Retinal Oxygen Delivery and Metabolism in Healthy and Sickle Cell Retinopathy Subjects

Mahnaz Shahidi,1 Anthony E. Felder,2 Ou Tan,3 Norman P. Blair,4 and David Huang3

1Department of Ophthalmology, University of Southern California, Los Angeles, California, United States
2Richard and Loan Hill Department of Bioengineering, University of Illinois at Chicago, Chicago, Illinois, United States
3Casey Eye Institute, Oregon Health & Science University, Portland, Oregon, United States
4Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago, Chicago, Illinois, United States

PURPOSE. Reduction in inner retinal oxygen delivery (DO2) can cause retinal hypoxia and impair inner retinal oxygen metabolism (MO2), leading to vision loss. The purpose of the current study was to establish measurements of DO2 and MO2 in healthy subjects and test the hypothesis that DO2 and MO2 are reduced in sickle cell retinopathy (SCR) subjects.

METHODS. Dual wavelength retinal oximetry and Doppler optical coherence tomography were performed in 12 healthy control and 12 SCR subjects. Images were analyzed to measure retinal arterial and venous oxygen content (O2A and O2V), venous diameter (DV), and total retinal blood flow (TRBF). Retinal arteriovenous oxygen content difference (O2AV), DO2, MO2, and oxygen extraction fraction (OEF) were calculated according to the following equations: O2AV = O2A - O2V; DO2 = TRBF * O2A; MO2 = TRBF * O2AV; OEF = MO2/DO2.

RESULTS. Retinal DV and TRBF were higher in the SCR group as compared to the control group, whereas, O2A, O2V and O2AV were lower in SCR group as compared to the control group. MO2 and DV were linearly related, such that higher MO2 was associated with higher O2A. There was an inverse relationship between TRBF and OEF such that lower TRBF was associated with higher OEF.

CONCLUSIONS. Increased blood flow compensated for decreased oxygen content, thereby maintaining DO2, MO2, and OEF at predominately lower stages of SCR. Quantitative assessment of these parameters has the potential to advance knowledge and improve diagnostic evaluation of retinal ischemic conditions.

Keywords: oxygen metabolism, oxygen extraction fraction, blood flow, sickle cell retinopathy

The retinal tissue requires oxygen and nutrients to maintain normal function for visual processing. Reduction in inner retinal oxygen delivery (DO2) can cause retinal hypoxia and impair inner retinal oxygen metabolism (MO2), leading to vision loss. Therefore, it is imperative to develop methods for quantitative assessment of these key parameters under normal and retinal ischemic conditions. To this end, methods have been developed for assessment of MO2 in humans by combining spectrophotometry to measure retinal vascular oxygen saturation (SO2) with Doppler optical coherence tomography (OCT) to measure total retinal blood flow (TRBF).

Various approaches to quantify TRBF using Doppler OCT have been described previously.1 In order to integrate flow in a blood vessel sectioned on an OCT B-scan, the angle between the OCT beam and the direction of blood flow was obtained using double circular scans,2,3 resampled from volume scans,4,5 or created by dual-beam OCT.6 Alternatively, to minimize the error associated with angle determination, a dual-angle Doppler OCT, dual-beam OCT with a known angular offset between beams was used.6–11 Furthermore, to obviate the need for angle calculation, an en face Doppler OCT approach employs high-speed three-dimensional volumetric OCT and integrates flow across an en face section of the blood vessel. However, to measure the high axial blood velocity in the central retinal vein or artery, high-speed (200 kHz or higher) OCT systems were necessary.12–14 In order to apply the en face method on slower Doppler OCT systems (70–100 kHz), measurements were obtained on vessel branches with lower blood velocity using tilted planes15 or multiple en face planes.16

