Regulation of Choroidal Blood Flow During Isometric Exercise at Different Levels of Intraocular Pressure

Alina Popa-Cherecheanu,1–3 Doreen Schmidl,1 René M. Werkmeister,4 Jacqueline Chua,5 Gerhard Garhöfer,1 and Leopold Schmetterer1,4–7

1Department of Clinical Pharmacology, Medical University of Vienna, Vienna, Austria
2Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
3Department of Ophthalmology, Emergency University Hospital, Bucharest, Romania
4Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Vienna, Austria
5Singapore Eye Research Institute, Singapore National Eye Centre, Singapore
6Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore
7Ophthalmology and Visual Sciences Academic Clinical Program, Duke-NUS Medical School, Singapore

Correspondence: Leopold Schmetterer, Department of Clinical Pharmacology, Medical University of Vienna, Waehringer Guertel 18-20, Vienna 1090, Austria; leopold.schmetterer@meduniwien.ac.at.
Submitted: June 11, 2018
Accepted: October 9, 2018

PURPOSE. There is evidence that choroidal blood flow (ChBF) is regulated in a complex way during changes in ocular perfusion pressure (OPP). We hypothesized that ChBF regulates better in response to changes in mean arterial pressure (MAP) than in intraocular pressure (IOP).

METHODS. Eighteen volunteers (mean age, 26 years) were recruited for a randomized, three-way crossover design. MAP was varied via isometric exercise. IOP was either kept normal or elevated by 10 or 20 mm Hg by using a suction cup. Subfoveal ChBF was measured continuously for 8 minutes with laser Doppler flowmetry and OPP was calculated as 2/MAP-IOP. For data analysis, values from all subjects were pooled according to either IOP or MAP values, and correlation analyses were done.

RESULTS. When data were grouped according to IOP, no correlation was observed between ChBF and MAP, but ChBF was lower the higher the IOP (P < 0.001). When data were grouped according to MAP, a significant correlation was found between ChBF and IOP (P < 0.001). When data were pooled according to IOP, the correlation between ChBF and OPP was weaker (P < 0.05). The OPP at which ChBF significantly increased from baseline was 61.3% ± 4.9% without suction cup, 65.2% ± 3.5% when IOP was increased by 10 mm Hg, and slightly lower when IOP was increased by 20 mm Hg (56.3% ± 4.8%, P = 0.07), but this effect did not reach the level of significance.

CONCLUSIONS. The present study provides further evidence that the regulation of ChBF during changes in OPP is controlled by complex mechanisms in humans and has less capacity to adapt to IOP elevation than to MAP increase.

Keywords: choroidal blood flow, ocular perfusion pressure, intraocular pressure, isometric exercise, laser Doppler flowmetry

There is evidence from several preclinical and clinical studies that blood flow regulation in the eye is dependent not only on the absolute levels of intraocular pressure (IOP) and systemic blood pressure (BP).1–6 This is in contradiction to the classical view of blood flow regulation where vascular tone in the resistance vessels is regulated only by the absolute value of OPP.7

OPP in a vascular bed is defined as the difference between arterial and venous pressure. In contrast to other vascular beds, venous pressure in the eye can reach high levels when IOP is elevated. This is because the venous pressure of the eye has to be slightly above the IOP in order to maintain sufficient outflow of blood.8 Whereas in healthy subjects venous pressure is only slightly higher than IOP, venous pressure can far exceed IOP in at least a subgroup of glaucoma patients.9 The relation between systemic arterial BP and glaucoma prevalence is also complex, showing a J-shaped dependence.10–12 As such there is considerable interest in the regulation of blood flow during changes in BP and IOP.

In humans, investigation of blood flow regulation is difficult, because of the challenges in measuring blood flow. Most studies have either used laser Doppler flowmetry (LDF)13–23 or laser Speckle flowgraphy (LSFG)24–27 for measuring choroidal perfusion. In addition, experimental modification of BP and IOP is difficult and such modifications often result not only in BP or IOP change, but also in other effects such as activation of the sympathetic and parasympathetic system. As such, the procedure that is used to manipulate IOP and BP may affect the blood flow response, particularly in a richly innervated vascular bed such as the choroid. In our previous study,7 we have used a period of isometric exercise during which we have induced a stepwise increase in IOP, using a suction cup. In this study we observed that the choroidal blood flow (ChBF) was independent of mean arterial pressure (MAP) when grouped according to IOP levels. In a subsequent study we followed the same
protocol but measured optic nerve head blood flow instead of ChBF. We reported that blood flow only becomes independent of MAP at high IOP levels above 25 mm Hg, which is consistent with the classical model of blood flow regulation. In addition, we observed that in this study variability of blood flow data was less pronounced than in the ChBF study. As such we were looking for a different experimental paradigm to obtain more reproducible data when IOP and MAP were changed concurrently. This was done in an effort to investigate whether ChBF is independent of MAP when grouped according to IOP under different experimental conditions.

