Effect of Latanoprostene Bunod on Optic Nerve Head Blood Flow

Dan Samaha, OD, MSc, FAAO,[1]* Vasile Diaconu, PhD,[1] Jean François Bouchard, PhD,[1] Charlene Desalliers, OD,[1] and Ariane Dupont, OD[1]

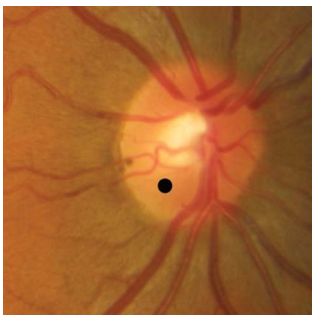
SIGNIFICANCE: Topical latanoprostene bunod increases capillary oxygen saturation and blood volume at the optic nerve head in healthy individuals.

PURPOSE: This study aimed to evaluate the effect of topical latanoprostene bunod on optic nerve blood volume and oxygen saturation in a population of healthy participants.

METHODS: In this prospective double-blind crossover study, 23 healthy participants aged from 21 to 62 years were recruited. Optic nerve head capillary blood volume (ONH_{vol}) and oxygen saturation (ONH_{SaO2}) baselines were measured over a period of 2 hours using multichannel spectroscopic reflectometry and were remeasured after a 7-day once-daily instillation regimen of either latanoprost 0.005% or latanoprostene bunod 0.024%. After a 30-day washout period, participants were crossed over to the alternate product, and measurements were repeated. Participants were used as their own baselines to calculate variation in ONH_{vol} and ONH_{SaO2} across time and pharmacological agents. The Friedman test was used to establish significant differences in optic nerve head parameters from baseline values, and Conover post hoc analysis was carried for multiple between-group comparisons.

RESULTS: Latanoprostene bunod 0.024% induced a significant increase of 4% in ONH_{SaO2} compared with latanoprost 0.005% ($P < .001$). Furthermore, latanoprostene bunod increased ONH_{vol} levels by more than twofold at all time points ($P < .001$ at T60, T90, and T120). The increase in ONH_{vol} was 66.2% higher than levels achieved with latanoprost at T60 ($P = .001$), 47% higher at T90 ($P < .001$), and 45% higher at T120 ($P < .01$).

CONCLUSIONS: Latanoprostene bunod 0.024% induces a significant increase in optic nerve head blood volume and oxygen saturation in healthy subjects, when compared with latanoprost 0.005%. Future studies are needed to evaluate whether similar responses are elicited in patients suffering from glaucomatous optic neuropathy.



Author Affiliations:
[1]Montreal University School of Optometry, Montreal, Quebec, Canada
*Dan.samaha@umontreal.ca

Optom Vis Sci 2022;99:172–176. doi:10.1097/OPX.0000000000001842

Prostaglandin analogues are regarded as the criterion standard in terms of topical treatment for most types of glaucoma.[1–3] Although intraocular pressure is the only modifiable risk factor and mainstay for glaucoma treatment, it is now generally accepted that ocular blood flow dysregulation at the optic nerve head is concurrent with glaucomatous optic neuropathy.[4] Several authors have thus put forth evidence supporting the role of vasoprotection in the treatment of glaucomatous optic neuropathy in both animal and human models.[5–8]

Specifically, nitric oxide or nitric oxide–containing agents have been thoroughly studied, as this compound is endogenously involved in blood flow regulation.[9] Authors have shown its involvement in regulating ocular blood flow in healthy and glaucomatous populations, as well as hinted to the potential neuroprotective property nitric oxide might confer.[10–13] The notion of neuroprotective properties of nitric oxide is of particular interest considering the recent addition to the drug arsenal available to treat glaucoma, Latanoprostene bunod (Vyzulta; Bausch & Lomb, Rochester, NY), which was approved for the treatment of glaucoma in the United States in 2017 and is the first innovation in topical therapy since the advent of prostaglandin analogues on the market more than 25 years ago. This topical agent uses the intraocular pressure–lowering mechanism specific to prostaglandin analogues, conferred by the latanoprost acid component, as well as donates a nitric oxide group allowing increased outflow through the conventional pathway.[14]

Authors have already demonstrated an intraocular pressure–independent effect of topical latanoprost on optic nerve head blood velocity on both animal and human eyes.[15] The present study sought to determine if the addition of a nitric oxide–donating group to a prostaglandin analogue might further enhance blood flow to the optic nerve head. We thus compared the effect of a 7-day, once-a-day instillation regimen of latanoprost and latanoprostene bunod on optic nerve head blood volume and capillary oxygen saturation in a population of healthy individuals, using multichannel spectroscopic reflectometry.

