Reactivity of the Human Retinal Circulation to Darkness: A Laser Doppler Velocimetry Study

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Bidirectional laser Doppler velocimetry (LDV) and fundus photography were applied to investigate the effects of light and darkness on retinal blood flow. Blood flow had increased by an average of 67% after 5 min of darkness. This increase persisted for periods of darkness as long as 80 min (the longest period tested). The magnitude of this elevation, its time course, and its neutralization by the breathing of 100% O₂ suggest that, in vivo, the retina consumes more O₂ in the dark than in light. This effect is, most probably, associated with the maintenance of the photoreceptor dark current. LDV in light and darkness may become a useful probe of retinal function. Invest Ophthalmol Vis Sci 24:737-740, 1983

The uptake of O₂ in the isolated frog and human retina is markedly higher during darkness than during light.1,2 The maintenance of high levels of active sodium transport in the photoreceptors appears to be mainly responsible for this increased metabolism.2 As the outer retina consumes more O₂ in the dark, P0₂ decreases in both the outer and, to some extent, the inner retinal layers. Two mechanisms could provide an additional supply of O₂ to the outer retina in vivo: an increase in O₂ extraction from the choroid3 and an increase in retinal blood flow.4,5 The present study was undertaken to test whether blood flow in the normal human retina depends indeed upon the conditions of illumination and, if so, to verify that the characteristics of the response to a sudden change of illumination from light to darkness correspond to those predicted by increased photoreceptor metabolism.

Our study reveals a significant increase in retinal blood flow in darkness. The magnitude of this increase, its time course, and its neutralization by the breathing of 100% O₂ suggest that increased retinal O₂ consumption is responsible for the increased retinal blood flow in darkness.

Materials and Methods

Bidirectional fundus laser Doppler velocimetry (LDV) was applied, as described in detail elsewhere,6-8 to determine the axial or maximum velocity, V_max, of red blood cells moving in large retinal veins of three healthy volunteers, whose ages ranged from 31–43. Following various periods of darkness, V_max was measured while an area of the posterior pole of the eye (30° in diameter) was illuminated with a retinal irradiance of approximately 0.03 mW/cm² at a wavelength of 570 nm. The calculated retinal irradiance of the 632.8 nm helium-neon laser measuring beam (approximately 150 μm in diameter at the retina) was about 80 mW/cm². Pairs of Doppler shift frequency spectra were recorded simultaneously with measurement times of less than 1.28 sec, and the difference, Δf, between the cutoff frequencies in each pair of spectra was determined. The calculation of V_max was based on an average value of at least five successive measurements of Δf. The fixation target was a particular speckle within the aperture of an optical fiber (about 200 μm in diameter) that was focused on the retina. The fiber was connected to a helium-neon laser and delivered a retinal irradiance of about 1.2 mW/cm². In addition to the measuring light focused on the vessel and the fixation light focused on the fovea, an extended speckle pattern was also present during the LDV measurements. This pattern originated from the scattering of laser light by the optics of the delivery system, by the ocular media, and by the illuminated red blood cells. We have estimated the retinal irradiance of this scattered light by illuminating the fundus uniformly with monochromatic light at 630 nm so that the speckle appeared barely visible. Corresponding retinal irradiance was approximately 0.23 mW/cm².

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Fig. 1. $V_{\text{max}}$ measured from a vein of a normal volunteer after approximately 5 min of fundus illumination and after 20 min of darkness. Each data point represents an average based on at least 10 pairs of Doppler spectra. Error bars indicate ±1 standard deviation.

The diameter, $D$, of the veins at the site of the LDV measurements was determined from photographic negatives taken with a Zeiss fundus camera in monochromatic light at 570 nm after 3 min of fundus illumination (0.2 mW/cm$^2$) and after 5 and 20 min of dark adaptation. Blood flow, $Q$, was calculated using the relation $Q = \pi/8D^2 \cdot V_{\text{max}}$, which is valid under the assumption of Poiseuille flow.7

Alignment of the Zeiss and LDV fundus cameras in darkness was achieved by inserting a 800 nm monochromatic filter in the fundus illumination beam and observing the path of this beam through the subject’s pupil with an infrared viewer.

Two subjects were given 100% O$_2$ to breathe during 8 min of dark adaptation and during the following LDV measurements. Fundus photographs were also taken under these conditions.

After 10 min of darkness, the right eye of two subjects (four experiments) was illuminated for 2 min with saturating white light (approximately 10 mW/cm$^2$) just prior to LDV measurements in the left eye.

Results

Figure 1 displays a typical time course of $V_{\text{max}}$ obtained from an inferior temporal vein after 20 min of dark adaptation. Data collected during the first seconds following darkness demonstrated that the diameter of the vein had increased by 5%, the velocity by approximately 65%, and the calculated blood flow by about 82% from the respective value in light. Concurrent with the LDV measurements taken after darkness, $V_{\text{max}}$ decreased quickly, reaching the baseline value within 3–4 min.

Laser Doppler velocimetry measurements taken after various periods of darkness demonstrate that $V_{\text{max}}$ increased quickly in darkness (Fig. 2) and reached a plateau within less than 5 min. This plateau was 47% ± 7% higher than the value obtained during fundus illumination. The diameter of the veins at the site of the LDV recordings, obtained from photographs taken after 5 or 20 min of darkness, was between 5% and 8% larger than in light. Assuming that the diameter remained constant for periods of darkness longer than 20 min, blood flow in darkness was between 62 and 71% higher than in light.

