Microcirculatory Responses to Hyperoxia in Macular and Peripapillary Regions

Huan Xu,[1,2] Guohua Deng,[1,3] Chunhui Jiang,[1,2,4] Xiangmei Kong,[1,2] Jian Yu,[1,2] and Xinghuai Sun[1,2]

[1]Department of Ophthalmology and Vision Science, Eye and ENT Hospital, Fudan University, Shanghai, People's Republic of China
[2]Key Laboratory of Myopia of the State Health Ministry, and Key Laboratory of Visual Impairment and Restoration of Shanghai, Shanghai, People's Republic of China
[3]Department of Ophthalmology, The Third People's Hospital of Changzhou, Changzhou, People's Republic of China
[4]Department of Ophthalmology, People's Hospital of Shanghai No. 5, Shanghai, People's Republic of China

Correspondence: Chunhui Jiang, Department of Ophthalmology and Vision Science, Eye and ENT Hospital, Fudan University, 83 Fenyang Road, Shanghai 200031, People's Republic of China; chhjiang70@163.com.
Xiangmei Kong, Department of Ophthalmology and Vision Science, Eye and ENT Hospital, Fudan University, 83 Fenyang Road, Shanghai 200031, People's Republic of China; kongxm95@163.com.

HX and GD contributed equally to the work presented here and should therefore be regarded as equivalent authors.

Submitted: March 21, 2016
Accepted: July 11, 2016

Citation: 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. DOI:10.1167/iovs.16-19603

Purpose. To evaluate the hyperoxia response of the retinal vascular system in different parts of the fundus using optical coherence tomography (OCT) angiography.

Methods. Using an OCT angiography system and a split-spectrum amplitude decorrelation angiography (SSADA) algorithm, perfused vessel density at the macular and peripapillary regions was measured before and after breathing 80% oxygen in 10 healthy Chinese subjects. The repeatability of the hyperoxia response was also tested.

Results. Hyperoxia provocation caused a significant decrease in retinal perfused vessel density in both the macular and peripapillary areas. The para- and perifoveal area had a mean reduction of 13.66% and 15.17%, respectively; these reductions were significantly greater than in the peripapillary area (9.52%; parafovea versus peripapillary, $P = 0.023$; perifovea versus peripapillary, $P = 0.006$). The coefficients of variation (CV), intraclass correlations (ICC), and Bland–Altman plots suggested strong repeatability of the hyperoxia response.

Conclusions. The optical coherence tomography system, in conjunction with the SSADA algorithm, recorded a significant reduction in retinal perfused vessel density after hyperoxia, and the reduction was greater in the macular area than in the peripapillary area.

Keywords: optical coherence tomography, angiography, hyperoxia, retinal microvasculature, autoregulation

The retinal vascular system can adjust to meet the metabolic demands of retinal tissues and provide sufficient blood flow to them under various conditions, an ability known as autoregulation.[1,2] Several methods have been used to assess the autoregulation of retinal blood vessels, of which gas challenge is one of the most common. Reductions in both retinal vessel diameter and blood flow velocity in response to hyperoxia have been reported.[3,4] However, most studies focused on the large vessels around the optic disc, and there has been relatively little research on small arteries and the capillary system. Notably, many retinal vascular diseases develop primarily in the capillaries.

Recently, using high-speed optical coherence tomography (OCT) and a split-spectrum amplitude-decorrelation angiography (SSADA) algorithm, Jia et al.[5] successfully quantified the vascular system of the retina at the capillary level with excellent repeatability and reproducibility. Meanwhile, differences in retinal vessels and differences in the autoregulation of retinal vessels in different parts of the fundus have been reported by others.[6,7] In the current study, we investigated the autoregulation of retinal microcirculation in healthy adults, particularly the response in different parts of the fundus.