METHODS

Participants

Twenty-five healthy participants were recruited for the purpose of this study. This research was reviewed by an independent ethical review board and conforms with the principles and applicable guidelines for the protection of human subjects in biomedical research. Written consent was received before any examination.

Strict inclusion criteria included no history of systemic disease, migraines, use of any oral medication, or oral contraception method. Participants were screened for glaucoma using optical coherence tomography, tendency-oriented perimetry with the Octopus standard automated perimeter (Haag-Streit International, Koeniz, Switzerland;

G-TOP test strategy), slit-lamp examination, and iCare tonometry (Icare, Helsinki, Finland). Specifically, optical coherence tomography was carried out using the Cirrus high-definition tomograph (Carl Zeiss Meditec Inc., Dublin, CA). Peripapillary retinal nerve fiber layer measurements were obtained using the optic disc cube 200 × 200 protocol, whereas ganglion cell complex measurements were obtained using the macular cube 200 × 200 protocol. Images with poor centration, segmentation errors, and scan quality less than 7/10 were excluded. Subjects demonstrating an intereye retinal nerve fiber layer asymmetry of 9 μm or more or an intereye macular thickness asymmetry of 9 μm or more were excluded.[16,17] Any suspicious findings on slit-lamp examination, such as asymmetrical optic disc cupping or intraocular pressure higher than 21 mmHg, also resulted in participant exclusion.

Resting arm systolic and diastolic pressures were measured before each spectroreflectometry measurement. Pupillary dilation was carried out with one drop of tropicamide 1%, after which baseline measurements of optic nerve head capillary volume and optic nerve head capillary oxygen saturation were obtained after 60 minutes of tropicamide 1% instillation, after 90 minutes, and after 120 minutes using multichannel spectroreflectometry.[18,19] Each participant went through a 10-minute dark adaptation in the spectroreflectometry examination room before each measurement (0.1 lux or less). The first spectroreflectometry measurements were scheduled between 9 and 10 AM for all participants. The start time for each participant was the same for all experimental days to account for individual diurnal changes in ocular blood flow.[20,21] Each time-point measurement consisted of acquiring 10 consecutive measurements from optic nerves capillaries in the temporal region, to maximize the signal-to-noise ratio. The system uses a multichannel spectroreflectometry technique with 1024 discrete wavelengths between 400 and 700 nm coupled with a fundus camera that captures light reflected from a specific zone of the optic nerve papilla through a pinhole aperture. This acts as a highly sensitive photon detector. The spectrum of the reflected light contains the hemoglobin and oxyhemoglobin spectral signature changes depending on whether the hemoglobin is associated with oxygen or not; the analysis of the reflected light absorption spectrum allows the oxygen saturation to be determined in capillary beds. Simply put, using a linear mathematical model described elsewhere,[18,19] reflectometry functions from capillaries of the optic nerve are used to derive oxygen saturation and blood volume represented by the optical density of the blood. The device is connected to a computer software that derives the rate of blood oxygenation and blood volume optical density in real time.

Patients were then randomized into receiving either latanoprost 0.005% (Xalatan; Pfizer, New York, NY) or latanoprostene bunod 0.024% (Vyzulta; Bausch & Lomb) once daily for 7 days. On the eighth day, the same measurement protocol was repeated, to have the first measurements exactly 60 minutes after the last topical instillation and at the same time of day as the baseline measurements. Participants then underwent a 30-day washout and were crossed over to the other topical agent, using the same regimen. Spectroreflectometry measurements were once again carried out, abiding to the same schedule as the previous sessions. Participants were instructed to refrain from consuming caffeine-containing foods or beverages for at least 8 hours before experimental days.

Drugs

Latanoprost 0.005% and latanoprostene bunod 0.024% were transferred under sterile conditions in identical masked bottles. Only the principal investigator was aware of the randomization key.

Data Analysis

Because there exists great interindividual variability in optic nerve head blood supply, multichannel spectroreflectometry parameters are not suited for direct interindividual comparisons.[22] Instead, owing to the excellent intraindividual reproducibility of this method, optic nerve head capillary volume and optic nerve head capillary oxygen saturation normalized to baseline values correlate to determine relative change in capillary blood volume and oxygen saturation, respectively.[18,23] The Friedman test was performed to evaluate ratio differences from baseline values in both treatment arms, followed by a two-way repeated-measures analysis of variance.

