Flicker-induced changes in retinal blood flow assessed by Doppler optical coherence tomography

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Abstract: We used Doppler Fourier-domain optical coherence tomography (OCT) to investigate flicker-induced changes of total retinal blood flow. Total retinal blood flow was measured by summing flows in veins imaged in double-circular scans around the optic disc. In 3 healthy volunteers, total retinal blood flow was measured before and 10-15 seconds after 30 seconds of flicker stimulation. The average blood flow increased 22.2% (p = 0.002). The total venous and arterial vessel cross-sectional area increased 11.3% (p < 0.001) and +2.7% (p = 0.28) respectively. The average venous and arterial flow velocity were calculated indirectly by dividing total retinal blood flow by total venous and arterial cross-sectional areas. They also increased by 8.8% (p = 0.046) and 18.3% (p = 0.004), respectively. These results show that human retinal blood flow increases after visible flicker stimulation, and this could be measured with OCT.

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1. Introduction

Experimental investigations of cerebral blood flow responses to stimuli have led to a better understanding of the relationship between neural activity and blood flow [1,2]. In the human eye, the retina and optic nerve are optically accessible extensions of the central nervous system. This provides the unique opportunity to study neurovascular coupling and other physiological phenomena of neural tissues. Animal studies have shown that retinal and optic nerve hemodynamics are modulated by visual stimulation [3,4]. In human subjects, functional hyperemia at the rim of the optic nerve head was found to be coupled with retinal activity [5].

Numerous techniques have been used to measure variations in hemodynamic parameters of retinal and optic nerve blood flow as a response to light stimuli. However, these techniques have limited ability to provide comprehensive information. Ultrasound color Doppler imaging has sufficient resolution to measure the larger retrobulbar vessels [6], but it requires corneal contact and repositioning of the transducer after light stimulation. The Heidelberg retina flowmeter can measure in arbitrary units the flicker-induced blood flow change in capillary beds over a small region [7], but it cannot assess global volumetric retinal blood flow. Laser Doppler velocimetry measures total retinal blood flow (TRBF) by summing blood flow measurements from individual vessels [8–10]. This requires many measurements over a lengthy session [9]. Animal studies have shown that flicker-induced blood flow change decayed to baseline within one minute after cessation of the stimulus [11]. Therefore, laser Doppler velocimetry can only evaluate blood flow response to light stimuli in specific vessels, not the TRBF that reflects the overall retinal response.

Optical coherence tomography (OCT) [12] is an imaging technique that has widespread applications for the diagnosis and management of retinal diseases [13–16]. It has the requisite resolution to image retinal blood vessels [17]. As a coherent detection technique, OCT can detect the Doppler frequency shift of the backscattered light that provides information on blood flow [18,19]. With the development of high-speed Fourier-domain OCT (FD-OCT) techniques [20–22], it has become possible to capture the pulsatile dynamics of blood flow [23,24].

Using Doppler FD-OCT, we recently developed a double circular scanning pattern that measures the TRBF with data sampled within 2 seconds [25]. We have demonstrated that flow measurements in normal subjects [26] and retinal patients [27] can be reproducibly obtained. Fast data acquisition makes it adequate to access stimuli-induced TRBF responses. In this article, we report the first use of this new technique to measure human TRBF changes in response to flicker stimuli.

2. Study population

This study was performed at the Doheny Eye Institute, University of Southern California (USC). The research protocol was approved by the USC institutional review board and carried out in accordance with the tenets of the Declaration of Helsinki. Three healthy volunteers participated in the study.