Methods

Participants

Ten normal Chinese subjects were recruited into the study. All underwent a thorough ophthalmologic examination including measurements of best corrected visual acuity (BCVA), slit-lamp biomicroscopy, refractive state measurement by an autorefractor, dilated fundus examination, intraocular pressure (IOP) measured via noncontact tonometer, and axial length (AL) measured with an optical biometry device (IOLMaster; Carl Zeiss AG, Jena, Germany). The subjects' medical and family histories were collected. The inclusion criteria were BCVA of 16/20 or better, IOP $<$ 21 mm Hg, and emmetropia or mild myopia (from +0.5 to −3 diopters [D]) without other eye diseases. Exclusion criteria were a history of ocular surgery or trauma, a condition that might affect ocular circulation such as

Investigative Ophthalmology & Visual Science

diabetes mellitus, hypertension, BCVA < 16/20, IOP > 21 mm Hg, myopia greater than 3 D, AL > 25 mm, history of glaucoma in a first-degree relative, or any other abnormal ophthalmic findings. The research was performed in accordance with the tenets of the Declaration of Helsinki, and was approved by the institutional review board of the Eye & ENT Hospital of Fudan University. Informed consent was obtained from each subject.

Study Protocol

All subjects refrained from consuming alcohol and caffeine for at least 12 hours, and were instructed to rest for 20 minutes in a sitting position before the trial. Their baseline data, including retinal perfused vessel density in the macular and peripapillary area, blood pressure, and pulse rate were then recorded. For the administration of oxygen, high concentration oxygen masks (Intersurgical EcoLite, Intersurgical, Berkshire, UK) were used. The oxygen flow rate was set to 15 L/minute to achieve an oxygen concentration of 80%. After hyperoxia provocation for 5 minutes, the retinal perfused vessel density, blood pressure, and pulse rate were assessed again. The subjects were then switched back to breathing room air, and the same parameters were recorded 5 and 10 minutes later. This entire procedure was performed again in each of the subjects after an interval of at least 1 week, to assess its repeatability.

OCT Data Acquisition and Processing

All OCT angiography scans were collected via a commercial spectral domain system (RTVue-XR Avanti, software version 2.0.5.39; Optovue, Inc.; Meridianville, AL, USA). The scans were acquired over a 6.0 × 6.0-mm region centered at the fovea, and a 4.5 × 4.5-mm region centered at the optic nerve head. For each area, four volumetric raster scans, two horizontal priority (x-fast) and two vertical priority (y-fast) scans, were obtained consecutively. The x- and y-fast scans with a higher signal-to-noise ratio were selected by the system automatically and processed via the SSADA algorithm. Motion artifacts were removed by 3D orthogonal registration and merging of the two scans. Signals from the internal membrane to the retinal pigment epithelium were projected, and en face retinal angiograms were created with the system's built-in software.

Measurement of Perfused Vessel Density at the Macular and Peripapillary

The perfused vessel density of both the para-/perifoveal and peripapillary areas was measured. The peripapillary region was defined as a 700-μm wide elliptical annulus extending outward from the optic disc boundary. The parafoveal area was defined as an annulus with an outer diameter of 3 mm and an inner diameter of 1 mm, and the perifoveal area was defined as an annulus with an outer diameter of 5 mm and an inner diameter of 3 mm. The perfused vessel density of the specific areas was defined as the proportion of the area occupied by vessels.[8]

Foveal Avascular Zone Measurements

The foveal avascular zone (FAZ) area was outlined and measured with ImageJ software (http://imagej.nih.gov/ij/; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA), as described in a previous report.[8]

Repeatability analysis

"Hyperoxia response" was defined as the percentile changes from baseline after breathing 80% oxygen. The hyperoxia test was conducted twice in each subject, and the coefficient of variation (CV), intraclass correlation (ICC), and Bland–Altman plots were used to assess the repeatability. Values of ICC (0.81–1.00) indicated strong agreement between the two visits, and values less than 0.40 indicated poor agreement.

