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 Falsini2

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 (Fonh) 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 Fonh to this stimulus (RFonh) was expressed as percentage change in Fonh between baseline and the last 20 seconds of flicker. Two to three temporal sites of the disc were tested, and the highest RFonh was considered for further analysis. RFonh 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, Fonh and RFonh 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 Fonh or RFonh and the other morphometric and functional parameters. The group-averaged time course of RFonh was not significantly different from that in the normal subjects.

CONCLUSIONS. Luminance flicker-evoked RFonh 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 RFonh can be independently altered early in the disease process. (Invest Ophthalmol Vis Sci. 2004;45:3662–3668) DOI:10.1167/iovs.04-0100

Several investigations in both animals and humans1–10 indicate that blood flow measured by laser Doppler flowmetry (LDF) at the neuroretinal rim of the optic nerve head (Fonh) increases when the neural retinal activity is increased by flicker. In normal subjects, this regulatory response of Fonh (RFonh), 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.11–15

Based on these considerations, a laser Doppler flowmetry (LDF) study was undertaken to investigate whether RFonh, 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 RFonh 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 50-2 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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Submitted for publication February 2, 2004; revised March 29, 2004; accepted April 6, 2004.

Disclosure: C.E. Riva, None; T. Salgarello, None; E. Logean, None; A. Colotto, None; E.M. Galan, None; B. Falsini, None.

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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 RFonh 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. 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 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 mm².

All patients underwent pattern electroretinogram (PERG) recordings according to a previously published technique. 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 Hz). The stimulus was delivered to the eye in 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.

**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 placed 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.

### Table 1. Summary of Demographic and Clinical Characteristics of the Study Population

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

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.
stimulus. At each site of the temporal disc, an average value of $R_{\text{baseline}}$ was approximately equal to the mean luminance of the response (top fl. response and no response (bottom fl. response). $R_{\text{baseline}}$ derived from the 18 central locations of the Humphrey 30-2 deviation plot), pattern SD deviation (MD), central mean deviation (MD values derived from the 18 measurements (visual 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 (CP SD). The rationale of using the MD derived from the 18 central field locations was based on the fact that, because $R_{\text{baseline}}$ 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 analysis of variance (ANOVA) with the Tukey honest significant difference (HSD) post hoc comparison test. The Pearson correlation analysis were considered for their relationship with morphologic and functional parameters. $R_{\text{baseline}}$ was defined as $100 \times (F_{\text{onh, st}} - F_{\text{onh, bl}})/F_{\text{onh, bl}}$, where $F_{\text{onh, st}}$ represents the average of $F_{\text{onh}}$ over the last 20 seconds of flicker and $F_{\text{onh, bl}}$ the average of $F_{\text{onh}}$ 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 $R_{\text{baseline}}$ 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. $R_{\text{baseline}}$ 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 $F_{\text{onh, bl}}$ and $R_{\text{baseline}}$ 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 (CP SD). The rationale of using the MD derived from the 18 central field locations was based on the fact that, because $R_{\text{baseline}}$ 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, $F_{\text{onh, bl}}$ and $R_{\text{baseline}}$ 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.\(^\text{10}\)

The variability of $R_{\text{baseline}}$ in normal subjects has been reported.\(^\text{10}\) 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 $R_{\text{onh,1}}$ and $R_{\text{onh,2}}$ for two successive recordings. The variability between the two recordings was defined as the group mean of $100 \times |R_{\text{onh,1}} - R_{\text{onh,2}}|/0.5(R_{\text{onh,1}} + R_{\text{onh,2}})^2$.

**RESULTS**

**F onh and R onh in Normal Subjects and Patients**

Figure 1 shows two time courses of $F_{\text{onh}}$ 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,\(^\text{6,10}\) and a response close to zero. The group average time course of $F_{\text{onh}}$ 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 $F_{\text{onh}}$ values were normalized to 1 AU. Each time and $F_{\text{onh}}$ value represents an average of 100 independent data points. Time constant $\tau$ for the increase in $F_{\text{onh}}$ 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 (non-paired $t$-test between both 1/\tau values of the exponential fits).\(^\text{10}\)

