# Oxygen Saturation in Human Retinal Vessels Is Higher in Dark Than in Light

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**Purpose.** Animal studies have indicated that retinal oxygen consumption is greater in dark than light. In this study, oxygen saturation is measured in retinal vessels of healthy humans during dark and light.

**Methods.** The oximeter consists of a fundus camera, a beam splitter, a digital camera and software, which calculates hemoglobin oxygen saturation in the retinal vessels. In the first experiment, 18 healthy individuals underwent oximetry measurements after 30 minutes in the dark, followed by alternating 5-minute periods of white light (80 cd/m$^2$) and dark. In the second experiment, 23 volunteers underwent oximetry measurements after 30 minutes in the dark, followed by light at 1, 10, and 100 cd/m$^2$. Three subjects were excluded from analysis in the first experiment and four in the second experiment because of poor image quality.

**Results.** In the first experiment, the arteriolar saturation decreased from 92% ± 4% ($n$ = 15; mean ± SD) after 30 minutes in the dark to 89% ± 5% after 5 minutes in the light ($P$ = 0.008). Corresponding numbers for venules are 60% ± 5% in the dark and 55% ± 10% ($P$ = 0.020) in the light. In the second experiment, the arteriolar saturation was 92% ± 4% in the dark and 88% ± 7% in 100 cd/m$^2$ light ($n$ = 19, $P$ = 0.012). The corresponding values for venules were 59% ± 9% in the dark and 55% ± 10% in 100 cd/m$^2$ light ($P$ = 0.065).

**Conclusions.** Oxygen saturation in retinal blood vessels is higher in dark than in 80 or 100 cd/m$^2$ light in human retinal arterioles and venules. The authors propose that this is a consequence of increased oxygen demand in the outer retina in the dark. (*Invest Ophthalmol Vis Sci.* 2009;50:2308–2311) DOI:10.1167/iovs.08-2576

Light is known to influence oxygen metabolism in the retina of living monkeys.[1] Partial pressure of oxygen ($Po_2$) in the outer retina at the level of the photoreceptors is lower in dark than in light in living cats[2–5] and monkeys.[6,7] On the other hand, $Po_2$ in the inner half of the retina in cats has been found to be higher in the dark,[5] although this was not confirmed in a study on monkeys.[7] In the dark, $Po_2$ decreases sharply from the choroid to a very low minimum at the outer retina and then rises toward the inner retina, where irregular peaks in the profiles indicate inner retinal capillaries.[5–8] In the dark, oxygen will diffuse from both the choroid and the inner retina to the minimum $Po_2$ at the outer retina. In the light, the minimum at the outer retina is absent or not as pronounced.[5–9] $Po_2$ measurements can be used to calculate oxygen consumption in different layers of the retina. Elevation of oxygen consumption of photoreceptors in the dark has been established, with various methods, in several animal species in vitro[10–14] and in vivo.[2–8,15–18] This elevation is believed to stem, at least in large part, from the increased activity of the $Na^+/K^+$-ATPase in photoreceptors,[10,13] which is needed to maintain transmembrane ion gradients, while cGMP-gated cation channels are open in the dark. In the inner retina, however, oxygen consumption seems to be similar in light and dark in cats[18] and rats.[14] Increased oxygen consumption in the outer retina in the dark and unchanged consumption in the inner retina add up to increased total consumption in the retina in the dark. Indeed, in studies of monkeys,[1] cats,[19] rats,[14] and rabbits[20–22] the total estimated retinal oxygen consumption has been found to be increased in the dark.

One study, published only in abstract form (Delori FC, et al. *IOVS* 1983;24:ARVO Abstract 1), described the effect of constant light and dark on retinal oxygen metabolism in humans and suggested an increase in oxygen saturation in retinal venules in the dark. In the present study, we use a novel noninvasive oximetry technique to measure oxygen saturation in retinal vessels of healthy human volunteers.