To date, calculated measurements of DO2 have not been reported in humans. Since DO2 is the product of TRBF and arterial oxygen content, TRBF alone provides only partial information about oxygen delivery to the retina. Similarly, MO2 is the product of TRBF and arteriovenous O2 difference,17 thus measuring either SO2 or TRBF individually would likely lead to incorrect conclusions with regard to MO2. Alternatively, MO2 can be expressed as the product of DO2 and the inner retinal oxygen extraction fraction (OEF).18,19 OEF quantifies how much of the oxygen available from the retinal vasculature is extracted by the retinal tissue for metabolism. Under physiologic or pathologic challenges, compensatory changes in DO2 and OEF respond in order to maintain MO2. Conversely, alterations in MO2 can play a role in regulating DO2. In healthy subjects, MO2 was measured under normoxia and hyperoxia.20,21 Furthermore, MO2 was shown to be reduced in subjects with diabetic retinopathy.22,23 However, MO2 has not been evaluated in sickle cell retinopathy (SCR), in which vision-threatening retinal capillary occlusions, nonperfusion, ische-
The purpose of the current study was to establish measurements of DO2 and MO2 in healthy subjects and test the hypothesis that DO2 and MO2 are reduced in SCR subjects.

**METHODS**

**Subjects**

The research study was approved by an institutional review board at the University of Illinois at Chicago. Prior to subject enrollment, the research study was explained to the subjects and informed consents were obtained according to the tenets of the Declaration of Helsinki. Twelve healthy control and 12 African American SCR subjects (eight with hemoglobin C disease, three with hemoglobin SS disease, and one with hemoglobin S-beta thalassemia disease) participated in the study. One, nine, and two subjects had retinopathy stages of 1, 2, and 3, respectively. The stages were defined according to their most severe feature: peripheral arteriolar occlusions (stage 1), peripheral arteriolar-venular anastomoses (stage 2), or neovascular/vascular proliferation (stage 3). Retinal arteriovenous oxygen content difference (O2AV) was determined by the following relation:

\[ O_{2AV} = O_{2A} - O_{2V} \]

where O2max is the oxygen-binding capacity of hemoglobin, Hgb is the hemoglobin concentration and hematocrit (HCT) were measured.

**Image Acquisition and Analysis**

Multimodal imaging was performed to derive DO2 and MO2 by measurements of TRBF and retinal vascular SO2. TRBF was measured using a commercially available 70-kHz Doppler OCT system (Avanti; Optovue Inc., Fremont, CA, USA) and a custom scan protocol that measured blood velocity in vein branches separately on multiple optimized en face planes. Three images covering a 2 × 2 mm area centered on the central retinal vein were acquired. Each image consisted of five consecutive volume scans. Each volume contained 80 B-scans within vein segments on the en face plane (Fig. 1). Veins were distinguished from arteries by the sign of the Doppler shift and direction of blood flow. TRBF was computed as the sum of flow in all individual detected retinal veins from different en face planes. To reduce measurement variability, the TRBF measurements from all volumes were averaged. A graded scale was used to divide volumes in which motion artifacts interfered with vein identification or flow calculation. Data were included from an average of nine volumes, range between 3 and 15.

For vascular SO2 measurements, dual wavelength (570 and 606 nm) retinal oximetry was performed using our custom-built slit lamp biomicroscope and previously published method. At each imaging wavelength, nine retinal images were acquired in a 5 × 5-mm area centered on the optic disk. An example of a retinal image acquired at 570 nm in a healthy control subject is shown in Figure 1. Retinal images were analyzed using a custom software written in MATLAB (Mathworks, Natick, MA, USA) to measure vessel diameter and SO2 in all retinal vessels within a circumpapillary region of interest extending between one and two disk radii from the perimeter of the optic disk (Fig. 1). Vessel diameter was determined by calculating the full width at half maximum of intensity profiles perpendicular to the vessel centerlines. At each imaging wavelength, nine retinal images were exported and a customized software was written in MATLAB (Mathworks, Natick, MA, USA) to analyze the Doppler phase-resolved images covering a 23-mm region (middle image). Blood flow was measured in the detected veins from different en face planes, projected here in one en face plane. (Right) En face OCT image of the optic nerve head acquired in the same subject.

