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# Diffuse luminance flicker increases retinal vessel diameter in humans

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## Abstract

**Purpose.** To determine retinal vessel diameter variations in response to neuronal activity induced by diffuse luminance flicker.

**Methods.** The diameter of retinal arteries and veins was measured in 9 normal subjects by computer analysis of fundus pictures taken in monochromatic light under normal conditions of illumination and after 1 min of sinusoidally varying diffuse luminance flicker at 10 Hz.

**Results.** The diameter immediately after flicker was significantly larger than the pre-stimulus diameter by  $4.2 \pm 2.2\%$  for the retinal arteries and  $2.7 \pm 1.7\%$  for the retinal veins (mean  $\pm$  SD). Six s after cessation of the flicker, arterial diameter was not significantly different from that of pre-flicker value.

**Conclusions.** Diffuse luminance flicker induces an increase in retinal vessel diameter. This increase most probably reflects an increase in retinal blood flow previously evidenced in humans by the blue field simulation technique. The technique needs to be optimized in terms of flicker parameters, to determine whether flicker-evoked retinal diameter changes could represent a useful clinical measure of the capability of the retinal vascular system to vasodilate. *Curr. Eye Res.* 16: 1252–1257, 1997.

**Key words:** retinal diameter; flicker; neuronal activity; retinal blood flow.

## Introduction

In their classical paper, published more than 100 years ago, Roy and Sherrington (1) hypothesized that the brain possesses

an intrinsic mechanism by which its vascular supply can be varied locally, in correspondence with local variations of functional activity. Using various techniques to measure regional cerebral blood flow, such as laser Doppler flowmetry, transcranial Doppler sonography and positron emission tomography, researchers have since amply verified this hypothesis (2–5). Evidence is accumulating that such a mechanism also operates in the optic nerve head (ONH) and retina in cats (6, 7), primates (8, 9) and humans (10–12).

Since retinal blood flow (RBF) in a main retinal vessel is equal to  $\pi D^2 V_{\text{mean}}/4$ , where  $D$  is the diameter of the vessel and  $V_{\text{mean}}$  the mean velocity of blood, a given change in  $D$  produces twice the percent change in RBF than an equal percent change in  $V_{\text{mean}}$ . This underlines the importance of measuring precisely changes in  $D$  when attempting to detect a change in RBF in response to a stimulus. The most favourable sites of a vascular bed to detect a change in diameter are the arterioles, which have a diameter below approximately 20  $\mu\text{m}$ , since these vessels are the major sites of resistance to flow (13) in the absence of precapillary sphincters. However, quantification of changes in  $D$  of retinal arterioles is difficult due to the limited resolution of present techniques. On the other hand, changes of less than 3% in the diameter of the larger retinal arteries are routinely detectable. Although they do not constitute the main component of vascular resistance, these arteries are known to vasoreact in response to various physiological stimuli. For example, they constrict in response to systemic hyperoxia or dilate during systemic hypoxia and hypercapnia (14, 15).

Based on the observation that retinal arteries in cats dilate during diffuse luminance flicker (11), we hypothesized that such an effect also occurs in the human retina. If this hypothesis is verified, flicker could provide a physiological test that is simple to apply in patients to assess the capability of the retinal vasculature to vasodilate in various pathologic conditions.