## Methods

### The Retinal Oximeter

The retinal oximeter[23] is based on a nonmydriatic fundus camera (Canon CR6-45NM; Canon Inc., Tokyo, Japan), which is coupled with a beam splitter (MultiSpec Patho-Imager; Optical Insights, Tucson, AZ) and a digital camera (SBIG ST-7E; Santa Barbara Instrument Group, Santa Barbara, CA). It estimates light absorption of retinal blood vessels at two wavelengths of light, one of which is sensitive to oxygen saturation and the other which serves as a reference. The beam splitter splits the image from the fundus camera into four optical channels and in each of the channels, a filter allows light of a desired wavelength to pass. In the present study, two channels were used and the center wavelengths of the filters were 586 and 605 nm. The half bandwidth of the filters was 5 nm. Specialized software was used to calculate the optical density (OD) of the retinal vessels at both 586 and 605 nm. Optical density is a measure of the light absorbance and can be calculated as

$$OD = \log (I_0/I) \quad (1)$$

where $I_0$ and $I$ are estimated by the brightness levels outside and inside the vessels, respectively. Light absorption of hemoglobin is dependent

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on oxygenation at 605 but not at 586 nm. For such wavelength pairs, it can be shown[24,25] that hemoglobin oxygen saturation ($SatO_2$) is approximately linearly related to the ratio of ODs:

$$SatO_2 = a + b(OD_{605}/OD_{586}) \quad (2)$$

where $OD_{605}$ and $OD_{586}$ are the optical densities calculated according to equation 1 at 605 and 586 nm, respectively, and $a$ and $b$ are constants. For calibration, $OD_{605}/OD_{586}$ was calculated for arterioles and venules in a group of healthy individuals and paired with $SatO_2$ values from earlier studies with a calibrated instrument,[26] which allowed solving for the constant $a$ and $b$ in equation 2. The results were $a = 116$ and $b = -122$. The linear relationship between $SatO_2$ and ODR is an approximation and does not yield accurate $SatO_2$ for every individual. However, the oximeter has been shown to be sensitive to changes in $SatO_2$[23] and comparison between $SatO_2$ in light and dark in the same vessels (paired statistics) is valid.

Light absorption in the retina, and therefore measured $SatO_2$, may change between light and dark adaptation due to mechanisms other than real changes in $SatO_2$, for example, because of bleaching of photoreceptor pigment in light. The ratios of light intensity at the two wavelengths inside retinal vessels ($I_{605}/I_{586}$) and outside vessels ($I_{0\text{-}605}/I_{0\text{-}586}$) were measured in both light and dark to evaluate the effect of confounding changes in light absorption. In short, changes in $I_{605}/I_{586}$ and $I_{0\text{-}605}/I_{0\text{-}586}$ indicated that such effect would attenuate rather than exaggerate the differences found in $SatO_2$ between light and dark. The influence of changes in absorption that is not related to $SatO_2$ therefore does not change the conclusions of the study.

## Light Levels

The retinal oximeter can use either infrared light for viewing the fundus of the subject or visible light from a tungsten lamp. In both experiments (see protocols later), the viewing light of the camera was used for light adaptation, but in the first experiment the overhead white fluorescent room lights (Philips 58W/830 New Generation; Royal Philips Electronics NV, Amsterdam, The Netherlands) were also switched on. The fundus camera of the oximeter was set to the 45° field of view setting. All measurements of light levels were made with a digital photometer (Mavolux; Gossen GmbH, Erlangen, Germany). The light levels reported were measured with the luminance attachment of the photometer in the same position as the eye of the subjects in the experiments (i.e., at approximately 4 to 5 cm distance from the lens of the fundus camera, pointing directly to the middle of the lens; maximum reading used). The aperture angle of the luminance attachment of the photometer is 20°; the light levels reported are approximate and do not represent equivalent Ganzfeld levels. In experiment 1 (described later), the light levels to the side of the fundus camera were approximately equal to the light levels measured in front of the lens.

To maintain dark adaptation, infrared light was used to view the fundus during measurements at the end of dark adaptation periods. Adjusting the oximeter before each measurement took less than 1 minute. A slight glow of light could be seen through the infrared filter of the fundus camera and from the screen of the fundus camera and computer. Both screens faced away from the volunteer being measured and, in the second experiment, the screens were dimmed as much as possible with neutral density filters (gray filters). Photometer reading at the lens of the oximeter (same setup as described earlier) was 0.0 cd/m$^2$ during dark adaptation.

The measurement itself requires the use of the xenon flash of the fundus camera, which lasts several milliseconds. A single flash is sufficient for each measurement. The minimum time between measurements was 5 minutes.

