

Short-Term Increase of Intraocular Pressure Does Not Alter the Response of Retinal and Optic Nerve Head Blood Flow to Flicker Stimulation

Gerhard Garbhöfer,^{1,2} Hemma Resch,¹ Günther Weigert,¹ Solveig Lung,¹ Christian Simader,² and Leopold Schmetterer^{1,3}

PURPOSE. It has been shown that stimulation with diffuse luminance flicker induces vasodilatation in the human retina and increases optic nerve head (ONH) blood flow. The present study was designed to investigate the influence of a short-term increase in intraocular pressure on flicker-induced changes in ONH blood flow and retinal vessel diameters.

METHODS. In a group of 15 healthy volunteers, IOP was increased by the episcleral suction cup technique. ONH blood flow was assessed with a laser Doppler flowmeter, and retinal vessel diameters were measured with the retinal vessel analyzer. Flicker responses of retinal vessel diameters and ONH blood flow were determined at baseline conditions and during suction of 70 and 140 mm Hg. Flicker light consisted of 8-Hz square-wave flashes at a wavelength below 550 nm and produced a retinal irradiance of 140 $\mu\text{W}/\text{cm}^2$.

RESULTS. Suction increased IOP from 12 ± 2 to 27 ± 4 mm Hg and 43 ± 4 mm Hg. Stimulation with diffuse luminance flicker induced an increase in ONH blood flow of $+24.0\% \pm 20.7\%$. Increased IOP did not significantly change the flicker response in the ONH ($+19.3\% \pm 26.6\%$ and $+22.1\% \pm 25.1\%$). In retinal veins, flicker induced an increase in vessel diameter of $+3.0\% \pm 2.0\%$. Flicker responses in retinal veins were not significantly altered after the IOP was increased, compared with those recorded at baseline IOP ($+2.8\% \pm 2.5\%$ and $+3.2\% \pm 2.2\%$). The flicker response in retinal arteries at baseline IOP was $+3.5\% \pm 2.0\%$. Again, the increase in IOP did not significantly alter this flicker response ($+2.8\% \pm 1.6\%$ and $+3.1\% \pm 2.4\%$).

CONCLUSIONS. A short-term increase in IOP does not alter the response of retinal vessel diameters and ONH blood flow to diffuse luminance flicker, which indicates that increased IOP does not alter retinal or ONH regulation during neuronal stimulation. (*Invest Ophthalmol Vis Sci.* 2005;46:1721-1725) DOI:10.1167/iovs.04-1347

A variety of studies have shown that blood supply in the retina as well as the optic nerve head (ONH) is very well regulated. These regulatory mechanisms assure sufficient blood supply over a wide range of ocular perfusion pressures¹⁻³ and regulate blood flow, depending on functional activity and the resultant metabolic demands.^{4,5} This regula-

tion system, however, may be impaired under several pathologic conditions. Especially in glaucoma, it has been hypothesized that besides increased intraocular pressure (IOP), impaired blood flow may contribute to the progression of the disease.⁶ However, the complex interaction among IOP, blood pressure, and blood flow demand, as well as methodological limitations, complicate the investigation of this risk factor.

Stimulation of the retina with diffuse luminance flicker light has been shown to increase blood flow in retinal vessels and in the ONH.^{4,5,7,8} This regulatory response of the retina and the ONH has recently been used as a new method to assess the regulatory capacity of the retinal and ONH circulation in healthy subjects and in those with pathologic conditions.⁹⁻¹¹ In glaucoma it has been demonstrated that this vascular response to flickering light is reduced.^{9,10} Whether this impaired blood flow response can be attributed to increased IOP in patients with glaucoma or represents vascular dysregulation should be clarified.

The present study was undertaken to investigate whether a short-term increase in IOP can affect the vascular regulation processes in the retina and the ONH. We therefore studied the response of retinal and ONH blood flow after stimulation with diffuse luminance flicker during an artificial IOP increase achieved with the suction cup technique in healthy subjects. Retinal vessel diameters and ONH blood flow were assessed with a retinal vessel analyzer and a laser Doppler flowmeter.