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## Materials and methods

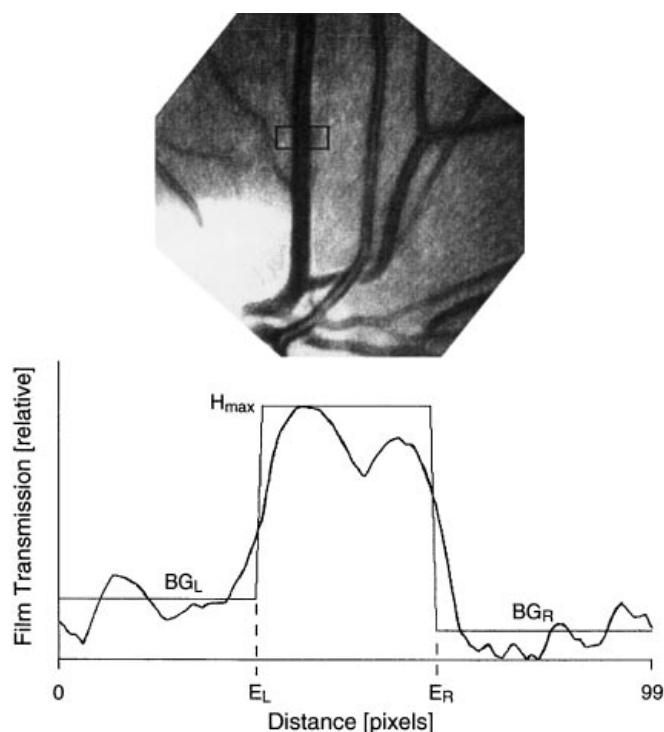
### Subjects

Nine subjects, 8 males and 1 female with a mean age of  $26 \pm 6$  SD years (range 18 to 39 years), each of whom had normal eye examination results, participated in this study. They had no history of systemic or ocular diseases and were under no medication. Refraction ranged from  $-5$  to  $1.5$  diopters. Mean diastolic and systolic brachial artery blood pressures were  $82 \pm 8$  SD mm Hg (range 65 to 90 mm Hg) and  $124 \pm 7$  SD mm Hg (115 to 135 mm Hg), respectively. Mean intraocular pressure measured by TonoPen (Mentor, USA) was  $13 \pm 1.2$  SD mm Hg (11 to 14 mm Hg). One eye chosen at random was selected for the measurements and dilated with 1% tropicamide. The study followed the tenets of the Declaration of Helsinki. Informed consent was obtained from all subjects after the nature and possible consequences of the study were explained. Approval by the institutional human experimentation committee of the University of Lausanne was obtained.

### Vessel diameter measurement

Ocular fundus photographs were taken in red-free light (centre wavelength = 540 nm, bandwidth at half height = 100 nm) with a Kowa Genesis fundus camera mounted on a support with chin and head rests. We used Kodak black and white TMAX 400 films, developed with Kodak TMAX for 6 min. The photos were synchronised with the end of systole of the cardiac pulse, which was obtained from an ear pulse plethysmograph. They were digitised on a Kodak Photo compact disk (CD). The CD was read and the digitised images were copied on the hard drive of a Power Macintosh 7300 computer using the maximum resolution of  $3072 \times 2048$  pixels. The fundus photographs were numbered and coded, and their sequence was mixed by a third party. The operator measuring the diameter using the NIH-Image software version 1.62 (16) was masked with regard to that sequence.

The diameter of straight segments of retinal vessels with a width of 20–40 pixels and located in the temporal half of the eye (3 in the superior and 6 in the inferior quadrant) was measured. These segments were placed vertically on the monitor, using a specifically developed macro software. An average vessel profile was obtained from 40 consecutive horizontal scans, each of 100 pixels. These scans define a rectangular window (see Fig. 1). This profile was then least-square fitted by a rectangular function (Fig. 1) defined by the maximum intensity,  $H_{\max}$ , and four parameters: the left and right vessel edges,  $E_L$  and  $E_R$ , the background level at the left,  $BG_L$ , the background level at the right,  $BG_R$ , representing the average values before  $E_L$  and after  $E_R$  respectively. The width  $E_R - E_L$  was documented as vessel diameter. The window was then shifted vertically by 5 pixels and a new width was obtained. This procedure was repeated 3 more times to obtain a total of 5 vessel widths. The mean  $\pm$  95% confidence limits of the 5 widths' values was determined and defined as  $D$  (in units of pixels).



**Figure 1.** **Top:** digitized fundus picture showing the window in which the mean retinal vessel profile is determined. **Bottom:** this profile is then least square fitted with a rectangular function by varying the four parameters  $BG_L$ ,  $BG_R$ ,  $E_L$  and  $E_R$  defined in the text. The width  $E_R - E_L$  was documented as diameter.