## Both Experiments

The study was approved by The Icelandic National Bioethics Committee and Data Protection Authority. Informed consent was obtained from all volunteers after explanation of the nature and possible consequences of the study. The study adhered to the tenets of the Declaration of Helsinki. One first-degree arteriole and one first-degree venule (first branch from central retinal artery and vein) were chosen for measurements in one eye of each subject. Measurements were made from outside the optic disc and along the vessel at all possible pixels up to or nearly up to branching of the vessel. The measurements were then averaged along the vessel. Statistical analyses were performed with paired *t*-tests.

## Experiment 1

Eighteen healthy volunteers participated in the first experiment. Three individuals were excluded from analysis because of poor image quality. After exclusion, the mean age was 30 years and the range was 21 to 57 years (13 men and 2 women). Tropicamide HCl (Mydriacyl; Alcon Inc., Fort Worth, TX) was used for dilation of the study eye, and the volunteers were placed in the dark for 30 minutes before the first measurement was made. The volunteers were then placed alternately in light (~80 cd/m$^2$) and dark. Each period of light or dark lasted for 5 minutes, and a measurement was performed with the oximeter at the end of each period.

## Experiment 2

Twenty-three volunteers participated in the second experiment. Four individuals were excluded from analysis due to poor image quality. After exclusion, mean age was 25 years, and the range was 22 to 30 years (11 men and 8 women). Tropicamide HCl (Mydriacyl; Alcon Inc, Fort Worth, TX) was used for dilation of the study eye, and the volunteers were placed in the dark for 30 minutes before the first measurement was made. The volunteers were then placed in light successively at approximately 1, 10, and 100 cd/m$^2$, each period lasting 5 minutes. Finally, the volunteers were adapted to dark for 5 minutes. A measurement was performed with the oximeter at the end of each period.

# Results

Figure 1A shows results of the first part of the study, in which the volunteers were initially placed in the dark for 30 minutes and then alternately in white light (80 cd/m$^2$) and dark, each period of light or dark lasting 5 minutes. After 30 minutes in the dark, retinal arteriolar oxygen saturation was 92% ± 4% ($n = 15$; mean ± SD) and venular oxygen saturation was 60% ± 5%. After a 5-minute exposure to 80-cd/m$^2$ light, oxygen saturation was significantly lower: 89% ± 5% in arterioles ($P = 0.008$) and 55% ± 10% in venules ($P = 0.020$). In subsequent measurements, oxygen saturation in both arterioles and venules increased when lights were turned off and decreased when lights were on (Fig. 1A). The arteriovenous difference in saturation did not change significantly between light and dark ($P > 0.17$ for paired comparison of subsequent measurements). In the second experiment (Fig. 1B), on another group of healthy subjects, light levels were increased in steps after 30 minutes of initial dark adaptation. Arteriolar and venular oxygen saturation had a slight downward trend when going from dark to light levels of 1 or 10 cd/m$^2$, but this was less than 1% in each step and was statistically insignificant (Fig. 1B). When the light level was raised to 100 cd/m$^2$, arteriolar oxygen saturation decreased from 92% ± 4% in the dark to 88% ± 7% ($n = 19$, $P = 0.012$) and venular oxygen saturation decreased from 59% ± 9% in the dark to 55% ± 10% in 100-cd/m$^2$ light ($P = 0.065$). Arteriovenous difference did not change significantly between dark and any light intensity (lowest $P > 0.61$).

# Discussion

Our results show that oxygen saturation in retinal arterioles and venules is higher in dark than in 80 and 100 cd/m$^2$ white

