Retinal Vascular and Oxygen Temporal Dynamic Responses to Light Flicker in Humans

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Purpose. To mathematically model the temporal dynamic responses of retinal vessel diameter (D), oxygen saturation (SO_2), and inner retinal oxygen extraction fraction (OEF) to light flicker and to describe their responses to its cessation in humans.

Methods. In 16 healthy subjects (age: 60 ± 12 years), retinal oximetry was performed before, during, and after light flicker stimulation. At each time point, five metrics were measured: retinal arterial and venous D (D_A, D_V) and SO_2 (SO_{2A}, SO_{2V}), and OEF. Intra- and intersubject variability of metrics was assessed by coefficient of variation of measurements before flicker within and among subjects, respectively. Metrics during flicker were modeled by exponential functions to determine the flicker-induced steady state metric values and the time constants of changes. Metrics after the cessation of flicker were compared to those before flicker.

Results. Intra- and intersubject variability for all metrics were less than 6% and 16%, respectively. At the flicker-induced steady state, D_A and D_V increased by 5%, SO_{2V} increased by 7%, and OEF decreased by 13%. The time constants of D_A and D_V (14, 15 seconds) were twofold smaller than those of SO_{2V} and OEF (39, 34 seconds). Within 26 seconds after the cessation of flicker, all metrics were not significantly different from before flicker values ($P \geq 0.07$).

Conclusions. Mathematical modeling revealed considerable differences in the time courses of changes among metrics during flicker, indicating flicker duration should be considered separately for each metric. Future application of this method may be useful to elucidate alterations in temporal dynamic responses to light flicker due to retinal diseases.

Keywords: retina, retinal vessels, oxygen extraction fraction, light flicker stimulation, temporal dynamics

The retina is one of the most metabolically active tissues in the human body, requiring a constant supply of oxygen from the retinal and choroidal vasculatures.[1,2] One method to investigate the retinal vascular and metabolic functions is by presenting a physiological challenge such as light flicker stimulation. Light flicker has been shown to increase neural activity,[3,4] which leads to augmentation of retinal vessel diameter (D),[5] increased blood flow (BF),[6] and alteration of oxygen saturation of hemoglobin (SO_2) in retinal veins.[7,8] As a result of these changes, inner retinal oxygen delivery (DO_2) and oxygen metabolism (MO_2) increase in humans,[9] whereas the inner retinal oxygen extraction fraction (OEF) decreases.[8]

To date, several studies have reported the static effect of light flicker on D, BF, SO_2, or OEF by either measuring these values before and during a light flicker-induced steady state,[8,10,11] or by averaging their temporal dynamic responses to light flicker.[6,12–14] Some studies have reported the nonlinear temporal dynamic responses of D and BF to light flicker[4,5,15–17] or its cessation,[5,6,13,15–17] and one study qualitatively reported the nonlinear responses of SO_2 to light flicker.[7] These temporal dynamic responses to light flicker and its cessation indicate how the retina accommodates physiological challenges during both the transient nonsteady state and the induced steady state, as opposed to the static effect of light flicker, which only provides information on the flicker-induced steady state. However, to the best of our knowledge, previous studies have only reported the mathematical modeling of temporal dynamic responses of D and BF to light flicker,[3,17,18] while the temporal dynamic responses of SO_2 and OEF to light flicker and their mathematical modeling have not been described. Therefore, the purpose of the current study was to simultaneously assess the temporal dynamic responses of retinal D, SO_2, and OEF to light flicker and its cessation, and to propose a mathematical model for the behavior of these temporal dynamics during light flicker.

Methods

Subjects

The research study was approved by an Institutional Review Board at the University of Illinois at Chicago. Prior to enrollment, the research study was explained to the subjects and informed consents were obtained according to the tenets of the Declaration of Helsinki. Sixteen healthy subjects (60 ± 12 years of age; 5 males, 11 females) participated in the study.