RESULTS

Twenty-three subjects (60% female; ages, 21 to 62 ± 11.5 years) completed the study. Subjects' ocular parameters are summarized in Table 1. Average spherical equivalent was –0.71 ± 2.1 D, and average visual acuity (logMAR) was 0.2 ± 0.1. Average untreated intraocular pressure was 14.7 mmHg (95% confidence interval [CI], 13.5 to 15.9 mmHg) and decreased to 12.8 mmHg after a 7-day regimen of latanoprost (95% CI, 11.6 to 14.1 mmHg). The decrease in intraocular pressure was statistically significant ($P < .001$). After the latanoprostene bunod regimen, the intraocular pressure decreased to 11.8 (95% CI, 10.7 to 12.9 mmHg), and this difference from baseline was statistically significant (two-sample $t_{23} = 10.3$; $P < .001$). Average intraocular pressure between both treatment arms differed significantly (two-sample $t_{23} = 4.8$; $P < .001$).

Optic nerve head capillary oxygen saturation was increased by an average of 4% using latanoprostene bunod (95% CI, 3 to 5.5%), and values differed from baseline across all time points ($P = .001$ at 60 minutes, $P < .001$ at 90 minutes, $P = .001$ at 120 minutes). The oxygen saturation changes observed in the latanoprostene bunod group were also statistically different from

TABLE 1. Comparison of systemic and ocular parameters (ONH_{SaO2} and ONH_{VOL}) at baseline and after a 7-day instillation of latanoprost 0.005% or latanoprostene bunod 0.0024%

	60 min	90 min	120 min
ONH_{SaO2} (baseline)	1.00	0.99 ± 0.02	1.00 ± 0.03
ONH_{SaO2} (latanoprost)	1.01 ± 0.03	1.00 ± 0.05	0.99 ± 0.02
ONH_{SaO2} (latanoprostene bunod)	1.05 ± 0.04 ($P = .001$)	1.05 ± 0.05 ($P < .001$)	1.03 ± 0.04 ($P = .001$)
ONH_{VOL} (baseline)	1.00	1.01 ± 0.09	1.01 ± 0.08
ONH_{VOL} (latanoprost)	1.57 ± 0.96 ($P = .03$)	1.45 ± 0.59 ($P = .01$)	1.24 ± 0.48
ONH_{VOL} (latanoprostene bunod)	2.61 ± 0.92 ($P < .001$)	2.14 ± 0.72 ($P < .001$)	1.81 ± 0.94 ($P < .001$)

Units are ratio of variation from baseline values. Data are mean ± SEM. *P* values are indicated where difference from baseline values was statistically significant. ONH_{SaO2} = optic nerve head capillary oxygen saturation; ONH_{VOL} = optic nerve head capillary volume; SEM = standard error of the mean.

the values obtained in the latanoprost group at all time points ($P = .02$ at 60 minutes, $P < .001$ at 90 minutes, $P = .004$ at 120 minutes). Values of optic nerve head capillary oxygen saturation obtained with latanoprost did not differ from baseline values at any time point ($P > .99$ for all time points). Experimental data for optic nerve head capillary oxygen saturation are reported in Fig. 1.

The change from baseline in optic nerve head capillary blood volume observed in the latanoprost group was significant at 60 and 90 minutes after instillation ($P = .03$ and $P = .01$, respectively) but not at the 120-minute mark ($P = .98$). In the latanoprostene bunod–treated group, the optic nerve head capillary blood volume was increased, on average, 2.18-fold from baseline (95% CI, 186 to 250%), and this change was statistically significant across all time points ($P < .001$ at 60 minutes, $P < .001$ at 90 minutes, $P < .001$ at 120 minutes). The change in optic nerve head blood volume seen with the latanoprostene bunod group was, on average, 53% larger than the change seen with the latanoprost group and was significant at all time points ($P < .001$ at 60 minutes, $P < .001$ at 90 minutes, $P < .01$ at 120 minutes). Experimental data for optic nerve head capillary blood volume are demonstrated in Fig. 2.

For either optic nerve head capillary oxygen saturation or blood volume, there was no effect of treatment order on the response to either drug ($P > .99$ for oxygen saturation and $P = .79$ for blood volume).

Finally, average systolic and diastolic pressures did not significantly differ from baseline measurements after either latanoprost or latanoprostene bunod treatments ($P = .91$ and $P = .71$, respectively, for systolic pressure and $P = .32$ and $P = .43$, respectively, for diastolic pressure). Average systolic pressure was 113.4 mmHg (95% CI, 111.9 to 114.9 mmHg), and average diastolic pressure was 72.8 mmHg (95% CI, 71.5 to 74.2 mmHg).