METHODS

Subjects

The study protocol was approved by the Ethics Committee of the Vienna University School of Medicine and complied with guidelines of the Declaration of Helsinki. Fifteen healthy, male, nonsmoking subjects were included. Volunteers signed a written informed consent and passed a screening examination that included medical history and an ophthalmic examination. Inclusion criteria were normal ophthalmic findings, ametropia of <3 D, and anisometropia of <1 D. Subjects abstained from alcohol and stimulating beverages containing xanthine derivatives (tea, coffee, and cola drinks) 12 hours before the study day. In all subjects, the right eye was studied.

Suction Cup

IOP was increased by means of the episcleral suction cup technique. With this technique, a suction cup (diameter, 11 mm) is applied to the sclera. It produces a negative pressure that is transferred onto the globe and induces a subsequent increase in IOP. In the present study a suction pressure of 70 and 140 mm Hg was applied. Topical anesthesia was obtained by oxybuprocaine eye drops before the suction cup was placed.

Experimental Paradigms

On the trial day, subjects arrived after sleeping at least 7 to 8 hours and having a light breakfast. After a 20-minute rest period to allow hemo-

From the Departments of ¹Clinical Pharmacology and ²Ophthalmology and the ³Center for Biomedical Engineering, Medical University of Vienna, Vienna, Austria.

Submitted for publication November 18, 2004; revised December 23, 2004; accepted January 19, 2005.

Disclosure: **G. Garbhöfer**, None; **H. Resch**, None; **G. Weigert**, None; **S. Lung**, None; **C. Simader**, None; **L. Schmetterer**, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Leopold Schmetterer, Department of Clinical Pharmacology, Währinger Gürtel 18-20, A-1090 Vienna, Austria; leopold.schmetterer@univie.ac.at.

dynamic values to stabilize, three consecutive experiments with a break of at least 30 minutes were performed.

The first experiment was designed to assess flicker responses in retinal vessels. Baseline measurements with the RVA were performed for 2 minutes. To measure the flicker response at baseline IOP, flicker stimulation was started for 60 seconds. Thereafter, the suction cup was applied to the sclera. Suction was started at 70 mm Hg and was performed for 2 minutes. After 60 seconds of constantly elevated IOP, flicker responses were determined as described for the baseline measurements. Thereafter, the suction was increased up 140 mm Hg, and after 60 seconds, responses were measured again.

After completion of this experiment a resting period of at least 30 minutes was scheduled and thereafter the experiments investigating ONH blood flow were started. Experimental procedures and the time schedule followed exactly the one described for the vessel diameter experiments. Another resting period of at least 30 minutes was scheduled to measure the final IOP during the suction experiments.

Laser Doppler Flowmetry

ONH blood flow was assessed with a fundus-camera-based laser Doppler flowmeter (Oculix 4000; Oculix Sarl, Arbas, Switzerland) developed by Riva et al.^{12,13} The principles of laser Doppler flowmetry (LDF) have been described in detail elsewhere.¹⁴ Briefly, the vascularized tissue is illuminated by coherent laser light. Scattering on moving blood cells (RBCs) leads to a frequency shift in the scattered light. In contrast, static scattering in tissue does not change the light frequency but leads to a randomization of light directions impinging on the RBCs and, consequently, to a broadening of the spectrum of scattered light (Doppler shift power spectrum [DSPS]). From the DSPS, the mean RBC velocity, the blood volume, and the blood flow can be calculated in relative units. Blood flow in the ONH was measured at temporal sites of the neuroretinal rim. Care was taken to ensure that the measurement location on the ONH did not include visible vessels. Sensitivity and reproducibility of LDF measurements in the ONH have been published previously.¹⁵