3. Methods

3.1. Doppler optical coherence tomography

A spectrometer-based FD-OCT system (RTVue system, Optovue Inc., Fremont, CA, USA) was used for Doppler imaging. It operated at a wavelength of 840 nm with an axial resolution of 5 μm and a transverse resolution of 20 μm in the human eye. The power incident on the eye was 750 μW, which was below the ANSI safety limit [28]. The time interval between two sequential axial scans was 36.7 μs. The maximum measurable Doppler shift was 13.6 kHz at the phase wrapping limit of 2π radian phase shift between sequential axial scans. This corresponds to a maximum measurable axial velocity component of 4.2 mm/s.
For blood flow measurements, a double circular scanning pattern was employed to determine the angle between the probe beam and blood flow [25]. The scanning circles were centered at the optic nerve head and had radii of $r_1 = 1.7$ mm and $r_2 = 1.88$ mm. The frame rate for Doppler FD-OCT imaging was 6 circles per second (3 double circles per second). Scans were recorded over consecutive 2-second intervals.

3.2. Stimulus apparatus

The flicker stimulus apparatus consisted of an array of white light emitting diodes (LEDs) arrayed in a ring with an inner diameter of 35 mm and outer diameter of 90 mm. It was mounted around the objective lens of a RTVue OCT system (Fig. 1). The front surface of the LED array was covered with a plastic film that diffused the LED light for uniform illumination of the retina.

The LED array was controlled by a programmable electrical unit that was connected to a computer through a USB port. A square wave driving signal (repetition rate 8 Hz, modulation depth 100%) with a 50% duty cycle was generated by the computer, amplified by the electrical unit, and sent to the LED array. The brightness of the LED light was controlled through programming the output current from the electrical unit. For each stimulation period, the LED array was on for 30 seconds to illuminate a ring area on the retina.

![Fig. 1. Experiment setup of light stimulation with ring LED array. EU, Electrical unit; OL, Objective lens](image)

3.3. Safe light exposure level

In this study, the participant’s eye was illuminated for 30 seconds for each measurement of retinal blood flow. According to the ANSI safety limit [28], at visible wavelengths, the maximum permissible 30 second exposure is 0.05 W/cm$^2$ = 33.4 lumen/cm$^2$ (using 1 Watt = 668 lumen) on the cornea. In this study, the maximum ocular exposure was less than 1.0 lumen/cm$^2$, which was well below the ANSI limit.
3.4. Doppler image acquisition and processing

One eye of each subject was examined. Following pupil dilation with 1% tropicamide and 2.5% phenylephrine eye drops, each subject was seated in front of the OCT scanner and instructed to look at the center of the objective lens. The subject’s head was stabilized by a chin and forehead rest during flicker stimulation and OCT scanning. During light stimulation, the OCT scanning head was pulled back to a distance of about 80 mm between the objective lens and the eye. For OCT scanning, the distance between the objective lens and the eye was approximately 2 cm. Four measurements were performed at each stimulation brightness. The time interval between each measurement was about one minute.

Retinal blood flow was measured in post-processing according to a previously described method [25]. Blood vessels were identified based on OCT Doppler and reflectance images. For the RTVue system, the pixel dimensions are 11.2 μm in the horizontal dimension and 3.1 μm in the vertical dimension. Vessel diameter $D$ was measured by computer caliper on the cross-sectional Doppler OCT images and used to compute lumen area ($\pi D^2/4$). The venous cross-sectional areas for all branch vessels around the optic disk were summed together to obtain the total venous cross-sectional areas (TVCA) for the eye. There were six Doppler images sampled at radius $r_1$. The other six images were sampled at radius $r_2$. The speed profiles of a single vessel in the twelve Doppler images were calculated. Twelve flow velocities from the twelve flow profiles were normalized to the maximum one and plotted against time to show the flow pulsation. This curve was integrated as the pulsation factor [25]. For some veins, the Doppler flow signal was too weak for accurate reading at diastole, the pulsation factor in the adjacent venules was used for flow calculation.