Table 1. Perfused Vessel Density at Baseline and After Hyperoxia

Groups	Baseline	Hyperoxia	*P* Value	Hyperoxia Response, %
Parafovea	76.5 ± 4.14	66.0 ± 3.62	<0.001*	13.66 ± 3.54
Perifovea	73.1 ± 4.33	61.9 ± 3.44	<0.001*	15.17 ± 4.71
Peripapillary	86.7 ± 6.35	78.2 ± 9.00	<0.01*	9.52 ± 7.24

The results are presented as mean ± SEM ($n = 10$).

* Statistical values of were calculated from repeated measures ANOVA (* $P < 0.01$).

Statistical Analyses

All statistical analyses were conducted using statistical software (SPSS version 20.0; SPSS, Inc., Chicago, IL, USA). The data from the first visit were used for further analysis, presented as tables and figures in our article. According to the One-Sample Kolmogorov-Smirnov Test, all the variables followed a normal distribution. Thus, the perfused vessel densities at the various time-points before and after hyperoxia provocation were compared via a repeated measures analysis of variance (ANOVA) and multivariate ANOVA. The differences between the relative percentile changes in the perfused vessel density among the three regions after 5 minutes of hyperoxia were compared using 1-way ANOVA. The FAZ areas before and after hyperoxia were analyzed using the paired samples *t*-test. The inter- and intravisit ICCs for baseline levels and hyperoxia responses were calculated using the reliability analysis function of SPSS, and CVs were calculated as the standard deviation divided by the mean of the measured values.

Results

Basic Information

Ten eyes ($n = 8$ right, 2 left) in 10 normal subjects ($n = 5$ male, 5 female) were included in the study, and all subjects had a BCVA of 20/20 or better. Their mean age was 27.36 ± 6.97 years (range, 23–44 years); mean IOP was 13.42 ± 3.29 mm Hg (range, 8.2–18.1 mm Hg); mean spherical equivalence was −1.50 ± 1.39 D (range, −2.75 to 0 D); and mean AL was 24.20 ± 0.72 mm (range, 23.23–25.04 mm).

Systemic Response

After breathing 80% oxygen for 5 minutes, the subjects' pulse rates decreased slightly, by a mean of 4.05% ($P = 0.068$), while their systolic blood pressure (SP), diastolic blood pressure (DP), and mean artery pressure (MAP) remained almost unchanged (Supplementary Table S1).

Changes in Retinal Vessels After Hyperoxia

After breathing 80% oxygen for 5 minutes, the perfused vessel densities at the macular and peripapillary area decreased in all subjects. The perfused vessel densities in the parafoveal and perifoveal areas were significantly lower than baseline ($P < 0.001$ for both comparisons), with mean reductions of 13.66% in the parafovea and 15.17% in the perifovea (Table 1; Fig. 1). After breathing room air for 5 minutes, the perfused vessel

Investigative Ophthalmology & Visual Science

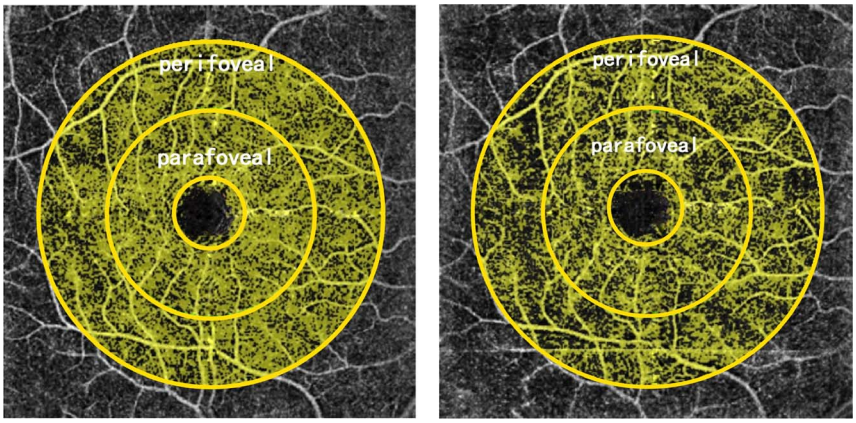

Figure 1. Angiogram images of the macular area at different time-points. (**A**) Baseline. (**B**) After 5 minutes of hyperoxia.

densities in the parafoveal and perifoveal areas were similar to baseline ($P > 0.05$ for both comparisons, Supplementary Table S2). Notably, the FAZ area did not change significantly throughout the entire observation period. The mean of the FAZ area was 0.309 mm^2 after hyperoxia, which did not differ significantly from the baseline value of 0.339 mm^2 ($P > 0.05$; Supplementary Fig. S1).