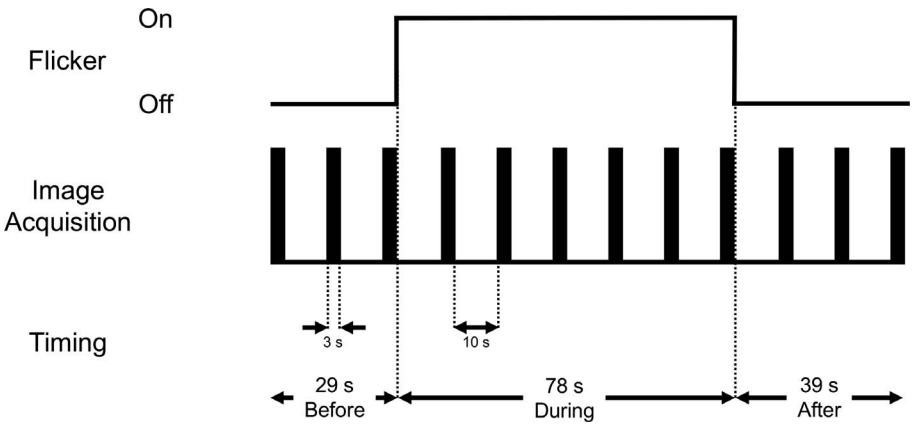

FIGURE 1. Schematic of the image acquisition protocol to assess the temporal dynamic responses of metrics to light flicker and its cessation.

Inclusion criteria were normal retinal examination and no history of eye disease. Subjects' pupils were dilated using 1% tropicamide and 2.5% phenylephrine, and subjects were seated in front of our modified slit lamp biomicroscope with their heads resting on a chin and forehead support. Light from a light emitting diode was presented to the fellow eye as a fixation target. Subjects were continuously light adapted during imaging due to the instrument's retinal illumination light. Imaging was performed in the right eyes of 12 subjects and the left eyes of four subjects. The left eye was selected because of subject's preference ($N = 2$), reduced visual acuity ($N = 1$), or choroidal nevus ($N = 1$) in the right eye. Subjects were excluded if the coefficient of variation of three repeated measures of D, SO_2, or OEF before light flicker was greater than 0.1.

Instrumentation

The image acquisition protocol and instrument control software of our previously established optical imaging system for the simultaneous quantification of metrics (D, SO_2, and OEF)[8] was modified to assess the temporal dynamic responses of these metrics to light flicker and its cessation. Briefly, a slit lamp biomicroscope was fitted with a rapid-switching filter wheel containing bandpass filters to illuminate the retina at multiple wavelengths. The optical imaging system provided light flicker stimulation at 10 Hz using light at 530 nm. In the current study, retinal reflectance images at 606 and 570 nm wavelengths were acquired periodically every 13 seconds over a time course consisting of 29 seconds before flicker (three time points), 78 seconds during flicker (six time points), and 39 seconds after the cessation of flicker (three time points). The schematic diagram of the image acquisition protocol is shown in Figure 1. The 13-second interval was chosen to allow 3 seconds for image acquisition followed by a 10-second period for the subject to blink comfortably, regain fixation, and allow the operator to optimize alignment before the next image acquisition.

At each time point, nine images were acquired at each of the two wavelengths, which were registered and averaged to generate two mean images. These two mean images were then also registered. A circumpapillary region of interest (ROI) extending between one and two disc radii from the perimeter of the optic disc was selected. Measurements of D and SO_2 from each major vessel within the ROI were obtained and averaged to yield a mean retinal arterial and venous D (D_A, D_V) and SO_2 (SO_{2A}, SO_{2V}), as previously described.[8]

Calculation of the Inner Retinal OEF

We also calculated the inner retinal OEF. OEF represents the fraction of oxygen from the retinal vasculature that is available for use by the inner retinal tissue, and is defined as $(BF^*(O_{2A}–O_{2V}))/(BF^*O_{2A})$, where O_{2A} is the arterial oxygen content, O_{2V} is the venous oxygen content, and BF is retinal blood flow. Since BF is a determinant of both the numerator and denominator, this term cancels. Further, since the dissolved oxygen content of blood is minimal,[19] oxygen content is closely approximated by SO_2. Thus, OEF was calculated as $(SO_{2A}–SO_{2V})/SO_{2A}$. According to the Fick principle, which applies to steady state conditions, OEF is also equal to the ratio of MO_2 to DO_2.[8,20] MO_2 is the rate that the inner retinal tissue consumes oxygen provided by the retinal circulation, and DO_2 is the rate that oxygen enters the retinal circulation. Therefore, OEF calculation under steady state conditions can be used to provide information on the ratio of MO_2 to DO_2, without calculating either directly. However, under nonsteady state conditions, such as those immediately after the initiation or cessation of light flicker, the ratio defined by MO_2 to DO_2 differs from OEF according to the accumulation or depletion of oxygen in the inner retina.

