Longitudinal Hemodynamic Changes Within the Optic Nerve Head in Experimental Glaucoma

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PURPOSE. To characterize longitudinal changes in basal blood flow (BF) of the optic nerve head (ONH) during progression of structural damage in experimental glaucoma (EG).

METHODS. Unilateral elevation of IOP was induced in 15 adult rhesus macaques by laser treatment to the trabecular meshwork. Prior to and after laser, retinal nerve fiber layer thickness (RNFLT) and ONH BF were measured biweekly by spectral-domain optical coherence tomography and a laser speckle flowgraphy device (LSFG), respectively.

RESULTS. Average postlaser IOP was 20.2 ± 5.9 mm Hg in EG eyes and 12.3 ± 2.6 mm Hg in control eyes ($P < 0.0001$). Longitudinal changes in basal ONH BF were strongly associated with changes in RNFLT as EG progressed from early through moderately advanced stages of damage, with Pearson correlation coefficients ranging from 0.64 to 0.97 (average = 0.81) and an average slope of 1.0. During early stage (RNFLT loss < 10%), basal ONH BF was mildly increased ($9\% \pm 10\%$, $P = 0.004$) relative to baseline and compared with fellow controls ($P = 0.02$). Basal ONH BF declined continuously throughout subsequent stages in EG eyes reaching $25.0\% \pm 9.6\%$ ($P < 0.0001$) below baseline at the final stage studied (RNFLT loss > 40%). In fellow control eyes, there was no significant change in basal ONH BF over time ($P = 0.27$).

CONCLUSIONS. In EG based on chronic mild-to-moderate IOP elevation, a two-phase pattern of ONH BF alteration was observed. ONH BF increased during the earliest stage (while RNFLT was within 10% of baseline) followed by a linear decline that was strongly correlated with loss of RNFLT.

Keywords: intraocular pressure, blood flow, optic nerve head, experimental glaucoma

Accumulating evidence has shown that blood flow (BF) in the optic nerve head (ONH) is compromised in glaucoma.^{1–12} This hemodynamic alteration has been proposed to play a role in the pathogenesis of glaucomatous optic neuropathy, either directly or indirectly by increasing the susceptibility of the ONH to IOP.^{13–16} A close relationship exists between reduced BF and deteriorated visual function in glaucoma,^{17–21} as well as with structural damage measured by retinal nerve fiber layer thickness (RNFLT) and other morphometric changes in the optic disc.^{4,20,22–24} However, since most previous studies about ONH BF in glaucoma were cross-sectional, the observed ONH BF differences represent only a “snapshot” during a given (or mixed) stage of glaucoma, providing little information about how hemodynamic changes evolve over the course of glaucoma development, leading to some controversy.²⁵ Another disadvantage of cross-sectional studies is that it is difficult to interpret from them whether reduced ONH BF is solely a consequence of axon loss (e.g., reduced metabolic demand) or whether it occurs as a preexisting ischemic condition that primarily contributes to the mechanism of neural and connective tissue damage. For example, some studies have enrolled patients who already had advanced visual field deficits, which makes it highly likely that a fraction of their ganglion cells and optic nerve axons had already degenerated.^{6,9,11,12} It is possible that for these patients, ONH BF was reduced secondary to reduced metabolic demand after neurodegeneration. On the other hand, some patients included in these studies had visual field deficits that were less severe,

such that ONH BF alterations may have greater primacy. Thus, longstanding questions about the role of vasogenesis in glaucoma persist.^{8,9,13,14} The persistence of these questions hinder a more complete understand of the pathogenic role that microcirculatory changes have in glaucoma and of the development of other potential therapeutic approaches to the disease.

The nonhuman primate (NHP) experimental glaucoma (EG) model used in our laboratories enables changes in structural and functional parameters to be monitored longitudinally with a corresponding estimate of their specificity.^{26,27} A longitudinal investigation of ONH hemodynamics in NHP EG may help clarify answers to some of these questions. Interestingly, previous studies using a similar NHP EG model failed to arrive at any consensus about ONH BF changes,^{28,29} perhaps because they too had a cross-sectional design with mixed severity at the time ONH BF was assayed, in addition to their relatively small sample size²⁸ and technical limitations of tracer used in the study for BF measurement.^{29,30}

Therefore, the current study was designed to evaluate longitudinal, rather than causal, basal ONH BF changes using a noninvasive technique. The studies measured BF during the development and progression of NHP EG and compared this with longitudinal estimates of axon loss determined by *in vivo* measures of RNFLT. In addition, because the EG model is induced by IOP elevation, which excludes ischemia as a primary, preexisting effect, any hemodynamic changes observed in this model would relate either to the direct effect of IOP and/or to consequence of chronically elevated IOP, such as

neural loss manifest as RNFLT changes. We postulated a priori that the BF requirement of the ONH ultimately diminishes with neural tissue loss or damage in EG.

