Flicker-Evoked Response Measured at the Optic Disc Rim Is Reduced in Ocular Hypertension and Early Glaucoma

Charles E. Riva,[1] *Tommaso Salgarello,*[2] *Eric Logean,*[1] *Alberto Colotto,*[2] *Elena M. Galan,*[2] *and Benedetto Falsini*[2]

Purpose. To determine in patients with ocular hypertension (OHT) or early glaucoma (EOAG) the change in blood flow measured at the neuroretinal rim of the optic disc in response to a 15-Hz diffuse green luminance flicker, a stimulus that activates predominantly the ganglion cell magnocellular pathway.

Methods. Thirteen patients with EOAG, 29 with OHT, and 16 age-matched control subjects, all with excellent fixation, were examined. Blood flow (F_{onh}) at the neuroretinal rim of the optic disc was continuously monitored by laser Doppler flowmetry before and during exposure to a 15-Hz, 30° field green luminance flicker. The response of F_{onh} to this stimulus ($\mathbf{RF}_{onh}$) was expressed as percentage change in F_{onh} between baseline and the last 20 seconds of flicker. Two to three temporal sites of the disc were tested, and the highest $\mathbf{RF}_{onh}$ was considered for further analysis. $\mathbf{RF}_{onh}$ results in patients were correlated with morphologic (cup-to-disc area ratio, cup shape neuroretinal rim area) and functional (perimetric mean deviation and pattern electroretinogram amplitude) clinical parameters.

Results. In the patients with OHT or EOAG, F_{onh} and $\mathbf{RF}_{onh}$ were both reduced compared with their respective values in the control group. Both quantities decreased significantly with neuroretinal rim area when the patients' data were pooled. No significant correlation was found between F_{onh} or $\mathbf{RF}_{onh}$ and the other morphometric and functional parameters. The group-averaged time course of $\mathbf{RF}_{onh}$ was not significantly different from that in the normal subjects.

Conclusions. Luminance flicker-evoked $\mathbf{RF}_{onh}$ is abnormally reduced in patients with OHT or EOAG, indicating an impairment of neurally mediated vasoactivity. The data suggest that PERG-derived neural activity and flicker-evoked $\mathbf{RF}_{onh}$ can be independently altered early in the disease process. (*Invest Ophthalmol Vis Sci.* 2004;45:3662–3668) DOI:10.1167/iovs.04-0100

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Several investigations in both animals and humans[1–10] indicate that blood flow measured by laser Doppler flowmetry (LDF) at the neuroretinal rim of the optic nerve head (F_{onh}) increases when the neural retinal activity is increased by flicker. In normal subjects, this regulatory response of F_{onh} ($\mathbf{RF}_{onh}$), when evoked by diffuse luminance flicker, shows physiological properties that are similar to those of magnocellular retinal ganglion cell neural activity, the latter derived from the electroretinogram.[8,9]

Glaucoma is a disease in which the measurement of ONH blood flow in response to flicker stimulation could provide important information on the pathogenesis of optic nerve damage. Indeed, a large body of anatomic evidence indicates that, in early glaucoma, large ganglion cells subserving primarily the magnocellular pathway are selectively or predominantly damaged.[11–13]

Based on these considerations, a laser Doppler flowmetry (LDF) study was undertaken to investigate whether $\mathbf{RF}_{onh}$ in response to luminance flicker is altered in patients with ocular hypertension (OHT) or early open-angle glaucoma (EOAG) and, if so, whether alterations in $\mathbf{RF}_{onh}$ are associated with morphologic and functional signs of early glaucomatous damage. Results from these two groups of patients were compared with those obtained from age-matched normal control subjects.

Materials and Methods

Subjects

Thirteen patients (seven men, six women, mean age 49 ± 8 (SD) years, range 36–62) with a diagnosis of EOAG and 29 OHT patients (15 men and 14 women, mean age 48 ± 7 years, range: 28–57) were included in the study. In the patients with EOAG, intraocular pressure (IOP) measured by Goldman tonometry was more than 21 mm Hg on two or more occasions. These patients had an open-angle, abnormal white-on-white perimetry test result (Humphrey 30-2; Humphrey Field Analyzer; Carl Zeiss Meditec, Dublin, CA) with a typical reproducible defect. Two experienced glaucoma specialists (AC, TS) evaluated the optic discs independently by slit lamp biomicroscopy and 78-D lens. They were in full agreement on the pathologic disc abnormalities. These discs had a cup-to-disc ratio greater than 0.6 or an interocular cup-to-disc ratio asymmetry greater than or equal to 0.2. Furthermore, the discs had one or more of the following abnormalities: excavation, thinning of the rim, notching, nerve fiber layer defects, or peripapillary atrophy. Peripapillary atrophy, associated with excavation, and thinning of the rim was observed in one EOAG eye. The remaining EOAG eyes had several of the other disc abnormalities, which were confirmed by a semiautomated analysis performed by using confocal scanning laser ophthalmoscopy (described later). Field loss was graded from early to moderate in all patients, with Humphrey 30-2 mean deviation ranging 1.2 to 6 dB.

Patients with OHT had an elevated IOP (>21 mm Hg on two or more occasions), normal Humphrey perimetry results, and normal clinical optic disc appearance. In all patients, central corneal thickness,

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TABLE 1. Summary of Demographic and Clinical Characteristics of the Study Population

	Normal	OHT	EOAG
Age (y)	49.00 ± 6.00	49.00 ± 8.00	48.00 ± 7.00
Sex (M/F)	8/8	15/14	7/6
IOP highest (mm Hg)	16.00 ± 2.00	24.00 ± 3.00	26.00 ± 5.00
IOP actual (mm Hg)	16.00 ± 2.00	18.00 ± 2.00	19.00 ± 3.00
Blood pressure (mm Hg)	98.00 ± 10.00	96.00 ± 8.00	96.00 ± 10.00
Mean perfusion pressure (mm Hg)*	48.00 ± 6.00	47.00 ± 5.00	45.00 ± 6.00
Cup-to-disk ratio	0.35 ± 0.10	0.40 ± 0.10	0.50 ± 0.15
Nerve fiber layer thickness, temporal	0.26 ± 0.05	0.27 ± 0.06	0.24 ± 0.09
Rim volume, temporal	0.41 ± 0.10	0.45 ± 0.16	0.33 ± 0.14
Humphrey 30-2 mean deviation (dB)	0.50 ± 0.50	0.65 ± 1.00	−3.00 ± 2.00
Topical treatment (yes/no)	0/16	16/13	13/0

Data are expressed as mean ± standard deviation.

