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 (MBRT) and clinical variables, including blood pressure, were recorded. We evaluated differences in MBRT alteration during systemic hyperoxia between the groups. Additionally, we calculated the mean % change in MBRT against baseline and determined contributing factors.

RESULTS. Despite similar clinical variables during systemic hyperoxia in both groups, the mean % change in MBRT against baseline was significantly lower in the POAG than control subjects (P < 0.0001). Multiple regression analysis revealed that baseline MBRT and systolic blood pressure (SBP) were contributing factors to mean % change in MBRT (β = 0.44, β = −0.32, respectively). Additionally, baseline MBRT and SBP were strongly correlated to mean % change in MBRT 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, CO2, 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 veins12–14 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.12 LSFG is already gaining popularity in Japan,23–25 and its clinical usability for estimating ocular perfusion has been validated in Caucasian


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Hyperoxia in Eyes With POAG

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. Here, we extend this protocol to include POAG patients to investigate the effect of POAG on vaso-reactivity.

Other background factors may influence the baseline/provocation vasoreactivity ratio. However, only factors influencing vasodilatation during flicker stimulation have been investigated, 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). When both eyes satisfied the inclusion criteria, one eye was randomly selected for the analysis. POAG was diagnosed by a glaucoma specialist.

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 nerve damage. The control group comprised the healthy fellow eyes of patients with dry eye, cataract, macular hole, or nerve damage. The control group comprised the healthy fellow eyes of patients with dry eye, cataract, macular hole, or nerve damage. The control group comprised the healthy fellow eyes of patients with dry eye, cataract, macular hole, or nerve damage. The control group comprised the healthy fellow eyes of patients with dry eye, cataract, macular hole, or nerve damage. The control group comprised the healthy fellow eyes of patients with dry eye, cataract, macular hole, or nerve damage.

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 = 2/5 MBP – IOP.

ONH BF Assessment With LSFG

The principles of LSFG have previously been described in detail. 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 x 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-NAV1 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. MBF 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.

Oxygen Inhalation Test

Clinical characteristics were measured before testing. The oxygen inhalation test itself was performed in the same way as our previous investigation. 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) = 2/5 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)
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 MBRT (%MBRT) 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 %MBRT 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 α = 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 MBRT and other ophthalmic and systemic variables. The independent variable in the multiple regression analysis was mean % change in MBRT, and the dependent variables were the variables that the univariate analysis showed were correlated with mean % change in MBRT. The value of mean % change in MBRT was defined as follows:

\[
\text{mean % change in MBRT(%) = 100 - \frac{1}{\sum_{t=1}^{12} \%MBRT(t)} (t = 1, 2, 3, 4, 8, 12)}
\]

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.

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 MBRT (P = 0.048) were significantly lower in the POAG patients.

Changes in BF Variables During Oxygen Inhalation Test

As shown in Table 2, SpO2 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, MBRT decreased significantly in the control group 2 minutes after the start of oxygen inhalation (%MBRT = 87.7 ± 6.5%, P = 0.0006) and after 3 minutes in the POAG patients (%MBRT = 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 MBRT and Other Variables

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>POAG, n = 15</th>
<th>Control, n = 15</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCVA, logMAR</td>
<td>−0.13 ± 0.06</td>
<td>0.12 ± 0.05</td>
<td>0.94</td>
</tr>
<tr>
<td>Refractive error, diopter</td>
<td>−2.1 ± 2.6</td>
<td>−2.2 ± 2.9</td>
<td>0.92</td>
</tr>
<tr>
<td>Axial length, mm</td>
<td>24.5 ± 1.1</td>
<td>24.8 ± 1.7</td>
<td>0.54</td>
</tr>
<tr>
<td>IOP, mm Hg</td>
<td>12.6 ± 1.5</td>
<td>14 ± 3.0</td>
<td>0.12</td>
</tr>
<tr>
<td>CnRNFLT, μm</td>
<td>88.8 ± 12.6</td>
<td>111.8 ± 9.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MBR, a.u.</td>
<td>11.3 ± 3.1</td>
<td>13.5 ± 2.7</td>
<td>0.048</td>
</tr>
<tr>
<td>Age, yr</td>
<td>54.7 ± 12.9</td>
<td>55.6 ± 7.6</td>
<td>0.82</td>
</tr>
<tr>
<td>Gender, male, female</td>
<td>7 : 8</td>
<td>8 : 7</td>
<td>1.0*</td>
</tr>
<tr>
<td>Diabetes mellitus, n</td>
<td>2</td>
<td>0</td>
<td>0.48*</td>
</tr>
<tr>
<td>Dyslipidemia, n</td>
<td>4</td>
<td>2</td>
<td>0.65*</td>
</tr>
<tr>
<td>Hypertension, n</td>
<td>3</td>
<td>1</td>
<td>0.60*</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>138.4 ± 5.9</td>
<td>131.1 ± 5.9</td>
<td>0.39</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>86.2 ± 3.3</td>
<td>87.4 ± 3.3</td>
<td>0.81</td>
</tr>
<tr>
<td>MBP, mm Hg</td>
<td>103.6 ± 15.4</td>
<td>101.9 ± 14.7</td>
<td>0.77</td>
</tr>
<tr>
<td>MOPP, mm Hg</td>
<td>56.6 ± 9.6</td>
<td>54.6 ± 10.2</td>
<td>0.60</td>
</tr>
<tr>
<td>PR, bpm</td>
<td>68.5 ± 7.7</td>
<td>71.0 ± 9.0</td>
<td>0.44</td>
</tr>
<tr>
<td>SpO2, %</td>
<td>97.6 ± 1.3</td>
<td>97.4 ± 0.9</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Unmarked P values, ANOVA test. * * χ² 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. 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, 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.

