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Variability of Retinal Oxygen Metrics in Healthy and Diabetic Subjects

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Purpose: Previous studies have reported alterations in total retinal blood flow (TRBF), oxygen delivery (DO_2), oxygen metabolism (MO_2), and oxygen extraction fraction (OEF) due to retinal diseases. The purposes of the current study were to determine variabilities and establish normal confidence intervals (CIs) for these metrics.

Methods: A total of 22 healthy and 14 diabetic subjects participated in the study. Retinal vascular oxygen saturation (SO_2) and TRBF were measured by oximetry and Doppler optical coherence tomography, respectively. DO_2, MO_2, and OEF were calculated from SO_2 and TRBF measurements. Means, standard deviations (SDs), and CIs of metrics were determined in healthy subjects. Intra-visit variability was determined by the mean SDs of repeated measurements. Inter-visit variability was determined by the difference of measurements between two visits.

Results: TRBF was 44 ± 15 µL/min (95% CI, 37–51) in healthy subjects. Intra-visit variabilities of TRBF were 5 µL/min and 6 µL/min in healthy and diabetic subjects, respectively. Inter-visit variability of TRBF was 3 µL/min in diabetic subjects. DO_2, MO_2, and OEF were 8.3 ± 2.9 µLO_2/min (95% CI, 7.0–9.6), 3.2 ± 0.9 µLO_2/min (95% CI, 2.8–3.6), and 0.40 ± 0.08 (95% CI, 0.36–0.43), respectively, in healthy subjects. Inter-visit variabilities of DO_2, MO_2, and OEF were 0.6 µLO_2/min, 0.1 µLO_2/min, and 0.03, respectively, in diabetic subjects.

Conclusions: The findings established variabilities and normal baselines for TRBF, DO_2, MO_2, and OEF measurements in a small cohort of subjects.

Translational Relevance: The variability and normal baselines of retinal oxygen metrics may be useful for diagnosing and monitoring patients with retinal diseases.

Introduction

The retina is one of the most metabolically active tissues, thus requiring a high oxygen demand. Retinal and choroidal circulations supply oxygen that is utilized by the retinal tissue for energy production. Adequate oxygen is essential to maintain retinal metabolism and visual function. Inadequate retinal blood flow (RBF) and oxygen delivery (DO_2), coupled with impaired oxygen metabolism (MO_2) and altered oxygen extraction fraction (OEF), have been implicated in various retinal diseases.[1–5] Specifically, changes in retinal microvasculature, RBF, and vascular oxygenation have been reported in diabetic retinopathy.[6–10] Furthermore, there are reports of retinal oxygenation changes due to retinal vascular occlusions,[11–13] retinopathy of prematurity,[14] and inherited retinal degenerations.[15,16] Moreover, measurements of retinal oxygen saturation and metabolism have been reported in exudative age-related macular degeneration,[17] glaucoma,[12,18–20] and sickle cell retinopathy.[1]

Evaluating the variabilities of total RBF (TRBF) and retinal oxygen metrics (DO_2, MO_2, and OEF) is essential to determine the sensitivity of measurements for detecting changes due to disease. Previous studies have reported variability of RBF in healthy[21–23] and diabetic[24] subjects and retinal vascular oxygen saturation in healthy subjects.[25] However, to the best of our knowledge, the variabilities of retinal DO_2,

MO_2, and OEF have not been reported previously. The purposes of the current study were to (1) determine intra- and inter-visit variabilities of TRBF, DO_2, MO_2, and OEF in healthy and diabetic subjects; and (2) establish normal confidence intervals (CIs) for the means of these metrics in healthy subjects.

Materials and Methods

Subjects

The study was performed following the tenets of the Declaration of Helsinki and was approved by the institutional review board of the University of Southern California. All subjects provided written informed consent, and the study was in accordance with Health Insurance Portability and Accountability Act regulations. Fourteen diabetic subjects diagnosed with either no diabetic retinopathy (DR) or untreated mild nonproliferative DR (NPDR) were included in the study. The clinical diagnosis was based on retinal examination performed by specialists according to the International Clinical Disease Severity Scale for Diabetic Retinopathy.[26] Twenty-two healthy subjects with normal clinical eye and retinal examinations participated in the study. Imaging was performed in one eye of each subject at one visit in healthy subjects and at two visits (6 ± 3 months apart) in diabetic subjects.

