Retinal Blood Flow in Healthy Young Subjects

Gerhard Garbofer,¹, Rene Werkmeister,² Nikolaus Dragostinoff,² and Leopold Schmetterer¹,²

PURPOSE. To characterize total retinal blood flow in a group of healthy subjects.

METHODS. Included in this study were 64 healthy volunteers. Retinal venous diameters were measured using a dynamic vessel analyzer. Retinal blood velocities were measured using bidirectional laser Doppler velocimetry. All vessels with a diameter of >60 μm entering the optic nerve head were measured. Total retinal blood flow was measured by summing up all data from the individually measured vessels. In a subgroup of 10 healthy subjects measurements were also taken from the arterioles, and results obtained for total retinal blood flow as measured both from retinal venules and from retinal arterioles were compared.

RESULTS. Total retinal blood flow was 44.0 ± 13.3 μL/min. Retinal blood flow was highest in the temporal inferior quadrant, followed by the temporal superior quadrant, the nasal inferior quadrant, and the nasal superior quadrant (P < 0.001 each). In all quadrants retinal blood velocities were linearly correlated to vessel diameters. Retinal blood flow as measured in retinal venules (42.1 ± 13.0 μL/min) and in retinal arterioles (45.3 ± 12.1 μL/min) was similar (P = 0.16).

CONCLUSIONS. The present study provides reference values for total retinal blood flow in 64 healthy subjects. The interindividual variability in retinal blood flow is high, making it unlikely that individual diagnostics can be based on measurements of retinal blood flow. Total retinal blood flow, however, may be important in risk stratification, which needs to be proven in future studies.

The inner retina is supplied by the retinal circulation. In recent years our knowledge on the perfusion of the retinal vascular bed has significantly improved and excellent overview publications on this topic are available.¹⁻³ Nevertheless, quantifying retinal blood flow is a difficult task. More than 25 years ago Riva and colleagues⁴ presented a method of measuring total retinal blood flow in absolute units. This approach is based on measuring vessel diameters from fundus photographs and retinal blood velocities using bidirectional laser Doppler velocimetry (LDV). If all vessels entering the optic nerve head were measured, absolute values of total retinal blood flow could be measured.

In their original publication, Riva and colleagues⁴ have shown that there is a good agreement between total blood flow values as measured from arterial and venous branch vessels relying on data from 12 healthy subjects. Given that the retina is an end organ, this indicates the validity of the technique. Nevertheless, this approach has later been used in only a limited number of studies because the procedure is time-consuming and requires significant observer experience as well as cooperation from the subject under study.

We set out to measure retinal blood flow in 64 healthy volunteers using this technique. This was done in an effort to characterize the physiology of retinal perfusion in more details, to look into the range of total retinal blood flow as it is observed in healthy young subjects, and to provide reference values for new methods aiming to measure total retinal blood flow.

METHODS

Research Design and Subjects

The study protocol was approved by the Ethics Committee of the Medical University of Vienna and followed the guidelines set forth in the Declaration of Helsinki. In all, 64 healthy male volunteers between 18 and 45 years of age were included in this study. All subjects signed written informed consent and passed a screening examination before the study day, including physical examination, assessment of visual acuity, slit-lamp biomicroscopy, funduscopy, and measurement of intraocular pressure (IOP). Exclusion criteria were ametropia ≥ 3 diopters, other ocular abnormalities and any clinically relevant illness, smoking, blood donation, or intake of a medication, a vitamin, or a mineral supplement in the 3 weeks before the study. Participants had to abstain from beverages containing alcohol or caffeine for 12 hours before the study day.

Protocol

After instillation of a single drop of tropicamide (mydriaticum, Agepha-Augentropfen eyedrops, 5 mg/mL tropicamide; Agepha GmbH, Vienna, Austria) into the study eye and after a resting period of at least 20 minutes, retinal blood flow was assessed by combining measurements of retinal vessel diameters and retinal blood velocities in all visible venules entering the optic nerve head. In a subgroup of 10 healthy subjects measurements were also taken from all retinal arterioles entering the optic nerve head. First, measurements of retinal vessel diameters were done with a dynamic vessel analyzer (DVA), which took approximately 5 to 10 minutes. After completion of these measurements an image obtained with the DVA was printed to guide measurements with the laser Doppler velocimeter. Thereafter, measurements with the laser Doppler velocimeter were done. Measurement times were variable between 15 and 45 minutes. During the whole study period blood pressure was measured in 5-minute intervals and pulse rate was monitored continuously to ensure stable hemodynamic conditions.