### Flicker stimulation of the retina

A sinusoidal photic stimulation was generated by a visual stimulator built, according to Rynders and Thibos's description (17). The light from a 75 watt Xenon arc was lowpass-filtered to remove the infrared component before being focused at the input of this stimulator, and the light at its exit was delivered to the illumination path of the Kowa Genesis fundus camera by means of a fibre optics bundle. The flicker light illuminated a region of approximately  $30^\circ$  in diameter, which was centred at the optic nerve head. The luminance spectrum is shown in Figure 2. Mean retinal luminance was  $5.4 \log Td$ , which, based on the ANSI 136.1 guidelines, is below the maximum permissible level for a duration of up to 16 min (18). The flicker stimulus had a frequency of 10 Hz and was applied for 1 min.

### Experimental paradigm

With the subject seated in a lighted room, 3 photographs of the fundus were taken at 3 s interval. After the third photograph the flicker stimulus was delivered and 3 photographs were taken immediately after, also at 3 s interval. This sequence was repeated twice at 3 min interval. Figure 3 illustrates the timing of photographs of the sequence *i*. From these photographs, we measured the diameters  $D_{bf1}^i$  to  $D_{bf3}^i$ , and  $D_{af1}^i$  to  $D_{af3}^i$ , where the subscripts "bf" and "af" indicate that the photographs were taken before and after flicker, respectively. The average vessel diameter of the 3 sequences  $D_{bf1}^i$  is calculated as  $1/3 \sum D_{bf1}^i$  ( $i =$

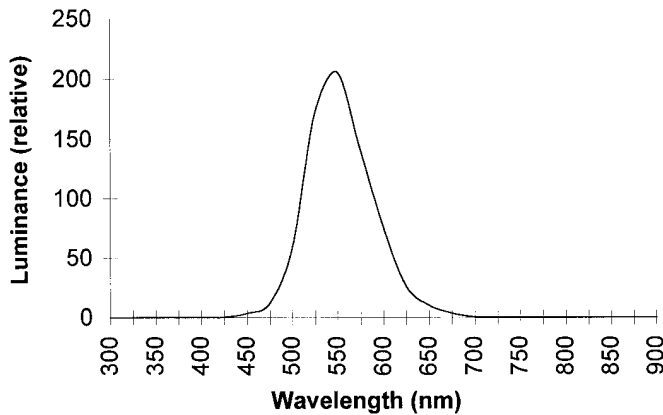


Figure 2. Luminance spectrum of the sinewave flicker stimulus, expressed in arbitrary units.

1, 2, 3). The same procedure was applied to calculate the other 5 diameters  $D_{bf2}$  to  $D_{af3}$ .

The reproducibility of the procedure was assessed using a Topcon model eye with pseudo vessels drawn at the fundus. Two groups of 3 fundus photos were taken. The same operator measured the diameter of a selected "vessel" on all photos. For each group, we determined the mean value  $D_1 = \sum D_{1i}/3$  and  $D_2 = \sum D_{2i}/3$  ( $i = 1, 2, 3$ ), respectively. The reproducibility was calculated as  $R = 1 - (2 * |D_1 - D_2|) / (D_1 + D_2)$ .

Interoperator variability of the diameter was determined as follows: three operators measured 5 times a given vessel at the same site on the same photograph. They were masked with regard to the results obtained by the others. For each operator we obtained a  $D$ . We then calculated the mean of the 3  $D$ -values and the coefficient of variation,  $100 \times SD/\text{mean}$ . Intraoperator variability was determined for 3 operators by having each one measure 5 times  $D$  of a vessel segment on the same photograph. From these values, we calculated the coefficient of variation.

In 3 eyes (5 arteries and 7 veins),  $D$  was obtained as average from 3 consecutive photographs during normal air breathing, and 3 photographs at 5 min of 100% oxygen breathing through a mask. The photographs were coded, their sequence mixed, and the operator measuring the diameters was masked with regard to their sequence.