The Doppler angle between the vessel and OCT beam was measured by the relative axial position of each vessel in the two concentric OCT images. Total flow velocity was computed from the Doppler frequency shift divided by the cosine of Doppler angle, with steps to account for the effect of background retinal motion and transverse scan step size. The standard error of Doppler angle measurements was 1.42° [25]. Thus if the incidence angle was less than that from perpendicular, the data was rejected to prevent excessive amplification of angle error from the cosine calculation. The Doppler angle in our study was small enough that the axial velocity component for the great majority of veins was within the range of the OCT system used. In the rare occasion where the peak axial velocity was between 2π and 4π at the center of the vessel, an unwrapping algorithm was applied to allow valid flow measurement [29]. Veins were identified by the flow direction toward the optic disc. Volumetric blood flow rate in each vein was calculated by integrating the velocity over the lumen cross-section. Flow measurements were averaged over each 2-second recording. Measurements from all valid scans were averaged. Total retinal blood flow was calculated by summing flow from all detectable veins. The average venous velocities were obtained by dividing the TRBF by TVCA. Retinal blood flow in arteries and veins should have an equal sum because in flow must equal outflow in any steady state system that obeys the law of conservation of mass. Thus, measuring total venous flow alone is sufficient to quantify the total retinal blood flow. The arterial cross-sectional areas for all branch arteries around the optic disk were summed together to obtain the total arterial cross-sectional areas (TACA) for the eye. Average arterial velocities were obtained by dividing the total retinal flow by the total arterial areas.

4. Statistical analysis

For measurements taken from each of the three subjects, a two-sided $t$-test was used to compare the parameters of flow, venous area, venous velocity, arterial area, and arterial velocity before and after light stimulation. Moreover, the generalized linear model with repeated measures was used to fit all three eyes in a single model for each of parameters compared. Results with $p < 0.05$ were considered significant. All of the statistical analyses were done with SAS 9.2 software (SAS Institute, Cary, NC, USA).
5. Results

In this study, the vessel diameter $D$ of retinal veins were measured from the Doppler OCT images and ranged from 45.02 to 150.55 $\mu$m. We calculated the relationship between baseline blood flow and $D$ by linear regression on a log-log scale for all veins (Fig. 2). The slope (mean $\pm$ SD) was $2.22 \pm 0.19$. This indicated that velocity increased with vessel diameter.

$$Y = (2.22 \pm 0.19) X - 3.63 \pm 0.38$$

$$R^2 = 0.88 \ (p < 0.001)$$

![Graph showing blood flow versus vessel diameter](image)

**Fig. 2.** Blood volume flow rate versus blood vessel diameter. Results are on a log-log scale. Solid line: best-fit result of linear regression ($P < 0.001$; $R^2 = 0.88$). Dotted lines: 95% confidence interval limits to the fitted line (solid).

![Graph showing variation of total retinal blood flow](image)

**Fig. 3.** Variation of total retinal blood flow at different stimulus brightness. The vertical line on each point shows the standard deviation (SD) of repeat measurements at each setting.
TRBF from three subjects was measured at different flicker brightness in ascending order (Fig. 3). For Subject #2 and #3, blood flow peaked at 2.88 and 5.0 Candela/cm², respectively. For Subject #1, TRBF was only measured at baseline and one flicker brightness.

We compared baseline TRBF with that after flicker stimulation at 2.88 Candela/cm² and above (Table 1). For the three participants, the average flow increased 22.2% (p = 0.002) after light stimulation.

For Subject #1, TRBF was only measured at baseline and one flicker brightness. Overall Group Subject 1 2 3

| Subject | Total Retinal Blood Flow |
|---------|--------------------------|
|         | Change (%) |
| 1       | 26.5 (p = 0.15) |
| 2       | 23.9 (p = 0.16) |
| 3       | 24.7 (p = 0.06) |

P values are in comparison with the base line. Blood flow shown is the total venous flow.

Retinal vessel size tended to increase on flicker stimulation (Table 2, flicker brightness ≥2.88 Candela/cm²). For venous vessels, the largest TVCA change was +16.7% in the subject #2. The average TVCA change for the three participants was 11.3% (p < 0.001). Because vessel diameter is proportional to the square root of the cross-sectional area, the average venous diameter change was +5.5%.