* Mean perfusion pressure was calculated as 2/3(BP) − IOP, where BP is mean brachial artery blood pressure measured by sphygmomanometry.

measured with a digital ultrasonic pachymeter (Altair; Optikon 2000, Rome, Italy) was between 520 and 570 μm. Sixteen normal subjects, whose sex and age distribution was comparable with that of the patients (mean age, 48 ± 6 years; range: 28–62), provided normative $\mathbf{R}F_{onh}$ values. Only subjects and patients with excellent target fixation were entered into the study.

In 22 of 29 OHT patients and in 12 of 13 EOAG patients, analysis of the optic disc was also performed by confocal scanning laser ophthalmoscopy (Heidelberg Retina Tomograph, HRT; Heidelberg Engineering, Heidelberg, Germany) according to a previously published protocol.[14] The other 7 OHT patients and 1 with EOAG did not consent to this additional examination. Among the various morphometric parameters obtained by HRT, those most sensitive and specific for glaucoma damage[15] were considered in the analysis: the neuroretinal rim area, the cup-to-disc area ratio, and the cup shape measure. In addition, the retinal nerve fiber layer thickness and the rim volume data were measured in each study eye. These measurements were compared with the 95% confidence limits established in 16 normal eyes (mean age, 44 ± 7 years, range 38–59), with disc areas in the ranges 1 to 2 and 2 to 3 mm^2.

All patients underwent pattern electroretinogram (PERG) recordings according to a previously published technique.[16,17] Briefly, PERGs were recorded in response to sinusoidal gratings of variable spatial frequency (1.7 and 2.6 cyc/deg), modulated in counterphase at 7.5 Hz and presented on a high-resolution video monitor (56% contrast; 80 cd/m^2 mean luminance; 24° × 14° field size). The peak-to-peak amplitude of the response second harmonic (isolated by Fourier analysis) was measured. Results were compared with those obtained from 36 age- and sex-matched control subjects (mean age 46 ± 8 years, range, 32–58).

Subjects providing normative $\mathbf{R}F_{onh}$, HRT, and PERG data belonged to independent groups. Table 1 summarizes demographic and clinical findings obtained in OHT and EOAG patients and in control subjects providing normative F_{onh} and $\mathbf{R}F_{onh}$ data. At the time of testing, all the EOAG patients were under medical treatment (with topical β-blocker alone or in combination with dorzolamide or latanoprost). Of the 29 OHT patients, 16 were not under treatment, whereas the remaining 13 were under topical β-blocker alone or in combination with dorzolamide or latanoprost. IOPs at the time of testing did not differ significantly between patients with EOAG or OHT, as well as between untreated or treated patients with OHT (independent *t*-test).

The study protocol was approved by the Ethics Committee of the Università Cattolica S. Cuore and followed the guidelines of the Declaration of Helsinki. Informed consent was obtained from every normal subject or patient after the procedures used in the study were fully explained.

Determination of RF_{onh}

Using a method previously described,[18] a probing laser beam (wavelength 810 nm; power at the cornea, 90 μW; diameter at the fundus, approximately 150 μm) was aimed at a temporal site of the neuroretinal rim. The laser light scattered by the tissue was collected by an optical fiber at the image plane of the fundus camera and guided to a photodetector. The aperture of the light-collecting optical fiber (diameter at the fundus approximately 180 μm) was centered on the site illuminated by the probing laser beam. An infrared video camera placed in the retinal image plane of the fundus camera allowed the operator to monitor the location of the beam and of the light-collecting aperture at the disc and, when necessary, reposition both at the desired site. The fundus camera was positioned in front of the eye in such a way that an edge of the pupil of the tested eye could also be observed on the video monitor. In this manner, the observer could maintain steady the position of the fundus camera relative to the subject's pupil eye. Guided by color photographs of the disc, we took care to avoid recording from large vessels. The output signal of the detector was analyzed using software implemented on a computer (NeXT; Redwood, CA)[19] and was monitored continuously in real time throughout the experiment. The relative flux of red blood cells in the volume sampled by the light-collecting fiber (F_{onh}) was obtained from the relationship $F_{onh} = k \times Vel_{onh} \times Vol_{onh}$, where k is an instrumental constant, Vel_{onh} is the first moment frequency of the Doppler shift spectrum, which is proportional to the mean velocity of the red blood cells in the volume sampled by the laser light and is expressed in units of frequency (hertz). Vol_{onh} is the relative number of red blood cells, expressed in arbitrary units (AU). Vel_{onh}, Vol_{onh}, and F_{onh} are commonly referred to as LDF parameters.[18] They were sampled at a rate of 21 data points per second. In addition to these LDF parameters, we recorded the DC of the photocurrent, which is proportional to the total amount of scattered light reaching the detector.[18]

Flicker Stimuli

The stimulus was a pure green luminance flicker generated by an array of ultrabright light-emitting diodes (LEDs) with dominant wavelengths at 524 nm and located in the plane of the light source of the fundus camera. The diodes were synchronously square-wave modulated at 15 Hz between 0 and 10 lux. The stimulus was delivered to the eye in Maxwellian view through the fundus illumination's optical system of the camera (Topcon Optical, Tokyo, Japan) and it uniformly illuminated a 25° diameter area that included both the fovea and the optic disc.