Retinal Vessel Analyzer

The retinal vessel analyzer (RVA; Imedos, Jena, Germany) is a commercially available system that comprises a fundus camera (FF 450; Carl Zeiss Meditec), a video camera, a high-resolution video recorder, a real-time monitor, and a computer with vessel diameter analysis software. The RVA allows for the precise measurement of retinal vessel diameter with a time resolution of 25 readings/s. For vessel diameter measurements, the fundus was illuminated through an interference filter placed in the illumination pathway of the RVA. This filter transmits light in the wavelength range between 567 and 587 nm. In this spectral range, the contrast between retinal vessels and the surrounding tissue is optimal. Retinal irradiance was approximately 220 $\mu\text{W}/\text{cm}^2$, which is approximately 50 times lower than the maximum level allowed for constant illumination of the retina at the mentioned wavelengths. Because of its adaptive algorithm, the system is largely independent of alterations in luminance as induced, for instance, by slight eye movements.

Flicker Light Stimulation

For flicker stimulation, a custom-built device was used, that produced stimulating light flashes at a frequency of 8 Hz. The flicker was generated by focusing the light of a 150-W halogen light source on a rotating sector disc, producing a square-wave light pattern with a modulation depth of 100%. Flicker stimuli were delivered to the eye through the illumination pathways of the fundus cameras of the RVA and the laser Doppler flowmeter, respectively. The flicker was centered in the macula with an angle of approximately 30°, producing a retinal irradiance of 300 $\mu\text{W}/\text{cm}^2$.

To spectrally separate the flicker light from that used to illuminate the fundus, a wavelength-separation technique was used. For flicker stimulation, white light in combination with a 550-nm low-pass cutoff

filter was used. This ensured that light with wavelengths below 550 nm was used for flicker stimulation. To separate flicker light from the light illuminating the fundus, we placed an interference filter with a center wavelength of 577 nm and a bandwidth of 10 nm (Laser Components, Olchingen, Germany) in front of the light source of the fundus camera. A second interference filter, exactly matching the one in the illumination pathway, was placed in front of the video camera. This ensured that the light used for flicker stimulation did not reach the charge-coupled device (CCD) chip of the video camera, but was perceived by the subject under study. This setup was chosen to allow for constant illumination conditions at the fundus image during flicker stimulation, a prerequisite for adequate vessel diameter determination.⁵

Blood Pressure Measurement

Mean arterial blood pressures were measured on the upper arm by an automated oscillometric device (HP-CMS patient monitor; Hewlett Packard, Palo Alto, CA). Pulse rate was automatically recorded from a finger pulse oximeter.

Statistics

Changes in ocular hemodynamic parameters were expressed as the percentage change over baseline values. Retinal vessel diameters were calculated as an average of the last 30 seconds of each measurement. Vessel diameter during flicker was calculated as an average of the last 30 seconds of light stimulation. ONH blood flow was calculated as the last 30 seconds of each measurement before and during light stimulation. Data are expressed as the mean \pm SD. A repeated-measures ANOVA was used for significance testing of retinal vessel diameters, LDF, and IOP. Planned comparison test was used for post hoc testing. The Shapiro-Wilk W Test was used for proving normal distribution. Because of the unknown distribution of the data, the Friedman ANOVA was used as a nonparametric test to compare flicker responses at the different IOP levels. $P < 0.05$ was considered to be the level of significance. Calculations were performed on computer (Statistica software package; Statsoft, Tulsa, OK).

RESULTS

IOP and Hemodynamics

As shown in Figure 1, suction increased IOP from 12 ± 2 mm Hg to 27 ± 4 and 43 ± 4 mm Hg (ANOVA, $P < 0.01$). Mean arterial pressure (MAP) and pulse rate remained unchanged during the whole experiment. Absolute MAPs and pulse rates are shown in Table 1.