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. 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. 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. 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, 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.

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. 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. Previous investigations have reported that endothelin receptors A and B are both expressed in the human ONH. 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.

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**Table 2. Changes in BF Variables During Oxygen Inhalation**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>5 min</th>
<th>10 min</th>
<th>16 min</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>POAG, $n = 15$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>138.4 ± 7.7</td>
<td>140.4 ± 7.7</td>
<td>140.2 ± 7.7</td>
<td>139.4 ± 7.7</td>
<td>0.99</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>86.2 ± 4.1</td>
<td>89.7 ± 4.1</td>
<td>86.5 ± 4.1</td>
<td>89.0 ± 4.1</td>
<td>0.91</td>
</tr>
<tr>
<td>MBRP, mm Hg</td>
<td>103.6 ± 15.4</td>
<td>104.4 ± 20.0</td>
<td>105.8 ± 19.8</td>
<td>106.6 ± 20.8</td>
<td>0.98</td>
</tr>
<tr>
<td>MOPP, mm Hg</td>
<td>56.6 ± 9.6</td>
<td>58.6 ± 13.1</td>
<td>57.2 ± 12.7</td>
<td>58.1 ± 12.5</td>
<td>0.97</td>
</tr>
<tr>
<td>PR, bpm</td>
<td>68.5 ± 7.7</td>
<td>66.8 ± 7.5</td>
<td>64.8 ± 7.2</td>
<td>69 ± 9.4</td>
<td>0.51</td>
</tr>
<tr>
<td>SpO$_2$, %</td>
<td>97.6 ± 1.3</td>
<td>99.1 ± 0.9</td>
<td>99.1 ± 0.6</td>
<td>97.1 ± 1.4</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

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 |
| MBRP, 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 ± 7.5  | 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.

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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. ET-1 has also been shown to induce astroglial proliferation in cultured human ONH astrocytes, acting via ET (A/B) receptor activation. Furthermore, in a pigmented rabbit-eye model, the intravitreous injection of ET-1 decreased ONH BF and increased optic disc cupping. 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 MBRT, and that there was a significant negative correlation between SBP and mean % change in MBRT 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. 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. High SBP can also cause chronic narrowing of the vessels, creating a pre-existing degree of vasoconstriction that might reduce constrictive capacity during the response to hyperoxia.

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, an adequate 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. 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. 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 MBRT. 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.

### Table 3. Dependent Factors Contributing to Mean % Change in MBRT

<table>
<thead>
<tr>
<th>Variable</th>
<th>Independent</th>
<th>Dependent</th>
<th>β</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean % change in MBRT&lt;sub&gt;T&lt;/sub&gt;</td>
<td>CpxRNFLT</td>
<td>0.23</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Baseline MBRT</td>
<td>0.44</td>
<td>0.0096*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Baseline SBP</td>
<td>−0.32</td>
<td>0.030*</td>
<td></td>
</tr>
</tbody>
</table>

β indicates the standard partial regression coefficient.

* Indicates statistical significance.

**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 %MBRT 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).
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 3.** The relationship of mean % change in MBRF to baseline MBRF and SBP in the two groups. Mean % change in MBRF was significantly correlated with baseline MBRF 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 MBRF 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 MBRF and baseline MBRF or SBP in the control group (r = 0.19, P = 0.48; r = -0.56, P = 0.40; Pearson’s correlation coefficient).

**FIGURE 4.** Representative fundus photographs, LSFG images, and %MBR alteration during the protocol. This figure shows representative fundus photographs, LSFG images, and %MBR alteration during the protocol. The upper panel shows a patient with high baseline MBRF, low SBP, and high mean % change in MBRF, while the lower panel shows a patient with low baseline MBRF, high SBP, and low mean % change in MBRF. 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 against baseline during hyperoxia was calculated; the gray double arrow indicates mean % change in MBRF. Upper panel: The left eye of a 45-year-old male glaucoma patient (MD: -7.88 dB, MBRF: 15.5 a.u., BP 126/86 mm Hg, PR 70 bpm, mean % change in MBRF: 10.2%). Lower panel: The left eye of a 66-year-old male glaucoma patient (MD: -2.81 dB, MBRF: 8.6 a.u., BP 180/103 mm Hg, PR 65 bpm, mean % change in MBRF: -5.1%).
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