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 (SO2), 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 (DA, DV) and SO2 (SO2A, SO2V), 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, DA and DV increased by 5%, SO2V increased by 7%, and OEF decreased by 13%. The time constants of DA and DV (14, 15 seconds) were twofold smaller than those of SO2V and OEF (39, 34 seconds). Within 26 seconds after the cessation of flicker, all metrics were not significantly different from before flicker values (P ≥ 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. 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, which leads to augmentation of retinal vessel stimulation. Light flicker has been shown to increase neural activity, which leads to augmentation of retinal vessel stimulation. Light flicker has been shown to increase neural activity, which leads to augmentation of retinal vessel stimulation.
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, SO2, 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, SO2, and OEF) 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 550 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 SO2 from each major vessel within the ROI were obtained and averaged to yield a mean retinal arterial and venous D (DA, DV) and SO2 (SO2A, SO2V), as previously described.

**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 \( BF*(O2A-O2V)/(BF*O2A) \), where \( O2A \) is the arterial oxygen content, \( O2V \) 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, oxygen content is closely approximated by SO2. Thus, OEF was calculated as \( (SO2A-SO2V)/SO2A \). According to the Fick principle, which applies to steady state conditions, OEF is also equal to the ratio of \( MO2 \) to \( DO2 \). \( MO2 \) is the rate that the inner retinal tissue consumes oxygen provided by the retinal circulation, and \( DO2 \) 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 \( MO2 \) to \( DO2 \), 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 \( MO2 \) to \( DO2 \) differs from OEF according to the accumulation or depletion of oxygen in the inner retina.

**Data Analysis**

Mean and standard deviation (SD) of metrics (DA, DV, SO2A, SO2V, and OEF) from the three repeated measurements acquired during the 29 seconds before light flicker were determined per subject. Intravariable 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 \( DA \) (DA(R)) at the first time point during light flicker was calculated as \( DA_{first\ time\ point}/DA_{mean\ before\ flicker} \). Data analyses were performed on metric ratios rather than metric values to normalize data in each subject. Metric ratios (DA(R), DVR, SO2A(R), SO2V(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
Inner Retinal Oxygen Extraction Fraction (OEFR) at 6 Time Points During Light Flicker

- **Temporal Dynamic Responses to Light Flicker:**
  - DAR, DVR, SO2AR, SO2VR, and OEFR during and after light flicker are provided in Tables 1 and 2.

**Results**

**Variability of Measurements**

Intrasubject variability of Da, Dv, SO2AR, SO2VR, and OEFR was 2%, 1%, 1%, 4%, and 5%, respectively. Mean Da, Dv, SO2AR, SO2VR, 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 Da, Dv, SO2AR, SO2VR, and OEF was 8%, 16%, 5%, 10%, and 16%, respectively. Metric ratios Da,R, Dv,R, SO2AR, SO2VR, and OEFR during and after light flicker are provided in Tables 1 and 2.

**Temporal Dynamic Responses to Light Flicker:**

- **DAR and DVR**
  - Figure 2 shows the temporal dynamic responses of Da,R and Dv,R. For both metrics, the exponential function was a good fit (R² ≥ 0.87). From the exponential functions, the flicker-induced steady state values of Da,R and Dv,R were 1.046 and 1.053, respectively, indicating vasodilatation of 5% during light flicker. Time constants of exponential fits for Da,R and Dv,R were 14 and 15 seconds, respectively, indicating relatively rapid vasodilatation 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 Da,R and Dv,R had reached over 99% of their maximal flicker-induced changes, as indicated by their exponential fits.

**Temporal Dynamic Responses to the Cessation of Light Flicker:**

Within 13 seconds after the cessation of light flicker, Da,R was not significantly different from the preflicker reference ratio (P = 0.4), whereas Da,R remained elevated by 3% (P < 0.001) (Fig. 2). However, for all following time points after the cessation of light flicker, Da,R and Dv,R were not significantly different from the preflicker reference ratio (P > 0.05).