Retinal Vessel Diameters

Absolute retinal vessel diameters at baseline and during suction are given in Table 1. Retinal venous diameters decreased by $-1.6\% \pm 1.1\%$ and $-1.9\% \pm 3.0\%$ (ANOVA, $P = 0.025$) after suction (Fig. 1). As shown in Figure 2, stimulation with flickering light induced a retinal vasodilatation of $+3.0\% \pm 2.0\%$ (ANOVA, $P < 0.01$) in baseline conditions. At an IOP of 27 and 43 mm Hg, flicker responses in retinal veins were not significantly different compared with those recorded at baseline IOP ($+2.8\% \pm 2.5\%$ and $+3.2\% \pm 2.2\%$; Friedman ANOVA, $P = 0.3$). Retinal arterial diameters tended to increase after application of the suction by $+2.5\% \pm 4.4\%$ and $+1.4\% \pm 5.8\%$ at the two pressure levels, respectively. This effect, however, did not reach significance (Fig. 1; ANOVA $P = 0.08$). The flicker response at baseline IOP in retinal arteries was $+3.5\% \pm 2.0\%$ (ANOVA, $P < 0.01$). Again, the increase of IOP did not significantly alter the flicker response ($+2.8\% \pm 1.6\%$ and $+3.1\% \pm 2.4\%$; Friedman ANOVA, $P = 0.7$).

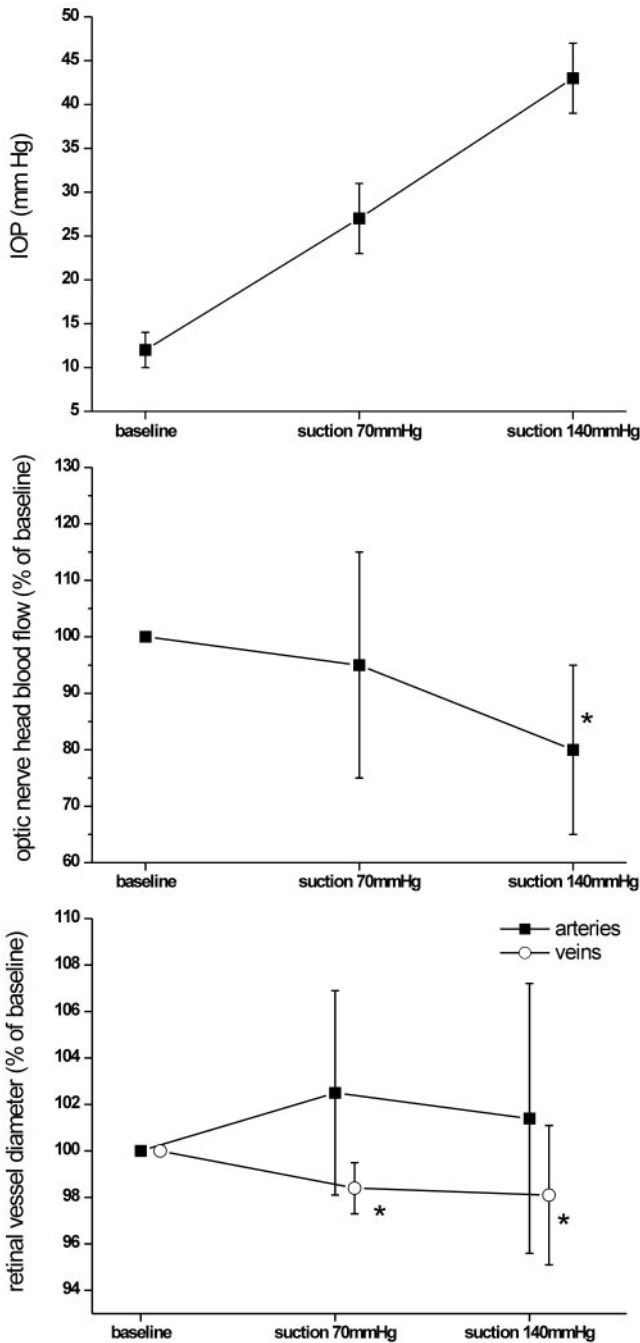


FIGURE 1. Intraocular pressure (mm Hg), ONH blood flow (percentage of baseline), and retinal vessel diameters (percentage of baseline) during suction are indicated in the three plots. Data are expressed as the mean \pm SD ($n = 15$). *Statistical significance versus baseline.

