Measurement of Retinal Blood Flow in Normal Chinese-American Subjects by Doppler Fourier-Domain Optical Coherence Tomography

Sowmya Srinivas,¹ Ou Tan,² Shuang Wu,³ Muneeswar Gupta Nittala,¹ David Huang,² Rohit Varma,³ and Srinivas R. Sadda^{1,4}

¹Doppler OCT Reading Center, Doheny Eye Institute, Los Angeles, California, United States

²Casey Eye Institute, Oregon Health & Science University, Portland, Oregon, United States

³Department of Ophthalmology, Keck School of Medicine, University of Southern California, Los Angeles, California, United States

⁴Department of Ophthalmology, David Geffen School of Medicine at UCLA, Los Angeles, California, United States

Correspondence: Srinivas R. Sadda, Department of Ophthalmology, Keck School of Medicine, University of Southern California, 1450 San Pablo Street, DEI 3623, Los Angeles, CA 90033, USA; SSadda@doheny.org.

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PURPOSE. To measure total retinal blood flow (TRBF) in normal, healthy Chinese Americans by using semi-automated analysis of Doppler Fourier-domain optical coherence tomography (FD-OCT) scans.

METHODS. Two hundred sixty-six normal, healthy Chinese-American participants (266 eyes) were enrolled from The Chinese American Eye Study. All participants underwent complete ophthalmic examination, including best-corrected visual acuity, indirect ophthalmoscopy, and Doppler FD-OCT imaging, using the circumpapillary double circular scan protocol. Total retinal blood flow and other vascular parameters (e.g., venous and arterial cross-sectional area and their velocities) were calculated by using Doppler OCT of Retinal Circulation software. Associations between TRBF and other clinical parameters were assessed by using bivariate correlations and linear regression.

RESULTS. The mean age of study participants was 57.40 ± 5.60 (range, 50–82) years. The mean TRBF was 49.34 ± 10.08 (range, 27.17–78.08, 95% confidence interval: 25.98–69.10) $\mu\text{L}/\text{min}$. The mean venous area was $0.0548 (\pm 0.0084) \text{ mm}^2$. Superior retinal hemispheric blood flow ($25.50 \pm 6.62 \mu\text{L}/\text{min}$) was slightly greater than inferior retinal hemispheric blood flow ($23.84 \pm 7.19 \mu\text{L}/\text{min}$, $P = 0.008$). The mean flow velocity was $15.16 \pm 3.12 \text{ mm}/\text{s}$. There was a weak but significant negative correlation between TRBF and age ($r = -0.15$, $P = 0.012$). No significant correlation was found between TRBF and axial length ($r = 0.11$, $P = 0.08$). Retinal blood flow was not significantly correlated with any other clinical parameters, including body mass index, systolic blood pressure, diastolic blood pressure, and intraocular pressure.

CONCLUSIONS. Normal Doppler OCT-derived total retinal blood values in a Chinese-American population showed considerable variability, some of which was explained by age. These observations should help design future studies evaluating TRBF in populations with eye disease.

Keywords: retinal blood flow, Doppler OCT, age

Measurement of retinal blood flow is an important clinical parameter to assess onset and progression of retinal diseases. Studies have shown abnormal retinal blood flow in age-related macular degeneration, diabetic retinopathy, glaucoma, and retinal vein occlusions.^{1–6} Previous research has used a variety of techniques to measure retinal blood flow, such as laser Doppler flowmeter,⁷ fluorescein and indocyanine green angiographies,⁸ ultrasound color Doppler imaging,⁹ and magnetic resonance imaging.¹⁰ Each of these techniques has its own limitations.