Image Acquisition and Analysis

TRBF was measured using a commercially available optical coherence tomography (OCT) instrument (Avanti; Optovue, Inc., Fremont, CA). A custom scan protocol and image analysis software were used to measure Doppler phase shifts and blood flow within retinal veins on multiple optimized en face planes, as previously described.[27,28] Briefly, an image set consisting of five consecutive volume scans covering a 2 × 2-mm area centered on the central retinal vein was acquired. Each volume contained 80 B-scans with 500 A-lines per B-scan and 195 en face planes. From each volume, TRBF was determined as the sum of flow in all detected retinal veins. Measurements from volumes within each image set were averaged to calculate TRBF. Repeated TRBF measurements were determined from multiple image sets and averaged to compute TRBF per subject. Figure 1A shows an example of an en face OCT image of the optic nerve head in a diabetic subject.

Retinal vascular oxygen saturation (SO_2) measurements were obtained by dual-wavelength oximetry using our custom-built slit-lamp biomicroscope as previously published.[2,29] Nine retinal images were acquired at each imaging wavelength in a 5 × 5-mm area centered on the optic disk. Retinal images were analyzed to measure 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. 1B). The following relation determined the oxygen content of retinal arteries (O_{2A}) and veins (O_{2V}): O_2 content = oxygen-binding capacity of hemoglobin (Hb) × Hb × SO_2. 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: $DO_2 = TRBF \times O_{2A}$; $MO_2 = TRBF \times O_{2AV}$; and $OEF = MO_2/DO_2$.

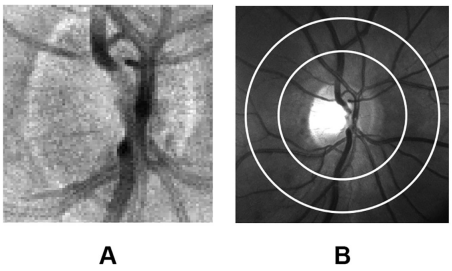

Figure 1. (**A**) Example of an en face OCT image of the optic nerve head in a diabetic subject. Blood flow measurements were obtained in veins detected in multiple en face planes. (**B**) A retinal image acquired in the same subject. Retinal vascular oxygen saturation was measured in blood vessel segments within the concentric rings.

Statistical Analysis

Descriptive statistics were used to compare demographic characteristics between groups. Data were compared using the unpaired *t*-test for the continuous variables and the χ^2 test or Fisher's exact test for categorical variables. Intra-visit variability of TRBF was determined by the mean of the standard deviation (SD) of multiple repeated measurements in each subject obtained on the same day, averaged in each group. Mean, SD, and 95% CIs for the metrics were established based on data in healthy subjects. We determined whether the means of metrics at each visit in the diabetic group were within the normal 95% CIs. Also, metrics were compared between groups by unpaired *t*-tests. Inter-visit variabilities of metrics were determined from the difference between measurements at two visits. Bland–Altman plots[30] were generated that displayed inter-visit differences plotted as a function of the average of measurements at the two visits. Mean difference (bias) and 95% upper and lower limits of agreement, defined by mean ± 2 × SD of differences, were determined. The D'Agostino–Pearson omnibus

test and Q–Q plots were used to assess the normality of data distributions. Pearson's correlation was performed to assess the associations of TRBF with age. A two-sided $P \leq 0.05$ was considered statistically significant. All analyses were performed using Prism 9.0.0 for Mac OS (GraphPad Software, San Diego, CA).

Results

Subject Demographics

The demographics of the subjects are presented in Table 1. Age and sex did not significantly differ between healthy and diabetic subjects ($P > 0.31$). Race was statistically different between groups ($P = 0.03$).