ONH Blood Flow

Increasing the IOP to 27 and 43 mm Hg decreased ONH blood flow by $-5.0\% \pm 20\%$ and $-20\% \pm 15\%$, respectively (Fig. 1; ANOVA, $P < 0.01$). At baseline conditions, stimulation with diffuse luminance flicker induced an increase in ONH blood flow of $24.0\% \pm 20.7\%$ (ANOVA, $P < 0.01$). However, as shown in Figure 3, increased IOP did not significantly change flicker response ($19.3\% \pm 27\%$ and $22.1\% \pm 25\%$; Friedman ANOVA, $P = 0.76$). No difference in DC was observed between the three measurement periods (baseline IOP, $2.7 V \pm$

TABLE 1. Absolute Retinal Vessel Diameters, MAP, and Pulse Rate ($n = 15$)

	Suction		
	Baseline	70 mm Hg	140 mm Hg
Retinal venous diameter (μm)	153 ± 21	153 ± 20	151 ± 20
Retinal arterial diameter (μm)	124 ± 15	128 ± 13	126 ± 13
MAP (mm Hg)	74 ± 10	75 ± 10	79 ± 8
Pulse rate (bpm)	76 ± 11	66 ± 10	65 ± 9

0.6 V; IOP 27 mm Hg, $2.7 V \pm 0.6 V$; and IOP 43 mm Hg, $2.6 V \pm 0.6 V$).

DISCUSSION

The present study provides evidence that the response of ONH blood flow and retinal vessel diameters to flicker stimulation is maintained up to an IOP of 43 mm Hg. This indicates that, even at high IOPs, blood flow responds to the neural stimulation caused by flickering light. Accordingly, it appears that the reduced flicker response recorded in patients with glaucoma in previous studies^{9,10} was not a direct consequence of increased IOP. One should be careful however, in directly applying the