The group means $F_{\text{onh}}$ and $R_{\text{onh}}$ for OD, OS, and OU (average of both eyes) are given in Table 2. Mean $F_{\text{onh}}$ was significantly reduced (Tukey HSD, $P < 0.05$) in EOAG compared with both OHT and normal control eyes (Table 2). Group mean $R_{\text{onh}}$ 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 $\pm$ 0.05 ($\pm 95\%$ confidence limits) in the control eyes, 0.44 $\pm$ 0.03 in the OHT group, and 0.41 $\pm$ 0.04 in the EOAG group, respectively. For Vol onh (AU) these mean values were 0.22 $\pm$ 0.09, 0.13 $\pm$ 0.03, and 0.08 $\pm$ 0.01, respectively. Mean Vel onh of the OHT and of the EOAG groups were both significantly different from the control Vol onh.

Figure 3 shows the $F_{\text{onh, bl}}$ and $R_{\text{onh}}$ distributions obtained from OD in the normal subjects and the two groups of patients. They differed significantly across groups (one-way ANOVA, $F_{2,31} = 16$, $P < 0.001$). Similar results were observed when the left eyes of patients were analyzed (not shown). $R_{\text{onh}}$ and
Fonh,bl did not correlate significantly in all the groups. Variability of $R_{\text{Fonh}}$ values, as defined in the Materials and Methods section, was $17.5\%$ in the patients with OHT and $15\%$ in the EOAG group.

In the normal subjects, approximately one third of the $R_{\text{Fonh}}$ was due to the change in $V_{\text{lonh}}$ ($R_{\text{Velonh}}$) and two thirds to that of $V_{\text{onh}}$ ($R_{\text{Volonh}}$). In the groups of OHT and EOAG patients, $R_{\text{Velonh}}$ and $R_{\text{Volonh}}$ were not significantly different, each contributing nearly equally to $R_{\text{Fonh}}$. The change in DC during flicker was $1\%/200$ to $4\%$ (mean $\pm$ SD) in normal subjects, $0.6\%/200$ to $3.5\%$ in the OHT patients, and $0.5\%/200$ to $2.3\%$ 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) $F_{\text{onh,bl}}$ and cup-to-disc area ratio, (2) $F_{\text{onh,bl}}$ and cup shape value, and (3) $R_{\text{Fonh}}$ 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. $F_{\text{onh,bl}}$ and $R_{\text{Fonh}}$ 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 $F_{\text{onh}}$ and $R_{\text{Fonh}}$ 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 $R_{\text{Fonh}}$ 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 $R_{\text{Fonh}}$ and PERG amplitudes and $R_{\text{Fonh}}$ 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 $R_{\text{Fonh}}$ 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 $R_{\text{Fonh}}$ were substantially below normal. No other statistically significant correlations were found between the $R_{\text{Fonh}}$ and corresponding functional indices (PSD, CPSD) of glaucomatous damage. These conclusions applied also when only the left eye data were used in every patient.

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

<table>
<thead>
<tr>
<th>Eye/Groups</th>
<th>Eyes (n)</th>
<th>$F_{\text{onh}}$ (AU)</th>
<th>$R_{\text{Fonh}}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normals</td>
<td>7.0</td>
<td>$8.2 \pm 5.3$</td>
<td>$44.0 \pm 16.0$</td>
</tr>
<tr>
<td>OHT</td>
<td>29.0</td>
<td>$4.8 \pm 1.4$</td>
<td>$17.5 \pm 6.0$</td>
</tr>
<tr>
<td>EOAG</td>
<td>13.0</td>
<td>$2.9 \pm 0.9$</td>
<td>$8.0 \pm 4.0$</td>
</tr>
<tr>
<td>OS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normals</td>
<td>9.0</td>
<td>$6.0 \pm 3.4$</td>
<td>$36 \pm 7.0$</td>
</tr>
<tr>
<td>OHT</td>
<td>29.0</td>
<td>$4.5 \pm 0.9$</td>
<td>$17.6 \pm 7.0$</td>
</tr>
<tr>
<td>EOAG</td>
<td>11.0</td>
<td>$5.0 \pm 0.7$</td>
<td>$13.0 \pm 11.0$</td>
</tr>
<tr>
<td>OU</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normals</td>
<td>16.0</td>
<td>$7.1 \pm 3.0$</td>
<td>$39.0 \pm 7.0$</td>
</tr>
<tr>
<td>OHT</td>
<td>58.0</td>
<td>$4.7 \pm 0.8$</td>
<td>$17.5 \pm 4.0$</td>
</tr>
<tr>
<td>EOAG</td>
<td>24.0</td>
<td>$2.9 \pm 0.5$</td>
<td>$10.4 \pm 5.0$</td>
</tr>
</tbody>
</table>