Data Analysis

Mean and standard deviation (SD) of metrics (D_A, D_V, SO_{2A}, SO_{2V}, and OEF) from the three repeated measurements acquired during the 29 seconds before light flicker were determined per subject. Intrasubject variability was assessed by coefficient of variation (SD/mean) and averaged over all subjects. Based on data in all subjects, mean and SD of metrics before light flicker were determined and intersubject variability was calculated by the coefficient of variation.

Metric ratios were defined as the metric values at each time point (during and after cessation of light flicker) divided by the mean of three repeated metric values obtained before initiation of light flicker. For example, the metric ratio of D_A (D_AR) at the first time point during light flicker was calculated as $D_{A\_first\ time\text{-}point}$ /$D_{A\_mean\ before\ flicker}$. Data analyses were performed on metric ratios rather than metric values to normalize data in each subject. Metric ratios (D_AR, DVR, $SO_{2A}R$, $SO_{2V}R$, OEFR) were averaged among subjects at each time point to generate mean temporal dynamic responses during light flicker and after its cessation. All statistical analyses were performed using SPSS software (version 22; SPSS, Chicago, IL, USA).

Temporal Dynamic Responses to Light Flicker. We reasoned that, for a well-regulated system like the retina, under

Table 1. Metric Ratios of Retinal Arterial and Venous Diameter (D_AR, D_VR), Retinal Arterial and Venous Oxygen Saturation ($SO_{2A}R$, $SO_{2V}R$), and Inner Retinal Oxygen Extraction Fraction (OEFR) at 6 Time Points During Light Flicker

	Time From Initiation of Light Flicker, s					
Metric Ratio	**13**	**26**	**39**	**52**	**65**	**78**
D_AR	1.035 ± 0.051	1.033 ± 0.037	1.045 ± 0.032	1.040 ± 0.029	1.044 ± 0.035	1.056 ± 0.043
D_VR	1.034 ± 0.044	1.049 ± 0.038	1.038 ± 0.040	1.051 ± 0.054	1.048 ± 0.047	1.065 ± 0.034
$SO_{2A}R$	0.997 ± 0.028	1.000 ± 0.027	0.996 ± 0.026	0.994 ± 0.024	0.994 ± 0.020	0.993 ± 0.022
$SO_{2V}R$	1.023 ± 0.057	1.041 ± 0.042	1.041 ± 0.058	1.052 ± 0.050	1.050 ± 0.050	1.069 ± 0.055
OEFR	0.946 ± 0.100	0.917 ± 0.070	0.921 ± 0.089	0.897 ± 0.076	0.899 ± 0.084	0.865 ± 0.086

Data are presented as mean ± SD.

a physiological perturbation like light flicker, the rate of change in a metric ratio would be inversely proportional to the deviation of the metric ratio from its value before light flicker. In other words, metric ratio changes occur rapidly at the initiation of light flicker and eventually slow with time as the metric ratios approach their flicker-induced steady states. Therefore, as a first approximation, we used a model in which metric ratios changed during light flicker according to an exponential function, similar to the approach used to evaluate the response of retinal tissue oxygen tension to changes in light levels.[21–24] General shape of this exponential change in metric ratios during light flicker was given by the following equation:

$$Metric\ Ratio(t) = A + \left(B * \left(1 - e^{-t/C}\right)\right) \qquad (1)$$

where t represents time during light flicker, A represents the fitted metric ratio at $t = 0$, B represents the difference in the fitted metric ratio from $t = 0$ to $t \rightarrow \infty$ (i.e., the maximal flicker-induced change in the metric ratio), and C represents the time constant; that is, the time for the fitted metric ratio to reach $1–e^{-1}$ (~63%) of the maximal change. Using this notation, the sum of A and B represents the metric ratio at the flicker-induced steady state.