In the present study, we therefore used a different approach, by presetting the IOP to a fixed level and thereafter beginning isometric exercise. Pilot data showed that this approach provides more reproducible data than our previous experimental paradigm (data not shown). We hypothesized that during this entire regimen, ChBF is dependent not only on OPP, but also on absolute values of IOP and BP.

Materials and Methods

The present study was performed according to the Declaration of Helsinki and the guidelines of Good Clinical Practice. The study protocol was approved by the Ethics Review Board of the Medical University Vienna. All volunteers gave written informed consent before participation after the study procedures were explained in detail. A prestudy screening was performed within 1 month of the first study day, including medical history and physical examination, 12-lead electrocardiogram, and an ophthalmic examination. Subjects were not included in the present study if any abnormality was found during the screening process unless the investigators considered it to be clinically irrelevant.

Experimental Design

The study was performed in a randomized three-way crossover design. Subjects were asked to refrain from alcohol and caffeine for at least 12 hours before the trial day. Before the experiments subjects were asked to have at least 7 to 8 hours of sleep and a light breakfast. For each subject, 3 study days were scheduled. After 20 minutes of rest, baseline measurements were taken. ChBF was measured continuously for 3 minutes at baseline. Thereafter three different IOP levels were adjusted on the 3 study days (basal IOP or suction with either 100 mm Hg corresponding to an elevation of approximately 10 mm Hg or suction with 200 mm Hg corresponding to an elevation of approximately 20 mm Hg). These levels of IOP were kept for 8 minutes. During the last 6 minutes an additional squatting period was performed. ChBF was recorded continuously throughout these experiments. After completion of these experiments a 60-minute resting period was scheduled. Thereafter the procedure described above with squatting and concomitant suction cup application was repeated and IOP was measured in 2-minute intervals instead of measuring ChBF. Systemic BP and pulse rate (PR) were recorded every minute. A break of at least 2 days was scheduled between the 3 study days.

Measurements

Systemic Hemodynamics. Systolic blood pressure, diastolic blood pressure, and MAP were measured on the upper arm by using an automated oscillometric device. The same device was used to measure PR based on finger pulse oximetry (HP-CMS patient monitor; Hewlett Packard, Palo Alto, CA, USA).

Laser Doppler Flowmetry. The measurement of subfoveal ChBF using LDF has been described in detail previously. With this technique the tissue is illuminated with coherent laser light that is scattered by moving red blood cells as well as nonmoving tissue structures. This leads to a broadening of the power spectrum because of Doppler shift and randomization of light directions. From the theory of light scattering in tissue, the velocity, the volume, and the flow (ChBF) of red blood cells can be determined. In the present study we used a compact LDF that has been previously shown to provide adequate reproducibility. All data were corrected for changes in direct current as described by Pemp and coworkers.

Intraocular Pressure. A Goldmann application tonometer mounted on a slit lamp was used for measuring IOP after instillation of 0.4% benoxinate hydrochloride combined with 0.25% sodium fluorescein.

Suction Cup Method. The IOP was elevated as described by Ulrich and Ulrich. An automatic suction pump connected by plastic tubing to a rigid plastic suction cup was placed on the temporal sclera. The anterior edge of this suction cup was placed at least 1 mm from the limbus. The vacuum was either set to 100 mm Hg to target an IOP increase of 10 mm Hg on one study day or to 200 mm Hg to target an IOP increases of 20 mm Hg on another study day.

Data Analysis

Ocular perfusion pressure was calculated as OPP = 2/3 MAP - IOP. The time course of the outcome parameters during squatting and/or suction application was analyzed by using a repeated measures ANOVA model to test for time and group interactions using the absolute data. Planned comparisons were used for post hoc testing. ChBF and OPP values were expressed as percentage change from baseline (%X) for further analysis. To obtain information on the MAP-ChBF relationship during combined changes in IOP and systemic arterial blood pressure, all values from all subjects were pooled independently of the time point of measurement. The data were independently analyzed for the 3 study days and as such divided into three groups of MAP and ChBF data pairs. To evaluate the IOP-ChBF relationship, data were sorted according to ascending MAP values and divided into three groups of IOP and ChBF data pairs. The first group included IOP and ChBF data pairs evaluated during MAP < 95 mm Hg, the second group during 95 mm Hg < MAP ≤ 115 mm Hg, and the third group during MAP > 115 mm Hg. Linear regression analysis was performed separately for each group. For this analysis the first value before any intervention was used as baseline. To assess significant differences between the groups, an ANOVA model was used and the slopes of the regression lines were compared.