Changes in diameter determined by the computer method described above were correlated with those determined manu-

ally by an operator, who, looking at magnified images of the fundus photographs projected on a screen, moved a thin wire from one edge of a straight segment of vessel to the other, as described elsewhere (20). This operator was masked with regard to the results provided by the computer method. The correlation was established based on 26 vessels where the changes in diameter were induced by flicker (14 vessels) and 5 min of 100%  $O_2$  (12 vessels).

### Statistical analysis

The data were analysed using a 2-way ANOVA test (SAS, mixed procedure) (19). The results are given as mean  $\pm$  SD%. They were considered significant when the  $p$ -value was  $<0.05$ .

## Results

Diameters  $D_{bf1}$  to  $D_{af3}$  obtained from retinal arteries and veins in the 9 subjects are shown in Table 1 and Table 2, respectively. Before the stimulus, there was no significant difference in the diameters between the 3 pictures. The diameter of the arteries after the stimulus, however, decreased significantly between the first and the third photograph ( $p < 0.001$ ), but not that of the veins. The diameter of the arteries measured at 6 s after cessation of the flicker was not significantly different from that of the pre-flicker value.

When the diameters  $D_{bf1}$ ,  $D_{bf2}$  and  $D_{bf3}$  were averaged and this average value compared with  $D_{af1}$ , it became apparent that flicker induced a significant increase in diameter amounting to  $4.2 \pm 2.2\%$  for the arteries ( $p < 0.014$ ) and  $2.7 \pm 1.7\%$  for the veins ( $p < 0.0001$ ), a difference that was significant (paired  $t$ -test,  $p < 0.03$ ).

Reproducibility,  $R$ , of the vessel diameter measurements based on the model eye and 3 series of measurements was found to be 0.989, 0.995 and 1. Interoperator and intraoperator variability were found to be  $<1.8\%$  and  $<1.7\%$ , respectively. The diameter changes in response to 5 min of oxygen breathing were  $-10.1 \pm 5.4\%$  in arteries, and  $-11.6 \pm 2.6\%$  in veins.

Figure 4 shows a plot of the changes in diameter obtained by the computer method versus those determined manually. Linear correlation coefficient of the fit was  $r = 0.97$  ( $p < 0.0001$ ).

## Discussion

Various methods have been proposed to measure relative retinal vessel diameter (20–24). In this paper, the changes in diameter of retinal vessels were determined by least-square fitting of a rectangular function to the vessel density profile. This method provides an interoperator and intraoperator variability of less than 1.8%. It demonstrates changes of diameter in response to oxygen breathing in agreement with previous findings (14). The results shown in Figure 4 correlate significantly with those obtained by a standard method (20) which, however, requires manual operation and subjective identification of the edges of a vessel. Our computer based method is less dependent upon particular features of the profile, such as the presence of clear kick points (23) and it is based on an average of 200 vertical

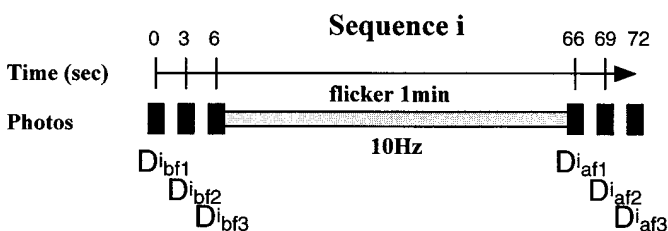


Figure 3. Timing of the photographs of the sequence  $i$ .  $D_{bf1}$  to  $D_{bf3}$ , and  $D_{af1}$  to  $D_{af3}$  are the diameters of the photographs taken before (bf) and after (af) the flicker stimulus, respectively.