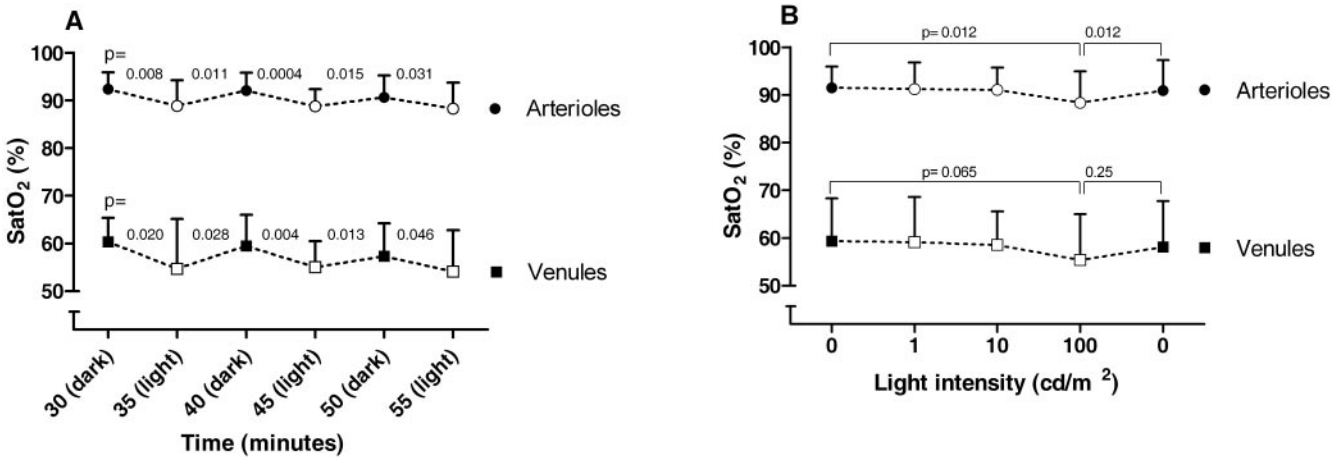


**FIGURE 1.** Oxygen saturation ($SatO_2$) in retinal arterioles in light (*open symbols*) and dark (*filled symbols*). (**A**) Alternating light and dark. Fifteen healthy volunteers were placed in the dark for 30 minutes and then alternately in 80 cd/m$^2$ light and dark, each period lasting 5 minutes. A measurement of $SatO_2$ was made at the end of each period of light or dark. *P* pertains to comparisons between successive measurements (paired *t*-tests). (**B**) Variable light intensity. Nineteen healthy individuals were first placed in the dark for 30 minutes, and the light intensity was then increased in steps (5-minute periods of light and then 5-minute dark period at the end). *P* pertains to comparisons between dark and 100 cd/m$^2$ (paired *t*-tests).

light, whereas the arteriovenous difference is stable. As explained later in the Discussion section, it is proposed that the results are a consequence of increased oxygen demand by the outer retina in the dark. Although variables such as intraocular pressure, blood pressure, and systemic oxygen saturation were not measured, they are not expected to change during in the 20 to 25 minutes that passed from the first to the last measurements.

Animal studies consistently show increased total[1,14,19–22] and outer[2–8,10–18] retinal oxygen consumption in the dark compared to light. Inner retinal consumption seems to be unaltered.[14,18] Oxygen delivery from the retinal vessels and/or choroid into the tissue must increase, to meet the increased total demand for oxygen in the dark. $Po_2$ gradients between the two blood circulations and the tissue must steepen, to increase diffusion in the dark. $Po_2$ in the outer retina decreases in the dark,[2–7] and changes in choroidal $Po_2$ in light and dark appear to be small in cats[2–5,8] and monkeys.[6,7] Therefore, more oxygen is delivered from the choroid in the dark.

According to a study on cats,[5] the inner retinal $Po_2$ is higher in the dark, although this finding was not confirmed in a recent study on monkeys.[7] Higher inner retinal $Po_2$ in the dark would facilitate diffusion of oxygen from the inner to the outer retina in the dark. Higher inner retinal $Po_2$ is also consistent with our results, which show higher saturation in the retinal vasculature in the dark. Oxygen is probably delivered from the choroid to the inner retina only in light[2,5,6] or not at all,[7] and the inner retinal consumption is similar in light and dark.[14,18] Therefore, the most likely explanation for increased inner retinal $Po_2$ and higher saturation in the retinal vasculature is increased retinal blood flow. Increased blood flow will raise the oxygen saturation in the retinal vasculature, because less oxygen will be lost from per unit volume of blood. This applies also to retinal arterioles because oxygen will diffuse through arteriolar walls