Optical coherence tomography (OCT)^{11–14} is a rapid, noninvasive, optical imaging technique that provides high-resolution cross-sectional imaging of the neurosensory retina.¹⁵ In addition to detailed morphologic information, OCT data also include information about the Doppler shift of reflected light, which provides an opportunity to assay blood flow.^{16,17} The

imaging speed of OCT has improved greatly with the development of Fourier-domain technique, facilitating the development of Doppler Fourier-domain optical coherence tomography (FD-OCT) approaches.^{18–22} The Doppler shift alone, however, is insufficient to calculate flow velocity within the vessel; the angle at which the incident light strikes the moving blood, termed “the Doppler angle,” is also required. To ascertain the Doppler angle, Wang and coworkers²³ have developed the circumpapillary double circular scan pattern, which transects all vessels entering and exiting the optic nerve in two locations, allowing determination of the vessel course within the retina (and hence its relationship to the incident light). This measures velocities over a short period of time in all veins entering the nerve. This technique has allowed rapid calculation of total (or hemispheric) retinal blood flow. Initial results using this technique have already been published for

small cohorts of diseased and normal eyes.^{24–28} But a much larger cohort is needed to truly understand the range of variability in normal blood flow and the factors that affect this variability. In the present study, we used Doppler OCT data collected as part of the Chinese American Eye Study (CHES) to establish these normative blood flow parameters in a healthy Chinese-American population.

MATERIALS AND METHODS

Overview of Chinese American Eye Study

The CHES is a population-based prevalence study funded by the National Eye Institute, which recruited healthy, noninstitutionalized Chinese-American adults, aged 50 years and older, in the City of Monterey Park in Los Angeles County. Clinical data collected from participants included age, sex, body mass index (BMI), systolic blood pressure, diastolic blood pressure, intraocular pressure, and axial length. In addition to FD-OCT and Doppler OCT data (acquisition described below), color fundus images were also collected from these subjects.

For inclusion in this Doppler OCT normative study, the eye must have been determined to be normal by ophthalmoscopic examination, with exclusion of any ocular disorder, including corneal opacity, glaucoma, ocular hypertension, diabetic retinopathy, or age-related macular degeneration. Individuals with diabetes mellitus, hypertension, or cardiovascular disease were excluded regardless of whether ocular abnormalities were present or not. The research protocol was approved by the Institutional Review Board of the University of Southern California, and the research adhered to the tenets set forth in the Declaration of Helsinki. Written informed consent was obtained from all participants.

Doppler FD-OCT Acquisition

All eligible normal participants underwent Doppler FD-OCT scans of both eyes. To be eligible for this analysis, subjects had to have no evidence of ocular disease (by ophthalmoscopic examination) or systemic disease. The eye with the better-quality valid scan (see below for definition of valid scan) was selected for inclusion in the study. Doppler OCT was obtained after dilation, by using a previously published double circular scan protocol consisting of two circumpapillary circles that transected all major blood vessels entering/exiting the optic nerve head. Scans were obtained by using an RTVue FD-OCT (Optovue, Inc., Fremont, CA, USA). The OCT system has a wavelength of 840 nm, with a scan rate of 26,000 A-scans/s. The time interval between two sequential axial scans is 36.7 ms. The Doppler phase shift is 13.6 kHz at the phase wrapping limit of $\pm\pi$ radian, which corresponds to the highest speed of 4.2 mm/s that can be unambiguously measured.^{20,24,29,30} The average blood flow velocity in the peripapillary veins is 15 mm/s. To solve this problem, the double-ring scan pattern was used.³¹ The scan consists of two concentric rings around the optic disc. The diameters of the two concentric ring scans were 3.4 and 3.75 mm, respectively. In the area, we found that the range of angles between beam and velocity vector in main veins was $75.4^\circ \pm 4.9^\circ$ or $78.6^\circ \pm 2.3^\circ$, depending on the scan protocol.^{32,33} Because the Doppler phase shift represents only the axial component of velocity in veins, the unambiguously measurable range of speed in veins is enlarged to 16.2 mm/s based on the following formula (if we use angle = 75°): velocity = axial speed/cos(α). Here α is the angle between laser beam and flow direction. In the calculation, the angle α can be estimated by using the vessel position in the inner (3.4-mm) and outer (3.75-mm) rings. Note that the flow result is not

precise when α is close to 90° . In practice, we consider the measurement of a vein unreliable if $|90 - \alpha|$ is less than 3° .