Experimental Protocols

The LDF parameters were recorded at two to three sites of the neuroretinal temporal rim of the optic disc in both eyes by an operator masked with regard to the diagnosis of the patients (OHT or EOAG). These recordings consisted of ~20 seconds at baseline, followed by ~50 seconds of flicker. At each site, we attempted to obtain at least two consecutive measurements. Only recordings on which the change in DC between baseline and stimulation was less than ±8% were used.

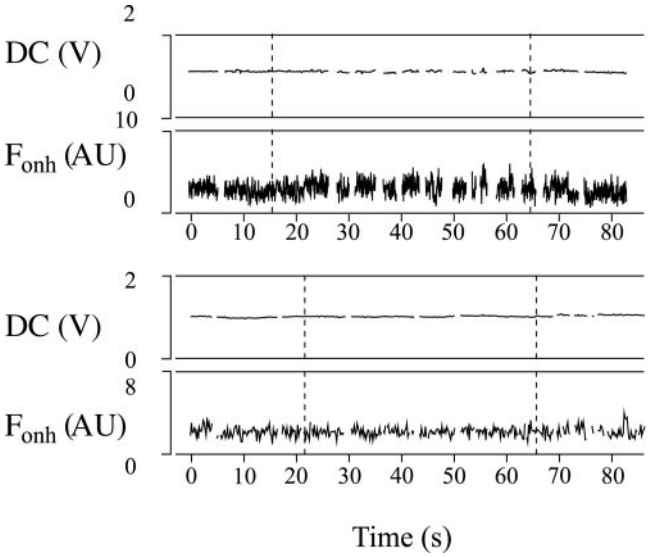

FIGURE 1. Time courses of F_{onh} and DC responses to a 15-Hz green flicker obtained from patients with EOAG. Recording demonstrating a response (*top*), albeit strongly reduced compared with the normal response and no response (*bottom*). *Vertical dashed lines* demarcate the period of stimulation. Spikes associated with microsaccadic motion have been removed from the recording, as described elsewhere.[30]

$\mathbf{RF}_{onh}$ was defined as $100 \times (F_{onh,st} - F_{onh,bl})/F_{onh,bl}$, where $F_{onh,st}$ represents the average of F_{onh} over the last 20 seconds of flicker and $F_{onh,bl}$ the average of F_{onh} during baseline. The luminance during baseline was approximately equal to the mean luminance of the flicker stimulus. At each site of the temporal disc, an average value of $\mathbf{RF}_{onh}$ over the successive recordings was calculated, and the largest of these average values between sites was retained.

Data Analysis

In normal subjects, one eye was randomly selected and evaluated, and its results were included in the statistical analysis. In patients, although both eyes were evaluated, only the LDF results from the right eyes were considered for their relationship with morphologic and functional parameters. $\mathbf{RF}_{onh}$ values were compared across the groups of the study population (normal, OHT and EOAG groups) by a one-way analysis of variance (ANOVA) with the Tukey honest significant difference (HSD) post hoc comparison test. The Pearson correlation analysis was used to evaluate the relationship between $F_{onh,bl}$ and $\mathbf{RF}_{onh}$ values of individual OHT and EOAG patients with the corresponding clinical measurements (visual field, optic disc tomography, and PERG parameters). Visual field parameters entered in the analysis were: mean deviation (MD), central mean deviation (MD values derived from the 18 central locations of the Humphrey 30-2 deviation plot), pattern SD (PSD), and corrected pattern SD (CPSD). The rationale of using the MD derived from the 18 central field locations was based on the fact that, because $\mathbf{RF}_{onh}$ measurements were obtained from the temporal neuroretinal rim, this "central" MD was expected to show a closer correlation with flowmetry data. Optic disc parameters included cup-to-disc area ratio, neuroretinal rim area and cup shape measure. The PERG parameter was the amplitude of the response. In this analysis, a Bonferroni-corrected $P < 0.05$ was considered statistically significant. Finally, $F_{onh,bl}$ and $\mathbf{RF}_{onh}$ were compared across treated and untreated OHT patients by independent *t*-tests with a significance level of $P < 0.05$ (two-tailed).

The time courses of Vel_{onh}, Vol_{onh}, and F_{onh} during stimulation were determined for the group of EOAG patients, and from them the time constants of the increases were calculated based on a three-parameter exponential increase to maximum (SigmaPlot; SPSS Science, Chicago, IL) and compared to those obtained previously in control subjects.[10]

The variability of $\mathbf{RF}_{onh}$ in normal subjects has been reported.[10] In the group of the patients it was assessed as follows: in every patient, from every site in the temporal part of the optic disc rim, we first determined $\mathbf{RF}_{onh,1}$ and $\mathbf{RF}_{onh,2}$ for two successive recordings. The variability between the two recordings was defined as the group mean of $100 \times |\mathbf{RF}_{onh,1} - \mathbf{RF}_{onh,2}|/0.5(\mathbf{RF}_{onh,1} + \mathbf{RF}_{onh,2})$.

Results

F_{onh} and RF_{onh} in Normal Subjects and Patients

Figure 1 shows two time courses of F_{onh} obtained from a patient with EOAG at baseline and during 15-Hz green luminance flicker. These recordings were selected among those that demonstrated a response, albeit strongly reduced compared with the normal response,[8,10] and a response close to zero. The group average time course of F_{onh} in OD during the first 40 seconds of stimulation for the patients with EOAG is shown in Figure 2. To obtain this time course, all baseline F_{onh} values were normalized to 1 AU. Each time and F_{onh} value represents an average of 100 independent data points. Time constant τ for the increase in F_{onh} was 9.9 seconds ($P = 0.049$). The range corresponding to $\tau \pm 1$ SE extended from 6.7 to 18.7 seconds. This time constant was not significantly different from the value previously published for the normal subjects (nonpaired *t*-test between both $1/\tau$ values of the exponential fits).[10]

The group means F_{onh} and $\mathbf{RF}_{onh}$ for OD, OS, and OU (average of both eyes) are given in Table 2. Mean F_{onh} was significantly reduced (Tukey HSD, $P < 0.05$) in EOAG compared with both OHT and normal control eyes (Table 2). Group mean $\mathbf{RF}_{onh}$ was also significantly reduced (Tukey test, $P < 0.01$) in both OHT and EOAG eyes compared with control subjects, whereas no significant difference was observed between the groups of OHT and EOAG eyes. At rest, mean Vel_{onh}, expressed in kHz, was 0.36 ± 0.05 ($\pm$95% confidence limits) in the control eyes, 0.44 ± 0.03 in the OHT group, and 0.41 ± 0.04 in the EOAG group, respectively. For Vol_{onh} (AU) these mean values were 0.22 ± 0.09, 0.13 ± 0.03, and 0.08 ± 0.01, respectively. Mean Vol_{onh} of the OHT and of the EOAG groups were both significantly different from the control Vol_{onh}.

