Retina

Inner Retinal Oxygen Extraction Fraction in Response to Light Flicker Stimulation in Humans

Anthony E. Felder, Justin Wanek, Norman P. Blair, and Mahnaz Shahidi

Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago, Chicago, Illinois, United States

Correspondence: Mahnaz Shahidi, Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago, 1855 West Taylor Street, Chicago, IL 60612, USA; mahnshah@uic.edu.

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Purpose. Light flicker has been shown to stimulate retinal neural activity, increase blood flow, and alter inner retinal oxygen metabolism (MO_2) and delivery (DO_2). The purpose of the study was to determine the change in MO_2 relative to DO_2 due to light flicker stimulation in humans, as assessed by the inner retinal oxygen extraction fraction (OEF).

Methods. An optical imaging system, based on a modified slit lamp biomicroscope, was developed for simultaneous measurements of retinal vascular diameter (D) and oxygen saturation (SO_2). Retinal images were acquired in 20 healthy subjects before and during light flicker stimulation. Arterial and venous D (D_A and D_V) and SO_2 (SO_{2A} and SO_{2V}) were quantified within a circumpapillary region. Oxygen extraction fraction was defined as the ratio of MO_2 to DO_2 and was calculated as $(SO_{2A} - SO_{2V})/SO_{2A}$. Reproducibility of measurements was assessed.

Results. Coefficients of variation and intraclass correlation coefficients of repeated measurements were $<5\%$ and ≥ 0.83, respectively. During light flicker stimulation, D_A, D_V, and SO_{2V} significantly increased ($P \leq 0.004$). Oxygen extraction fraction was 0.37 ± 0.08 before light flicker and significantly decreased to 0.31 ± 0.07 during light flicker ($P = 0.001$).

Conclusions. Oxygen extraction fraction before and during light flicker stimulation is reported in human subjects for the first time. Oxygen extraction fraction decreased during light flicker stimulation, indicating the change in DO_2 exceeded that of MO_2. This technology is potentially useful for the detection of changes in OEF response to light flicker in physiological and pathological retinal conditions.

Keywords: retina, oxygen extraction fraction, oxygen saturation, vessel diameter, oxygen metabolism, oxygen delivery, human

The retina is one of the most metabolically active tissues in the human body, requiring a constant supply of oxygen to meet its energy demand.[1,2] Diffuse light flicker stimulation has been shown to stimulate neural activity[3] which dilates retinal vessels,[4] increases blood flow,[5] and alters the vascular oxygen saturation (SO_2) of hemoglobin,[6] implying an increase in oxygen delivery from the retinal circulation. This process is known as functional hyperemia.[7] Moreover, light flicker stimulation has been reported to increase retinal glucose[8,9] and oxygen metabolism[9,10] in animals. However, the change in inner retinal oxygen metabolism (MO_2) relative to inner retinal oxygen delivery (DO_2) due to light flicker stimulation has not been reported in humans. This change in MO_2 relative to DO_2 is indicative of the capacity of the retinal circulation to address alterations in tissue metabolic demand due to light flicker stimulation.

Inner retinal oxygen extraction fraction (OEF) quantifies the ratio of MO_2 to DO_2, although it does not provide an absolute measurement of either quantity. In this study, MO_2 is defined as the rate that oxygen is extracted from the retinal circulation for energy metabolism in units of volume of oxygen per unit time, and DO_2 is defined as the rate that oxygen becomes available from the retinal circulation in the same units. According to Fick's principle,[11] MO_2 is the product of retinal blood flow (BF) and the arteriovenous oxygen content difference, and DO_2 is the product of BF and the arterial oxygen content. Since the dissolved oxygen content of blood is minimal,[12] SO_2 can be used to estimate oxygen content. Furthermore, since BF is a determinant of both MO_2 and DO_2, the ratio defined by OEF is independent of BF. Thus, as shown in Equation 1, OEF can be calculated by measurement of arterial and venous SO_2 (SO_{2A} and SO_{2V}, respectively),[13] without determining either MO_2 or DO_2:

$$OEF = \frac{MO_2}{DO_2} = \frac{BF * (SO_{2A} - SO_{2V})}{BF * (SO_{2A})} = \frac{SO_{2A} - SO_{2V}}{SO_{2A}} \qquad (1)$$

In the current study, we report an optical imaging system developed for simultaneous measurement of retinal vascular diameter (D) and SO_2 before and during light flicker stimulation in human subjects. We tested the hypothesis that OEF remains unchanged during light flicker stimulation, indicating the change in DO_2 matches that of MO_2 in healthy human subjects.