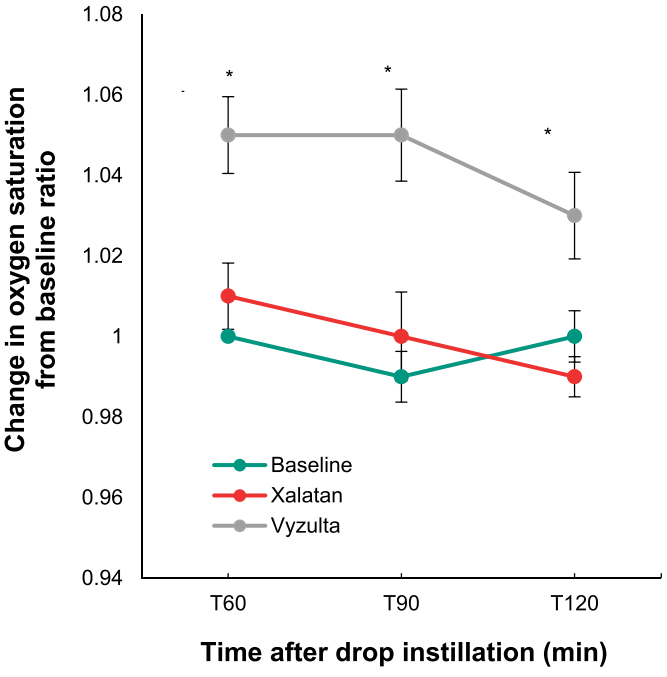


FIGURE 1. Optic nerve head capillary oxygen saturation (ONH_{SaO2}) change. Changes from baseline of ONH_{SaO2} after a 7-day, once-a-day instillation of latanoprost 0.004% and latanoprostene bunod 0.024%. Each plot represents the average of ONH_{SaO2} or the ratio, with a bar denoting standard error. *Significant statistical difference from baseline.

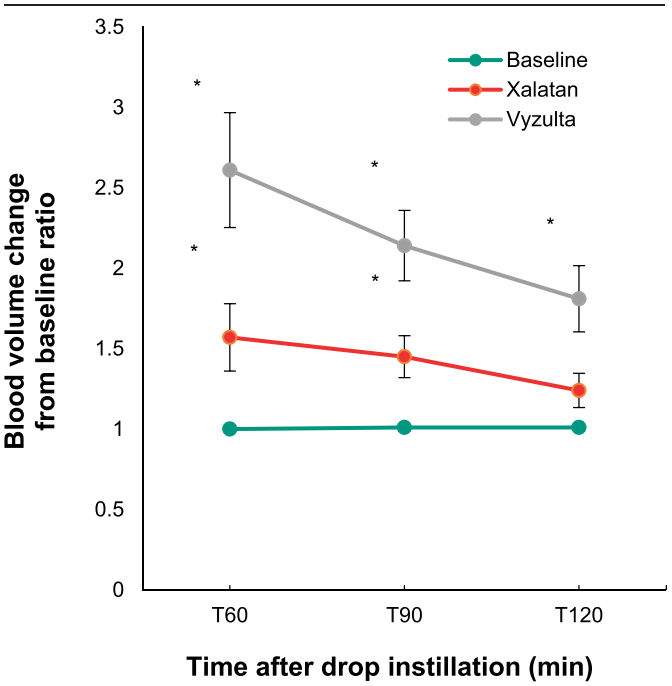


FIGURE 2. Optic nerve head capillary blood volume change (ONH_{VOL}). Changes from baseline of ONH_{VOL} after a 7-day, once-a-day instillation of latanoprost 0.004% and latanoprostene bunod 0.024%. Each plot represents the average of ONH_{VOL} or the ratio, with a bar denoting standard error. *Significant statistical difference from baseline.

DISCUSSION

The current study demonstrates the increase in optic nerve head capillary oxygen saturation and optic nerve head capillary blood volume after topical administration of latanoprostene bunod 0.024%. After instillation, latanoprostene bunod undergoes rapid hydrolysis into latanoprost acid and butanediol mononitrate, a nitric oxide–donating moiety.[14] The VOYAGER study demonstrated the effectiveness of a topical nitric oxide–donating group in lowering intraocular pressure by reducing outflow resistance through the trabecular meshwork. However, nitric oxide is also known to be an important homeostatic agent for ocular blood flow. Indeed, optic nerve head arteries are known to dilate in response to nitric oxide release by neurons.[13,24–26] Our results are analogous to the outcomes put forth by Grunwald et al.,[27] who documented an intraocular pressure–independent increase in blood flow at the optic nerve head after oral administration of isosorbide mononitrate, also a nitric oxide donor. A 33% increase in optic nerve head flow was reported in their study, which was mainly mediated by an increase in blood volume at the optic nerve head.