Figure 3 shows the $F_{onh,bl}$ and $\mathbf{RF}_{onh}$ distributions obtained from OD in the normal subjects and the two groups of patients. They differed significantly across groups (one-way ANOVA, $F_{2,41} = 16$, $P < 0.001$). Similar results were observed when the left eyes of patients were analyzed (not shown). $\mathbf{RF}_{onh}$ and

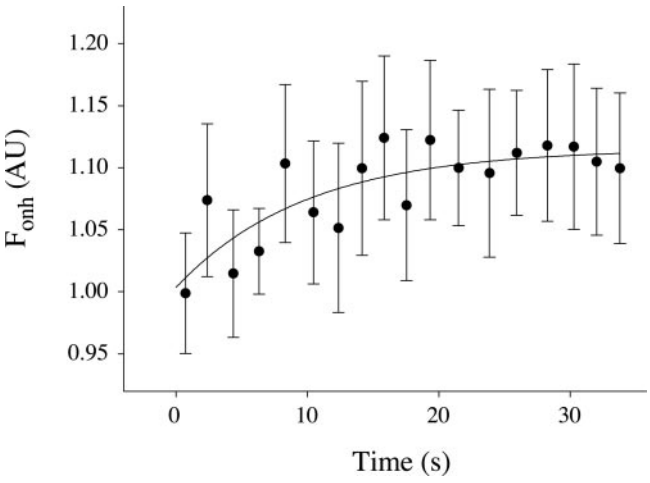

FIGURE 2. Group average time course of F_{onh} in response to flicker obtained from the right eyes of the patients with EOAG. The stimulus was applied at time 0. Error bars, $\pm$95% confidence intervals.

TABLE 2. Group-Averaged ± 95% Confidence Limits Baseline Values

Eye/Groups	Eyes (*n*)	F_{onh} (AU)	RF_{onh} (%)
OD			
Normals	7.0	8.2 ± 5.3	44.0 ± 16.0
OHT	29.0	4.8 ± 1.4	17.5 ± 6.0
EOAG	13.0	2.9 ± 0.9	8.0 ± 4.0
OS			
Normals	9.0	6.0 ± 4.3	36 ± 7.0
OHT	29.0	4.5 ± 0.9	17.6 ± 6.0
EOAG	11.0	3.0 ± 0.7	13.0 ± 11.0
OU			
Normals	16.0	7.1 ± 3.0	39.0 ± 7.0
OHT	58.0	4.7 ± 0.8	17.5 ± 4.0
EOAG	24.0	2.9 ± 0.5	10.4 ± 5.0

Data are shown separately for OD and OS (and average of both eyes, OU) of F_{onh} and $\mathbf{R}F_{onh}$ for the groups of normal subjects, patients with OHT, and patients with EOAG.

$F_{onh,bl}$ did not correlate significantly in all the groups. Variability of $\mathbf{R}F_{onh}$ values, as defined in the Materials and Methods section, was 17.5% in the patients with OHT and 15% in the EOAG group.

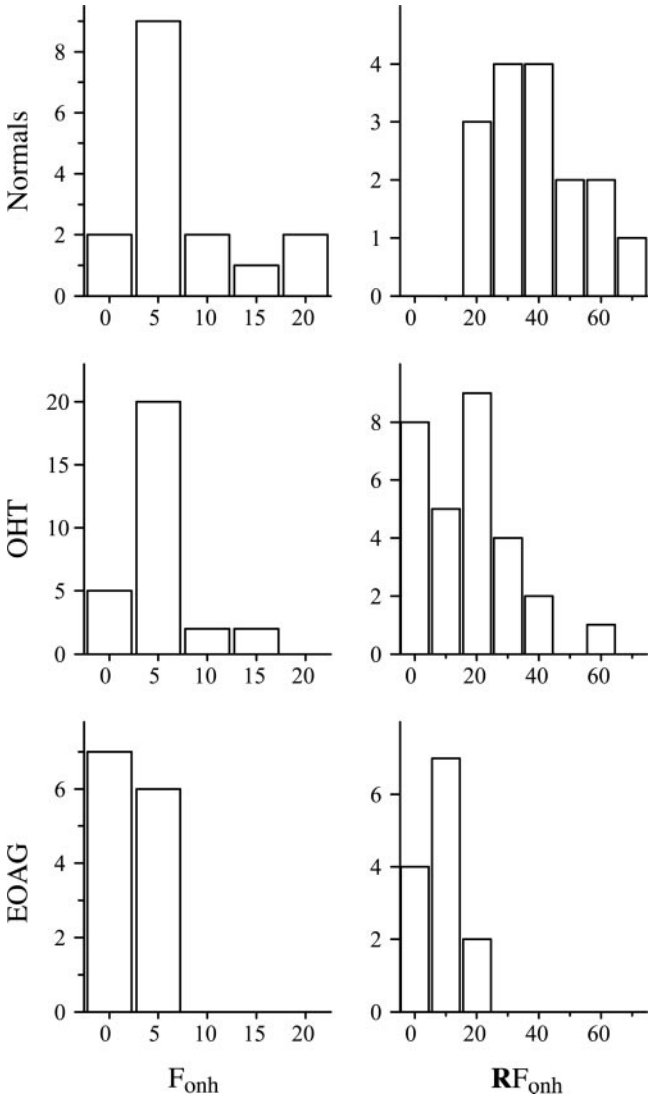

FIGURE 3. Frequency distribution histograms of F_{onh} and $\mathbf{R}F_{onh}$ in right eyes of the normal subjects and patients with OHT or EOAG.