The perfused vessel density in the peripapillary region also decreased significantly after 5 minutes of hyperoxia (9.52%, $P < 0.05$), and returned to baseline after breathing room air for 5 minutes (Table 1, Supplementary Table S2; Fig. 2).

A significant difference was found among the reduction in perfused vessel density at different parts of the fundus ($P = 0.013$). The parafoveal and perifoveal area had a significantly higher reduction in perfused vessel density than the peripapillary area (parafovea versus peripapillary, $P = 0.023$; perifovea versus peripapillary, $P = 0.006$; Table 2).

Repeatability of the Hyperoxia Response and Baseline Level

At baseline levels, the intra- and intervisit ICC values for perfused vessel density were 98.8% to 99.9% and 99.5% to 99.8%, respectively. The intra- and intervisit CV values were 0.40% to 0.62% and 1.16% to 1.81% (Supplementary Table S3). The mean ICCs derived from two hyperoxia responses measured in the same subjects were high (76.6%–93.9%) and the CVs were relatively low (7.70%–9.86%; Table 3). The Bland–Altman plots demonstrated good reliability of all measurements (Supplementary Figs. S2, S3, S4).

Discussion

In the current study, retinal vascular autoregulation in a group of healthy Chinese subjects was assessed after hyperoxia provocation. A significant and reproducible reduction in perfused vessel density in both the macular as well as peripapillary areas was observed via OCT angiogram. In humans, the vasculature is regulated by autonomic innervation, humoral regulation, and local autoregulation. Autonomic innervation of the retinal vasculature terminates as the central retinal artery passes through the lamina cribosa.[9] Furthermore, the effects of neurotransmitters and hormones derived from the circulatory system on the retinal vascular bed has been suggested to be minimal, due to the blood–retinal barrier.[10] However, the retina has an autoregulatory system that can adjust blood flow in response to various conditions. It has been

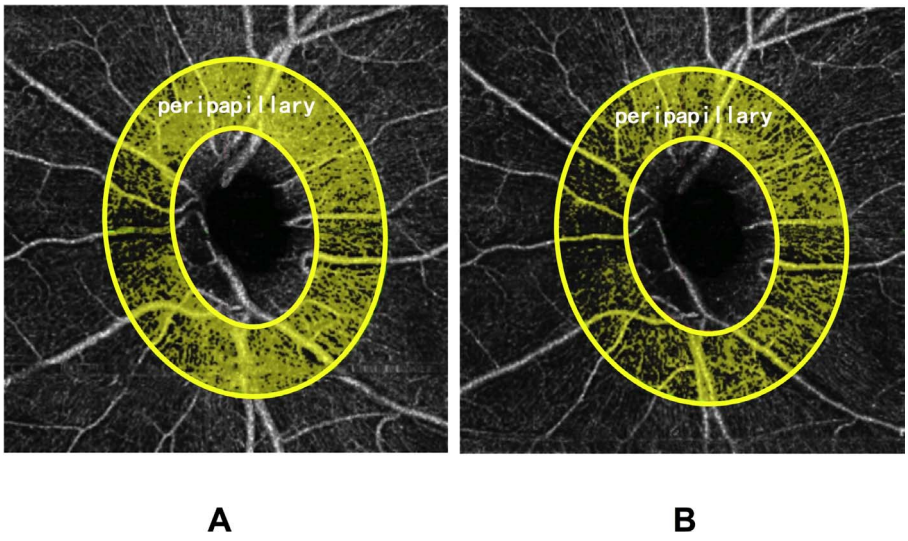

Figure 2. Angiogram images of the peripapillary area at different time-points. (**A**) Baseline. (**B**) After 5 minutes of hyperoxia.