The average $V_{\text{max}}$ obtained from a vein after 8 min of both darkness and 100% O$_2$ breathing was the same as that obtained under conditions of light and room-air breathing (Fig. 3), whereas the diameter of the vessel and the calculated blood flow were respectively 19 and 35% smaller. A 19% decrease in the diameter of this vein was also obtained after the subject breathed 100% O$_2$ for 15 min in light. Therefore, darkness by itself did not alter the diameter change.

Illumination of the contralateral eye prior to the LDV recordings in the experimental eye under normal breathing conditions did not influence the increase in blood flow observed in darkness.

Discussion

Our investigation demonstrates that retinal blood flow increases significantly during darkness and confirms the findings recently reported by Feke et al.9 This increase originates primarily from an increase in blood velocity rather than from an alteration in the diameter of the large vessels. Presumably, the smaller retinal vessels, which are not measurable by fundus photography, must dilate relatively more than the large ones to decrease the vascular resistance. Two
observations support the assumption that these ves- 
sels play an important role in controlling retinal vas-

cular resistance. First, within minutes after normal 

subjects breathe a gas mixture containing 7% CO 2, 

retinal blood flow increases, although there are no 

significant changes in the diameter of the large retinal 

vessels. Second, in subjects breathing pure O 2, retinal 

volume and, consequently, retinal vascular resistance 

decrease much more than predicted by the change in 

the diameter of the large retinal vessels.

The time course of the blood flow response to dark-

ness is very similar to that observed when light-

adapted subjects start breathing O 2 or CO 2 (our own 

unpublished observation), ie, immediate and com-

pleted within a few minutes. This suggests that local 

changes in gas tensions are either directly or indirectly 

responsible for the blood flow increase during dark-

ness. Furthermore, this increase is neutralized by 

100% O 2 breathing during darkness, suggesting that 

O 2 is the agent most likely responsible for the in-

creased blood flow observed when breathing condi-

tions are normal.

An increase in the metabolism required for regen-

erating the photopigment may be responsible for the 

increased O 2 uptake by the retina following exposure 

to light. Although the immediate increase in blood 

flow with the onset of darkness could be related to 

pigment regeneration, our finding that this increase 

persists long after the period required for pigment 

regeneration refutes this hypothesis. Rather, our find-

ings support the hypothesis that increased retinal 

blood flow during darkness results from an increased 

metabolism necessary to maintain the photoreceptor 

dark current. If one assumes that retinal venous O 2 

saturation remains constant between light and dark, 

the additional amount of O 2 (62-71%) delivered by 

the retinal circulation in darkness appears, indeed, 

to agree relatively well with the results of studies in 

the isolated retina, which demonstrate a 57% increase 

in retinal O 2 consumption in darkness. This agree-

ment should not be overemphasized, however, be-

cause in vivo the retina may consume a different 

amount of O 2 in the dark than does the excised retina, 

which is deprived of the pigment epithelium. Also, 

the response of the choroid to the increased demand 

of O 2 is not known.

Recent findings suggest that a decrease in cho-

roidal blood flow could occur during darkness. Such 

a decrease could lead, presumably, to a decrease in 

the supply of O 2 from the choroid to the retina caus-

ing retinal blood flow to increase in order to maintain 

constant retinal PO 2. Previous studies by Alm and Bill 
in cats, demonstrating that O 2 delivery to the cho-

roid is largely independent from blood flow, seem to 

exclude this possibility. More recent investigations by 

these same authors indicate, however, that a de-

crease in choroidal blood flow may cause a small shift 

in O 2 delivery from the retinal vessels to the retina. 

Based on the data shown in Figures 4 and 5 of Alm 
and Bill’s paper, we have estimated that a 47% de-

crease in choroidal blood flow induced by a decrease 

in the perfusion pressure would cause retinal blood 

flow, and consequently O 2 delivery, to increase by 

approximately 27%. This value is markedly below the 

range of increases that take place during darkness. In 

addition, the retinal blood flow reactivity to darkness, 

obtained after illuminating the contralateral eye (right 

eye in our experiment) with an intensity and duration 

previously shown to have produced a reflexive in-

crease in choroidal blood flow in the tested eye (left 

eye), was similar to that obtained when the contra-

lateral eye was kept in the dark. Clearly, this obser-

vation does not support the hypothesis that a decrease 

in choroidal blood flow is responsible for the increase 

in retinal blood flow in darkness.

The return of the blood flow to its value in light, 

which occurs during the LDV measurements (Figs. 

1, 3), can be attributed to the effect of the laser light 

illumination. This light, which corresponded to 6.75 • 

10 8 incident photons/rod/sec, was clearly above the 

level of saturation of about 25 • 10 4 incident photons/ 

rod/sec at 632.8 nm (approximately 800 photons ab-

sorbed/rod/sec).
Measuring retinal blood flow in light and darkness may provide a new, sensitive means of investigating the regulatory capacity of the retinal circulation under normal and pathologic conditions. In conditions characterized by dilated retinal vessels and full use of the vascular autoregulatory capacity, the retinal circulation may not be able to provide the additional O₂ required in darkness. Whether this could lead to long-term damage of some retinal structures remains to be investigated. The technique needs to be improved, however, before it can be used as a clinical tool. Presently, the light-adapting effect of the laser measuring beam rapidly abolishes the blood flow increase that occurs during darkness. Measurements must be done quickly after shining the laser beam into the eye, a procedure that requires rapid, precise target fixation and instrument alignment, currently possible only with trained subjects.

Key words: retinal blood flow, laser Doppler velocimetry, oxygen uptake, photoreceptors, dark current, retinal regulation

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References