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Mean blood pressure, mm Hg 89.8
Diastolic blood pressure, mm Hg 70.6
Systolic blood pressure, mm Hg 128.2
Sex, M/F 40/24
Age, y 31.8

The diameters of retinal arterioles and venules within one to two disc diameters from the center of the optic disc were measured in mydriasis using the DVA (Imedos GmbH, Jena, Germany). This system comprises a fundus camera (FF450; Carl Zeiss Meditec AG, Jena, Germany), a high-resolution digital video camera, and a personal computer with analyzing software. To determine retinal vessel diameters, recorded images were digitized and analyzed in real time, with a frequency of 50 Hz. Retinal irradiance during measurements was approximately 150 μW · cm⁻². The system provided excellent reproducibility and sensitivity. After selection of the measurement location the DVA was able to follow the vessels during movements within the measurement window. In the present study vessels < 60 μm in diameter were not included.

**Dynamic Vessel Analyzer**

The diameters of retinal arterioles and venules were measured in mydriasis using the DVA (Imedos GmbH, Jena, Germany). This system comprises a fundus camera (FF450; Carl Zeiss Meditec AG, Jena, Germany), a high-resolution digital video camera, and a personal computer with analyzing software. To determine retinal vessel diameters, recorded images were digitized and analyzed in real time, with a frequency of 50 Hz. Retinal irradiance during measurements was approximately 150 μW · cm⁻². The system provided excellent reproducibility and sensitivity. After selection of the measurement location the DVA was able to follow the vessels during movements within the measurement window. In the present study vessels < 60 μm in diameter were not included.

**Statistical Analysis**

All data are given as mean ± SD. Retinal blood flow in the four quadrants was compared using ANOVA and planned comparisons were used for post hoc analysis. In the 10 subjects in which total retinal blood flow was measured in both arterioles and venules, a paired t-test was used to compare the data. In addition, a Bland-Altman graph was plotted to characterize the association between the two methods. For the largest venules in each quadrant a linear correlation between blood velocities and vessel diameters was performed. This was done separately for the temporal superior, the temporal inferior, the nasal superior, and the nasal inferior quadrant. Fisher’s r to z transformation was used to calculate the significance of the difference between two correlation coefficients. The association between retinal blood flow and MAP, IOP, and OPP was also calculated by linear correlation analysis. A value of P < 0.05 was set as the level of significance.

**RESULTS**

Patient’s characteristics are presented in Table 1. Although all subjects were young and healthy and had normal blood pressures and IOPs, the range of retinal blood flows was extremely wide. Retinal blood flow data as shown in Table 1 were taken from either 4 venules (n = 4), 5 venules (n = 13), 6 venules (n = 18), 7 venules (n = 17), or 8 venules (n = 12). Table 2 presents the data according to the number of vessels that were studied. ANOVA analysis revealed that the total retinal blood flow values were not dependent on the number of vessels studied (P = 0.45). As mentioned earlier, vessels with a diameter of <60 μm were not included due to methodologic problems. In 24 subjects this was not the case, whereas in 13 subjects 1 visible vessel was not measured, in 15 subjects 2 vessels were not measured, and in 12 subjects 3 vessels were not measured. As shown in Table 3 the values of total retinal blood flow, however, did not depend on the number of vessels that were excluded from measurement (P = 0.93). Table 4 summarizes retinal blood flow according to the four quadrants of the fundus. Retinal blood flow was highest in the temporal inferior quadrant, followed by the temporal superior quadrant, the nasal inferior quadrant and the nasal superior quadrant (P < 0.001, ANOVA). Post hoc analysis revealed that retinal blood flow was higher in the temporal inferior quadrant than in the temporal superior quadrant (P < 0.001 post hoc analysis).

**TABLE 2. Total Retinal Blood Flow According to the Number of Vessels Studied (n = 64)**

<table>
<thead>
<tr>
<th>Number of Vessels Studied</th>
<th>Retinal Blood Flow* (μL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 vessels (n = 4)</td>
<td>34.9 ± 8.4 (29.3–47.2)</td>
</tr>
<tr>
<td>5 vessels (n = 13)</td>
<td>48.5 ± 14.9 (21.1–80.8)</td>
</tr>
<tr>
<td>6 vessels (n = 18)</td>
<td>42.0 ± 13.3 (23.5–72.5)</td>
</tr>
<tr>
<td>7 vessels (n = 17)</td>
<td>45.2 ± 14.6 (23.0–68.0)</td>
</tr>
<tr>
<td>8 vessels (n = 12)</td>
<td>43.3 ± 10.5 (26.6–64.5)</td>
</tr>
</tbody>
</table>

* Data are presented as mean ± SD (range).
Table 3. Total Retinal Blood Flow (RBF) According to the Number of Vessels with a Diameter of <60 µm that Were Not Studied (n = 64)

<table>
<thead>
<tr>
<th>Number of Vessels Not Studied</th>
<th>RBF* (µL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 vessels not studied (n = 12)</td>
<td>44.6 ± 13.7 (29.3–63.7)</td>
</tr>
<tr>
<td>2 vessels not studied (n = 15)</td>
<td>41.9 ± 16.8 (23.5–80.8)</td>
</tr>
<tr>
<td>1 vessel not studied (n = 13)</td>
<td>44.1 ± 11.0 (30.3–64.4)</td>
</tr>
<tr>
<td>All vessels studied (n = 24)</td>
<td>44.9 ± 12.9 (23.0–68.0)</td>
</tr>
</tbody>
</table>

* Data are presented as mean ± SD (range).