Blood Flow

TRBF data from image sets ranging between three and seven in healthy subjects and three and six in diabetic subjects were included. The number of image sets was five ± one in each group of subjects. Intra-visit variability of TRBF measurements (mean of SDs of repeated measurements) was 5 µL/min and 6 µL/min in healthy and diabetic subjects, respectively. TRBF (mean ± SD) was 44 ± 15 µL/min (95% CI, 37–51) in healthy subjects. Normal 95% CIs and TRBF measured in diabetic subjects are shown in Table 2. TRBF measurements at both visits in diabetic subjects were within normal 95% CIs and not significantly different than the mean in healthy subjects ($P > 0.30$). Inter-visit variability of TRBF (mean of measurement differences) was 3 µL/min in diabetic subjects. Figure 2 shows the Bland–Altman plot of TRBF. Upper and lower limits of agreement for differences were 18 and –12 µL/min, respectively. There was an inverse correlation between mean TRBF and age in healthy subjects ($r = -0.45$; $P = 0.03$; $n = 22$).

Oxygen Metrics

The means, SDs, and 95% CIs for DO_2, MO_2, and OEF in healthy subjects are shown in Table 2. DO_2 and MO_2 were 8.3 ± 2.9 μLO_2/min (95% CI, 7.0–9.6) and 3.2 ± 0.9 μLO_2/min (95% CI, 2.8–3.6), respectively. OEF was 0.40 ± 0.08 (95% CI, 0.37–0.43). As shown in Table 2, DO_2, MO_2, and OEF measurements obtained at both visits in diabetic subjects were within the normal 95% CIs and not significantly different than the means in healthy subjects ($P > 0.30$). In diabetic subjects, inter-visit variabilities of DO_2 and MO_2 were 0.6 μLO_2/min and 0.1 μLO_2/min, respectively. Inter-visit variability of OEF was 0.03. Figure 3 shows Bland–Altman plots for DO_2, MO_2, and OEF. The upper and lower limits of agreement for differences were 3.6 and –2.4, 2.1 and –2.0, and 0.22 and –0.15 for DO_2, MO_2, and OEF, respectively.

Table 1. Subject Demographics

Demographic	Healthy Subjects ($n = 22$)	Diabetic Subjects ($n = 14$)	P
Age (yr), mean ± SD	54 ± 9	56 ± 16	0.31
Sex, *n*			0.50
Male	7	6	
Female	15	8	
Race, *n*			0.03
Asian	10	1	
White	1	3	
Hispanic/Latino	11	10	

Table 2. Retinal Blood Flow and Oxygen Metrics in Diabetic Subjects ($n = 14$) at Two Visits and in Healthy Subjects ($n = 22$) at One Visit

	Diabetic Subjects, Mean ± SD		Healthy Subjects, Mean ± SD (95% CI)
Metric	Visit 1	Visit 2	Visit 1
TRBF (µL/min)	45 ± 14	48 ± 13	44 ± 15 (37–51)
DO_2 (μLO_2/min)	8.0 ± 2.6	8.6 ± 2.1	8.3 ± 2.9 (7.0–9.6)
MO_2 (μLO_2/min)	3.2 ± 1.4	3.2 ± 1.2	3.2 ± 0.9 (2.8–3.6)
OEF	0.40 ± 0.07	0.38 ± 0.10	0.40 ± 0.08 (0.37–0.43)

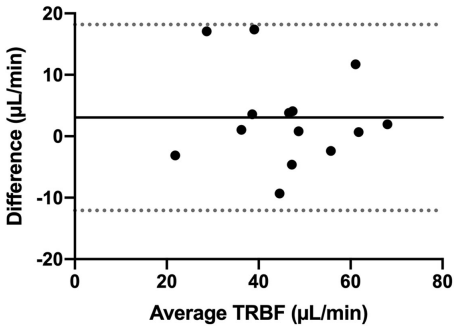

Figure 2. Inter-visit variability of TRBF displayed using the Bland–Altman plot. The difference in measurements between visits is plotted as a function of the average of measurements at two visits. The *solid line* shows the mean difference (bias) and the *dashed lines* denote upper and lower 95% limits of agreement.