Table 1. Mean Arterial Diameter (pixels) before ( $D_{bf_i}$ ) and after ( $D_{af_i}$ ) flicker

Subjects	$D_{bf1}$	$D_{bf2}$	$D_{bf3}$	$D_{af1}$	$D_{af2}$	$D_{af3}$	% Variation*
1	24.48	24.17	23.75	24.90	24.01	23.80	3.18
2	23.49	23.75	24.74	24.64	24.32	23.07	2.70
3	22.50	22.71	22.71	22.92	22.19	22.35	2.99
4	26.72	26.99	27.81	28.70	27.97	26.98	5.62
5	28.44	29.27	29.32	29.69	29.58	29.84	2.34
6	26.82	26.98	27.09	29.54	28.13	26.56	9.37
7	24.48	24.53	24.48	25.78	25.47	25.31	5.20
8	23.33	24.32	23.85	24.79	23.33	23.60	4.01
9	25.99	25.99	26.52	27.11	26.80	26.09	2.74
						Mean $\pm$ SD	4.2 $\pm$ 2.2

\*Calculated as  $100 \times 3 \times (D_{af1} - 1/3 \sum_i D_{bf1}) / \sum_i D_{bf1}$ .

scans compared to 3 used by others (24). A disadvantage of this method is that a band of about 2 vessel diameter width on each side of the measured vessel segment must be free of any other large vessel.

The photographs were taken in synchronisation with the cardiac pulse at the end of systole, a procedure that optimizes detection of small vessel diameter changes (21). With 9 subjects, our method enables detection of diameter changes of approximately 2%, a value similar to the precision obtained with other methods which also used synchronisation of the photographs with the cardiac cycle (24). A higher precision might be obtained from fluorescein angiograms (22), but we deliberately opted for a totally non-invasive technique.

Our investigation show that 1 min of diffuse luminance flicker at a mean luminance well below the maximum permissible level induce a significant increase in both retinal arterial and venous diameters. Similar results were obtained from the cat retina. In the cat, however, this increase was markedly larger (12). In the context of previous work in cats (12), pri-

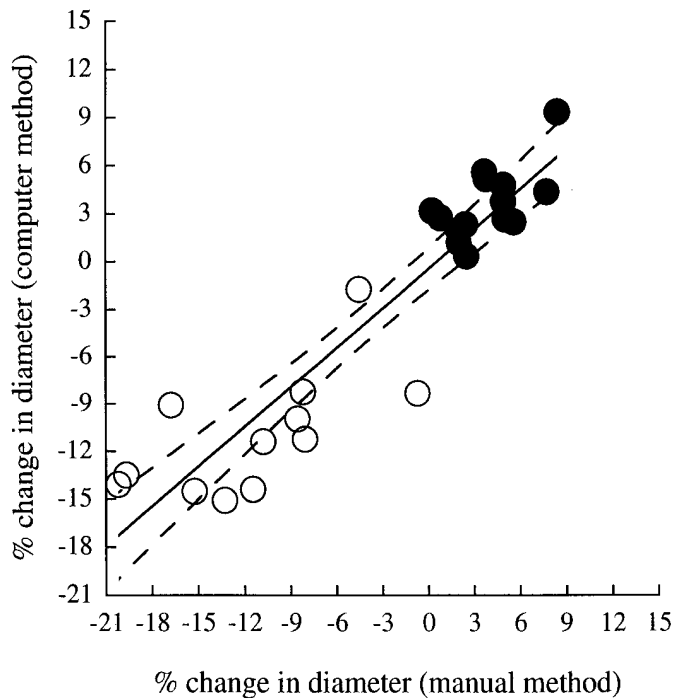
mates (8, 9) and humans (10, 11), this increase in vessel diameter most probably reflects an increase in retinal blood flow. However, this presumption remains to be tested by measuring the response of  $V_{mean}$  to flicker under the same experimental conditions. The increase in arterial diameter was significantly more pronounced than that of the veins. Assuming blood flow in the arteries and veins to be equal at the end of flicker, this suggests that a relative flicker-induced increase in blood velocity must occur in the veins that is larger than in the arteries by about 3%.

