia in normal tension glaucoma [in German]. *Ophthalmologe*. 2012;109:250–256.
12. Garhöfer G, Zawinka C, Resch H, Huemer KH, Schmetterer L, Dorner GT. Response of retinal vessel diameters to flicker stimulation in patients with early open angle glaucoma. *J Glaucoma*. 2004;13:340–344.
13. Gugleta K, Kochkorov A, Waldmann N, et al. Dynamics of retinal vessel response to flicker light in glaucoma patients and ocular hypertensives. *Graefe's Arch Clin Exp Ophthalmol*. 2012;250:589–594.
14. Gugleta K, Waldmann N, Polunina A, et al. Retinal neurovascular coupling in patients with glaucoma and ocular hypertension and its association with the level of glaucomatous damage. *Graefes Arch Clin Exp Ophthalmol*. 2013;251:1577–1585.
15. Riva CE, Salgarello T, Logean E, Colotto A, Galan EM, Falsini B. Flicker-evoked response measured at the optic disc rim is reduced in ocular hypertension and early glaucoma. *Invest Ophthalmol Vis Sci*. 2004;45:3662–3668.
16. Venkataraman ST, Hudson C, Rachmiel R, et al. Retinal arteriolar vascular reactivity in untreated and progressive primary open-angle glaucoma. *Invest Ophthalmol Vis Sci*. 2010;51:2043–2050.
17. Riva CE, Geiser M, Petrig BL; for the Ocular Blood Flow Research Association. Ocular blood flow assessment using continuous laser Doppler flowmetry. *Acta Ophthalmol*. 2010;88:622–629.
18. Xu H, Deng G, Jiang C, Kong X, Yu J, Sun X. Microcirculatory responses to hyperoxia in macular and peripapillary regions. *Invest Ophthalmol Vis Sci*. 2016;57:4464–4468.
19. Pechauer AD, Jia Y, Liu L, Gao SS, Jiang C, Huang D. Optical coherence tomography angiography of peripapillary retinal blood flow response to hyperoxia. *Invest Ophthalmol Vis Sci*. 2015;56:3287–3291.
20. Wei E, Jia Y, Tan O, et al. Parafoveal retinal vascular response to pattern visual stimulation assessed with OCT angiography. *PLoS One*. 2013;8:e81343.
21. Suh MH, Zangwill LM, Manalastas PIC, et al. Optical coherence tomography angiography vessel density in glaucomatous eyes with focal lamina cribrosa defects. *Ophthalmology*. 2016;123:2309–2317.
22. Wang L, Cull GA, Piper C, Burgoyne CF, Fortune B. Anterior and posterior optic nerve head blood flow in nonhuman primate experimental glaucoma model measured by laser speckle imaging technique and microsphere method. *Invest Ophthalmol Vis Sci*. 2012;53:8303–8309.
23. Tsuda S, Kunikata H, Shimura M, et al. Pulse-waveform analysis of normal population using laser speckle flowgraphy. *Curr Eye Res*. 2014;39:1207–1215.
24. Kiyota N, Shiga Y, Takahashi H, Nakazawa T. Large vessel area of the optic nerve head, measured with laser speckle flowgraphy, is significantly reduced in eyes with preperimetric glaucoma. *Clin Experiment Ophthalmol*. 2015;43:841–843.
25. Shiga Y, Omodaka K, Kunikata H, et al. Waveform analysis of ocular blood flow and the early detection of normal tension glaucoma. *Invest Ophthalmol Vis Sci*. 2013;54:7699–7706.
26. Luft N, Wozniak PA, Aschinger GC, et al. Measurements of retinal perfusion using laser speckle flowgraphy and doppler optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2016;57:5417–5425.
27. Shiga Y, Sato M, Maruyama K, et al. Assessment of short-term changes in optic nerve head hemodynamics in hyperoxic conditions with laser speckle flowgraphy. *Curr Eye Res*. 2015;40:1055–1062.
28. Takahashi M, Omodaka K, Maruyama K, et al. Simulated visual fields produced from macular RNFLT data in patients with glaucoma. *Curr Eye Res*. 2013;38:1133–1141.
29. van Ooij P-JAM, Sterk PJ, van Hulst RA. Oxygen, the lung and the diver: friends and foes? *Eur Respir Rev*. 2016;25:496–505.
30. Ferrari R, Ceconi C, Curello S, et al. Oxygen-mediated myocardial damage during ischaemia and reperfusion: role of the cellular defences against oxygen toxicity. *J Mol Cell Cardiol*. 1985;17:937–945.
31. Langhans M, Michelson G, Groh MJM. Effect of breathing 100% oxygen on retinal and optic nerve head capillary blood flow in smokers and non-smokers. *Br J Ophthalmol*. 1997;81:365–369.
32. Draijer M, Hondebrink E, van Leeuwen T, Steenbergen W. Review of laser speckle contrast techniques for visualizing tissue perfusion. *Lasers Med Sci*. 2009;24:639–651.
33. Aizawa N, Nitta F, Kunikata H, et al. Laser speckle and hydrogen gas clearance measurements of optic nerve circulation in albino and pigmented rabbits with or without optic disc atrophy. *Invest Ophthalmol Vis Sci*. 2014;55:7991–7996.
34. Nakazawa T, Yokoyama, Aizawa N, et al. Significant correlations between optic nerve head microcirculation and visual field defects and nerve fiber layer loss in glaucoma patients

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with myopic glaucomatous disk. *Clin Ophthalmol*. 2011;5: 1721–1727.