In the normal subjects, approximately one third of the $\mathbf{R}F_{onh}$ was due to the change in Vel_{onh} ($\mathbf{R}Vel_{onh}$) and two thirds to that of Vol_{onh} ($\mathbf{R}Vol_{onh}$). In the groups of OHT and EOAG patients, $\mathbf{R}Vel_{onh}$ and $\mathbf{R}Vol_{onh}$ were not significantly different, each contributing nearly equally to $\mathbf{R}F_{onh}$. The change in DC during flicker was 1% ± 4% (mean ± SD) in normal subjects, −0.6% ± 3.5% in the OHT patients, and −0.5% ± 2.3% in the EOAG patients.

Correlations were sought between the LDF parameters and the morphologic and functional data in the right eye. No significant correlation was observed between (1) $F_{onh,bl}$ and cup-to-disc area ratio, (2) $F_{onh,bl}$ and cup shape value, and (3) $\mathbf{R}F_{onh}$ and cup-to-disc area ratio, cup shape value, retinal nerve fiber layer thickness, and rim volume in both groups of patients and when all patients were grouped together. $F_{onh,bl}$ and $\mathbf{R}F_{onh}$ increased significantly as a function of neuroretinal rim area (Fig. 4) when the OHT and EOAG patients' data were pooled ($r = 0.41$, $P = 0.02$). Separate correlations for each group showed similar positive trends, although they did not reach statistical significance. It should be noted that the distribution of the F_{onh} and $\mathbf{R}F_{onh}$ values of the seven patients with OHT who did not consent to have an HRT examination was not significantly different from that of the other 22 OHT patients.

Figure 5 shows scatterplots of $\mathbf{R}F_{onh}$ in response to luminance flicker obtained from the right eyes of individual OHT and EOAG patients, plotted as a function of corresponding central perimetric MD (Fig. 5A) and PERG amplitudes (Fig. 5B). No significant correlations were found between $\mathbf{R}F_{onh}$ and PERG amplitudes and $\mathbf{R}F_{onh}$ and central MD when the data in the OHT and EOAG patients were analyzed separately or grouped together. In some OHT patients, PERG amplitudes and MDs were within normal limits or similar to the normal mean, whereas the corresponding $\mathbf{R}F_{onh}$ values were substantially reduced with respect to the mean value in the control group. Opposite results were observed in some other patients, in whom PERG and MD amplitudes but not $\mathbf{R}F_{onh}$ were substantially below normal. No other statistically significant correlations were found between the $\mathbf{R}F_{onh}$ and corresponding functional indices (PSD, CPSD) of glaucomatous damage. These conclusions applied also when only the left eye data were used in every patient.

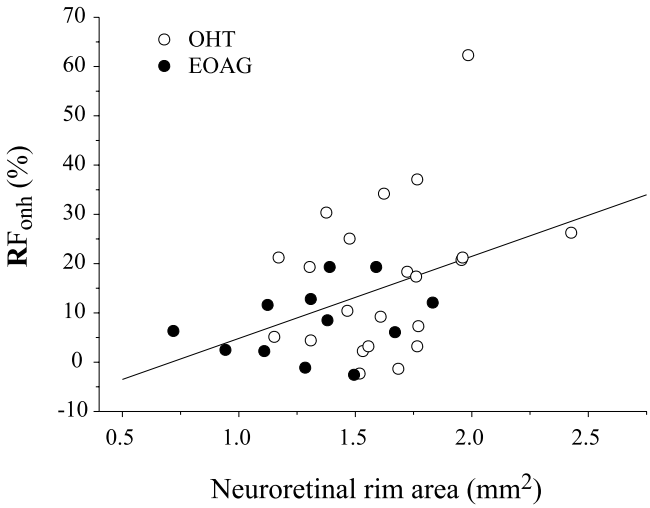

FIGURE 4. Scatterplot showing $\mathbf{R}F_{onh}$ data in OD obtained from patients with OHT or EOAG, as a function of corresponding neuroretinal rim area. The correlation coefficient of the linear regression line is significant ($r = 0.41$, $P = 0.02$).