The return of the arterial diameter to pre-flicker value occurs in less than 6 s. This process appears to be somewhat faster than the post-flicker return of macular blood flow (between 13 and 20 s), as evaluated by the index  $V \times D$ , where  $V$  and  $D$  are the velocity and density of the white blood cells as determined using the blue field simulation technique (10). It is similar, however, to the duration of the return of  $V$ , which was approximately 8 sec. Such a short time is typical of responses obtained also from the cerebral circulation when the brain is activated

Table 2. Mean Venous Diameter (pixels) before ( $D_{bf_i}$ ) and after ( $D_{af_i}$ ) flicker

Subjects	$D_{bf1}$	$D_{bf2}$	$D_{bf3}$	$D_{af1}$	$D_{af2}$	$D_{af3}$	% Variation*
1	32.40	32.66	32.65	33.80	32.86	33.23	3.78
2	32.34	33.28	31.82	32.60	33.23	32.76	0.37
3	30.11	32.40	32.19	31.30	32.03	32.27	0.98
4	35.84	36.20	36.04	36.93	36.82	36.56	2.51
5	35.68	35.10	35.89	35.99	36.46	35.37	1.22
6	33.07	33.12	32.60	34.51	33.85	34.01	4.80
7	38.59	37.60	37.76	39.69	40.62	40.05	4.39
8	36.09	35.78	36.25	37.66	36.88	37.60	4.50
9	32.86	32.76	32.78	33.21	32.97	32.86	1.99
						Mean $\pm$ SD	4.2 $\pm$ 2.2

\*See Table 1.



**Figure 4.** Change in diameter measured with the computer method versus those determined by the manual method. The data points were obtained from the oxygen breathing (○) and flicker (●) experiments; linear correlation  $r = 0.93$  ( $p < 0.0001$ ).

by visual or somatosensory stimulation (4, 5). The lack of a significant decrease in venous diameter after flicker suggests that the increased venous volume has not returned to normal during the 6 s in which the arterial diameter decreases back to pre-flicker value, an effect which might be due to the high capacitance of these vessels.

The mechanism underlying the flicker induced increase in retinal blood flow has been discussed in a previous paper (10). A number of studies have attempted to unravel the mechanisms involved in the modulation of vascular tone during increased neural activity. Although the putative role of various substances in the vasodilatation induced by increased retinal activity, such as  $K^+$  (7), nitric oxide (NO) (25),  $pO_2$ , pH,  $pCO_2$ , circulating hormones and others (26), has been investigated, the mechanism(s) involved in the coupling between activity and blood flow in the retina still remain to be fully elucidated.

In the present investigation, we used a photic sine wave stimulator. Previous studies suggest, however, that this may not be needed. Flashes of 20  $\mu$ sec duration from a Grass Model PS-22 device or a rotating sector disk should provide a similar if not larger vasodilatation of the retinal vessels (12). Further investigations with different stimulations (sinewave, rectangular waves, flashes) will attempt to determine which parameters (mean luminance, contrast, frequency and wavelength) provide the largest change in vessel diameter.

Compared with the decreases in retinal vessel diameter induced by breathing pure oxygen, the increases in diameter induced by flicker are small. Thus, from a clinical point of view, flicker is a less powerful test of vasoactivity than oxygen.

It allows, however, to test the capability of the retinal vessels to dilate, without causing the systemic effects and inconvenience to the patient produced by the commonly used maneuver of breathing carbon dioxide. Furthermore, the flicker-induced dilatation may involve a different control mechanism than the oxygen-induced vasoconstriction or carbon dioxide dilatation. Presumably, the various mechanisms of regulation may be differently affected by diverse pathologies.

Measuring retinal vessel diameter being today a routine procedure in most ophthalmic laboratories, the method presented in this paper, when optimized, could become a useful functional clinical test. For example, in glaucoma, a disease where the large nerve fibers, which carry the higher temporal frequencies, are known to be the first altered by the disease (27), flicker at various frequencies may reveal an subnormal response of vessel diameter at the high frequencies. Further investigations are needed to assess the clinical potential of this flicker test.

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