To gain information on the OPP-ChBF relationship during isometric exercise, the data were analyzed for the 3 study days separately. The relative OPP values as obtained during isometric exercise were sorted according to ascending values as described previously. For this analysis the value as obtained before isometric exercise (2-3 minutes) when the suction cup was already in place was used for analysis. In the present study 18 healthy subjects participated. Given that for each subject six values were obtained, 108 OPP-ChBF values were used for analysis. Data were then pooled into nine groups for the OPP-ChBF relationship, each consisting of 12 values. The OPP level at which ChBF increased significantly over baseline was defined when the 95% confidence interval did not overlap with the baseline ChBF value.

A P value below 0.05 was used as significance level. Statistical analysis was performed by using CSS Statistica for Windows (Version 6.0; Statsoft, Inc., Tulsa, CA, USA).
RESULTS

Demographics

Nine healthy male and nine healthy female subjects were included. The experiments were completed in all subjects as scheduled. The mean age of the subjects was 26.1 ± 3.9 years.

The Table summarizes the baseline values of the outcome parameters on the 3 study days.

### Table. Demographic and Baseline Characteristics of the Participating Subjects (Mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mm Hg</td>
<td>74 ± 7</td>
<td>76 ± 7</td>
<td>72 ± 6</td>
</tr>
<tr>
<td>PR, beats/min</td>
<td>71 ± 9</td>
<td>70 ± 11</td>
<td>75 ± 12</td>
</tr>
<tr>
<td>IOP, mm Hg</td>
<td>15 ± 2</td>
<td>13 ± 2</td>
<td>13 ± 2</td>
</tr>
<tr>
<td>OPP, mm Hg</td>
<td>37 ± 6</td>
<td>38 ± 7</td>
<td>36 ± 7</td>
</tr>
<tr>
<td>ChBF, arbitrary units</td>
<td>34 ± 16</td>
<td>37 ± 14</td>
<td>34 ± 17</td>
</tr>
</tbody>
</table>

Ocular Perfusion Pressure

The results are summarized in Figure 1. As expected the respective increase in IOP on the study days, using the suction cup, was close to 10 mm Hg and to 20 mm Hg. During the 8 minutes of suction cup application the IOP remained relatively stable. Isometric exercise induced a significant increase in MAP (between +52% and +54%) and PR (+56% to +61%) during the squatting periods on the different study days (P < 0.001 each versus baseline). The increase in MAP was not significantly different between the 3 study days (P = 0.56). These changes are also mirrored in the relative changes in OPP as presented in Figure 1. During the day without suction cup, OPP showed an increase during the period of isometric exercise. When IOP was elevated by 10 mm Hg, OPP initially dropped by −22.6% (P < 0.001 versus baseline), but then increased by 45.8% (P < 0.001 versus baseline) by the end of the study period. When IOP was increased by 20 mm Hg, the decrease in OPP was more pronounced (−51.3%; P < 0.001 versus baseline), but by the end of the study period OPP was higher than at baseline (25.1%; P < 0.001 versus baseline).

Choroidal Blood Flow

Isometric exercise induced an increase in ChBF when IOP was normal (+15.1% versus baseline; P < 0.001). This increase was, however, less pronounced than the increase in OPP, indicating ChBF regulation. When IOP was raised by 10 mm Hg this was accompanied by a decrease in ChBF (−10.3%, P < 0.001) before isometric exercise. Thereafter ChBF continuously increased during isometric exercise. At the end of the experiment ChBF was not different from baseline (4.8%, P = 0.44). When IOP was increased by 20 mm Hg, the initial drop in ChBF was more pronounced (−28.4%, P < 0.001). Although ChBF increased continuously during isometric exercise, ChBF still tended to be lower than at baseline after 8 minutes (−9.3%; P = 0.15) although OPP was elevated by more than 25%.