The temporal dynamic response of each metric ratio was fitted with the aforementioned exponential function using seven data points: a preflicker reference ratio (1.00) and six metric ratio values acquired during light flicker. All data were fit using Matlab 2015 (Mathworks, Natick, MA, USA) to determine values of A, B, C, and R^2 for each fitted exponential function.

Temporal Dynamic Response to the Cessation of Light Flicker. To elucidate the recovery of metrics after the cessation of light flicker, metric ratios from all subjects at each of the three time points were compared to the preflicker reference ratio using 1-sample t-tests.

Results

Variability of Measurements

Intrasubject variability of D_A, D_V, SO_{2A}, SO_{2V}, and OEF was 2%, 1%, 1%, 4%, and 5%, respectively. Mean D_A, D_V, SO_{2A}, SO_{2V}, and OEF before light flicker stimulation was 86 ± 7 μm, 105 ± 16 μm, 92% ± 4%, 60% ± 6%, and 0.35 ± 0.05, respectively (N = 16). Intersubject variability of D_A, D_V, SO_{2A}, SO_{2V}, and OEF was 8%, 16%, 5%, 10%, and 16%, respectively. Metric ratios D_AR, D_VR, $SO_{2A}R$, $SO_{2V}R$, and OEFR during and after light flicker are provided in the Tables 1 and 2.

Temporal Dynamic Responses to Light Flicker: D_AR and D_VR

Figure 2 shows the temporal dynamic responses of D_AR and D_VR. For both metrics, the exponential function was a good fit ($R^2 \geq 0.87$). From the exponential functions, the flicker-induced steady state values of D_AR and D_VR were 1.046 and 1.053, respectively, indicating vasodilation of 5% during light flicker. Time constants of exponential fits for D_AR and D_VR were 14 and 15 seconds, respectively, indicating relatively rapid vasodilation in response to light flicker. Further, at the last time point during light flicker (i.e., 78 seconds after the initiation of light flicker), the changes in D_AR and D_VR had reached over 99% of their maximal flicker-induced changes, as indicated by their exponential fits.

Temporal Dynamic Responses to Light Flicker: $SO_{2A}R$, $SO_{2V}R$, and OEFR

Figure 3 shows the temporal dynamic responses of both $SO_{2A}R$ and $SO_{2V}R$, while Figure 4 shows the temporal dynamic response of OEFR. The exponential functions were excellent fits for both $SO_{2V}R$ and OEFR ($R^2 \geq 0.93$), whereas the fit for $SO_{2A}R$ had a lower R^2 of 0.77. From the exponential functions, the flicker-induced steady state values of $SO_{2A}R$, $SO_{2V}R$, and OEFR were 0.991, 1.071, and 0.868, respectively. Time constants of exponential fits for $SO_{2A}R$, $SO_{2V}R$, and OEFR were 70, 39, and 34 seconds, respectively. At the last time point during light flicker (i.e., 78 seconds after the initiation of light flicker), changes in $SO_{2V}R$ and OEFR from the exponential fits had reached nearly 90% of their maximal flicker-induced changes, whereas the change of the $SO_{2A}R$ fit had only reached 70% of its maximal change.

Temporal Dynamic Responses to the Cessation of Light Flicker: D_AR and D_VR

Within 13 seconds after the cessation of light flicker, D_AR was not significantly different from the preflicker reference ratio (P = 0.4), whereas D_VR remained elevated by 3% ($P < 0.001$) (Fig. 2). However, for all following time points after the cessation of

Table 2. Metric Ratios of Retinal Arterial and Venous Diameter (D_AR, D_VR), Retinal Arterial and Venous Oxygen Saturation ($SO_{2A}R$, $SO_{2V}R$) and Inner Retinal Oxygen Extraction Fraction (OEFR) at 3 Time Points After the Cessation of Light Flicker

	Time From Cessation of Light Flicker, s		
Metric Ratio	**13**	**26**	**39**
D_AR	1.008 ± 0.035	0.993 ± 0.049	0.984 ± 0.048
D_VR	1.033 ± 0.040	1.023 ± 0.046	1.012 ± 0.024
$SO_{2A}R$	0.998 ± 0.026	1.004 ± 0.022	1.005 ± 0.013
$SO_{2V}R$	1.020 ± 0.065	1.026 ± 0.063	1.006 ± 0.054
OEFR	0.965 ± 0.094	0.963 ± 0.094	0.997 ± 0.085

Data are presented as mean ± SD.