35. Shiga Y, Kunikata H, Aizawa N, et al. Optic nerve head blood flow, as measured by laser speckle flowgraphy, is significantly reduced in preperimetric glaucoma. *Curr Eye Res*. 2016;41: 1447–1453.
36. Omodaka K, Horii T, Takahashi S, et al. 3D evaluation of the lamina cribrosa with swept-source optical coherence tomography in normal tension glaucoma. *PLoS One*. 2015;10: e0122347.
37. Venkataraman ST, Flanagan JG, Hudson C. Vascular reactivity of optic nerve head and retinal blood vessels in glaucoma–a review. *Microcirculation*. 2010;17:568–581.
38. Takagi C, King GL, Takagi H, Lin YW, Clermont AC, Bursell SE. Endothelin-1 action via endothelin receptors is a primary mechanism modulating retinal circulatory response to hyperoxia. *Invest Ophthalmol Vis Sci*. 1996;37:2099–2109.
39. Dallinger S, Dorner GT, Wenzel R, et al. Endothelin-1 contributes to hyperoxia-induced vasoconstriction in the human retina. *Invest Ophthalmol Vis Sci*. 2000;41:864–869.
40. Rao VR, Krishnamoorthy RR, Yorio T. Endothelin-1, endothelin A and B receptor expression and their pharmacological properties in GFAP negative human lamina cribrosa cells. *Exp Eye Res*. 2007;84:1115–1124.
41. Shoshani YZ, Harris A, Shoja MM, et al. Endothelin and its suspected role in the pathogenesis and possible treatment of glaucoma. *Curr Eye Res*. 2012;37:1–11.
42. Wang L, Fortune B, Cull G, Dong J, Cioffi GA. Endothelin B receptor in human glaucoma and experimentally induced optic nerve damage. *Arch Ophthalmol (Chicago, Ill 1960)*. 2006;124:717–724.
43. Prasanna G, Krishnamoorthy R, Clark AF, Wordinger RJ, Yorio T. Human optic nerve head astrocytes as a target for endothelin-1. *Invest Ophthalmol Vis Sci*. 2002;43:2704–2713.
44. Oku H, Sugiyama T, Kojima S, Watanabe T, Azuma I. Experimental optic cup enlargement caused by endothelin-1-induced chronic optic nerve head ischemia. *Surv Ophthalmol*. 1999;44Suppl 1:S74–S84.
45. Costa VP, Harris A, Anderson D, et al. Ocular perfusion pressure in glaucoma. *Acta Ophthalmol*. 2014;92:e252–e266.
46. Pinsky JL, Klein R. Retinal arteriolar diameters and elevated blood pressure. *Am J Epidemiol*. 1999;150:263–270.
47. Wong TY, Hubbard LD, Klein R, et al. Retinal microvascular abnormalities and blood pressure in older people: the Cardiovascular Health Study. *Br J Ophthalmol*. 2002;86: 1007–1013.
48. Wong TY, Klein R, Sharrett AR, et al. Retinal arteriolar diameter and risk for hypertension. *Ann Intern Med*. 2004; 140:248–255.
49. Wong TY, Shankar A, Klein R, Klein BEK, Hubbard LD. Prospective cohort study of retinal vessel diameters and risk of hypertension. *BMJ*. 2004;329:79.
50. Takahashi H, Sugiyama T, Tokushige H, et al. Comparison of CCD-equipped laser speckle flowgraphy with hydrogen gas clearance method in the measurement of optic nerve head microcirculation in rabbits. *Exp Eye Res*. 2013;108:10–15.
51. Sugiyama T, Azuma I. Effect of UF-021 on optic nerve head circulation in rabbits. *Jpn J Ophthalmol*. 1995;39:124–129.
52. Kurashima H, Watabe H, Sato N, Abe S, Ishida N, Yoshitomi T. Effects of prostaglandin F-2 alpha analogues on endothelin-1-induced impairment of rabbit ocular blood flow: comparison among tafluprost, travoprost, and latanoprost. *Exp Eye Res*. 2010;91:853–859.
53. Fuchsjäger-Mayrl G, Wally B, Rainer G, et al. Effect of dorzolamide and timolol on ocular blood flow in patients with primary open angle glaucoma and ocular hypertension. *Br J Ophthalmol*. 2005;89:1293–1297.
54. Liu CJ-L, Ko Y-C, Cheng C-Y, Chou JC, Hsu W-M, Liu J-H. Effect of latanoprost 0.005% and brimonidine tartrate 0.2% on pulsatile ocular blood flow in normal tension glaucoma. *Br J Ophthalmol*. 2002;86:1236–1239.
55. Kaiser HJ, Flammer J, Wenk M, Lüscher T. Endothelin-1 plasma levels in normal-tension glaucoma: abnormal response to postural changes. *Graefes Arch Clin Exp Ophthalmol*. 1995; 233:484–488.
56. Kunimatsu S, Mayama C, Tomidokoro A, Araie M. Plasma endothelin-1 level in Japanese normal tension glaucoma patients. *Curr Eye Res*. 2006;31:727–731.

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