Investigative Ophthalmology & Visual Science

Table 2. Difference of Hyperoxia Response Among Three Regions

Relative Percentile			*P* Values			
Parafovea (A)	**Perifovea (B)**	**Peripapillary (C)**	**A vs. B**	**A vs. C**	**B vs. C**	**Overall Analysis**
–13.66%	–15.17%	–9.52%	0.556	0.023*	0.006*	0.013*

* Statistical values were calculated from 1-way ANOVA (* $P < 0.05$).

reported that this autoregulatory system is damaged in diabetic retinopathy patients,[11] and even in diabetic patients before the clinical manifestation of retinopathy.[12] This suggests that the autoregulatory system could have already been compromised before any structural damage to the retina occurs. The early identification of such cases, and thorough follow-ups or early interventions, may delay or even prevent the development of pathologic retinal vascular changes in these patients.

In the current study, hyperoxia induced a reproducible reduction in retinal perfused vessel density in healthy subjects. Retinal vascular responses have previously been tested via other methods, including elevated IOP,[13] cold stimulation/the "cold test",[14] and isometry exercises.[1] However, these methods either entail potential risks or are too cumbersome for routine clinical use, especially in older patients. Hyperoxia has proven to be a safe and relatively comfortable treatment modality,[15] and can also be used to test the retinal vascular response.[4] Kiss et al.[16] reported that vasoconstriction occurred during the first 5 minutes of hyperoxia; therefore, this duration was used in the current study. We also found that the retinal perfused vessel density returned to baseline after 5 minutes of breathing room air, which suggested that 5 minutes might be suitable for the test of hyperoxia response. In addition, the OCT angiogram proved to be an effective noninvasive in vivo method for recording perfused retinal vessels at the capillary level. Finally, the results of the repeatability tests in the current and previous studies were promising.[17] Thus, the methods described herein are safe and reliable for the evaluation of autoregulation in the retinal vascular system, may also be useful for detecting subjects with an abnormal vascular response, as well as for assessing the effectiveness of early interventions.

The perfused vessel density was reduced after breathing a high concentration of oxygen in the current study. These results are similar to those of previous reports. Riva et al.[4] and Tomic et al.[18] both reported reductions in retinal vessel diameter and retinal vessel blood flow velocity in response to hyperoxia. On the other hand, compared with results reported by Pechauer et al.[19] in a study using a similar system and breathing an oxygen concentration of 60%, we observed a slightly higher reduction in the peripapillary area. This is in accordance with previous findings suggesting that higher oxygen concentrations may induce stronger responses.[20]

Compared with the peripapillary area, the para- and perifoveal area exhibited a significantly higher reduction in perfused vessel density (all $P < 0.05$). It has been reported that vessels in different parts of the retina may respond differently to the same stimulus.[6,7] Rassam et al.[7] reported that the response to hyperoxia was lower in nasal vessels than in temporal vessels. This is similar to the findings of the current study, in which a greater reduction in perfused vessel density was noted in the macula.

Table 3. Repeatability of Hyperoxia Response at Parafovea, Perifovea, and Peripapillary

Groups	Intervisit ICCs	Intervisit CVs
Parafovea	76.6%	7.70%
Perifovea	89.2%	8.59%
Peripapillary	93.9%	9.86%