Relationship Between MAP, IOP, and Choroidal Blood Flow

The MAP-ChBF relationship on the 3 study days is presented in Figure 2. Regression lines of the three groups were almost parallel and showed a small but significant association (day without IOP increase: slope = 0.25, r = 0.27, P < 0.001; day with IOP increase of 10 mm Hg: slope = 0.28, r = 0.25, P < 0.001; day with IOP increase of 20 mm Hg: slope = 0.31, r = 0.43, P < 0.001). The slope of the regression lines was not significantly different between the different IOP levels (P = 0.68).
0.72). On the day without IOP increase the average change in ChBF during isometric exercise versus baseline was $+2.6\% \pm 16.6\%$; on the day with IOP increase of 10 mm Hg, $-7.1\% \pm 16.3\%$; and on the day with IOP increase of 20 mm Hg, $-20.2\% \pm 13.8\%$. These differences were statistically significant between the study days ($P < 0.001$), indicating that ChBF is influenced by the absolute level of IOP. In Figure 3 the IOP-ChBF relationship is shown when data are categorized according to MAP. In all three groups a significant negative association was observed that was more pronounced than the association between MAP and ChBF shown in Figure 2. Again regression lines of the three groups were almost parallel (lowest MAP values: slope $=-1.21$, $r = -0.58$, $P < 0.001$; medium MAP values: slope $=-1.08$, $r = -0.53$, $P < 0.001$; highest MAP values: slope $=-1.49$, $r = -0.65$, $P < 0.001$) with no significant differences between the slopes ($P = 0.67$).

**Choroidal Pressure-Flow Relationship**

The OPP-ChBF relationship during isometric exercise periods is presented in Figure 4. There was no significant difference between the curves at the different levels of IOP ($P = 0.56$). The OPP value at which ChBF significantly increased from baseline level was not significantly different between the day without suction cup (61.3% ± 4.9%) and the day when IOP was increased by 10 mm Hg (65.2% ± 3.5%, $P = 0.32$). On the study day when IOP was increased by 20 mm Hg, the OPP values at which ChBF increased from baseline were slightly lower (56.3% ± 4.8%, $P = 0.07$), but this effect did not reach the level of significance.

**DISCUSSION**

We investigated the regulation of ChBF during combined changes in systemic BP and IOP. Our data support our previous studies that perfusion in the choroid in response to changes of OPP is dependent on the absolute values of BP and IOP. In particular, our data indicated that at the same level of OPP, ChBF is negatively correlated to IOP. Moreover, the data as presented in Figures 2 and 3 indicate that ChBF is less sensitive to changes in BP than in IOP.

There are some similarities but also important differences between the present data and our previously published observations, in which we used a different protocol to induce changes in MAP and IOP. With both protocols it was found that ChBF is strongly dependent on IOP, and Figures 2 and 3 confirm that the regulatory capacity of the choroid in response to an increase in IOP is limited. Nevertheless some degree of regulatory ability is seen because the percentage change in ChBF is smaller to the percentage change in OPP during these conditions. In contrast, MAP-induced changes in ChBF do differ between the present study and our previous experiments. In the latter study, ChBF values are independent of MAP when
grouped according to IOP. The present study identified a small but significant dependence of ChBF values on MAP after grouping according to IOP. The reason behind this is not entirely clear and requires further investigation. One difference between the two data sets is that the present values show less scattering. This is also obvious when calculating the coefficient of determination for the MAP-ChBF relationship from the two studies. In the present study, coefficient of determination was between 0.06 and 0.18 at different levels of IOP, whereas it is as low as 0.0001 to 0.0016 in our previous study. This indicates that perfusion and its regulation in the human choroid is highly modulated. Nowadays a wide variety of data support this concept, including several studies in humans. The present study has several limitations that need to be addressed. Since the LDF technique used in the present study does not allow for scanning, only the subfoveal ChBF was measured. In addition, the fovea does not contain any retinal vessels and a contribution from the retinal vasculature to the Doppler shift power spectrum can be excluded. When techniques like LDF or LSFG are, however, used at other retinal locations both retinal and choroidal vessels will contribute to the signal. Little is known about local regulation of ChBF in response to changes in OPP. In humans the number of noninvasive interventions that can be used to alter IOP is limited. Even though isometric exercise and suction cup are well tolerated in general, it is difficult for the participating subjects to fixate for the entire 8 minutes study period. As such, data may be less reproducible than with protocols where only MAP is increased. We tried to overcome this problem by correcting for changes in direct current.

In conclusion the present study supports the previous evidence that ChBF regulation in response to changes in OPP is complex and depends on the absolute levels of MAP and IOP. Blood flow is less sensitive to exercise-induced increase in MAP than to suction cup-induced elevation in IOP. This indicates that perfusion and its regulation in the human choroid is highly sensitive to the level of IOP.

Acknowledgments
Supported by Austrian Science Fund (Fonds zur Förderung der wissenschaftlichen Forschung), Project No. KLIF 340.

Disclosure: A. Popa-Cherecheanu, None; D. Schmidl, None; R.M. Werkmeister, None; J. Chua, None; G. Garhöfer, None; L. Schmetterer, None

References
6. Wang L, Cull GA, Fortune B. Optic nerve head blood flow response to reduced ocular perfusion pressure by alteration...