The phase unwrapping technique was also applied to correct the Doppler phase shift in the center of veins where the axial speed might be larger than the phase-wrapping limit.²⁰ The algorithm took advantage of the fact that the flow velocity was near zero close to the vessel wall and increased toward the center of the vessel, forming a parabolic distribution in ideal laminar flow. Therefore, the Doppler phase shift near the vessel wall provided information on the correct direction of flow (sign of the Doppler phase shift) within the entire lumen. The phase shift near the center of the lumen was adjusted by multiples of 2π to be consistent in sign and continuous in value with the phase shift near the vessel wall. Phase unwrapping is less reliable when the phase wrapping is more than 2π , that is, where axial speed is more than twice the phase-wrapping limit. But in general we found that multiple phase wrapping occurred only in arteries and not in veins in peripapillary area. Therefore, total retinal blood flow (TRBF) was measured by summing veins only in this study.

Six dual circular scans were obtained in a single scan acquisition sequence. This acquisition sequence was repeated five or six times so that 30 to 36 frames were obtained for each ring to provide a higher probability of having sufficient high-quality scans for flow calculation.³³ A volume OCT optic disc cube scan (101×512 A-scans over a 6-mm $\times$ 6-mm square) and color fundus photograph were also obtained. Using the RTVue Doppler transfer output software on the OCT instrument, the Doppler OCT scans and optic disc cube scans were exported and submitted, along with infrared and color disc photographs, to the Doheny Doppler OCT Reading Center for masked grading and calculation of blood flow.³²

Doppler OCT Blood Flow Calculation

Unfortunately, reliable automated calculation of retinal blood flow from Doppler FD-OCT scans is not yet possible owing to inaccuracies in vessel segmentation and motion artifacts. Thus, reliable blood flow calculation requires a semi-automated, human grader-supervised process that has been shown to yield good reproducibility.³² For this study, we used the previously described Doppler Optical Coherence Tomography of Retinal Circulation (DOCTORC)³⁴ software, which performs an initial automatic processing of candidate vessel locations. Manual grading and correction was then performed by a certified Doppler OCT reading center grader (SS), whose intragrader reproducibility has been previously established to be excellent.³²

The grading procedure was separated into three steps: preprocessing, manual editing, and flow calculation. In the preprocessing step, the DOCTORC software registered the Doppler OCT scans with the OCT projection image (from the cube scan), infrared scanning laser ophthalmoscope disc image, and the color disc photograph. This allows the grader, in subsequent steps, to correlate vessel positions on the Doppler OCT B-scans with the vessels on the en face OCT image. Using the hue (bright red versus darker red) of the vessel on the color photograph, the grader is able to more confidently determine whether the vessel is an artery or vein. In the preprocessing step, the veins and arteries were also automatically detected and classified by DOCTORC.

In the manual editing step, the grader adjusted the positions and sizes of the automatically identified candidate vessels, added or deleted vessels as needed, and classified the vessel as an artery or vein. In addition, the grader identified the corresponding vessel on the two circular B-scans, thereby establishing the vessel trajectory and allowing determination of

TABLE 1. Descriptive Statistics for Blood Flow and Other Parameters

	Mean $\pm$ SD (Range)
Blood flow, $\mu\text{L}/\text{min}$	49.34 $\pm$ 10.08 (27.17–78.08)
Superior flow, $\mu\text{L}/\text{min}$	25.50 $\pm$ 6.62 (8.98–53.73)
Inferior flow, $\mu\text{L}/\text{min}$	23.84 $\pm$ 7.19 (9.56–52.75)
Venous area, mm^2	0.0548 $\pm$ 0.0084 (0.0335–0.0816)
Venous velocity, mm/s	15.16 $\pm$ 3.12 (8.07–30.88)
Arterial area, mm^2	0.03664 $\pm$ 0.0097 (0.0154–0.1270)
Arterial velocity, mm/s	23.43 $\pm$ 6.16 (4.61–52.29)
Age, y	57.40 $\pm$ 5.60 (50–82)
BMI, kg/m^2	23.44 $\pm$ 3.16 (16.00–32.5)
Systolic BP, mm Hg	116.17 $\pm$ 11.92 (80–140)
Diastolic BP, mm Hg	73.84 $\pm$ 7.79 (50–90)
IOP, mm Hg	14.51 $\pm$ 2.28 (6.67–20.33)
Axial length, mm	23.90 $\pm$ 1.24 (21.27–27.15)