In contrast to our findings, Kiss et al.[16] reported that a less pronounced response to hyperoxia was observed in the fovea than in the peripheral retina. However, they used the blue field technique, which measures the speed of white blood cells to quantify blood flow in the central retina, while the peripheral flow was assessed via laser Doppler velocimetry, which calculates retinal blood flow based on erythrocyte velocity. It has been reported that white blood cells travel much slower than red blood cells in the retinal capillary system,[21,22] and Kiss et al.[16] noted that this may have affected the results of their study. In the current study, responses in the macular and peripapillary regions were recorded via OCT angiogram. However, the reasons for the differences in the vascular response at different part of the fundus are not fully understood. Whether or not the primary site of blood flow regulation is at the capillary level remains controversial[23–25]; however, recent evidence suggests that capillary responses in the brain may be responsible for more than 80% of the changes in cerebral blood flow.[25] Though there are dense radical peripapillary capillaries in the peripapillary area, the four principal intraretinal arteries and veins are also located there. On the other hand, the vessels in the macular area are exclusively capillaries or small vessels, so the higher reductions in the macular area may reflect a more prominent response at the capillary level. The high metabolic rate of the macula may also explain this observation.

Notably, the FAZ area did not change significantly in response to hyperoxia. This may have been because most of the oxygen supplied to the retina from the FAZ area is derived from the choroidal vessel, rather than the retinal circulation. On the other hand, a study by Yu et al.[26] in monkeys found that the oxygen consumption of the foveal retina increased under systemic hyperoxia. This might explain our findings—under systemic hyperoxia, the capillaries around the fovea had not be significantly changed because of the oxygen tension in the inner retina was only slightly increased due to the remarkably increased oxygen consumption of the inner retina at the foveal region. They also found that the oxygen levels within the inner retina showed a relative immunity while the oxygen tension in the choroid was significantly increased to systemic hyperoxia at the parafoveal and inferior areas. This could also indicate that the regulatory capability of the vasculature to hyperoxia is different between retinal and choroidal circulations.

These results suggest that the response of the retinal vascular system to hyperoxia differs at different parts of the fundus. Therefore, when comparing such responses in different subjects, or the responses at different time-points in the same subject, investigators must ensure that the measurements are derived from the same area of the fundus.

The current study was limited in scope, in that it included only a relatively small number of normal, adult, Chinese subjects. Additionally, it has been conclusively demonstrated that retinal perfused vessel density is closely related to age.[8] Further studies with more subjects of different ages and ethnic backgrounds may yield additional information on the autoregulation of the retinal vessel.

Investigative Ophthalmology & Visual Science

Acknowledgments

The authors thank Jian Gao and Jianfeng Luo, Fudan University, Shanghai, China, for assistance with the statistical analyses in the study.

Supported in part by research grants from the National Major Scientific Equipment Program (2012YQ12008003) and the Shanghai Committee of Science and Technology (13430710500, 15DZ1942204).

Disclosure: **H. Xu**, None; **G. Deng**, None; **C. Jiang**, None; **X. Kong**, None; **J. Yu**, None; **X. Sun**, None