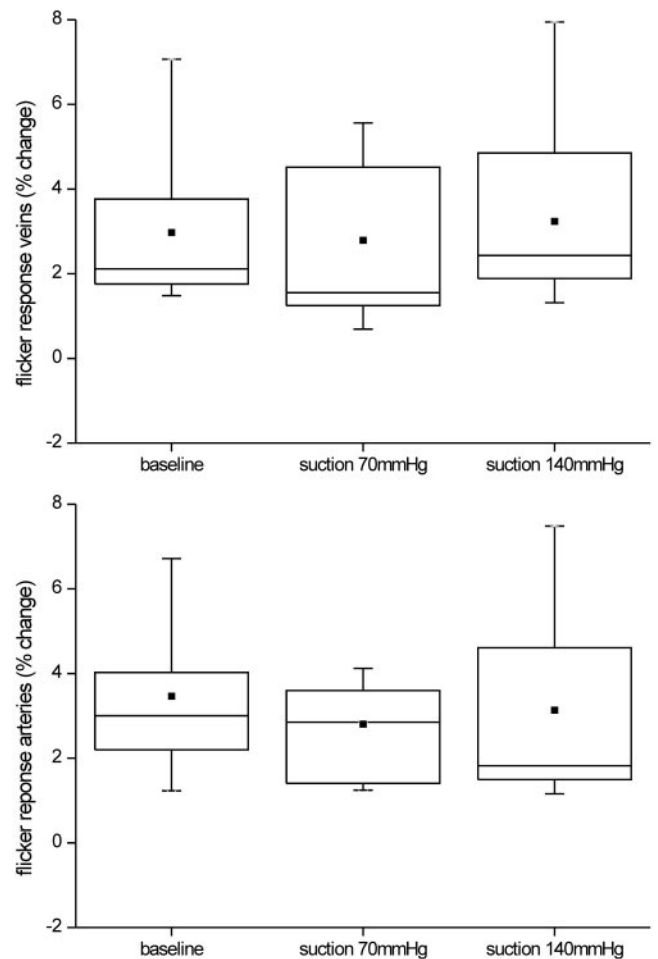


FIGURE 2. Flicker responses in retinal vessels. Box plots depict flicker responses as percentage change during flicker stimulation of retinal arteries and veins. Horizontal lines, top to bottom: 25th percentile, the median and 75th percentile. Error bars: 5th and 95th percentiles. (■) The mean ($n = 15$).

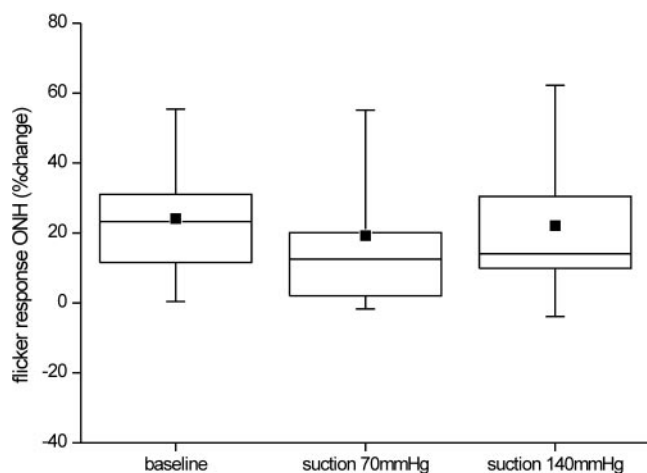


FIGURE 3. Flicker responses in ONH blood flow. Box plots depict flicker responses as percent change of ONH blood flow during flicker stimulation in the ONH. The plots are constructed as described in Figure 2 ($n = 15$).

data of the present study to the results observed in patients with glaucoma, because long-term changes in IOP were not examined in the present study.

It has been shown previously in numerous animal and human experiments that ONH and retinal blood flow increase after flickering light provocation, indicating the ability of the retina to adapt its blood flow to changing energy demands caused by increased neural activity.^{4,5,8,16-21} The response in ONH blood flow and retinal vessel diameters to flicker stimulation under baseline conditions in the present study is comparable to that reported previously in healthy humans.^{4,5,20} Our data are also in agreement with previous studies in animal models,¹⁶⁻¹⁸ in which the vascular response to flicker stimulation remained normal, even at low ocular perfusion pressure.²²

In the retina we found a slight constriction in retinal veins and a tendency for retinal arteries to dilate when the IOP was increased. These results are in keeping with previous studies showing comparable vessel behavior under experimentally induced ocular hypertension.^{23,24} It has been hypothesized that the constriction of retinal veins is a primarily passive effect, caused by the weaker wall construction and the lower intravascular pressure of the venous system. In contrast, the reaction of retinal arteries appears to be part of an autoregulatory response to counteract the decreased perfusion pressure.²³

Both retinal arteries and veins reacted normally during flicker stimulation despite altered vascular tone. A complete description of the vascular reaction occurring during combined experimental increase in IOP and flicker stimulation in the retina is not possible based on our data, because of the lack of data from the microvasculature. We have developed a method of measuring retinal blood velocity data during flicker stimulation based on a commercially available bidirectional laser Doppler velocimeter.⁷ This instrument was not used in the present study, however, because pilot experiments showed that we were not able to get reliable flicker response data when the suction cup was applied.