Superior and inferior flows are hemispheric values. BP, blood pressure; IOP, intraocular pressure.

the Doppler angle by the DOCTORC software. Because of misregistration from fixation/motion artifact, small Doppler angles, or suboptimal image quality, confidence in vessel identification might vary from vessel to vessel and case to case. To contribute to an overall determination of the reliability of a retinal blood flow result, the grader also provided a subjective rating of XX grading confidence for each vessel on each scan on a scale of 0 to 5 (0 being worst, and 5 being best).

In the next step, DOCTORC calculated blood flow from the corrected vessel position, vessel type, and subjective rating. In DOCTORC, only the flow in veins was used for TRBF calculation because the blood flow speed in arteries was out of the measurable range of the OCT system.²⁴ For each vessel, the variance of the Doppler angle and the subjective quality rating were combined to decide if the flow is reliable, as described in previous reports.^{32,34} The flow in each vessel from all scans was averaged. Similar to previous studies, if a vein did not have a reliable measurement from all scans, the flow for that particular vein was defined as a product of the cross-sectional area of that vein and the estimated velocity averaged from other nearby veins. The estimated velocity was also adjusted according to vessel diameter because velocity is known to increase with vessel diameter.²⁴ The TRBF was the summation of flow in all veins. A reliability factor of TRBF, “valid cross-section area,” was defined as a ratio between the summed total vessel area of all reliable veins and the total venous area. Accordingly, if a particular eye had a valid cross-sectional area of $<50\%$, the flow result was considered unreliable. Such unreliable eyes were excluded from the subsequent analyses. Since only one eye was used for the summary normative statistics, at least one reliable eye was available for every subject. In cases where both eyes were of equal quality (with respect to this reliability/validity standard), the eye to be analyzed was randomly chosen.

Finally, the TRBF is corrected by axial length. In Doppler OCT, the scan is actually a fan scan. The angle of the fan is fixed, based on a model eye with axial length of 24 mm. Thus, the real scan area varies with different eye lengths. The magnification factor can be corrected.^{35–37} The length of an arc on the retina can be corrected with the ratio of axial length to default length. In DOCTORC, a vein's blood flow is equal to integration of Doppler phase shift/ $\cos(\alpha)$ in the vessel area ($X \cdot Z$). Here, α is the angle between laser beam and vessel; X and Z are the size of the vessel in the transverse and axial directions. Though Z needs no correction, the correction ratio still needs to be squared. One is for correction of the transverse size in the area calculation. The other is for correction of angle

TABLE 2. Univariate Regression Analysis to Study Correlations Between Blood Flow and Clinical Parameters

	<i>r</i>	<i>P</i> Value
Age	−0.15	0.012
BMI	0.10	0.10
Systolic BP	0.05	0.38
Diastolic BP	0.06	0.30
IOP	−0.01	0.86
Axial length	0.11	0.08

calculation because $1/\cos(\alpha)$ = (distance of vessel centers in transverse direction/distance of vessel centers in axial direction). Therefore, we obtained the formula for blood flow correction: axial length-corrected TRBF = $\text{oTRBF} \cdot (\text{axial length/default axial length})^2$. Here, oTRBF is the original total blood flow before eye length correction. For the default axial length, we used a value of 24 mm. A similar formula should also be applied to the following: vessel area = original vessel area $\cdot (\text{axial length/default axial length})$, and velocity = original velocity $\cdot (\text{axial length/default axial length})$. Note that the correction ratio was not squared. The axial length-corrected TRBF, vessel area, and velocity were used in later statistical analyses.