Other studies using nipradilol, a topical hypotensive drug used in Japan that also carries a nitric oxide–donating group, determined that rats with induced retinal damage suffered from significantly less ganglion cell loss when compared with controls.[12,28] Significant amounts of topically applied nipradilol were also detected at the retinal-choroid interface, implying that topical medication

can sufficiently penetrate ocular media, via corneal and scleral routes.[29,30]

The effect of nitric oxide at the retinal level has been greatly studied, and outcomes remain uncertain.[31–34] Increasing evidence points to its possible neuroprotective properties.[35–38] These results may contradict the general concept that nitric oxide possesses neurotoxic properties, particularly in regard to glaucomatous optic neuropathy, be it causative or resulting of the optic neuropathy.[39] More precisely, conflicting evidence exists concerning the different nitric oxide isoforms in retinal function, and the significance of this component is still under study.[28,40]

The role of hypoxia in the development of glaucomatous optic neuropathy is well known.[4,41–43] Essentially, transcription of hypoxia-inducible factor 1α, an indicator of hypoxic stress, has been found to be higher at the level of retinas of glaucoma patients, suggesting its role in the pathogenic mechanism of glaucomatous optic neuropathy (Singh S, Husain S. IOVS 2019;60:ARVO E-Abstract 22).[44]

Furthermore, oxygen saturation in the juxtapapillary regions has been shown to be significantly lower in both low-tension and high-tension glaucoma groups when compared with healthy controls.[45] This is of particular interest considering the disturbed ocular blood flow regulation that is known to occur in glaucomatous patients.[43,46,47] Because the association between glaucoma incidence and decreased retinal vessel oxygen saturation has been established, it is, however, still unknown whether an increase in saturation at the capillary level may translate into an increased oxygen extraction toward retinal tissue and the optic nerve head.

Concerning the increased capillary blood volume observed in the latanoprostene bunod group and considering Poiseuille's formula, it can be asserted that, as the vascular resistance is decreased and thus blood volume is increased, oxygen delivery to retinal tissues may be facilitated. Retinal arteriolar diameters have been measured to be narrower in glaucoma patients; one can then hypothesize that reversing this narrowing and increasing blood flow in retinal arterioles and capillaries may help provide a positive effect to retinal tissues.[48]

Although the use of latanoprost yielded no change in oxygen saturation at the optic nerve head, a statistically significant but momentaneous increase in optic nerve head capillary volume was observed after 60 and 90 minutes of drop instillation (57 and 45% increase from baseline, respectively; $P < .01$). These results are in line with a similar study evaluating optic nerve head blood velocity after a 7-day regimen of latanoprost in healthy humans.[15] It is theorized that latanoprost, a prostaglandin analogue, may exert vasodilating properties on the optic nerve head, as only small concentrations of prostanoids are needed to induce such effects.[49] It is thus possible, however, that the measured increase in optic nerve head capillary blood volume may have been too low to enable detection of any increase in optic nerve head capillary oxygen saturation.

Most oximeters use two to four wavelengths to derive oxygen saturation levels from spectroreflectometry readings.[50–52] It was demonstrated that increasing the number of wavelengths used in oximetry increases the precision of the blood oxygenation evaluation by about the square root of the considered wavelength number.[53] This means that the accuracy of multichannel technology, which uses 1024 discrete wavelengths, versus a technology that uses two to four wavelengths can offer approximately 30 times the precision of other oximeters.

Study limitations were related to patient adherence to instructions pertaining to drop instillation, time of instillation, and food consumption on experimental days. Researchers sought to obtain homogenous data (measurements were to begin precisely 60 minutes after the last drop administration) and to limit any external factors that could potentially affect data collection and quality. Participants were to refrain from consuming foods or beverages known to affect retinal and optic nerve head circulation, such as caffeine and foods with high salt content, but ultimately, there was no control over what participants consumed before measurement sessions.[54,55] Smoking was considered an exclusion criterion, and patients were asked to abstain from exercising on experimental days.[56,57] Although systolic and diastolic pressures and physical activity were monitored on data sampling days, oxygen saturation and pulse rate were not confirmed to be stable between sessions. Lastly, although participants acted as their own control, a vehicle control group was not included in the study protocol.