**Statistical Analysis**

Compiled retinal oxygen metrics (DV, TRBF, O2A, O2V, O2AV, DO2, MO2, OEF), demographic, and systemic physiologic data were compared between groups using unpaired t-tests. Linear regression analysis was performed to determine the significance of associations between metrics. Statistical analyses were performed using statistical software (SSPS Statistics, version 24; IBM Armonk, New York, USA). Statistical tests were two-sided and significance was accepted at \( P \leq 0.05 \).

**RESULTS**

Age of control and SCR subjects were 41 ± 15 years and 38 ± 14 years (mean ± standard deviation), respectively (\( P = 0.62 \)). MAP of control and SCR subjects was 95 ± 10 mm Hg and 86 ± 12 mm Hg, respectively (\( P = 0.06 \)). HCT of SCR subjects (33% ± 7%) was lower than that of control subjects (45% ± 6%) (\( P < 0.001 \)).

The Table lists retinal oxygen metrics in control and SCR groups. Retinal DV and TRBF were higher in the SCR group as compared to the control group. O2A, O2V, and O2AV were lower in the SCR group as compared to the control group. DO2, MO2, and OEF were not significantly different between control and SCR groups. As shown in Figure 2, there was a linear relationship between MO2 and DO2, such that higher MO2 was associated with higher DO2 (\( r = 0.67; P < 0.001; N = 24 \)). As shown in Figure 3, lower TRBF was associated with higher OEF, with a significant linear fit to the data (\( r = -0.55; P = 0.005; N = 24 \)).
**TABLE.** Comparison of Retinal Oxygen Metrics Between Control (N = 12) and SCR (N = 12) Groups

<table>
<thead>
<tr>
<th>Oxygen Metric</th>
<th>Control</th>
<th>SCR</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dv, µm</td>
<td>97 ± 9</td>
<td>117 ± 18</td>
<td>0.004</td>
</tr>
<tr>
<td>TRBF, µL/min</td>
<td>48 ± 16</td>
<td>69 ± 27</td>
<td>0.03</td>
</tr>
<tr>
<td>O₂A, mL O₂/dL</td>
<td>19 ± 4</td>
<td>15 ± 4</td>
<td>0.008</td>
</tr>
<tr>
<td>O₂V, mL O₂/dL</td>
<td>15 ± 2</td>
<td>10 ± 2</td>
<td>0.005</td>
</tr>
<tr>
<td>O₂AV, mL O₂/dL</td>
<td>7 ± 2</td>
<td>5 ± 2</td>
<td>0.06</td>
</tr>
<tr>
<td>DO₂, µL O₂/min</td>
<td>9.0 ± 3.0</td>
<td>9.7 ± 2.4</td>
<td>0.52</td>
</tr>
<tr>
<td>MO₂, µL O₂/min</td>
<td>3.1 ± 1.2</td>
<td>3.0 ± 1.2</td>
<td>0.88</td>
</tr>
<tr>
<td>OEF</td>
<td>0.34 ± 0.06</td>
<td>0.32 ± 0.10</td>
<td>0.39</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation. Bold type indicates statistical significance at P < 0.05.

**DISCUSSION**

The feasibility of our methodology to quantitatively measure retinal DO₂ and MO₂ in humans was demonstrated. In control subjects, diameter measurements in major retinal veins were in agreement with previously reported values. Similarly, TRBF was consistent with previously published values. SO₂ measurements in retinal arteries and veins were in agreement with values obtained from previously published studies. MO₂ measurements obtained in the present study were similar to those reported previously in control subjects. Of note, MO₂ depends on both the tissue consumption rate and the amount of tissue supplied by the retinal circulation. In the current study, the amount of tissue supplied in healthy and SCR subjects was likely similar, thus MO₂ is indicative of oxygen consumption rate. However, under certain conditions, such as hyperoxia, a portion of the inner retina may be supplied by the choroidal circulation, thus MO₂ can decrease even if the consumption per amount of tissue is unchanged. Although measurements of TRBF and SO₂ have been previously published in human subjects, we report here, for the first time to our knowledge, calculated values of DO₂. Moreover, OEF values derived from direct measurements of MO₂ and DO₂ indicate approximately 35% of the available oxygen is metabolized by the retinal tissue under normal physiological condition, which is consistent with previous OEF values obtained by oximetry alone.