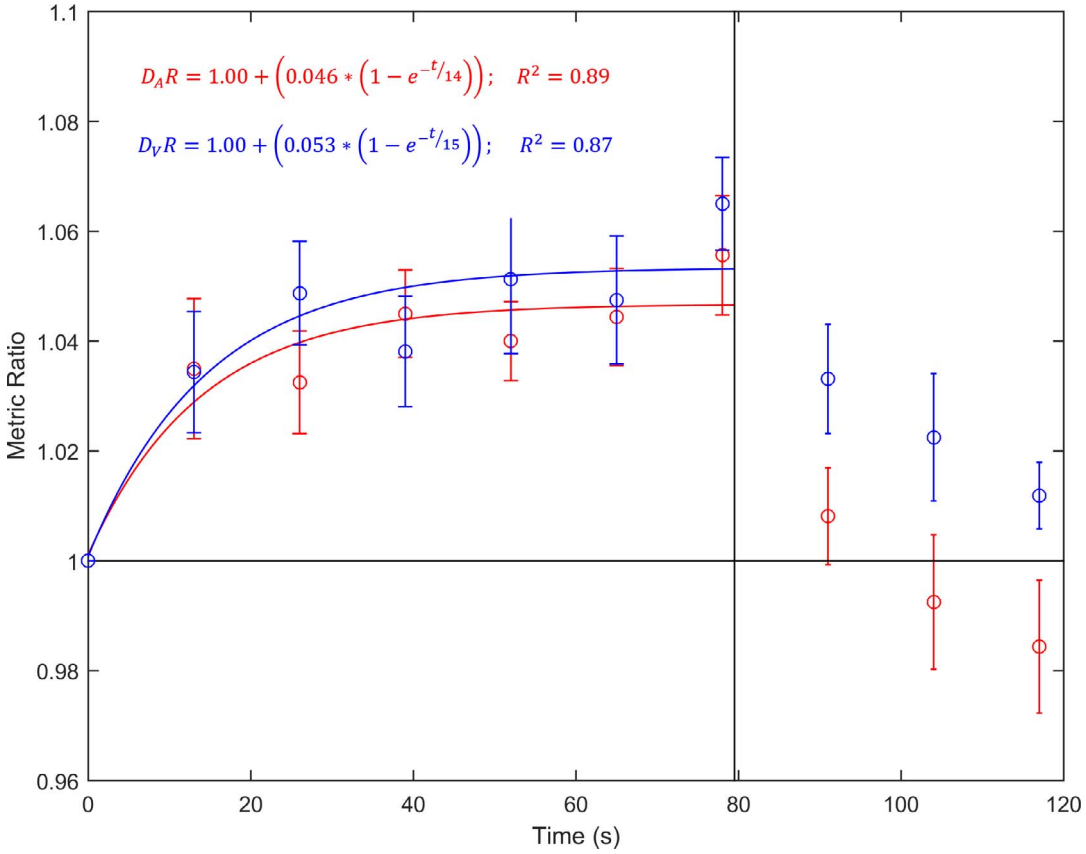

Figure 2. Mean metric ratio measurements of retinal arterial and venous diameter (D_AR – *red circles*; D_VR – *blue circles*) during light flicker and after its cessation from all subjects ($N = 16$). Best-fit exponential functions to both D_AR (*red curve*) and D_VR (*blue curve*) during light flicker are shown. The legend provides the exponential functions and R^2 values of D_AR (*red*) and D_VR (*blue*). Light flicker begins at $t = 0$, and the *vertical black line* indicates the time of cessation of light flicker. The *horizontal black line* indicates a reference ratio of 1.00. *Error bars* indicate standard error of the mean.

light flicker, both D_AR and D_VR were not significantly different from the preflicker reference ratio ($P \geq 0.07$).

