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

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Purpose. Reduction in inner retinal oxygen delivery (DO_2) can cause retinal hypoxia and impair inner retinal oxygen metabolism (MO_2), leading to vision loss. The purpose of the current study was to establish measurements of DO_2 and MO_2 in healthy subjects and test the hypothesis that DO_2 and MO_2 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 (O_{2A} and O_{2V}), venous diameter (D_V), and total retinal blood flow (TRBF). Retinal arteriovenous oxygen content difference (O_{2AV}), DO_2, MO_2, and oxygen extraction fraction (OEF) were calculated according to the following equations: $O_{2AV} = O_{2A} - O_{2V}$; $DO_2 = TRBF * O_{2A}$; $MO_2 = TRBF * O_{2AV}$; $OEF = MO_2/DO_2$.

Results. Retinal D_V and TRBF were higher in the SCR group as compared to the control group, whereas, O_{2A}, O_{2V}, and O_{2AV} were lower in SCR group as compared to the control group. DO_2, MO_2, and OEF were not significantly different between control and SCR groups. MO_2 and DO_2 were linearly related, such that higher MO_2 was associated with higher DO_2. 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 DO_2, MO_2, 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 (DO_2) can cause retinal hypoxia and impair inner retinal oxygen metabolism (MO_2), 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 MO_2 in humans by combining spectrophotometry to measure retinal vascular oxygen saturation (SO_2) 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 planes[15] or multiple en face planes.[16]

To date, calculated measurements of DO_2 have not been reported in humans. Since DO_2 is the product of TRBF and arterial oxygen content, TRBF alone provides only partial information about oxygen delivery to the retina. Similarly, MO_2 is the product of TRBF and arteriovenous O_2 difference,[17] thus measuring either SO_2 or TRBF individually would likely lead to incorrect conclusions with regard to MO_2. Alternatively, MO_2 can be expressed as the product of DO_2 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 DO_2 and OEF respond in order to maintain MO_2. Conversely, alterations in MO_2 can play a role in regulating DO_2. In healthy subjects, MO_2 was measured under normoxia and hyperoxia.[20,21] Furthermore, MO_2 was shown to be reduced in subjects with diabetic retinopathy.[22,23] However, MO_2 has not been evaluated in sickle cell retinopathy (SCR), in which vision-threatening retinal capillary occlusions, nonperfusion, ische-
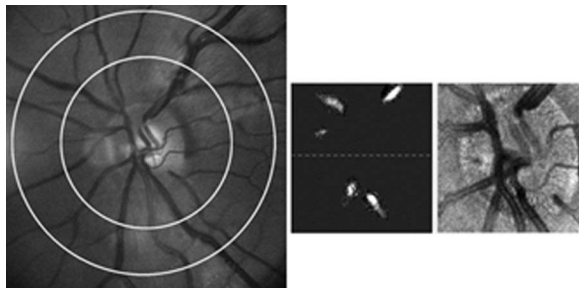
FIGURE 1. (*Left*) Example of a retinal image in a healthy control subject. Retinal arterial and venous diameter and oxygen saturation were measured in blood vessel segments within the concentric rings. (*Middle*) Blood flow was measured in the detected veins from different en face planes, projected here in one en face plane. (*Right*) En face OCT image of the optic nerve head acquired in the same subject.

mia, and neovascularization are known to occur.[24-32] The purpose of the current study was to establish measurements of DO_2 and MO_2 in healthy subjects and test the hypothesis that DO_2 and MO_2 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/fibrous proliferation (stage 3).[33] Prior to imaging, subjects' pupils were dilated using 1% tropicamide and 2.5% phenylephrine. Mean arterial pressure (MAP) and hematocrit (HCT) were measured.

Image Acquisition and Analysis

Multimodal imaging was performed to derive DO_2 and MO_2 by measurements of TRBF and retinal vascular SO_2. 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.[16] 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 with 500 A-lines/B-scan, and the three-dimensional volumetric data set contained 195 en face planes. TRBF was measured by our previously published method.[16,34] The raw spectral data were exported and a customized software was written in MATLAB (Mathworks, Natick, MA, USA) to analyze the Doppler OCT images, detect vessels, and compute TRBF. An example of an en face OCT image of the optic nerve head in a healthy control subject is shown in Figure 1. A phase-resolved technique was used to calculate Doppler phase shift and flow 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 grader excluded 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 SO_2 measurements, dual wavelength (570 and 606 nm) retinal oximetry was performed using our custom-built slit lamp biomicroscope and previously published method.[18,35] 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 SO_2 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.[18,36] Retinal arterial and venous diameter (D_A and D_V, respectively) and SO_2 (SO_{2A} and SO_{2V}, respectively) were determined for each blood vessel by averaging multiple values along the vessel segment. Blood oxygen content of retinal arteries and veins (O_{2A} and O_{2V}) were determined by the following relation: $O_2 = O_{2max} * Hgb * SO_2$, where O_{2max} is the oxygen-binding capacity of hemoglobin[37] and Hgb is the hemoglobin concentration calculated from the measured HCT values. Mean values of D_V, O_{2A}, and O_{2V} were determined per eye by averaging measurements in all retinal arteries and veins.

Retinal arteriovenous oxygen content difference (O_{2AV}) was calculated as $O_{2A} - O_{2V}$. DO_2, MO_2, and OEF were calculated according to the following equations: $DO_2 = TRBF * O_{2A}$; $MO_2 = TRBF * O_{2AV}$; $OEF = MO_2/DO_2$.