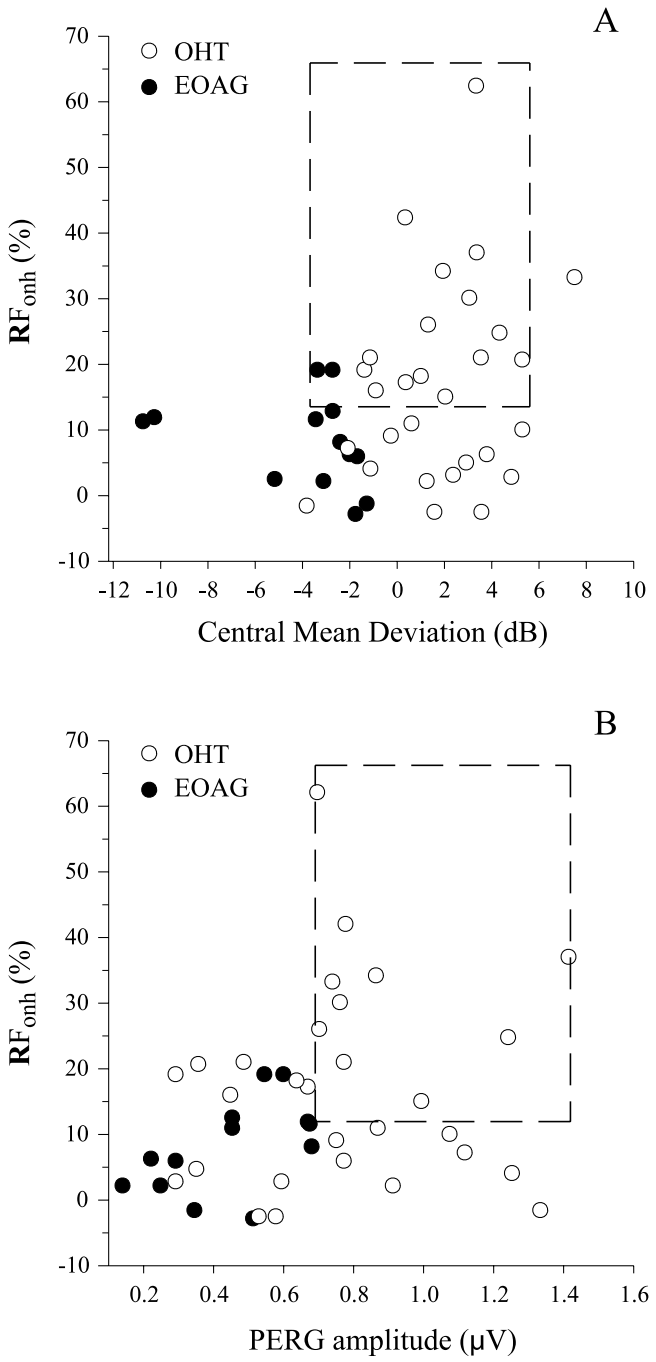

FIGURE 5. Scatterplots of $\mathbf{RF}_{onh}$ to luminance flicker in patients with OHT or EOAG versus perimetric central MD (**A**) and PERG amplitude (**B**). *Dashed rectangles*: mean ±2 SD range of the normal population.

Comparing F_{onh} and $\mathbf{RF}_{onh}$ values obtained from the 16 untreated OHT eyes with those from the 13 OHT eyes that were receiving IOP-lowering treatment provided average F_{onh} and $\mathbf{RF}_{onh}$ that tended to be greater in the untreated eye (independent *t*-tests, $P = 0.10$ and 0.18, respectively). Thus, mean F_{onh} was 5.8 ± 3 AU (95% confidence interval) and 4 ± 1 AU and $\mathbf{RF}_{onh}$ was 21% ± 12% and 14% ± 6% in the untreated and treated OHT, respectively.

DISCUSSION

The present study focuses on the changes in blood flow measured in the anterior optic nerve of OHT and EOAG patients in response to diffuse luminance flicker. It demonstrated that blood flow is diminished in both groups of patients compared with normal control subjects, even in the case of using the patients' largest $\mathbf{RF}_{onh}$ value between sites of the temporal disc. The rationale for using the largest $\mathbf{RF}_{onh}$ was to increase our confidence that an alteration in $\mathbf{RF}_{onh}$ indeed occurs, should a reduction be found in the patients' groups.

All EOAG and some OHT patients were receiving IOP-lowering treatment at the time of testing. Therefore, a possible confounding effect of drugs on the results cannot be ruled out in these patients. In contrast, a substantial fraction of the patients with OHT did not use any treatment at the time of examination, and in them the $\mathbf{RF}_{onh}$ was, on average, still smaller than in the control subjects (more than 1 SD). This suggests that the disease has a deteriorating effect per se on the optic nerve flow regulation in response to flicker.

The group-averaged time course of the flicker-induced increase in F_{onh} in EOAG was not significantly different from normal. Presumably, in EOAG the flicker-induced vasodilatation in the apparently healthy rim tissue of the anterior optic nerve has a normal temporal evolution. The fact that $\mathbf{RF}_{onh}$ is due to almost equal changes in Vel_{onh} and Vol_{onh} indicates that both arteriolar dilatation (reflected in $\mathbf{RVel}_{onh}$) and capillary engagement and/or erythrocyte recruitment (reflected in $\mathbf{RVol}_{onh}$) are involved in the activity-induced blood-flow response.[10]

In the framework of Roy and Sherrington's hypothesis of a tight coupling between neural activity, blood flow, and metabolism,[20] the flicker-evoked response at the temporal rim of the optic disc could be due to two processes: a local increase of the metabolism of the rim tissue produced by the flicker or a conduction increase in the axons at the rim as a consequence of the increased activity in the perifoveal ganglion cells. Convincing evidence for either process remains to be established. However, support for the prevalence of the second process is available from previous data obtained in a normal subject.[18] An $\mathbf{RF}_{onh}$ of ~25% was obtained in this subject when only the macula was flickered compared with an $\mathbf{RF}_{onh}$ of 14% when only the region of the disc was stimulated with the same stimulus. Our results strongly suggest therefore that the decrease in $\mathbf{RF}_{onh}$ reflects a disturbance of this second process—namely, altered ganglion cell response to the flicker. Nevertheless, other possible explanations of this decrease must be considered. One is that resting blood flow in the rim tissue of the OHT and EOAG patients could be too low in comparison with the amount of tissue it supplies to satisfy the metabolic needs. Consequently, the vessels in this rim region would be unable to respond fully to the additional stress evoked by the flicker stimulation. However, the lack of correlation between $\mathbf{RF}_{onh}$ and F_{onh} does not support this hypothesis. Another explanation would be that, because of decreased rim tissue volume (see Table 1) in the EOAG group compared with the control and OHT groups, the choroid would contribute more to the LDF signal. This is unlikely, because the markedly higher flow velocities in the choriocapillaris did not result in a significantly greater Vel_{onh} in the patients with EOAG. Furthermore, $\mathbf{RF}_{onh}$ was found to be reduced in the OHT group, although the rim tissue volume in this group was not significantly different from that in the control group.

Studies in the human eye using a range of flicker frequencies and modulations suggest that the $\mathbf{RF}_{onh}$ loss observed in our patients when using a 15-Hz luminance flicker occurs predominantly at the level of the ganglion cells magnocellular pathway.[8,9] This hypothesis agrees with the large body of anatomic evidence indicating that, in early glaucoma, large ganglion cells subserving primarily this pathway are selectively or predominantly damaged.[11–13] in contrast, patients' $\mathbf{RF}_{onh}$ were poorly correlated with functional indicators of early dam-

age, such as pattern electroretinogram and Humphrey perimetric indices. Thus, the data obtained from the OHT patients show that, in some of these patients, the pattern electroretinogram was normal whereas $\mathbf{RF}_{onh}$ was substantially diminished. In other OHT patients, the opposite was found. This poor correlation may be related to the inherent variability of the techniques used in this study, or, alternatively, may reflect an altered neurovascular coupling in the early stages of disease.