References

1. Dumskyj MJ, Eriksen JE, Doré CJ, Kohner EM. Autoregulation in the human retinal circulation: assessment using isometric exercise, laser Doppler velocimetry, and computer-assisted image analysis. *Microvasc Res*. 1996;51:378–392.
2. Riva CE, Grunwald JE, Petrig BL. Reactivity of the human retinal circulation to darkness: a laser Doppler velocimetry study. *Invest Ophthalmol Vis Sci*. 1983;24:737–740.
3. Werkmeister RM, Palkovits S, Told R, et al. Response of retinal blood flow to systemic hyperoxia as measured with dual-beam bidirectional Doppler Fourier-domain optical coherence tomography. *PLoS One*. 2012;7:e45876.
4. Riva CE, Grunwald JE, Sinclair SH. Laser Doppler velocimetry study of the effect of pure oxygen breathing on retinal blood flow. *Invest Ophthalmol Vis Sci*. 1983;24:47–51.
5. Jia Y, Morrison JC, Tokayer J, et al. Quantitative OCT angiography of optic nerve head blood flow. *Biomed Opt Express*. 2012;3:3127–3137.
6. Schmetterer L, Wolzt M, Lexer F, et al. The effect of hyperoxia and hypercapnia on fundus pulsations in the macular and optic disc region in healthy young men. *Exp Eye Res*. 1995;61:685–690.
7. Rassam SM, Patel V, Chen HC, Kohner EM. Regional retinal blood flow and vascular autoregulation. *Eye (Lond)*. 1996;10:331–337.
8. Yu J, Jiang C, Wang X, et al. Macular perfusion in healthy Chinese: an optical coherence tomography angiogram study. *Invest Ophthalmol Vis Sci*. 2015;56:3212–3217.
9. Ye XD, Laties AM, Stone RA. Peptidergic innervation of the retinal vasculature and optic nerve head. *Invest Ophthalmol Vis Sci*. 1990;31:1731–1737.
10. Delaey C, Van De Voorde J. Regulatory mechanisms in the retinal and choroidal circulation. *Ophthalmic Res*. 2000;32:249–256.
11. Grunwald JE, Riva CE, Petrig BL, Sinclair SH, Brucker AJ. Effect of pure O2-breathing on retinal blood flow in normals and in patients with background diabetic retinopathy. *Curr Eye Res*. 1984;3:239–241.
12. Lott MEJ, Slocomb JE, Shivkumar V, et al. Impaired retinal vasodilator responses in prediabetes and type 2 diabetes. *Acta Ophthalmol*. 2013;91:e462–e469.
13. Riva CE, Sinclair SH, Grunwald JE. Autoregulation of retinal circulation in response to decrease of perfusion pressure. *Invest Ophthalmol Vis Sci*. 1981;21(1 pt 1):34–38.
14. Ikemura T, Someya N, Hayashi N. Autoregulation in the ocular and cerebral arteries during the cold pressor test and handgrip exercise. *Eur J Appl Physiol*. 2012;112:641–646.
15. Bitterman H. Bench-to-bedside review: oxygen as a drug. *Crit Care*. 2009;13:205.
16. Kiss B, Polska E, Dorner G, et al. Retinal blood flow during hyperoxia in humans revisited: concerted results using different measurement techniques. *Microvasc Res*. 2002;64:75–85.
17. Wang X, Jiang C, Ko T, et al. Correlation between optic disc perfusion and glaucomatous severity in patients with open-angle glaucoma: an optical coherence tomography angiography study. *Graefes Arch Clin Exp Ophthalmol*. 2015;253:1557–1564.
18. Tomic L, Bjärnhall G, Mäepea O, Sperber GO, Alm A. Effects of oxygen and carbon dioxide on human retinal circulation: an investigation using blue field simulation and scanning laser ophthalmoscopy. *Acta Ophthalmol Scand*. 2005;83:705–710.
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. Strenn K, Menapace R, Rainer G, Findl O, Wolzt M, Schmetterer L. Reproducibility and sensitivity of scanning laser Doppler flowmetry during graded changes in PO2. *Br J Ophthalmol*. 1997;81:360–364.
21. Wolf S, Arend O, Toonen H, Bertram B, Jung F, Reim M. Retinal capillary blood flow measurement with a scanning laser ophthalmoscope. Preliminary results. *Ophthalmology*. 1991;98:996–1000.
22. Ben-nun J. Comparative flow velocity of erythrocytes and leukocytes in feline retinal capillaries. *Invest Ophthalmol Vis Sci*. 1996;37:1854–1859.
23. Vanzetta I, Hildesheim R, Grinvald A. Compartment-resolved imaging of activity-dependent dynamics of cortical blood volume and oximetry. *J Neurosci*. 2005;25:2233–2244.
24. Hillman EMC, Devor A, Bouchard MB, et al. Depth-resolved optical imaging and microscopy of vascular compartment dynamics during somatosensory stimulation. *Neuroimage*. 2007;35:89–104.
25. Hall CN, Reynell C, Gesslein B, et al. Capillary pericytes regulate cerebral blood flow in health and disease. *Nature*. 2014;508:55–60.
26. Yu D-Y, Cringle SJ, Su E-N. Intraretinal oxygen distribution in the monkey retina and the response to systemic hyperoxia. *Invest Ophthalmol Vis Sci*. 2005;46:4728–4733.

Investigative Ophthalmology & Visual Science