Temporal Dynamic Responses to the Cessation of Light Flicker: $SO_{2A}R$, $SO_{2V}R$, and OEFR

Within 13 seconds after the cessation of light flicker, $SO_{2A}R$, $SO_{2V}R$, and OEFR were not significantly different from the preflicker reference ratio ($P \geq 0.13$) (Figs. 3, 4).

Discussion

In the current study, the simultaneous temporal dynamic responses of D_AR, D_VR, $SO_{2A}R$, $SO_{2V}R$, and OEFR during light flicker and after its cessation were reported in human subjects. To the best of our knowledge, this is the first study to mathematically model the temporal dynamic responses of $SO_{2A}R$, $SO_{2V}R$, and OEFR to light flicker stimulation.

The flicker-induced steady state values of D_AR and D_VR were 1.046 and 1.053, consistent with previous studies that found vasodilation of a similar magnitude during light flicker.[5,10,12,15–17] The flicker-induced steady state values of $SO_{2V}R$ and OEFR were 1.071 and 0.868, in agreement with previous studies that found an increase in SO_{2V}[7,8] and a decrease in OEF[8] with light flicker. The flicker-induced steady state value in $SO_{2A}R$ was 0.991 and represents essentially no change in SO_{2A} during light flicker, consistent with previous studies.[7,8]

The time constants of changes in both D_AR and D_VR in response to light flicker were similar to those reported by previous studies,[3,17] which substantiates the exponential modeling of the temporal dynamic responses in the current study. The rapid rise time of these metrics is also in agreement with a previous study that reported a 10-second time constant for the response of BF at the optic disk to light flicker.[3,18] Taken together, the time constants of D_AR, D_VR, and BF indicate that DO_2 would likely have a similar time constant, indicating a rapid increase of DO_2 at the initiation of light flicker. Indeed, the ability of DO_2 to increase rapidly during light flicker has been previously described as a result of complex neurovascular coupling mechanisms.[1,25,26] In contrast, the time constants of changes in $SO_{2V}R$ and OEFR in response to light flicker were more than twofold larger than those of D_AR, D_VR, and BF. The apparent mismatch between the supposed time constant of DO_2 and that of OEFR may have important implications concerning the temporal dynamic response of MO_2 to light flicker. However, OEF is the ratio of MO_2 to DO_2 only under steady state conditions,[8,20] and thus we cannot infer relative changes in MO_2 to DO_2 from OEFR measured during light flicker prior to the establishment of a flicker-induced steady state. Ultimately, future studies that directly measure the temporal dynamic responses of MO_2 and DO_2 to light flicker are necessary to determine the relationship between OEFR and the ratio of MO_2 to DO_2 in the nonsteady state. Nevertheless, this study demonstrates, for the first time, that the time courses of changes in $SO_{2V}R$ and OEFR to light flicker are considerably different from those of D_AR and D_VR.