Further longitudinal research is needed in glaucoma patients, in which dysfunction of optic nerve head autoregulation plays a key role in the development and progression of glaucomatous optic neuropathy.[4] Based on the improvements in optic nerve capillary blood flow and oxygen saturation seen in this study, the nitric oxide–donating property of latanoprostene bunod 0.024% may well prove useful as a neuroprotective agent for glaucoma.

ARTICLE INFORMATION

Submitted: May 18, 2021

Accepted: September 4, 2021

Funding/Support: None of the authors have reported funding/support.

Conflict of Interest Disclosure: None of the authors have reported a financial conflict of interest.

Study Registration Information: Ethics certificate CERC-20-016-P.

Author Contributions: Conceptualization: DS, VD; Data Curation: VD, CD, AD; Formal Analysis: DS; Investigation: DS, JFB; Methodology: DS, VD, JFB, CD, AD; Project Administration: DS, JFB; Resources: DS, JFB; Software: VD; Supervision: DS, JFB; Validation: DS; Writing – Original Draft: DS; Writing – Review & Editing: DS, JFB.

REFERENCES

1. Harasymowycz P, Birt C, Gooi P, et al. Medical Management of Glaucoma in the 21st Century from a Canadian Perspective. J Ophthalmol 2016;2016:6509809.

2. European Glaucoma Society Terminology and Guidelines for Glaucoma, 4th Edition—Chapter 3: Treatment Principles and Options Supported by the EGS Foundation: Part 1: Foreword; Introduction; Glossary; Chapter 3 Treatment Principles and Options. Br J Ophthalmol 2017;101:130–95.

3. Prum BE, Rosenberg LF, Gedde SJ, et al. Primary Open-angle Glaucoma Preferred Practice Pattern® Guidelines. Ophthalmology 2018;123:41–111.

4. Flammer J, Orgül S, Costa VP, et al. The Impact of Ocular Blood Flow in Glaucoma. Prog Retin Eye Res 2002; 21:359–93.

5. Tomita G, Niwa Y, Shinohara H, et al. Changes in Optic Nerve Head Blood Flow and Retrobular Hemodynamics following Calcium-channel Blocker Treatment of Normal-tension Glaucoma. Int Ophthalmol 1999;23:3–10.

6. 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;89:1293–7.

7. Guymer C, Wood JP, Chidlow G, et al. Neuroprotection in Glaucoma: Recent Advances and Clinical Translation. Clin Experiment Ophthalmol 2019;47:88–105.

8. Harris A, Ciulla TA, Kagemann L, et al. Vasoprotection as Neuroprotection for the Optic Nerve. Eye Lond 2000; 14:473–5.

9. Boo YC, Jo H. Flow-dependent Regulation of Endothelial Nitric Oxide Synthase: Role of Protein Kinases. Am J Physiol Cell Physiol 2003;285:C499–508.

10. Koriyama Y, Kamiya M, Arai K, et al. Nipradilol Promotes Axon Regeneration through S-nitrosylation of PTEN in Retinal Ganglion Cells. Adv Exp Med Biol 2014;801:751–7.

11. Watanabe M, Tokita Y, Yata T. Axonal Regeneration of Cat Retinal Ganglion Cells Is Promoted by Nipradilol, an Anti-glaucoma Drug. Neuroscience 2006;140:517–28.

12. Mizuno K, Koide T, Yoshimura M, et al. Neuroprotective Effect and Intraocular Penetration of Nipradilol, a Beta-blocker with Nitric Oxide Donative Action. Invest Ophthalmol Vis Sci 2001;42:688–94.

13. Dorner GT, Garhofer G, Kiss B, et al. Nitric Oxide Regulates Retinal Vascular Tone in Humans. Am J Physiol Heart Circ Physiol 2003;285:631–6.

14. Weinreb RN, Ong T, Scassellati Sforzolini B, et al. A Randomised, Controlled Comparison of Latanoprostene Bunod and Latanoprost 0.005% in the Treatment of Ocular Hypertension and Open Angle Glaucoma: The VOYAGER Study. Br J Ophthalmol 2015;99:738–45.

15. Ishii K, Tomidokoro A, Nagahara M, et al. Effects of Topical Latanoprost on Optic Nerve Head Circulation in Rabbits, Monkeys, and Humans. Invest Ophthalmol Vis Sci 2001;42:2957–63.

16. Sullivan-Mee M, Ruegg CC, Pensyl D, et al. Diagnostic Precision of Retinal Nerve Fiber Layer and Macular Thickness Asymmetry Parameters for Identifying Early Primary Open-angle Glaucoma. Am J Ophthalmol 2013;156:567–77.