Alteration of the neurovascular coupling caused by an impaired functional hyperemia could occur at various points of the chain of events coupling the activity to the local vasodilatation,[21] such as flicker-induced release of nitric oxide (NO), K^+, or other substances[5,21,22]; glutamate release from axonal terminals; activation of astrocytes and subsequent release of vasodilating products; and possibly others. Clearly, future work should be aimed at resolving this important question.

$\mathbf{RF}_{onh}$ correlated positively with neuroretinal rim area when the data of the two groups of patients were pooled. In patients with EOAG, although this correlation did not reach significance, possibly because of the low number of patients, both neuroretinal rim area and $\mathbf{RF}_{onh}$ were smaller on average than the corresponding values in OHT patients and normal control subjects. It has been shown that a reduction in neuroretinal rim area, together with an increase in cup-to-disc area ratio and cup shape value, may be highly accurate in detecting early glaucomatous damage.[15,23] These results indicate that a reduction in ONH vasoactivity assessed by flicker stimulation is associated with early loss of nerve fiber layer in eyes with EOAG.

A number of studies have compared F_{onh} measurements obtained in normal subjects with those in patients with glaucoma. Although the results vary considerably between studies,[24] F_{onh} has been generally found to be reduced in patients with primary open-angle glaucoma compared with that in normal control subjects.[25,26] The data of the present study of EOAG are in agreement with those results. In addition, F_{onh} also tends to be reduced in patients with OHT. In our study, these conclusions apply only to the sites of measurements where $\mathbf{RF}_{onh}$ was found to have the largest value. Whether they are valid for the other sites of the disc remains to be assessed.

This decrease in F_{onh} in glaucoma has been interpreted by previous investigators as evidence of an actual reduction in blood flow, although some caution about the validity of this interpretation has been expressed in view of the limitations of the LDF technique. As discussed elsewhere,[6] comparisons between F_{onh} values in terms of actual blood flow is strictly valid only if the scattering properties of the tissue from which F_{onh} is measured are identical. This is because the Doppler shift power spectrum depends not only on the number and velocity of red blood cells in the sampled volume, but also on the optical characteristics (i.e., the absorption and scattering) of the tissue (nonmoving scatterers) sampled. In general, increased tissue scattering broadens the power spectrum, causing an artificial increase in the measured flow. To our present knowledge, it is not clear yet how the scattering of light by the glaucomatous rim tissue differs from that of the healthy eye and therefore how it may affect F_{onh}.

In contrast to F_{onh}, $\mathbf{RF}_{onh}$ is not affected by the scattering properties of the tissue. Changes in light-scattering may be expected during increased neural activity, but these changes are too fast and too small to have a notable effect on the LDF spectrum and F_{onh}.[27] Furthermore, the changes in F_{onh} are proportional to the actual flow changes, as they are within the range of linearity of the LDF technique.[18] Both of these aspects make the LDF technique most appropriate for investigating the regulation of F_{onh} in response to various physiological stimuli.

Using flicker to investigate F_{onh} regulation offers additional advantages over previously used physiological stimuli, such as decreases in mean ocular perfusion pressure (PP_m = ophthalmic artery blood pressure − IOP) achieved by increasing the IOP with a suction cup,[28,29] increases in PP_m by means of isometric exercises,[30] and the breathing of various gas mixtures.[31] Flicker is not invasive and is a more physiological stimulus because modulation of light exposure is the most natural stimulus for the visual system, leaving the systemic circulation unperturbed.

Our LDF measurements were obtained by directing the probing laser beam at the temporal site of the neuroretinal rim of the optic disc. In humans, as in monkeys,[32] LDF is probably predominantly sensitive to blood flow changes occurring only in the most superficial layers (supplied by the retinal circulation) of the optic nerve head. However, regardless of which layer of the optic nerve head circulation is in fact sampled by the LDF technique, our data may be considered to reflect the blood flow changes (elicited by a visual stimulus) in a limited area of the microcirculatory district belonging to the neuroretinal rim, a key anatomic component of the optic nerve.[33] Although implications of the present data for the pathophysiology of glaucomatous optic neuropathy remains a matter of speculation, the finding of an abnormal flicker-evoked flow regulation in the superficial layers of the optic nerve recorded in both OHT and EOAG eyes clearly merits further investigation.

In conclusion, this LDF study demonstrated a decreased blood flow response to flicker stimulation measured at the temporal neuroretinal rim of the optic disc in both OHT and EOAG. This decrease, which in EOAG is associated with early loss of the nerve fiber layer, could be due to a decrease of the flicker-induced activity of the magnocellular pathway. Furthermore, the relation of blood flow changes with visual function data suggests that neural activity and vascular response can be independently altered early in the disease process.