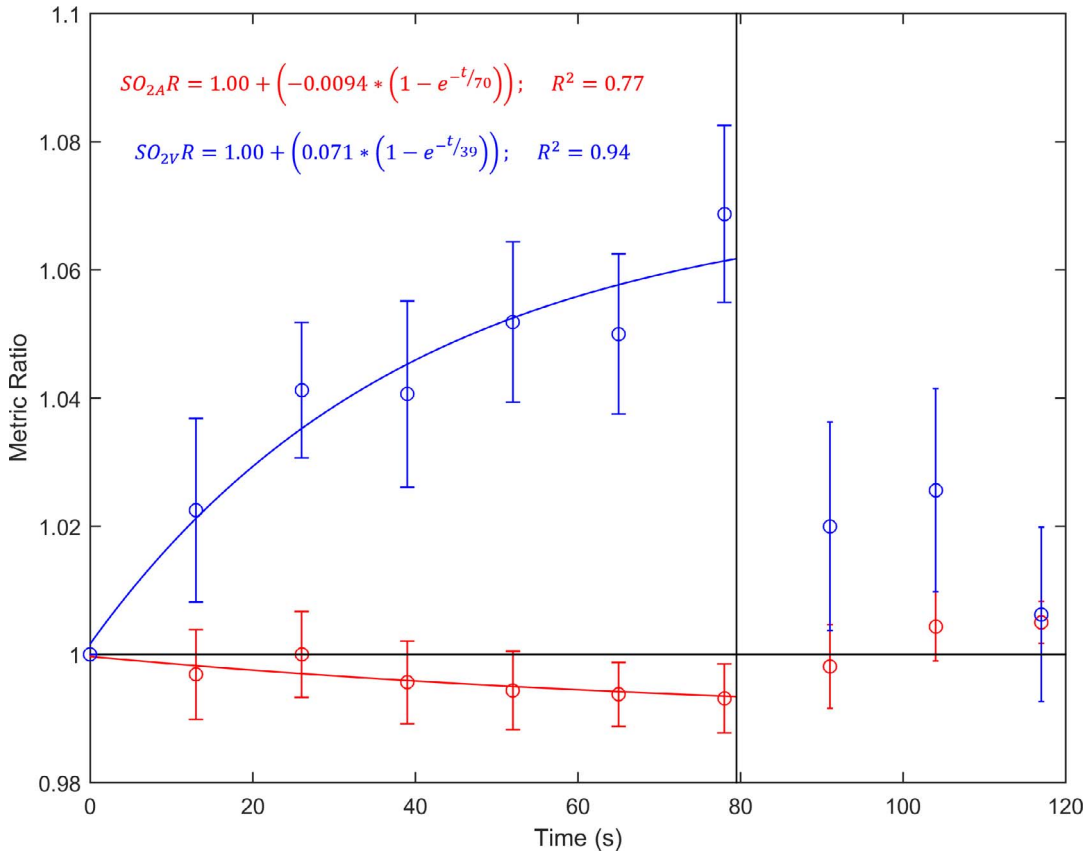

Figure 3. Mean metric ratio measurements of retinal arterial and venous oxygen saturation ($SO_{2A}R$ – *red circles*; $SO_{2V}R$ – *blue circles*) during light flicker and after its cessation from all subjects ($N = 16$). Best-fit exponential functions to both $SO_{2A}R$ (*red curve*) and $SO_{2V}R$ (*blue curve*) during light flicker are shown. The legend provides the exponential functions and R^2 values of $SO_{2A}R$ (*red*) and $SO_{2V}R$ (*blue*). Light flicker begins at $t = 0$, and the *vertical black line* indicates the time of cessation of light flicker. The *horizontal black line* indicates a reference ratio of 1.00. *Error bars* indicate standard error of the mean.

Indeed, to achieve 95% of the maximal flicker-induced change in a metric, a flicker duration of thrice the time constant is necessary. From the current study, a flicker duration of 45 seconds would be necessary for changes in D_AR and D_VR to reach 95% of their maximal flicker-induced changes, whereas 117 seconds would be necessary for $SO_{2V}R$. Thus, the duration of light flicker should be carefully considered when comparing the results of previous studies, particularly for metrics that have longer time constants.

Within 13 seconds after the cessation of light flicker, D_AR, $SO_{2V}R$, and OEFR had returned to the preflicker reference ratio, whereas D_VR remained elevated. These findings in D_AR and D_VR are consistent with previous studies that reported minimal arterial vasoconstriction and slight venous vasodilation within 10 seconds after the cessation of light flicker.[15–17] Although D_AR returned to baseline within 13 seconds after the cessation of light flicker, the continued elevation of D_VR may correspond to the phenomenon of delayed venous compliance.[27] Nevertheless, within 26 seconds after the cessation of light flicker, all metric ratios were not significantly different from the reference ratio, and the retina had essentially returned to its preflicker steady state.