17. Budenz DL. Symmetry between the Right and Left Eyes of the Normal Retinal Nerve Fiber Layer Measured with Optical Coherence Tomography (an AOS Thesis). Trans Am Ophthalmol Soc 2008;106:252–75.

18. Diaconu V. Multichannel Spectroreflectometry: A Noninvasive Method for Assessment of On-line Hemoglobin Derivatives. Appl Optics 2009;48:52–61.

19. Vucea V, Bernard PJ, Sauvageau P, et al. Blood Oxygenation Measurements by Multichannel Reflectometry on the Venous and Arterial Structures of the Retina. Appl Optics 2011;50:5185–91.

20. Rommel F, Rothe M, Kurz M, et al. Evaluating Diurnal Variations in Retinal Perfusion Using Optical Coherence Tomography Angiography. Int J Retina Vitreous 2020;6:22.

21. Claridge KG, Smith SE. Diurnal Variation in Pulsatile Ocular Blood Flow in Normal and Glaucomatous Eyes. Surv Ophthalmol 1994;38:198–205.

22. Hayreh SS. Inter-individual Variation in Blood Supply of the Optic Nerve Head. Its Importance in Various Ischemic Disorders of the Optic Nerve Head, and Glaucoma, Low-tension Glaucoma and Allied Disorders. Doc Ophthalmol 1985;59:217–46.

23. Palsson O, Geirsdottir A, Hardarson SH, et al. Retinal Oximetry Images Must Be Standardized: A Methodological Analysis. Invest Ophthalmol Vis Sci 2012;53:1729–33.

24. Stamer WD, Lei Y, Boussommier-Calleja A, et al. eNOS, a Pressure-dependent Regulator of Intraocular Pressure. Invest Ophthalmol Vis Sci 2011;52:9438–44.

25. Schmetterer L, Krejcy K, Kastner J, et al. The Effect of Systemic Nitric Oxide–synthase Inhibition on Ocular Fundus Pulsations in Man. Exp Eye Res 1997;64:305–12.

26. Deussen A, Sonntag M, Vogel R. L-arginine–derived Nitric Oxide: A Major Determinant of Uveal Blood Flow. Exp Eye Res 1993;57:129–34.

27. Grunwald JE, Iannaccone A, DuPont J. Effect of Isosorbide Mononitrate on the Human Optic Nerve and Choroidal Circulations. Br J Ophthalmol 1999;83:162–7.

28. Karim MZ, Sawada A, Mizuno K, et al. Neuroprotective Effect of Nipradilol [3,4-dihydro-8-(2-hydroxy-3-isopropylamino)-propoxy-3-nitroxy-2*H*-1-benzopyran] in a Rat Model of Optic Nerve Degeneration. J Glaucoma 2009;18:26–31.

29. Ross A, Bengani L, Tulsan R, et al. Topical Sustained Drug Delivery to the Retina with a Drug-eluting Contact Lens. Biomaterials 2019;217:119285.

30. Himawan E, Ekström P, Buzgo M, et al. Drug Delivery to Retinal Photoreceptors. Drug Discov Today 2019;24:1637–43.

31. Eldred WD, Blute TA. Imaging of Nitric Oxide in the Retina. Vision Res 2005;45:3469–86.

32. Izumi N, Nagaoka T, Sato E, et al. Role of Nitric Oxide in Regulation of Retinal Blood Flow in Response to Hyperoxia in Cats. Invest Ophthalmol Vis Sci 2008;49:4595–603.

33. Kalamkarov GR, Bugrova AE, Konstantinova TS, et al. Endogenous Nitric Oxide Content in the Cellular Layers of the Retina. Neurosci Behav Physiol 2016;46:291–5.

34. Opatrilova R, Kubatka P, Caprnda M, et al. Nitric Oxide in the Pathophysiology of Retinopathy: Evidences from Preclinical and Clinical Researches. Acta Ophthalmol 2018;96:222–31.

35. Nakazawa T, Tomita H, Yamaguchi K, et al. Neuroprotective Effect of Nipradilol on Axotomized Rat Retinal Ganglion Cells. Curr Eye Res 2002;24:114–22.

36. Okamura T, Kitamura Y, Uchiyama M, et al. Canine Retinal Arterial and Arteriolar Dilatation Induced by Nipradilol, a Possible Glaucoma Therapeutic. Pharmacology 1996;53:302–10.