References

1. Riva CE, Harino S, Shonat RD, Petrig BL. Flicker evoked increase in optic nerve head blood flow in anesthetized cats. *Neurosci Lett*. 1991;128:291–296.
2. Riva CE, Harino S, Petrig BL, Shonat RD. Laser Doppler flowmetry in the optic nerve. *Exp Eye Res*. 1992;55:499–506.
3. Toi VV, Riva CE. Variations of blood flow at optic nerve head induced by sinusoidal flicker stimulation in cats. *J Physiol*. 1995; 482:189–202.
4. Buerk DG, Riva CE, Cranstoun SD. Frequency and luminance dependent blood flow and K^+ ion changes during flicker stimuli in cat optic nerve head. *Invest Ophthalmol Vis Sci*. 1995;36:2216–2227.
5. Buerk DG, Riva CE. Cranstoun SD. Nitric oxide has a vasodilatory role in cat optic nerve head during flicker stimuli. *Microvasc Res*. 1996;52:13–26.
6. Buerk DG, Riva CE. Adenosine enhances functional activation of blood flow in cat optic nerve head during photic stimulation independently from nitric oxide. *Microvasc Res*. 2002;2432:1–11.
7. Kiryu J, Asrani S, Shahidi M, Mori M, Zeimer R. Local response of the primate retinal microcirculation to increased metabolic demand induced by flicker. *Invest Ophthalmol Vis Sci*. 1995;36: 1240–1246.
8. Riva CE, Falsini B, Logean E. Flicker-evoked responses of human optic nerve head blood flow: luminance versus chromatic modulation. *Invest Ophthalmol Vis Sci*. 2001;42:756–762.
9. Falsini B, Riva CE, Logean E. Flicker-evoked changes in human optic nerve blood flow: relationship with retinal neural activity. *Invest Ophthalmol Vis Sci*. 2002;43:2309–2316.
10. Riva CE, Logean E, Falsini. Temporal dynamics and magnitude of the blood flow response at the optic disc in normal subjects during functional retinal flicker-stimulation. *Neurosci Lett*. 2004;356:75–78.
11. Quigley HA, Dunkelberger GR, Green WR. Retinal ganglion cell atrophy correlated with automated perimetry in human eyes with glaucoma. *Am J Ophthalmol*. 1989;107:453–464.

12. Glovinski Y, Quigley HA, Dunkelberger GR. Retinal ganglion cells loss is size dependent in experimental glaucoma. *Invest Ophthalmol Vis Sci*. 1991;32:484–491.
13. Kerrigan-Baumrind LA, Quigley HA, Pease ME, Kerrigan DF, Mitchell RS. Number of ganglion cells in glaucoma eyes compared with threshold visual field tests in the same persons. *Invest Ophthalmol Vis Sci*. 2000;41:741–748.
14. Salgarello T, Colotto A, Falsini B, et al. Correlation of pattern electroretinogram with optic disk cup shape in ocular hypertension. *Invest Ophthalmol Vis Sci*. 1999;40:1989–1997.
15. Uchida H, Brigatti L, Caprioli J. Detection of structural damage from glaucoma with confocal laser image analysis. *Invest Ophthalmol Vis Sci*. 1996;37:2393–2401.
16. Porciatti V, Falsini B, Brunori S, Colotto A, Moretti G. Pattern electroretinogram as a function of spatial frequency in ocular hypertension and early glaucoma. *Doc Ophthalmol*. 1987;65:349–355.
17. Falsini B, Colotto A, Porciatti V, Porrello G. Follow-up study with pattern ERG in ocular hypertension and glaucoma patients under timolol maleate treatment. *Clin Vision Sci*. 1992;7:341–347.
18. Riva CE, Petrig BL, Falsini B, Logean E. Neuro-vascular coupling at the optic nerve head studied by laser Doppler flowmetry. In: Lakshminarayanan V, ed. *Vision Science and its Applications*. Vol. 35. Washington, DC: Optical Society of America; 2000:64–76.
19. Petrig BL, Riva CE. Optic nerve head laser Doppler flowmetry: principles and computer analysis. In: Kaiser HJ, Flammer J, Hendrickson P, eds. *Ocular Blood Flow*. Basel: Karger; 1996:120–127.
20. Roy CS, Sherrington CS. On the regulation of the blood supply of the brain. *J Physiol*. 1890;11:85–108.
21. Zonta M, Angulo MC, Gobbo S, et al. Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. *Nat Neurosci*. 2003;6:43–50.
22. Kondo M, Wang L, Bill A. The role of nitric oxide in hyperaemic response to flicker in the retina and optic nerve in cats. *Acta Ophthalmol Scand*. 1997;75:232–235.
23. Blumenthal EZ, Weinreb RN. Assessment of the retinal nerve fiber layer in clinical trials of glaucoma neuroprotection. *Surv Ophthalmol*. 2001;45(suppl 3):305–312.
24. Piltz-Seymour JR, Grunwald JE, Hariprasad SM, DuPont J. Optic nerve blood flow is diminished in eyes of primary open-angle glaucoma suspects. *Am J Ophthalmol*. 2001;132:63–69.
25. Michelson G, Langhans MJ, Groh MJM. Perfusion of the juxtapapillary retina and the neuroretinal rim area in primary open angle glaucoma. *J Glaucoma*. 1996;5:91–98.
26. Hafez AS, Bizzarro RL, Lesk MR. Evaluation of optic nerve head and peripapillary retinal blood flow in glaucoma patients, ocular hypertensives, and normal subjects. *Am J Ophthalmol*. 2003;136:1022–1031.
27. Gratton G, Fabiani M. The event-related optical signal: a new tool for studying brain function. *Int J Psychophys*. 2001;42:109–121.
28. Pillunat LE, Stodtmeister R, Wilmanns I. Pressure compliance of the optic nerve head in low tension glaucoma. *Br J Ophthalmol*. 1987;71:181–187.
29. Riva CE, Hero M, Titze P, Petrig B. Autoregulation of human optic nerve head blood flow in response to acute changes in ocular perfusion pressure. *Graefes Arch Clin Exp Ophthalmol*. 1997;235:618–626.
30. Movaffaghy A, Chamot SR, Petrig BL, Riva CE. Blood flow in the human optic nerve head during isometric exercise. *Exp Eye Res*. 1998;67:561–568.
31. Harris A, Anderson DR, Pillunat L, et al. Laser Doppler flowmetry measurement of changes in human optic nerve head blood flow in response to blood gas perturbations. *J Glaucoma*. 1996;5:258–265.
32. Petrig BL, Riva CE, Hayreh SS. Laser Doppler flowmetry and optic nerve head blood flow. *Am J Ophthalmol*. 1999;127:413–425.
33. Jonas JB, Budde WM. Diagnosis and pathogenesis of glaucomatous optic neuropathy: morphological aspects. *Prog Retin Eye Res*. 2000;19:1–40.