As expected, blood O₂ contents of retinal arteries and veins were reduced in SCR subjects due to the lower systemic HCT. The observed vasodilation and increased TRBF in SCR suggests a regulatory mechanism to compensate for the reduced O₂ contents. The finding of increased TRBF is consistent with a recent study that reported increased retinal arterial and venous flow velocities measured by retinal function imaging. However, a previous study showed blood velocity is decreased in central retinal artery, but increased in the ophthalmic artery of sickle cell patients with no retinopathy. A previous study in subjects with Eisenmenger syndrome, a cardiac condition resulting in systemic hypoxia, showed arterial and venous SO₂ difference was not altered; however, no information about changes in MO₂ was reported since TRBF was not measured.

In the current study, SO₂ was not corrected for oxygen loss across the vessel walls and not weighted by blood flow in each vessel. Furthermore, for calculation of the blood O₂ content, the low level of fraction of dissolved oxygen was not included. Nevertheless, the values of arterial and venous O₂ contents obtained in control subjects (0.19 and 0.13 mL O₂/mL) are similar to previously reported corrected values (0.181 and 0.128 mL O₂/mL). The oxygen-hemoglobin dissociation curves may differ between healthy and SCR subjects, although a previous study showed the difference was small at high SO₂ (>92%) but shifted to the right at lower levels. Finally, hemoglobin absorption spectra may not be the same in healthy and SCR subjects. Differences in healthy and sickle cell hemoglobin absorption spectra have been reported based on blood samples, although measurements under physiological conditions are not readily available. In the current study, adjustments were not made for any potential differences in hemoglobin absorption spectra.

DO₂ was not found to be impaired in predominate lower stages of SCR. This finding is attributed to the increased TRBF which adequately compensated for the decreased O₂A. Similarly, MO₂ was maintained due to the countereffects of increased TRBF and reduced O₂AV. A reduction in MO₂ was expected in SCR due to peripheral arteriolar occlusions and arteriolar-venular anastomoses (shunting). The finding of unchanged MO₂ suggests a higher metabolic rate per unit volume in the central retina than in the thinner peripheral retina and potentially a limited contribution of shunting in peripheral retina to altering MO₂.

A linear relationship between MO₂ and DO₂ was established based on compiled data from all subjects. This finding indicates the rate at which oxygen is delivered by the inner retinal circulation is highly correlated with the oxygen metabolic rate. Furthermore, TRBF and OEF were shown to be inversely related, similar to findings in the brain. This result demonstrates the presence of a compensatory increase in OEF under conditions of decreased TRBF in order to maintain...
MO2. Nevertheless, the maximum value for OEF was 0.48, which indicates OEF augmentation to supply oxygen in these SCR subjects.

Overall, our method will allow investigation of the relationships among retinal blood flow, vascular oxygen levels, and oxygen consumption under physiologically challenged and pathologic conditions. Quantitative assessment of retinal MO2, DO2, and OEF has the potential to advance knowledge of oxygen dynamics and serve as new biomarkers for disease diagnosis, progression, and treatment monitoring.

Acknowledgments

Supported by National Institutes of Health grants: DK104393 (MS), EY023285 (DH), EY01792, EY010572, Antonio Champalimaud Vision Award (DH), Senior Scientific Investigator Award (MS) and a departmental award from Research to Prevent Blindness.

Disclosure: M. Shahidi, None; A.E. Felder, None; O. Tan, Optovue, Inc. (F); N.P. Blair, None; D. Huang, Optovue, Inc. (F, I, R)

References

34. Pechauer AD, Tan O, Liu L, et al. Retinal blood flow response to hyperoxia measured with en face Doppler optical...
coherence tomography. *Invest Ophthalmol Vis Sci*. 2016;57:
OCT141–OCT145.
5586–5592.
119–127.
5064–5067.
55–60.