37. Kanno M, Araie M, Tomita K, et al. Effects of Topical Nipradilol, a Beta-blocking Agent with Alpha-blocking and Nitroglycerin-like Activities, on Aqueous Humor Dynamics and Fundus Circulation. Invest Ophthalmol Vis Sci 1998;39:736–43.

38. Zurakowski D, Vorwerk CK, Gorla M, et al. Nitrate Therapy May Retard Glaucomatous Optic Neuropathy, Perhaps through Modulation of Glutamate Receptors. Vision Res 1998;38:1489–94.

39. Neufeld AH, Hernandez MR, Gonzalez M. Nitric Oxide Synthase in the Human Glaucomatous Optic Nerve Head. Arch Ophthalmol 1997;115:497–503.

40. Goldstein IM, Ostwald P, Roth S. Nitric Oxide: A Review of Its Role in Retinal Function and Disease. Vision Res 1996;36:2979–94.

41. Stefánsson E, Pedersen DB, Jensen PK, et al. Optic Nerve Oxygenation. Prog Retin Eye Res 2005;24:307–32.

42. Bonomi L, Marchini G, Marraffa M, et al. Vascular Risk Factors for Primary Open Angle Glaucoma: The Egna-Neumarkt Study. Ophthalmology 2000;107:1287–93.

43. Hardarson SH, Gottfredsdottir MS, Halldorsson GH, et al. Glaucoma Filtration Surgery and Retinal Oxygen Saturation. Invest Ophthalmol Vis Sci 2009;50:5247–50.

44. Tezel G, Wax MB. Hypoxia-inducible Factor 1alpha in the Glaucomatous Retina and Optic Nerve Head. Arch Ophthalmol 2004;122:1348–56.

45. Ito M, Murayama K, Deguchi T, et al. Oxygen Saturation Levels in the Juxta-papillary Retina in Eyes with Glaucoma. Exp Eye Res 2008;86:512–8.

46. Garzozi HJ, Shoham N, Chung HS, et al. Ocular Blood Flow Measurements and Their Importance in Glaucoma and Age-related Macular Degeneration. Isr Med Assoc J 2001;3:443–8.

47. Sekeroglu MA, Irkec M, Mocan MC, et al. The Association of Ocular Blood Flow with Haemorheological Parameters in Primary Open-angle and Exfoliative Glaucoma. Acta Ophthalmol 2011;89:429–34.

48. Yap ZL, Verma S, Lee YF, et al. Glaucoma Related Retinal Oximetry: A Technology Update. Clin Ophthalmol 2018;12:79–84.

49. Kimura T, Yoshida Y, Toda N. Mechanisms of Relaxation Induced by Prostaglandins in Isolated Canine Uterine Arteries. Am J Obstet Gynecol 1992;167:1409–16.

50. Hardarson SH, Harris A, Karlsson RA, et al. Automatic Retinal Oximetry. Invest Ophthalmol Vis Sci 2006;47:5011–6.

51. Hammer M, Vilser W, Riemer T, et al. Retinal Vessel Oximetry-calibration, Compensation for Vessel Diameter and Fundus Pigmentation, and Reproducibility. J Biomed Opt 2008;13:054015.

52. Ramm L, Jentsch S, Peters S, et al. Investigation of Blood Flow Regulation and Oxygen Saturation of the Retinal Vessels in Primary Open-angle Glaucoma. Graefes Arch Clin Exp Ophthalmol 2014;252:1803–10.

53. Schweitzer D, Hammer M, Kraft J, et al. *In Vivo* Measurement of the Oxygen Saturation of Retinal Vessels in Healthy Volunteers. IEEE Trans Biomed Eng 1999;46:1454–65.

54. Wenstedt EF, Beugelink L, Schrooten EM, et al. High-salt Intake Affects Retinal Vascular Tortuosity in Healthy Males: An Exploratory Randomized Cross-over Trial. Sci Rep 2021;11:801.

55. Okuno T, Sugiyama T, Tominaga M, et al. Effects of Caffeine on Microcirculation of the Human Ocular Fundus. Jpn J Ophthalmol 2002;46:170–6.

56. Tamaki Y, Araie M, Nagahara M, et al. Acute Effects of Cigarette Smoking on Tissue Circulation in Human Optic Nerve Head and Choroid-retina. Ophthalmology 1999;106:564–9.

57. Witkowska KJ, Bata AM, Calzetti G, et al. Optic Nerve Head and Retinal Blood Flow Regulation during Isometric Exercise as Assessed with Laser Speckle Flowgraphy. PLoS One 2017;12:e0184772.