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

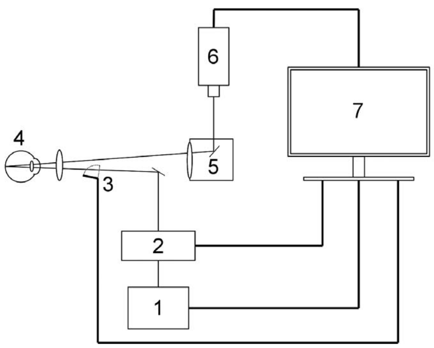

FIGURE 1. Schematic diagram of the optical imaging system for simultaneous measurement of retinal vascular diameter and oxygen saturation before and during light flicker stimulation. *Thick lines* represent physical connections between system components and *thin lines* represent the optical path.

of the Declaration of Helsinki. Subjects' pupils were dilated using 1% tropicamide and 2.5% phenylephrine. Subjects were seated in front of a modified slit lamp biomicroscope with their heads resting on a chin and forehead support and a light-emitting diode was presented to the fellow eye as a fixation target. While the room lights were turned off, retinal imaging was performed in the study eye before and during light flicker stimulation. Since subjects were exposed to the instrument's retinal illumination light throughout imaging, they were continuously light adapted. Twenty healthy subjects (age: 53 ± 17 years; 11 male, 9 female) participated in the study. In four subjects, three sets of repeated images were acquired to determine the reproducibility of the measurements.

Instrumentation

An optical imaging system (Fig. 1) was developed for simultaneous measurement of retinal vascular D and SO_2 before and during light flicker stimulation. A slit lamp biomicroscope (Carl Zeiss Microscopy GmbH, Jena, Germany) was modified to incorporate a rapid-switching filter wheel (FW103H, Thorlabs, Newton, NJ, USA) which integrated three bandpass filters with transmission wavelengths of 606 ± 5 nm, 570 ± 5 nm, and 530 ± 5 nm (Edmund Optics, Barrington, NJ, USA). Light at 606 nm and 570 nm corresponded to oxygen-sensitive and oxygen-insensitive imaging wavelengths, respectively,[14] whereas the 530-nm wavelength was chosen for light flicker stimulation to elicit a maximal vasodilatory response.[15,16] A solenoid with an attached shutter was also integrated into the optical imaging system to provide light flicker at a frequency of 10 Hz[17] with a 50% duty cycle. Prior to image acquisition, image alignment was performed in less than 2 minutes using light at 530 nm and 45 μW. Image acquisition before light flicker was achieved within 3 seconds by capturing 9 retinal reflectance images at 606 (230 μW) and 570 nm (180 μW), using an electron multiplying charge coupled device (Rolera EM-C2; QImaging, Surrey, British Columbia). Then light flicker was provided with light at 530 nm and 25 μW for 60 seconds. Image acquisition during light flicker was then performed through continued light flicker and rapid filter wheel adjustment to acquire images at 606 and 570 nm at approximately 50% of the light level before light flicker. A customized computer program was developed using a visual programming language (LabView 2013, National Instruments, Austin, TX, USA) and incorporated an Arduino microprocessor (Arduino Duemilanove; Arduino, Ivrea, Italy) to synchronize filter changes, light flicker stimulation and image acquisition.