DISCUSSION

The present study is by far the largest study quantifying total retinal blood flow in a group of healthy young subjects. Generally, our results are in the same range as those observed in previous studies that measured total retinal blood flow. In their previous studies that measured total retinal blood flow, in a group of healthy young subjects. Gen-

must keep in mind that Riva and colleagues used a factor of 1.6 instead of 2 when converting Vmax to Vmean. It appears, however, based on measurements of velocity profiles in human retinal vessels that a factor of 2 is more appropriate. The reason for the differences in total retinal blood flow, as measured in different studies, is unknown and requires further attention. Generally two types of bidirectional LDV systems for the measurement of red blood flow have been commercialized. Whereas the system applied in the present study (Oculix Sarl, Arbaz, Switzerland) uses the principle to analyze the Doppler shifts of the back-scattered light in two distinct directions, the Canon system illuminates the vessels with laser light from two directions. To solve the issue that different absolute values have been reported comparative measurements using the two systems may be required.

The observation that retinal blood flow to the temporal side of the retina is much higher than that to the nasal part is in good agreement with previous studies. This was observed for measurements in retinal venules as well as for measurements in retinal arterioles. Obviously, this may be related to the number of retinal ganglion cells that need to be nourished. Interpreting these results, however, one needs to keep in mind that the larger vessels studied in our experiments do not necessarily supply capillaries in the same quadrant only and that all blood that is supplied through one specific arteriole is not necessarily drained through an adjacent venule. An interesting observation of the present study is the difference in correlations between blood velocities and vessel diameters in superior and inferior vessels. Since this was found for both nasal and temporal vessels, we deem it unlikely to be a chance finding. Neither the reason nor the physiologic basis for this behavior is known. A correlation as seen in the inferior venules would be expected from Murray’s law, which proposes that flow should vary with D³ in the vascular bed in which it seeks an optimum compromise between blood volume and vascular resistance. Murray’s law predicts the vessel radius that requires minimum expenditure of energy by a vascular bed. In larger vessels the pressure drop in the vessels reduces with increasing diameter according to the Hagen–Poiseuille’s law and flow varies with D⁴. Murray’s law, however, includes the best-recognized minimum dissipation principle, in which the metabolic consumption in a single vessel segment is proportional to the blood volume. Whether this means that the physiologic behavior of the superior and inferior retinal vasculature is different remains unknown. To the best of our knowledge no differences in blood flow regulation between the inferior and the superior retina have been previously reported.

The validity of data is supported by the relatively good association between retinal blood flow values as assessed in all arterioles and retinal blood flow values as assessed in all venules. The Bland–Altman plot presented in Figure 2 indicates that there is almost no difference between these measurements and that the differences rather arise from random errors. This is in good agreement with the data of Riva and colleagues, who also found a good agreement between total retinal blood flow measured on the arterial and venous side.

Table 4. Retinal Blood Flow (RBF) in the Four Quadrants (n = 64)

<table>
<thead>
<tr>
<th>Quadrant</th>
<th>Mean ± SD (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBF temporal superior, µL/min</td>
<td>12.2 ± 7.0 (3.6–34.0)</td>
</tr>
<tr>
<td>RBF temporal inferior, µL/min</td>
<td>17.9 ± 7.1 (5.8–33.9)</td>
</tr>
<tr>
<td>RBF nasal superior, µL/min</td>
<td>6.5 ± 2.2 (2.5–12.8)</td>
</tr>
<tr>
<td>RBF nasal inferior, µL/min</td>
<td>7.3 ± 2.0 (3.4–13.4)</td>
</tr>
</tbody>
</table>
The interindividual variability in measurements was high. As such, it is doubtful whether such measurements can be used on an individual diagnostic level. It seems more attractive to use such data for risk stratification. This has been extensively done for measurements of retinal vessel diameters as discussed only recently in several excellent review publications.\textsuperscript{17–19} In the present study retinal vessel diameters were measured using the DVA. Compared with previous methods for assessing retinal vessel caliber based on fundus photography, this provides a significant improvement.\textsuperscript{6} An unsolved problem with all techniques measuring retinal vessel diameters, however, is the correction for refractive error and eye length.\textsuperscript{6} We have limited the influence of this problem in the present study by including only subjects with refractive errors of $<1$ diopters.