Table 2. Change in Total Vessel Cross-Sectional Area with Flicker Stimulation

| Subject | Total Venous Cross Section | Total Arterial Cross Section |
|---------|-----------------------------|------------------------------|
|         | Base line (mm²) | Stimulated (mm²) | Change (%) | Base line (mm²) | Stimulated (mm²) | Change (%) |
| 1       | 0.065 ± 0.001 | 0.074 ± 0.003 | +13.8 (p = 0.02) | 0.047 ± 0.001 | 0.048 ± 0.001 | +2.1 (p = 0.13) |
| 2       | 0.048 ± 0.001 | 0.056 ± 0.001 | +16.7 (p = 0.01) | 0.039 ± 0.001 | 0.032 ± 0.001 | +6.7 (p = 0.12) |
| 3       | 0.047 ± 0.001 | 0.049 ± 0.001 | +4.3 (p = 0.02) | 0.032 ± 0.002 | 0.033 ± 0.001 | +3.1 (p = 0.97) |
| Group   | 0.053 ± 0.010 | 0.059 ± 0.013 | +11.3 (p<0.001) | 0.036 ± 0.009 | 0.037 ± 0.010 | +2.7 (p = 0.28) |

We compared baseline TRBF with that after flicker stimulation at 2.88 Candela/cm² and above (Table 1). For the three participants, the average flow increased 22.2% (p = 0.002) after light stimulation.

Table 3. Change in Average Flow Velocity with Flicker Stimulation.

| Subject | Average Venous Velocity | Average Arterial Velocity |
|---------|-------------------------|---------------------------|
|         | Base line (mm/s) | Stimulated (mm/s) | Change (%) | Base line (mm/s) | Stimulated (mm/s) | Change (%) |
| 1       | 14.6 ± 1.3 | 16.0 ± 1.7 | +9.8 (p = 0.39) | 20.0 ± 1.3 | 24.6 ± 3.4 | +23.0 (p = 0.18) |
| 2       | 14.7 ± 1.9 | 16.8 ± 1.4 | +14.3 (p = 0.16) | 23.9 ± 4.3 | 29.8 ± 2.5 | +24.7 (p = 0.06) |
| 3       | 14.9 ± 0.6 | 15.3 ± 0.5 | +2.7 (p = 0.51) | 21.5 ± 0.8 | 23.0 ± 1.6 | +7.0 (p = 0.26) |
| Group   | 14.7 ± 0.2 | 16.0 ± 0.8 | +8.8 (p = 0.046) | 21.8 ± 1.9 | 25.8 ± 3.6 | +18.3 (p = 0.004) |

Arterial velocity is calculated by dividing the flow by the total arterial area in each eye.

We compared baseline average venous and arterial velocities with those after flicker light stimulation (Table 3, flicker brightness ≥2.88 Candela/cm²). The average venous flow velocity increased significantly, 8.8% (p = 0.046), and the average arterial velocity increased 18.3% (p = 0.004).

6. Discussion

Our results show that human TRBF increases after flicker light stimulation. This is the first study using Doppler Fourier-domain OCT to investigate the response of TRBF to visual stimulation. In the past, several approaches have been tried to measure changes in the retinal vascular circulation following visual light stimulation. Michelson et al. assessed by pulsed Doppler sonography blood flow velocities in response to flicker in the central retinal artery and vein [30]. They observed increases on the order of 60%. Scheiner et al., using the blue-
field entopic technique, described a transient increase in retinal white blood cell flux after flicker stimulation [31]. White blood cell flow should be proportional to retinal blood flow, though the coupling may vary between individuals. In another study using laser Doppler velocimetry, blood flow increased by 50% in individual retinal arteries and veins after light stimulation [32]. With laser targeted angiography, investigators observed a 30% increase of retinal blood flow [33].