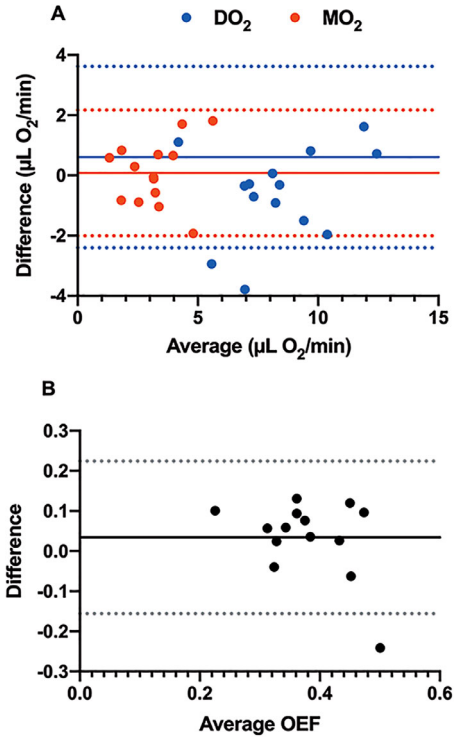

Figure 3. Inter-visit variability of DO_2 and MO_2 (**A**) and OEF (**B**) displayed using Bland–Altman plots. The differences in measurements between visits are plotted as a function of the average of measurements at two visits. The *solid line* indicates mean difference (bias), and the *dashed lines* denote upper and lower 95% limits of agreement.

Discussion

Determining the variabilities of TRBF and oxygen metrics (DO_2, MO_2, and OEF) is necessary to determine alterations due to certain diseases and monitor changes over time. In the current study, intra-visit variability of TRBF and inter-visit variability of TRBF, DO_2, MO_2, and OEF were reported. Additionally, normal 95% CIs for these metrics were established in healthy subjects.

Previous studies have reported intra-visit coefficient of variation of TRBF to be 4% in diabetic subjects[24] and 8% to 11% in healthy subjects.[22,31] These values are slightly lower than 10% in healthy subjects and 12% in diabetic subjects in the current study. Multiple factors can contribute to differences in measurement variabilities, such as subjects' fixation and eye motion, the numbers of subjects evaluated and measurements obtained per subject, as well as previously demonstrated differences in technical instrumentation,[32] and age of subjects.[33] On the other hand, inter-visit variability of TRBF in the current study (3 μL/min) was lower than previously reported variabilities of 6 μL/min and 11 μL/min in young and elderly healthy subjects, respectively.[22]

TRBF measurements in the current study were in agreement with previously reported values obtained using Doppler OCT methods.[24,27,31,34,35] Additionally, the current study showed no significant difference in TRBF between healthy and diabetic subjects with no DR or untreated mild NPDR. Consistent with our results, other studies also reported no significant decrease in TRBF at the early stages of DR.[24,36] However, one study reported lower TRBF in mild-to-moderate NPDR subjects compared to control subjects.[4]

Finally, our finding of an inverse correlation between TRBF and age in healthy subjects agrees with published reports of decreases in retinal vessel density, venular blood flow velocity, and artery blood column diameter associated with aging.[33,37–39] The reduction in TRBF with age can be attributed to both vascular constriction and reduced vessel density, as previously suggested.[33]

DO_2, MO_2, and OEF measurements in healthy subjects in the current study were comparable to those reported in previous studies.[1,3,5,29] Additionally, normal 95% CIs established in healthy subjects provide a baseline for evaluating changes due to diseases. The current study also reported inter-visit variability of these metrics in diabetic subjects with no DR or mild NPDR. These results may be potentially used in future longitudinal studies to determine progressive changes in oxygen metrics over time or evaluate treatment outcomes.

The current study had limitations. First, inter-visit variability of retinal oxygen metrics was assessed in diabetic subjects. Although the diabetic subjects had no or minimal retinopathy with oxygen metrics within the normal CIs at both visits and the time interval between visits was relatively short, the potential for changes over

time may not be eliminated. Second, the sample size for this study was small, which may have limited the accurate establishment of normal baselines. Additionally, due to the small sample size, the potential effects of age, race, and sex on the variability of measurements were not evaluated, and differences in race could not be accounted for. Future studies with larger cohorts and multiple visits are necessary to determine more accurately the variabilities and normal CIs of metrics according to race, sex, and age.

Overall, the findings established variabilities and normal baselines for TRBF, DO_2, MO_2, and OEF measurements, providing a basis for detecting and monitoring changes due to retinal diseases.

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MS designed the study. MR and SL performed image analysis. MR and MS analyzed the data. MR wrote and edited the manuscript. MR, SL, NPB, and MS read and revised the manuscript. MS approved the manuscript for submission.

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