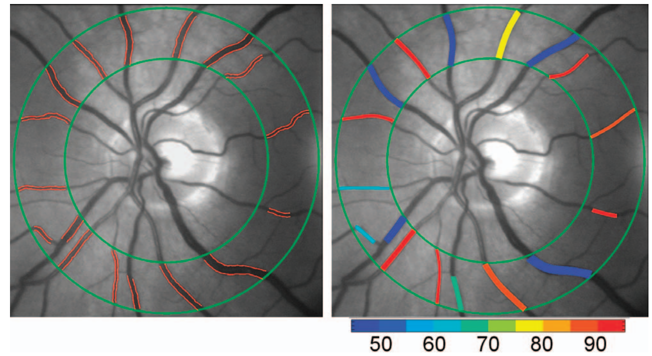

FIGURE 2. Example of a retinal image displaying vessel boundaries (*red lines*), obtained from multiple diameter measurements along blood vessels within a circumpapillary region of interest enclosed by two concentric *green circles* (*left*). Mean retinal vascular oxygen saturation measurements overlaid on the retinal image in pseudocolor (*right*). *Color bar* indicates oxygen saturation in percent.

Image Analysis

Images which displayed low contrast, reduced focus, large eye movements or blinks were excluded by visual inspection. The remaining images were registered using ImageJ (National Institutes of Health, Bethesda, MD, USA) and then averaged to generate a mean image at each imaging wavelength ($image_{606}$ and $image_{570}$). These mean images were manually registered to compensate for any minor eye motion that occurred between filter changes. Our previously reported customized image analysis algorithm[18] programmed in a computing environment (MATLAB 2013b; MathWorks, Natick, MA, USA) was modified to segment retinal vessels using a Hessian-based Frangi "vesselness" filter[19] within a circumpapillary region of interest which extended between one and two optic disk radii from the optic disk edge (Fig. 2). Vessel centerlines within the region of interest were automatically generated for each segmented vessel using a Euclidian distance transformation. Every seven pixels (~45 μm) along the vessel centerlines, a perpendicular intensity profile (PIP) was generated from both mean images.

Vessel D and boundaries were determined from the full width at half maximum of the PIPs generated from $image_{570}$, as previously described.[20] Gross boundary errors were manually removed by visual inspection and D measurements were averaged along each blood vessel segment. Measurements of D in units of pixels were converted to microns (μm) using a constant calibration factor of 6.63 μm/pixel, which was derived based on previously published optic disk size in healthy human subjects.[21]

Optical densities (OD) were calculated for each vessel using the PIPs from both mean images. As previously reported,[22] optical density was calculated as $\log(I_{outside}/I_{inside})$, where I_{inside} and $I_{outside}$ represent the average pixel intensity inside and outside the vessel along the PIP, respectively. We measured I_{inside} by averaging the lowest 50% of pixel values within the vessel boundaries to help eliminate specular reflection.[23] Based on the vessel diameter, $I_{outside}$ was determined by averaging a percentage of background pixel values at locations corresponding to the maximum negative curvatures of PIPs. These locations were determined based on the minima of a second order derivative of the PIPs, as previously described.[20]

Optical density ratios were calculated as OD_{606}/OD_{570}, where OD_{606} and OD_{570} are the optical densities calculated from $image_{606}$ and $image_{570}$, respectively.[22] Previous studies have described a linear relationship between optical density ratio and vessel size which is due to imaging artifacts[24] and

Table. Effects of Light Flicker Stimulation on D_A, D_V, SO_{2A}, SO_{2V}, and OEF in 20 Healthy Subjects

	Before Light Flicker	During Light Flicker	*P* Value	During/Before Light Flicker
D_A, µm	84 ± 7	90 ± 8	<0.001	1.07 ± 0.05
D_V, µm	109 ± 17	115 ± 16	<0.001	1.06 ± 0.04
SO_{2A}, %	92 ± 6	92 ± 6	0.5	0.99 ± 0.04
SO_{2V}, %	58 ± 10	63 ± 9	0.004	1.10 ± 0.16
OEF	0.37 ± 0.08	0.31 ± 0.07	0.001	0.86 ± 0.19

Data are presented as mean ± SD.

physiological factors.[25] Since artifactual factors account for most of the linear dependence, a previously described calibration method which did not differentiate between the two factors was employed.[23] Adjusted optical density ratio values were converted to SO_2[23,26] by performing a linear regression with previously published retinal arterial and venous SO_2 values (92.2% and 57.9%, respectively).[27] Measurements of SO_2 were averaged along each blood vessel segment.