Statistical Analysis

Compiled retinal oxygen metrics (D_V, TRBF, O_{2A}, O_{2V}, O_{2AV}, DO_2, MO_2, 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 $\pm$ 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 ($33\% \pm 7\%$) was lower than that of control subjects ($45\% \pm 6\%$) ($P < 0.001$).

The Table lists retinal oxygen metrics in control and SCR groups. Retinal D_V and TRBF were higher in the SCR group as compared to the control group. O_{2A}, O_{2V}, and O_{2AV} were lower in the SCR group as compared to the control group. DO_2, MO_2, and OEF were not significantly different between control and SCR groups. As shown in Figure 2, there was a linear relationship between MO_2 and DO_2, such that higher MO_2 was associated with higher DO_2 ($r = 0.67$; $P < 0.001$; $N = 24$). 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

Oxygen Metric	Control	SCR	P Value
D_V, µm	97 ± 9	117 ± 18	**0.004**
TRBF, µL/min	48 ± 16	69 ± 27	**0.03**
O_{2A}, mL O_2/dL	19 ± 4	15 ± 4	**0.008**
O_{2V}, mL O_2/dL	13 ± 2	10 ± 2	**0.005**
O_{2AV}, mL O_2/dL	7 ± 2	5 ± 2	0.06
DO_2, µL O_2/min	9.0 ± 3.0	9.7 ± 2.4	0.52
MO_2, µL O_2/min	3.1 ± 1.2	3.0 ± 1.2	0.88
OEF	0.34 ± 0.06	0.32 ± 0.10	0.39

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 DO_2 and MO_2 in humans was demonstrated. In control subjects, diameter measurements in major retinal veins were in agreement with previously reported values.[18,38–40] Similarly, TRBF was consistent with previously published values.[16,20,22,23] SO_2 measurements in retinal arteries and veins were in agreement with values obtained from previously published studies.[18,39,41] MO_2 measurements obtained in the present study were similar to those reported previously in control subjects.[20,22] Of note, MO_2 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 MO_2 is indicative of oxygen consumption rate. However, under certain conditions, such as hyperoxia,[21,42,43] a portion of the inner retina may be supplied by the choroidal circulation, thus MO_2 can decrease even if the consumption per amount of tissue is unchanged. Although measurements of TRBF and SO_2 have been previously published in human subjects,[20] we report here, for the first time to our knowledge, calculated values of DO_2. Moreover, OEF values derived from direct measurements of MO_2 and DO_2 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.[18]

As expected, blood O_2 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 O_2
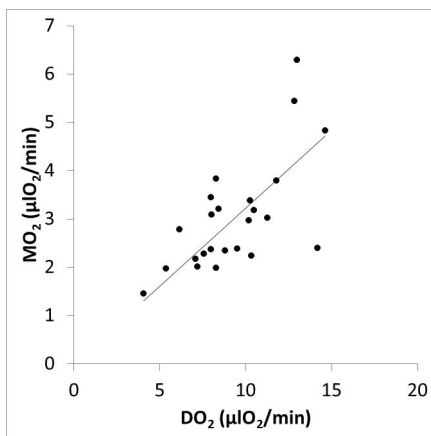
FIGURE 2. Relationship between inner retinal MO_2 and DO_2. The best fit line to the data is shown.
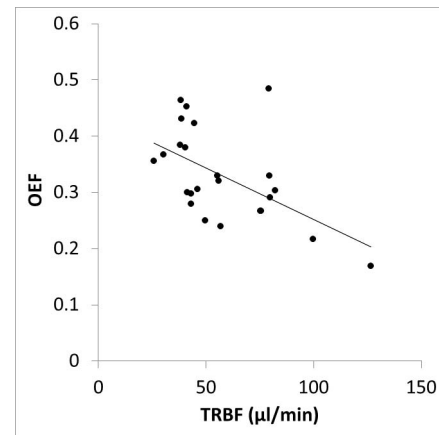
FIGURE 3. Relationship between inner retinal OEF and TRBF. The best fit line to the data is shown.

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.[44] 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.[45] A previous study in subjects with Eisenmenger syndrome, a cardiac condition resulting in systemic hypoxia, showed arterial and venous SO_2 difference was not altered[46]; however, no information about changes in MO_2 was reported since TRBF was not measured.

In the current study, SO_2 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 O_2 content, the low level of fraction of dissolved oxygen was not included. Nevertheless, the values of arterial and venous O_2 contents obtained in control subjects (0.19 and 0.13 mL O_2/mL) are similar to previously reported corrected values (0.181 and 0.128 mL O_2/mL).[20] The oxygen-hemoglobin dissociation curves may differ between healthy and SCR subjects, although a previous study showed the difference was small at high SO_2 (>92%) but shifted to the right at lower levels.[47] 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,[48,49] 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.

DO_2 was not found to be impaired in predominately lower stages of SCR. This finding is attributed to the increased TRBF, which adequately compensated for the decreased O_{2A}. Similarly, MO_2 was maintained due to the countereffects of increased TRBF and reduced O_{2AV}. A reduction in MO_2 was expected in SCR due to peripheral arteriolar occlusions and arteriolar-venular anastomoses (shunting). The finding of unchanged MO_2 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 MO_2.

A linear relationship between MO_2 and DO_2 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.[50] This result demonstrates the presence of a compensatory increase in OEF under conditions of decreased TRBF in order to maintain

MO_2. 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 MO_2, DO_2, and OEF has the potential to advance knowledge of oxygen dynamics and serve as new biomarkers for disease diagnosis, progression, and treatment monitoring.

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