Data are shown separately for OD and OS (and average of both eyes, OU) of $F_{\text{onh}}$ and $R_{\text{Fonh}}$ for the groups of normal subjects, patients with OHT, and patients with EOAG.
and treated OHT, respectively. 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 RF\textsubscript{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 RF\textsubscript{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 RF\textsubscript{onh} is due to almost equal changes in Velonh and Volonh, indicates that both arteriolar dilatation (reflected in RVel\textsubscript{onh}) and capillary engagement and/or erythrocyte recruitment (reflected in RVol\textsubscript{onh}) are involved in the activity-induced blood-flow response.\textsuperscript{10}

In the framework of Roy and Sherrington’s hypothesis of a tight coupling between neural activity, blood flow, and metabolism,\textsuperscript{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.\textsuperscript{18} An RF\textsubscript{onh} of ~25% was obtained in this subject when only the macula was flickered compared with an RF\textsubscript{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 RF\textsubscript{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 RF\textsubscript{onh} and Fonh 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\textsubscript{onh} in the patients with EOAG. Furthermore, RF\textsubscript{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 RF\textsubscript{onh} loss observed in our patients when using a 15-Hz luminance flicker occurs predominantly at the level of the ganglion cells magnocellular pathway.\textsuperscript{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.\textsuperscript{11–15} In contrast, patients’ RF\textsubscript{onh} were poorly correlated with functional indicators of early dam-

**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 RF\textsubscript{onh} value between sites of the temporal disc. The rationale for using the largest RF\textsubscript{onh} was to increase our confidence that an alteration in RF\textsubscript{onh} indeed occurs, should a reduction be found in the patients’ groups.

**FIGURE 5.** Scatterplots of RF\textsubscript{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 Fonh and RF\textsubscript{onh} values obtained from the 16 untreated OHT eyes with those from the 13 OHT eyes that were receiving IOP-lowering treatment provided average Fonh and RF\textsubscript{onh} that tended to be greater in the untreated eye (independent t-tests, \( P = 0.10 \) and 0.18, respectively). Thus, mean Fonh was 5.8 ± 3 AU (95% confidence interval) and 4 ± 1 AU and RF\textsubscript{onh} was 21% ± 12% and 14% ± 6% in the untreated and treated OHT, respectively.
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 \( \text{RF}_{\text{ono}} \) 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 vasodilation, such as flicker-induced release of nitric oxide (NO), \( K^+ \), or other substances, 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.

\( \text{RF}_{\text{ono}} \) 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 \( \text{RF}_{\text{ono}} \) 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. 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 \( \text{F}_{\text{onh}} \) measurements obtained in normal subjects with those in patients with glaucoma. Although the results vary considerably between studies, \( \text{F}_{\text{onh}} \) has been generally found to be reduced in patients with primary open-angle glaucoma compared with that in normal control subjects. The data of the present study of EOAG are in agreement with those results. In addition, \( \text{F}_{\text{onh}} \) also tends to be reduced in patients with OHT. In our study, these conclusions apply only to the sites of measurements where \( \text{RF}_{\text{ono}} \) 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 \( \text{F}_{\text{onh}} \) 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, comparisons between \( \text{F}_{\text{onh}} \) values in terms of actual blood flow is strictly valid only if the scattering properties of the tissue from which \( \text{F}_{\text{onh}} \) 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 \( \text{F}_{\text{onh}} \).

In contrast to \( \text{F}_{\text{ono}} \), \( \text{RF}_{\text{ono}} \) 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. Furthermore, the changes in \( \text{F}_{\text{ono}} \) are proportional to the actual flow changes, as they are within the range of linearity of the LDF technique. Both of these aspects make the LDF technique most appropriate for investigating the regulation of \( \text{F}_{\text{ono}} \) in response to various physiological stimuli. Using flicker to investigate \( \text{F}_{\text{ono}} \) regulation offers additional advantages over previously used physiological stimuli, such as decreases in mean ocular perfusion pressure (\( \text{PP}_{\text{m}} = \text{ophthal-}

References