As can be seen from Figure 3 there was no association between retinal blood flow and OPP. This may also be expected for an autoregulated vascular bed.\textsuperscript{20} Autoregulation of retinal blood flow has been documented in a large variety of previous studies during both an increase and a decrease in OPP.\textsuperscript{1,21–25}

A number of limitations have to be considered in interpreting the present results. LDV is a time-consuming procedure and requires very good fixation abilities from the subject under study. This is particularly true if all venules entering the optic nerve head are measured. As such, only subjects with excellent fixation abilities were included. In addition, one needs to consider that only vessels with a diameter $<60\mu m$ were included for our measurements. A total of 73 visible vessels were not measured accordingly, but in none of the subjects there were more than three small vessels that were not measured. Based on the correlation graphs presented in Figure 2 one would assume that blood flow through a $60\mu m$ retinal venule is approximately 1 $\mu L/min$. As such, the maximum error should not exceed 3 to 4 $\mu L/min$. Analysis of the present data does not indicate that the number of included vessels is responsible for the wide interindividual variability because

![Figure 1](image_url)

**Figure 1.** Linear correlation analysis between mean blood velocities and vessel diameters for the largest venules in each quadrant ($P<0.001$ each). Data are shown separately for the nasal inferior, nasal superior, temporal inferior, and temporal superior quadrant ($n=64$).

<table>
<thead>
<tr>
<th>Subject</th>
<th>$Q_V$ (µL/min)</th>
<th>$Q_A$ (µL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29.3</td>
<td>32.1</td>
</tr>
<tr>
<td>2</td>
<td>47.2</td>
<td>46.4</td>
</tr>
<tr>
<td>3</td>
<td>29.4</td>
<td>31.7</td>
</tr>
<tr>
<td>4</td>
<td>33.8</td>
<td>36.2</td>
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<tr>
<td>5</td>
<td>63.7</td>
<td>60.6</td>
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<tr>
<td>6</td>
<td>40.4</td>
<td>43.2</td>
</tr>
<tr>
<td>7</td>
<td>60.9</td>
<td>61.9</td>
</tr>
<tr>
<td>8</td>
<td>33.0</td>
<td>37.1</td>
</tr>
<tr>
<td>9</td>
<td>51.8</td>
<td>54.7</td>
</tr>
<tr>
<td>10</td>
<td>31.7</td>
<td>29.3</td>
</tr>
</tbody>
</table>

Total RBF as measured in retinal venules ($42.1 \pm 13.0$ $\mu L/min$) was not significantly different from RBF as measured in retinal arterioles ($45.3 \pm 12.1$ $\mu L/min$; $P=0.16$).

Table 5. RBF (µL/min) as Measured in Retinal Venules ($Q_V$) and Retinal Arterioles ($Q_A$) in a Subgroup of 10 Healthy Subjects
neither the number of vessels studied (Table 2) nor the number of small vessels that were not included for measurements (Table 3) were related to the values obtained. Together with the good agreement between values, as obtained from the arteriolar and the venular site indicates that in the present study reproducibility of total retinal blood flow may even be higher, although it was not formally studied. One must not forget, however, that the subjects for the present study were selected based on excellent target fixation. Variability in retinal branch venule diameter measurements is less critical and was reported to be 1.6% using DVA measurements in our laboratory.26 Another critical issue arises from the fact that the laser beam has to hit the center of the vessel when LDV is used because, otherwise, the centerline velocity is underestimated.

Finally, one needs to consider that vessel diameter measurements were not corrected for magnification errors. Based on the method of Littmann (1982)27 and its modification by Bennett and colleagues (1994)28 one can estimate the error that is introduced by this limitation given that only subjects with ametropia $< 3$ diopters were included. As such, we calculated that the maximum error due to magnification errors is 4%.

As such, it is clear that the method used in this study is not feasible for clinical routine measurements. Only recently, however, have different techniques based on OCT been developed that may be capable of measuring retinal blood flow with acceptable speed and reproducibility.29–33 As such, the present data may be a valuable database for comparison. Careful validation of new techniques, however, is required before they can be used in clinical studies.

In conclusion the data of the present study indicate that reliable data on retinal blood flow can be obtained using combined LDV and vessel diameter measurement, although the technique is not suitable for clinical routine measurements. Because of the very high interindividual variability in total retinal blood flow values it is unlikely that retinal blood flow itself is of high diagnostic value. It may well be, however, that
such data can be used for risk stratification, which has to be proven in future studies.

References