Effect of Light Flicker

To study the effect of light flicker stimulation, the fractional change in each metric was assessed as the ratio of the metric value during light flicker to before light flicker, and was denoted by the inclusion of an *R* to the end of the metric name. As such, the fractional change in OEF (OEFR) was defined in terms of the fractional changes in MO_2 and DO_2.

$$\mathrm{OEFR} = \frac{\text{OEF during light flicker}}{\text{OEF before light flicker}} = \frac{MO_2R}{DO_2R} \quad (2)$$

The ratio of MO_2R to DO_2R can result in one of three conditions as reflected by the value of OEFR: 1) if OEFR > 1, then $MO_2R > DO_2R$; 2) if OEFR = 1, then $MO_2R = DO_2R$; or 3) if OEFR < 1, then $MO_2R < DO_2R$. Thus, OEFR provides information about whether the light flicker-induced change in DO_2 is lower than, matches, or exceeds that of MO_2.

Data Analysis

Reproducibility was determined by calculating the coefficients of variation and intraclass correlation coefficients from three repeated measurements before light flicker in four subjects. Mean arterial D (D_A), venous D (D_v), SO_{2A}, and SO_{2V} were calculated from data in at least four vessels and then compiled from all subjects. We calculated OEF from mean SO_{2A} and SO_{2V} according to Equation 1. Mean values of D_AR, D_VR, $SO_{2A}R$, $SO_{2V}R$, and OEFR were calculated from data averaged in all subjects. Paired *t*-tests were used to evaluate the statistical significance of differences due to vessel type and light flicker. Statistical significance was accepted at $P < 0.05$.

Results

Mean coefficients of variation for D_A, D_V, SO_{2A}, SO_{2V}, and OEF were 2.1%, 1.3%, 1.6%, 3.9%, and 4.9%, respectively ($n = 4$). Intraclass correlation coefficients of D_A, D_V, SO_{2A}, SO_{2V}, and OEF were 0.85, 0.99, 0.96, 0.93, and 0.83 ($n = 4$), respectively. As expected, D_V was significantly larger than D_A and SO_{2A} was significantly greater than SO_{2V}, both before and during light flicker stimulation ($P < 0.001$; $n = 20$).

Measurements of D_A, D_V, SO_{2A}, SO_{2V}, and OEF before and during light flicker are summarized in the Table. Both D_A and D_v significantly increased during light flicker ($P < 0.001$, $n = 20$). D_AR and D_VR were 1.07 ± 0.05 (mean ± SD) and 1.06 ± 0.04, indicating average flicker-induced vasodilations of 7% and 6%, respectively. During light flicker, SO_{2A} remained unchanged ($P = 0.5$, $n = 20$), while SO_{2V} significantly increased ($P = 0.004$, $n = 20$). The value of $SO_{2A}R$ was 0.99 ± 0.04, representing no significant change, whereas $SO_{2V}R$ was 1.10 ± 0.16, indicating an average flicker-induced increase of 10%. Inner retinal OEF was 0.37 ± 0.08 and 0.31 ± 0.07 ($P = 0.001$, $n = 20$) before and during light flicker stimulation, respectively. The fractional change in OEF was 0.86 ± 0.19, indicating that OEF decreased on average by 14% during light flicker stimulation.

Discussion

In the current study, inner retinal OEF was determined before and during light flicker stimulation in healthy human subjects for the first time, using a custom designed optical imaging system. OEF decreased with light flicker stimulation, indicating the change in DO_2 exceeded that of MO_2, thus our hypothesis was rejected.

Values of retinal vessel D, SO_2, and OEF obtained by our optical imaging system were repeatable to within 5%. As expected, D_V was significantly larger than D_A, and SO_{2A} was significantly greater than SO_{2V}. In response to light flicker stimulation, both D_A and D_V significantly dilated, in agreement with previous studies.[5,6,17,28,29] Furthermore, SO_{2A} did not significantly change with light flicker, while SO_{2V} significantly increased, consistent with the findings of a previous study.[6] Therefore, our optical imaging system was able to detect the physiological perturbations caused by light flicker stimulation.