There were several limitations in the current study. First, since data were derived based on an optical imaging technique, image quality may have affected measurements. However, inter- and intrasubject variability was low, indicating consistency in measurements. Second, the current study did not account for any potential effects of age on the temporal dynamic responses. Previous studies found no significant correlation between retinal vessel dilation during light flicker and age,[28–31] and one study reported a correlation between maximal vessel constriction after the cessation of light flicker and age.[29] Since the current study reported findings only in older subjects, future studies in younger individuals are necessary to elucidate any potential effects of age on temporal dynamics responses, particularly those after the cessation of light flicker. Third, the acquisition of images every 13 seconds limited the temporal resolution of data obtained in the current study. Future studies with finer temporal resolutions may permit the modeling of temporal dynamic responses after the cessation of light flicker, as well as better modeling of those during light flicker. Last, we modeled the complex temporal dynamic responses of metrics to light flicker with a relatively simple exponential function. Future studies may reveal better models of the temporal dynamic responses to light flicker.

In summary, the temporal dynamic responses of OEFR to light flicker and its cessation were reported in human subjects for the first time. Additionally, the temporal dynamic responses of all metrics to light flicker were fit with an exponential function allowing calculation of their flicker-induced steady state values and time constants. The time constant of the inner retinal OEF was more than twofold larger than those of the retinal vascular diameters, indicating considerable differences in the time courses of responses in these metrics to light flicker. Thus, the duration of light flicker should be carefully considered when comparing the results of previous studies. Future application of this technique is potentially useful to

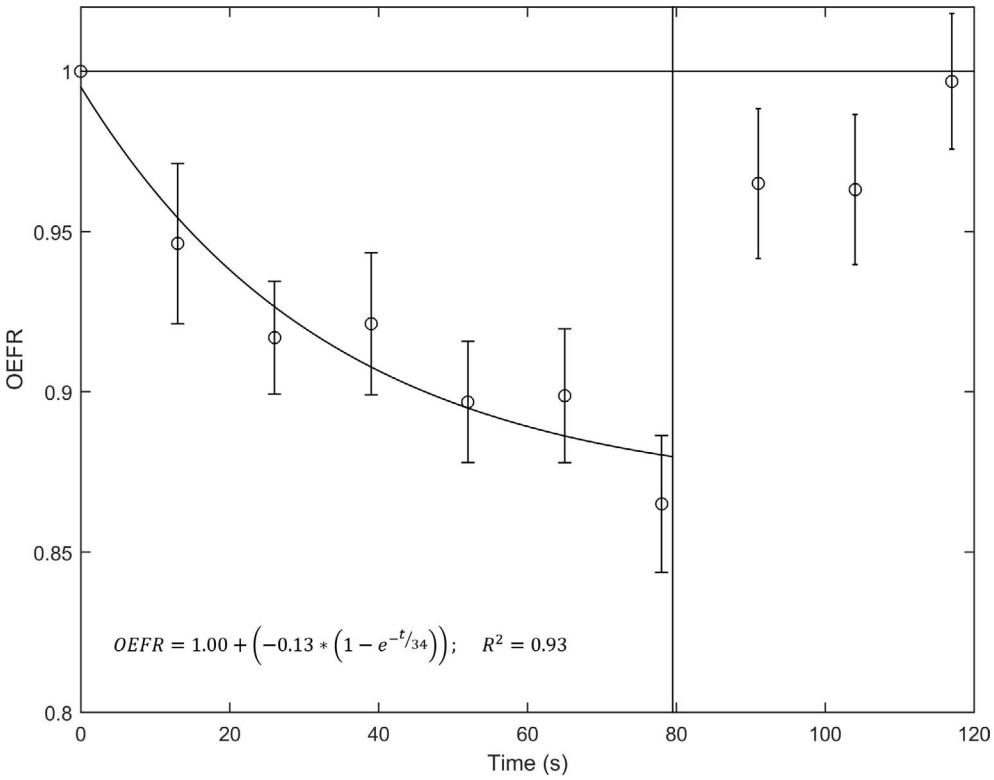

Figure 4. Mean metric ratio measurements of inner retinal OEF (OEFR – *circles*) during light flicker and after its cessation from all subjects ($N = 16$). A best-fit exponential function to OEFR (*curve*) during light flicker is shown. The legend provides the exponential function and R^2 value of OEFR. Light flicker begins at $t = 0$, and the *vertical black line* indicates the time of cessation of light flicker. The *horizontal black line* indicates a reference ratio of 1.00. *Error bars* indicate standard error of the mean.

elucidate normal physiology and the pathophysiology of retinal diseases.

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