**Table 1.** Metric Ratios of Retinal Arterial and Venous Diameter (Da,R, Dv,R), Retinal Arterial and Venous Oxygen Saturation (SO2AR, SO2VR), and Inner Retinal Oxygen Extraction Fraction (OEFR) at 6 Time Points During Light Flicker

| Metric Ratio | Time From Initiation of Light Flicker, s |
|--------------|-----------------------------------------|
|              | 13           | 26   | 39   | 52   | 65   | 78   |
| Da,R         | 1.035 ± 0.051 | 1.033 ± 0.037 | 1.045 ± 0.032 | 1.040 ± 0.029 | 1.044 ± 0.035 | 1.056 ± 0.043 |
| Dv,R         | 1.054 ± 0.049 | 1.049 ± 0.038 | 1.038 ± 0.040 | 1.051 ± 0.054 | 1.048 ± 0.047 | 1.065 ± 0.034 |
| SO2AR,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 |
| SO2VR,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.

**Table 2.** Metric Ratios of Retinal Arterial and Venous Diameter (Da,R, Dv,R), Retinal Arterial and Venous Oxygen Saturation (SO2AR, SO2VR), and Inner Retinal Oxygen Extraction Fraction (OEFR) at 3 Time Points After the Cessation of Light Flicker

| Metric Ratio | Time From Cessation of Light Flicker, s |
|--------------|-----------------------------------------|
|              | 13           | 26   | 39   |
| Da,R         | 1.008 ± 0.035 | 0.993 ± 0.049 | 0.984 ± 0.048 |
| Dv,R         | 1.053 ± 0.040 | 1.023 ± 0.046 | 1.012 ± 0.024 |
| SO2AR,R      | 0.998 ± 0.026 | 1.004 ± 0.022 | 1.005 ± 0.013 |
| SO2VR,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.
light flicker, both DAR and DVR were not significantly different from the preflicker reference ratio ($P \geq 0.07$).

**DISCUSSION**

In the current study, the simultaneous temporal dynamic responses of DAR, DVR, SO2AR, SO2VR, 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 SO2AR, SO2VR, and OEFR to light flicker stimulation.

The flicker-induced steady state values of DAR and DVR 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 SO2R and OEFR were 1.071 and 0.868, in agreement with previous studies that found an increase in SO2R7,8 and a decrease in OEF8 with light flicker. The flicker-induced steady state value in SO2AR was 0.991 and represents essentially no change in SO2A during light flicker, consistent with previous studies.7,8

The time constants of changes in both DAR and DVR in response to light flicker were similar to those reported by previous studies,5,17 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 DAR, DVR, and BF indicate that DO2 would likely have a similar time constant, indicating a rapid increase of DO2 at the initiation of light flicker. Indeed, the ability of DO2 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 SO2VR and OEFR in response to light flicker were more than twofold larger than those of DAR, DVR, and BF. The apparent mismatch between the supposed time constant of DO2 and that of OEFR may have important implications concerning the temporal dynamic response of MO2 to light flicker. However, OEF is the ratio of MO2 to DO2 only under steady state conditions,8,20 and thus we cannot infer relative changes in MO2 to DO2 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 MO2 and DO2 to light flicker are necessary to determine the relationship between OEFR and the ratio of MO2 to DO2 in the nonsteady state. Nevertheless, this study demonstrates, for the first time, that the time courses of changes in SO2R and OEFR to light flicker are considerably different from those of DAR and DVR.
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 DAR and DVR to reach 95% of their maximal flicker-induced changes, whereas 117 seconds would be necessary for SO2VR. 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, DAR, SO2VR, and OEFR had returned to the preflicker reference ratio, whereas DVR remained elevated. These findings in DAR and DVR 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 DAR returned to baseline within 13 seconds after the cessation of light flicker, the continued elevation of DVR 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 3](image-url)

**Figure 3.** Mean metric ratio measurements of retinal arterial and venous oxygen saturation (SO2AR – red circles; SO2VR – blue circles) during light flicker and after its cessation from all subjects (N = 16). Best-fit exponential functions to both SO2AR (red curve) and SO2VR (blue curve) during light flicker are shown. The legend provides the exponential functions and $R^2$ values of SO2AR (red) and SO2VR (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.

\[
SO_{2AR} = 1.00 + \left( -0.0094 \times \left( 1 - e^{-t/\tau} \right) \right); \quad R^2 = 0.77
\]

\[
SO_{2VR} = 1.00 + \left( 0.071 \times \left( 1 - e^{-t/\tau} \right) \right); \quad R^2 = 0.94
\]
elucidate normal physiology and the pathophysiology of retinal diseases.

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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.

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