In contrast to these previous studies, the present work allowed us to determine the response of the entire retinal circulation after light stimulation in absolute volumetric terms. For the three participants, the average TRBF increased by 22.2% compared to baseline. The average venous and arterial velocity also increased significantly. Flicker light brightness range, 1.5 to 7.6 candela/cm², was close to the brightness level, 5.5 candela/cm², used in Michelson’s study [30].

Using a fundus photograph-based vessel analyzer, Polak et al. assessed flicker stimulation-induced retinal vasodilatation [34]. They found an increase of 2.4% in retinal arterial diameters. In another flicker stimulation study, increases of 4.2% in retinal arterial diameters and 2.7% in retinal venous diameters were reported [35]. In our study, we observed a significant increase in venous diameters and a nonsignificant increase in arterial diameters.

In this study, the volumetric flow rate varied with the vessel diameter with a power coefficient of 2.22 ± 0.19, which agreed with our previous value of 2.13 [27]. This indicated that velocity increased with vessel diameter, as shown by Riva [10].

One important limitation of this study was the timing for retinal blood flow measurement after cessation of stimulation light. Due to the absence of fixation during the stimulus, we performed blood flow and vessel diameter measurement soon after the LEDs were turned off. It took about 10–15 seconds to reposition the probe beam for the OCT retinal scan. Light stimulation study showed that flicker-induced blood flow and vessel diameter change decayed to baseline within one minute after cessation of the stimulus [11,34]. The time delay before blood flow measurement introduced an uncertainty regarding the blood flow and vessel diameter decay after stimulation light off. Compared with 60% blood flow increase [30], the smaller blood flow increase (group average 22.2%) in this study might have been due to a longer interval between the flicker stimulation and OCT flow measurement. Garhofer et al., [32] showed that after cessation of flicker light, retinal arterial vessel diameter decreased more than that of veins. The smaller arterial vessel diameter response in this study might be also due to the measurement time delay. We are developing a new apparatus to enable OCT measurement of blood flow and maintenance of gaze fixation while the visible light stimulation continues concurrently.

Another limitation is that the measurement result is noisy on a vessel by vessel basis, especially for small vessels. Volumetric flow and velocity analysis showed that for small vessels, the results were dominated by noise, and measuring change on single vessel basis was simply too noisy. In our earlier study [27], we reported that global retinal blood flow was mainly contributed by large retinal vessels, and its measurement repeatability was 10.9%. Therefore, we only evaluated global blood flow result in this study, which showed significant trend.

Many of the retinal diseases are related to abnormalities in ocular blood flow, such as glaucoma and diabetic retinopathy. It is important to explore potential activity-induced vascular changes in assessing ocular pathophysiology. Such considerations motivated studies that were aimed at identifying potential mediators and modulators of functional hyperemia. Physiological stresses, such as hyperglycemia and increased intraocular pressure, as well as pathological conditions, such as glaucoma and diabetes, disrupt the neurovascular coupling. Thus, methods for visually assessing the induced vascular responses could be valuable for early detection of retinal and neuro-ophthalmic disorders.

The physiology of blood flow increase by light stimulation is not completely clear. An increase of glucose consumption in monkey retina was reported with flicker light stimulation
[36]. In the anesthetized rabbit, retinal glucose consumption and lactate formation increased significantly after light stimulation [37], thereby affecting retinal blood flow. Nitric oxide (NO) is also considered a possible modulator for neurovascular coupling [38]. An increase of NO was measured between retinal vessels through flicker stimulation of the dark-adapted pig retina [39]. To access the pathophysiology of ocular diseases having an activity-induced hemodynamical response, the mechanism underlying the blood flow change during flicker stimulation needs to be better understood.

7. Conclusion

In summary, we have demonstrated for the first time the feasibility of using Doppler Fourier-domain OCT to measure TRBF variation due to flicker visual stimulation. We found that the flicker light stimulus significantly increased volumetric blood flow, flow velocity, and vessel caliber. This study could greatly expand the utility of OCT in the study of mechanisms linking functional neural activity and retinal blood flow.

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