In the current study, mean OEF before light flicker stimulation was 0.37, indicating that 37% of oxygen available from the retinal circulation was extracted for energy metabolism by the inner retinal tissue. This value is consistent with previous studies which measured an OEF of 0.44 in human brain[30] and 0.46 in rat inner retina.[13] Mean OEF during light flicker was 0.31, resulting in a mean OEFR < 1, which indicates the change in DO_2 exceeded that of MO_2. The change in DO_2 likely occurs due to an increase in BF during functional hyperemia as supported by our finding of vasodilation. Although the exact vasoregulatory mechanisms[7,31,32] involved in functional hyperemia remain to be elucidated, they result in unequal alterations in DO_2 and MO_2. This may be necessary to raise venous capillary PO_2 in order to drive oxygen to regions of the retina which are farther away from the blood vessels.

Recently, efforts have been made to directly quantify MO_2 in humans using combined measurements of SO_2 and BF.[33] Although, light flicker has been shown to increase MO_2 in animals,[9,10] its effect on MO_2 in humans has not been reported. MO_2R can be expressed as the product of OEFR and DO_2R, where DO_2R is the product of BFR and $SO_{2A}R$. Hence, MO_2R can be expressed as follows:

$$MO_2R = \mathrm{OEFR} * DO_2R = \mathrm{OEFR} * \mathrm{BFR} * SO_{2A}R \quad (3)$$

Since DO_2R was not measured in the current study, MO_2R cannot be directly quantified by our measurements of OEFR alone. However, it is possible to use our values of mean OEFR (0.86) and $SO_{2A}R$ (0.99) to calculate the value of BFR at which $MO_2R = 1$, namely, BFR = 1.17. Thus, for MO_2 to increase during light flicker, there must be an associated increase in BF of at least 17%. Consequently, an increase in MO_2 with light flicker is very likely since Garhofer et al.[5] have measured BFR in major retinal arteries to be 1.59. Nevertheless, future studies that simultaneously measure vascular SO_2 and BF in the same human subjects are needed to determine MO_2 before and during light flicker stimulation.

While OEF provides information about MO_2 relative to DO_2 based on oxygen saturation measurements in the retinal circulation, it inherently does not account for the potential contribution of oxygen supply from the choroidal circulation. Hence, measurements of OEFR may have been influenced by flicker-induced changes in the relative proportion of the retinal mass supplied by the retinal and choroidal circulations. This relative proportion is determined by oxygen gradients through the retinal depth, which depend on oxygen consumption rates of the inner and outer retina, inner retinal oxygen delivery, and choroidal oxygen delivery. Therefore, without empirical data on intraretinal oxygen tension profiles during light flicker, the effects of dual circulatory beds on OEFR measurements remain to be elucidated.

There were several limitations in the current study. First, as with all optical imaging techniques, image quality may affect measurements. However, the reproducibility of measurements was sufficient for detection of changes due to light flicker. Second, light levels before and during light flicker were not matched. A reduction in the mean light level during the 1-minute duration of light flicker may have induced a small change in the photoreceptor oxygen consumption. However, this change is primarily addressed by the choroidal circulation. Therefore, the reduction in mean light levels between image alignment and light flicker is expected to minimally influence measurements of OEFR which predominately reflects the retinal circulation and inner retinal neural activity. Third, the effects of pigmentation on measurements of SO_2[23] and optical properties on measurements of D were not accounted for. However, since flicker-induced changes were evaluated within subjects and the statistical significance of the differences was high, these factors likely contributed minimally to the results. Fourth, absolute measurements of MO_2 and DO_2 were not possible since BF was not measured, but their ratio was derived based on measurements of OEF. Consequently, the findings based on OEFR measurements cannot address the adequacy of any light flicker-induced compensatory changes in BF in response to tissue need. Finally, the light flicker–induced change in OEF was calculated based on theoretical considerations which assumed the volume of retinal tissue consuming oxygen that was supplied by the retinal vasculature was identical before and during light flicker stimulation. Direct measurements of MO_2 and DO_2 are needed to substantiate the findings of the current study.

In summary, with the use of an optical imaging system for simultaneous measurement of retinal vascular D and SO_2, OEF was determined before and during light flicker stimulation in human subjects. As indicated by the measured decrease in OEF, the light flicker–induced change in DO_2 exceeded that of MO_2 in healthy human subjects. Future application of this technique is potentially useful for detection of alterations in inner retinal OEF and OEFR due to physiological and pathological conditions.

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