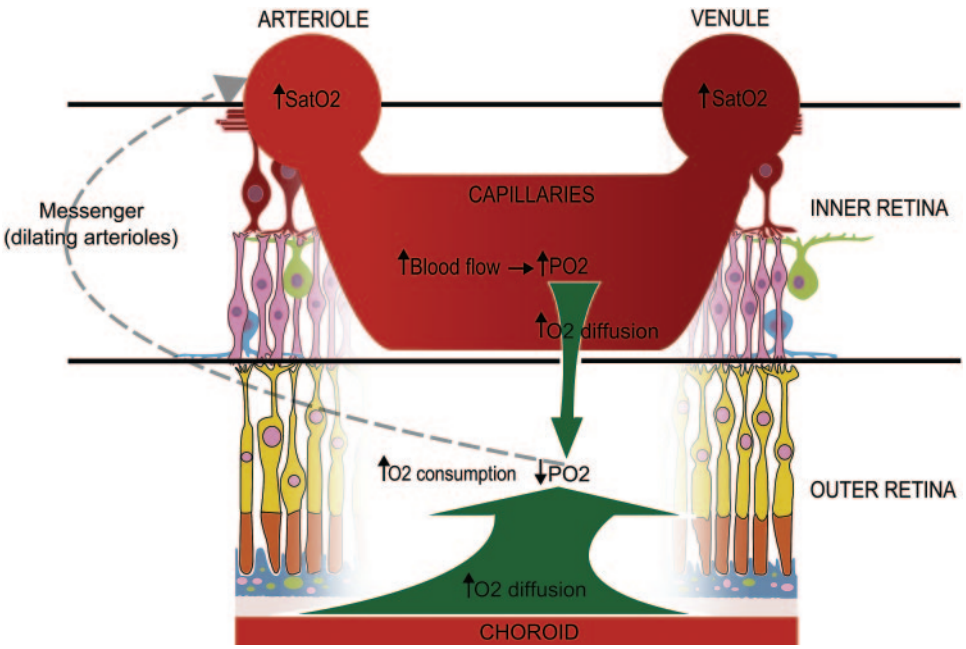


**FIGURE 2.** Representation of a thesis that explains the increased oxygen saturation ($SatO_2$) in retinal arterioles and venules in the dark. We propose that increased oxygen consumption and lower $Po_2$ in the outer retina (photoreceptors) lead to dilation of retinal vessels, which helps to increase the $Po_2$ gradient and thereby diffusion of oxygen from the inner retinal vasculature to the outer retina. The increased $SatO_2$ is a consequence of the increased blood flow (i.e., less oxygen diffuses from per unit volume of blood).

as the blood flows along the length of the arterioles and the saturation measurements are averaged over a long vessel segment (almost the entire first-degree vessel).

In two studies performed with laser Doppler flowmetry,[27,28] the investigators have reported that the retinal blood flow in human subjects increased in the dark. However, in a later study, performed with near infrared laser Doppler,[29] this increase was found to be transient, raising the question of whether the first results could have been a consequence of a transient rise in blood flow in response to visible measured light. Other studies have found increased blood flow velocity in the central retinal artery[30] or small changes in vessel width[31] in the dark. Dilation of vessels in the dark[27,28] may affect our measured oxygen saturation slightly. However, if such an effect is present, it is likely to attenuate the measured difference between light and dark and not to change the conclusions of this study.[25]

Although the results in studies on retinal blood flow do not all agree, we propose that increased oxygen consumption and lower $Po_2$ in the outer retina causes the release of a messenger to the retinal vasculature to stimulate vasodilation and increase blood flow. Identifying this putative messenger was beyond the scope of this study. A possible messenger is adenosine, which is known to dilate retinal vessels.[32] The increased retinal blood flow raises the $Po_2$ and the oxygen saturation in the retinal vessels and steepens the gradient from blood to tissue, thereby increasing the oxygen diffusion. According to this thesis, which is summarized in Figure 2, it is the outer retina that calls for more oxygen from the inner retinal vasculature. Such a response is in agreement with studies on cats[5,8] and monkeys,[6,7] which have shown that the photoreceptors receive more oxygen from the inner retinal vasculature in the dark (7%–15% of their consumption) than in the light (0%–11%).

A more complete understanding of basic human physiology is essential for understanding pathophysiology in retinal diseases. Our study shows for the first time the change in human retinal oxygen metabolism in light and dark. These changes may be vital to our understanding of retinal oxygen metabolism in health and in ischemic eye diseases, such as diabetic retinopathy.[33]

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