In the ONH, our data can be more easily interpreted, because we measured relative changes in blood flow instead of vessel diameters. We found that, at an IOP of 27 mm Hg, ONH blood flow was maintained, indicating that we were still in the autoregulatory range. This finding is in agreement with several previous reports investigating regulation of ONH blood flow.^{3,25} By contrast, at 43 mm Hg ONH blood flow had already declined by approximately 20%, which is again compatible

with findings in previous studies.^{3,25} Even in these experimental conditions, it was possible to induce an increase in ONH blood flow of >20%, on average. This clearly indicates that, even at ocular perfusion pressures that are below the lower autoregulatory limit, ONH vessels are not in a state of complete vasodilatation. Obviously, this indicates that ONH blood vessels predilated in response to the decrease in perfusion pressure still have a vasodilator reserve, which can, at least in part, be activated by neural stimulation. Accordingly, different vasodilator mechanisms appear to be responsible for autoregulatory vasodilatation and flicker-induced vasodilatation in the ONH. In addition, this finding provides evidence that the autoregulatory response in the ONH is not purely myogenic. This is in agreement with a previous study investigating the autoregulatory response in the retina.²⁶

As mentioned previously, the present data should be interpreted with caution when applying them to the situation in glaucoma. In the present study, an experimental short-term increase of IOP was used. Whether our results also hold true for a long-term increase in IOP with all associated functional and anatomic alterations as seen in patients with glaucoma has yet to be investigated. A comparison of flicker responses in patients with open-angle glaucoma and patients with ocular hypertension could be helpful in clarifying this question. Furthermore, one has to consider that young, male volunteers participated in our study. Thus, we cannot exclude that the flicker response under increased IOP is age dependent. This is, however, unlikely, because it has recently been shown that flicker-induced vasodilatation of retinal vessels is independent of age.²⁷ In addition, we cannot exclude that because of the limited sensitivity of the LDF measurements and the intraindividual variation of flicker responses, small changes in flicker responses may have been missed. The present study was designed to allow for detecting differences in flicker responses of 25% at the three experimental IOP levels. The standard deviation of the flicker response in the ONH (32%; Garhöfer G, Schmetterer L, unpublished data, 2004), an α error of 0.05, and a power of 0.8 lead to a sample size of 15. Accordingly, the present study was not capable of detecting differences in the flicker response smaller than 25% under the different experimental conditions.

In conclusion, we report that an increase of IOP up to 43 mm Hg does not alter the flicker response in young, healthy volunteers. These findings support the concept that short-term ocular hypertension does not affect ONH and retinal blood flow regulation during neural stimulation. In addition, our data indicate that even at the lower limit of ONH blood flow, autoregulation ONH vessels are not in a state of maximum vasodilatation.

References

- Movaffaghy A, Chamot SR, Petrig BL, Riva CE. Blood flow in the human optic nerve head during isometric exercise. *Exp Eye Res.* 1998;67:561-568.
- Dumskyj MJ, Eriksen JE, Dore 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.
- Riva CE, Hero M, Titze P, Petrig B. Autoregulation of human optic nerve head blood flow in response to acute changes in ocular perfusion pressure. *Graefes Arch Clin Exp Ophthalmol.* 1997;235:618-626.
- Riva CE, Falsini B, Logean E. Flicker-evoked responses of human optic nerve head blood flow: luminance versus chromatic modulation. *Invest Ophthalmol Vis Sci.* 2001;42:756-762.
- Polak K, Schmetterer L, Riva CE. Influence of flicker frequency on flicker induced changes of retinal vessel diameters. *Invest Ophthalmol Vis Sci.* 2002;43:2721-2726.