Statistical Analyses

The main outcome measures for analysis were blood flow, venous area and velocity, and arterial area and velocity. Descriptive statistics were calculated for all variables and expressed as mean ($\pm$ SD). Pearson correlations and linear regression were performed by using SAS 9.2 statistical software (The SAS Institute, Cary, NC, USA). The level of significance was set at 0.05.

RESULTS

Summary of Descriptive Statistics

A total of 266 eyes (122 right, 144 left) of 266 normal, healthy participants (79 men, 187 women) were included in this analysis (Table 1). Average age was 57.40 ($\pm$ 5.60) years with a range of 50 to 82 years. The mean ($\pm$ SD) TRBF was 49.34 $\pm$ 10.08 $\mu\text{L}/\text{min}$ (range, 27.17–78.08 $\mu\text{L}/\text{min}$, 95% confidence interval [CI]: 25.98–69.10 $\mu\text{L}/\text{min}$). There was a small but statistically significant difference between blood flow in the superior retinal hemisphere (25.50 $\pm$ 6.62 $\mu\text{L}/\text{min}$) and the inferior hemisphere (23.84 $\pm$ 7.19 $\mu\text{L}/\text{min}$), with a mean difference of 1.66 $\mu\text{L}/\text{min}$ (P = 0.008). There was no significant difference in blood flow between men (48.58 $\pm$ 9.84 $\mu\text{L}/\text{min}$) and women (49.66 $\pm$ 10.19 $\mu\text{L}/\text{min}$), P = 0.445, or between right and left eyes (48.47 $\pm$ 10.20 vs. 50.07 $\pm$ 9.96, respectively, P = 0.217), although this was not a comparison of the blood flow between both eyes of the same subject. The mean ($\pm$ SD) for inner diameter (D) of retinal veins was 0.0548 ($\pm$ 0.0084) mm^2 (range, 0.0335–0.0816 mm^2). The average venous velocity was 15.16 ($\pm$ 3.12) mm/s (range, 8.07–30.88 mm/s). The mean axial length was 23.90 ($\pm$ 1.24) mm with a range of 21.27 to 27.15 mm.

Correlation Analyses

Univariate regression (Pearson correlation) analysis revealed that TRBF was significantly correlated with age (Table 2). Total retinal blood flow declined with advancing age. The slope of total retinal blood flow versus age indicates an annual decline

TABLE 3. Univariate Regression Analysis to Study Correlations Between Blood Flow and Parameters

	<i>r</i>	<i>P</i> Value
Venous area	0.43	<0.0001
Venous velocity	0.70	<0.0001
Arterial area	0.13	0.03
Arterial velocity	0.58	<0.0001

of 0.27 $\mu\text{L}/\text{min}$ (95% CI: 0.03–0.51 $\mu\text{L}/\text{min}$) between the ages of 50 to 75 years. After correcting for magnification (axial length), there was no significant correlation although it showed a positive trend. The analysis did not demonstrate any significant correlation between blood flow and other clinical parameters such as BMI, systolic blood pressure, diastolic blood pressure, or intraocular pressure.

Total retinal blood flow also showed significant correlations with venous area and velocity and with arterial area and velocity (Table 3).

DISCUSSION

In the current study, we present the Doppler FD-OCT-derived retinal blood flow values in normal eyes of a Chinese-American population. The retinal blood flow values and ranges from this study would appear to corroborate previous smaller case series. For example, we observed a mean TRBF of $49.34 \pm 10.08 \mu\text{L}/\text{min}$ in our population, which falls within the range of values (40.8–52.9 $\mu\text{L}/\text{min}$) published in prior reports (only 10 normal values in previous report).³¹ Our result falls between the previously published values by Riva and colleagues,³⁸ which showed the venous blood flow of $34.0 (\pm 6.3) \mu\text{L}/\text{min}$, and those of Garcia et al.,⁷ with total venous blood flow of $64 (\pm 12.8) \mu\text{L}/\text{min}$ by laser Doppler flowmeter. The relationship between blood flow, velocity, and area measurements agrees with the results validated in the previous publication by Wang et al.²³

Although the mean ranges appear consistent, an important finding of our study, which has implications for interpretation of retinal blood values in future studies of disease, is that there appears to be considerable variability in retinal blood flow in normal subjects (95% CI: 26–69 $\mu\text{L}/\text{min}$). Moreover, the demographic, systemic, and ocular factors included in our analyses appeared to explain very little of this variability. We observed a weak negative correlation between age and retinal blood flow ($P = 0.012$), analogous to the findings from another study using laser Doppler flowmetry, which observed a 6% to 11% decrease in blood flow per decade.³⁹ The laser Doppler flowmetry study showed that increased age causes an increase in the retinal artery resistivity index, which causes a decrease in retinal blood flow. Lee et al.⁴⁰ have described morphologic changes of retinal vessels with myocyte alteration, which may be involved in age-related reduction of retinal blood flow. Longer axial length decreases the magnification of fundus imaging, making transverse dimensions appear smaller on the OCT scan, in inverse proportion to axial length.¹⁴ This would reduce the apparent transverse diameter of blood vessels (making vessel cross-sections artifactually lower) and increase the apparent Doppler angle (making flow velocity artifactually lower). Thus, if the magnification correction was not performed by using the square of axial length, then longer eyes would artifactually appear to have lower flow. In our study, without axial correlation, the TRBF was significantly correlated to axial length ($P < 0.001$). Based on both theoretical considerations and actual results, correction of magnification effects is necessary to accurately compare

Doppler OCT measurement of retinal blood flow between individual eyes. Thus, magnification correction is needed for research studies and diagnostic applications.

Other factors, such as BMI, intraocular pressure, and systemic blood pressure, had no apparent effect on blood flow. There was a statistically significant difference between the blood flow in the superior retinal hemisphere and that in the inferior retinal hemisphere. Superior retinal hemispheric blood flow was greater than inferior retinal hemispheric blood flow. This finding is consistent with the fact that the fovea is located inferiorly to the optic nerve head, the entry site of retinal arteries and veins.⁴¹ Therefore, the superior retinal arteries and veins serve a larger retinal area than the inferior retinal arteries and veins. The larger area of the superior retinal hemisphere would naturally lead to a greater perfusion need and greater flow.

A host of other physiological variables that could alter blood flow were not considered in our study, including the possibility of temporal/diurnal variations or the impact of the status of the systemic autonomic system or other unmeasured systemic variables. Other known nonphysiological sources of variability have also previously been studied and reported, including human grading variability (coefficient of variation of up to 10%) and the imputation method for nonvalid vessels. The total variability presented here represents a sum of population, physiologic, and technical measurement variabilities.

Our findings have implications for the design and interpretation of retinal blood flow studies of diseased eyes, using current methods. Age should be taken into account, but the residual variability remains large. The significant variability means that a large cohort size may be necessary to detect a biological signal. Small differences in mean blood flow, such as the difference between hemispheres in our study, require a relatively large sample size to be detected.

In addition to the technical issues discussed above, our study has other limitations. Although it is a prospective, cross-sectional epidemiologic study, only a subset of normal eyes with high-quality valid/reliable scans was included in the analysis cohort. Thus, there is a potential for selection bias. In addition, the cohort was restricted to Chinese Americans. As a result, the generalizability to other populations is uncertain. Our study, however, has many strengths, including a much larger normative cohort than any prior study and the use of a standardized grading protocol by certified experienced dedicated Doppler OCT reading center graders.

In summary, total retinal blood values in our normal Chinese-American population appeared to be similar to those described in previous reports. Blood flow values, however, can vary dramatically, with most of the factors that contribute to the variability remaining unidentified. Given that retinal blood flow may be an important quantitative parameter for a number of posterior segment diseases, our observations should be of value in guiding the design of future studies.

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