6. Flammer J, Haefliger IO, Orgul S, Resink T. Vascular dysregulation: a principal risk factor for glaucomatous damage? *J Glaucoma*. 1999;8:212-219.
7. Garhöfer G, Zawinka C, Resch H, Huemer KH, Dorner GT, Schmetterer L. Diffuse luminance flicker increases blood flow in major retinal arteries and veins. *Vision Res*. 2004;44:833-838.
8. Formaz F, Riva C, Geiser M. Diffuse luminance flicker increases retinal vessel diameter in humans. *Curr Eye Res*. 1997;16:1252-1257.
9. 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.
10. 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.
11. Garhöfer G, Zawinka C, Resch H, Kothly P, Schmetterer L, Dorner GT. Reduced response of retinal vessel diameters to flicker stimulation in patients with diabetes. *Br J Ophthalmol*. 2004;88:887-891.
12. Riva CE, Harino S, Petrig BL, Shonat RD. Laser Doppler flowmetry in the optic nerve. *Exp Eye Res*. 1992;499-506.
13. Riva CE, Cranston SD, Grunwald JE, Petrig BL. Choroidal blood flow in the foveal region of the human ocular fundus. *Invest Ophthalmol Vis Sci*. 1994;35:4273-4281.
14. Bonner R, Nossal R. Principles of laser-Doppler flowmetry. In: Shepherd AP, Öberg PA, eds. *Developments in Cardiovascular Medicine: Laser Doppler Blood Flowmetry*. Boston: Kluwer Academic Publishers; 1990;17-45.
15. Joos KM, Pillunat LE, Knighton RW, Anderson DR, Feuer WJ. Reproducibility of laser Doppler flowmetry in the human optic nerve head. *J Glaucoma*. 1997;6:212-216.
16. Riva CE, Harino S, Shonat RD, Petrig BL. Flicker evoked increase in optic nerve head blood flow in anesthetized cats. *Neurosci Lett*. 1991;128:291-296.
17. Buerk DG, Riva CE, Cranston SD. Frequency and luminance-dependent blood flow and K⁺ ion changes during flicker stimuli in cat optic nerve head. *Invest Ophthalmol Vis Sci*. 1995;36:2216-2227.
18. Buerk DG, Riva CE, Cranston SD. Nitric oxide has a vasodilatory role in cat optic nerve head during flicker stimuli. *Microvasc Res*. 1996;52:13-26.
19. Scheiner A, Riva C, Kazahaya K, Petrig B. Effect of flicker on macular blood flow assessed by the blue field simulation technique. *Invest Ophthalmol Vis Sci*. 1994;35:3436-3441.
20. Garhöfer G, Huemer KH, Zawinka C, Schmetterer L, Dorner GT. Influence of diffuse luminance flicker on choroidal and optic nerve head blood flow. *Curr Eye Res*. 2002;24:109-113.
21. Michelson G, Patzelt A, Harazny J. Flickering light increases retinal blood flow. *Retina*. 2002;22:336-343.
22. Riva CE, Cranston SD, Petrig BL. Effect of decreased ocular perfusion pressure on blood flow and the flicker-induced flow response in the cat optic nerve head. *Microvasc Res*. 1996;52:258-269.
23. Nagel E, Vilser W. Autoregulative behavior of retinal arteries and veins during changes of perfusion pressure: a clinical study. *Graefes Arch Clin Exp Ophthalmol*. 2004;42:13-17.
24. Riva CE, Grunwald JE, Sinclair SH, Petrig BL. Blood velocity and volumetric flow rate in human retinal vessels. *Invest Ophthalmol Vis Sci*. 1985;26:1124-1132.
25. Pillunat LE, Anderson DR, Knighton RW, Joos KM, Feuer WJ. Autoregulation of human optic nerve head circulation in response to increased intraocular pressure. *Exp Eye Res*. 1997;64:737-744.
26. Riva CE, Grunwald JE, Petrig BL. Autoregulation of human retinal blood flow: an investigation with laser Doppler velocimetry. *Invest Ophthalmol Vis Sci*. 1986;27:1706-1712.
27. Nagel E, Vilser W, Lanzl I. Age, blood pressure, and vessel diameter as factors influencing the arterial retinal flicker response. *Invest Ophthalmol Vis Sci*. 2004;45:1486-1492.