METHODS

Animals and Anesthesia

Fifteen adult rhesus monkeys (*Macaca mulatta*) were included in the study, 14 females and 1 male; their average age at the beginning of the study ($\pm$ SD) was 9.0 ± 2.6 years (range, 5–14). All procedures were performed with the animals under general anesthesia, adhered to the Association for Research in Vision and Ophthalmology's Statement for the Use of Animals in Ophthalmic and Vision Research, and were approved and monitored by the Institutional Animal Care and Use Committee (IACUC) at Legacy Research Institute. In all cases, anesthesia was induced with intramuscular ketamine (15 mg/kg) and xylazine (1.5 mg/kg), along with a single subcutaneous injection of atropine sulfate (0.05 mg/kg). Animals were intubated and breathed air plus 10% oxygen spontaneously. Heart rate, end tidal CO₂, and arterial oxygenation saturation were monitored continuously. Body temperature was maintained with a heating pad at 37°C. Pupils were fully dilated with 1.0% tropicamide (Alcon Laboratories, Inc., Fort Worth, TX). One of the superficial branches of a tibial artery was cannulated with a 27-gauge needle, which was connected to a pressure transducer (BLPR2; World Precision Instruments, Sarasota, FL) and a four-channel amplifier system (Lab-Trax-4/24T; World Precision Instruments) for continuous arterial blood pressure (BP) recording. Anesthesia was maintained by continuous administration of pentobarbital (8–12 mg/kg/h, IV) using an infusion pump (Aladdin; World Precision Instruments) for all procedures except during trabecular meshwork laser sessions.

IOP Measurement and RNFLT Imaging Protocol

IOP was measured at each session by rebound tonometry (Tonopen XL; Reichert, Inc., Depew, NY) in both eyes of each animal (mean of three measurements per eye) within 30 minutes of general anesthesia induction. After the initial IOP measurements, IOP was set in both eyes manometrically to 10 mm Hg. Two 27-gauge needles were inserted into the anterior chamber of each eye. One needle was connected to a custom-made manometer set at 10 mm Hg, the other needle was connected to a pressure transducer (BLPR2; World Precision Instruments) to record actual eye pressure, as previously described.³¹

Peripapillary RNFLT was measured in both eyes of each animal using a commercial spectral-domain optical coherence tomography (SDOCT) instrument (Spectralis; Heidelberg Engineering GmbH, Heidelberg, Germany). For this study, the average peripapillary RNFLT is reported from a single circular B-scan (12° diameter) consisting of 1536 A-scans. Nine to 16 individual sweeps were averaged in real time to comprise the final stored B-scan at each session. At the initial imaging session, the operator centered the position of the scan on the ONH and all subsequent scans were pinned (identical) to this location using the instrument's eye-tracking software. A trained technician manually corrected the accuracy of the instrument's native automated layer segmentations when the algorithm had obviously erred from the inner and outer borders of the RNFLT to an adjacent layer (such as a refractive element in the vitreous instead of the internal limiting membrane, or to the inner plexiform layer instead of the outer border of the retinal nerve fiber layer). SDOCT data, including segmentations, were then exported for extraction of RNFLT values by custom software.²⁷

Induction of Chronic Unilateral Experimental IOP Elevation

Laser treatment to one eye of each animal was performed under ketamine and xylazine anesthesia. One hundred eighty degrees of the trabecular meshwork (50- μ m spot size, 1.0-second duration, 600- to 750-mW power) were treated in each of two separate sessions at least 2 weeks apart. After each treatment, a sub-Tenon's injection of 0.5 mL dexamethasone (10 mg/mL) was given in the inferior fornix of the treated eye. Laser treatments were repeated (but limited to a 45° or 90° sector) on subsequent occasions as necessary to achieve sustained IOP elevation.

BF Measurement With Laser Speckle Flowgraphy

The principles of the laser speckle flowgraphy (LSFG) technique (Softcare, Iizuka, Japan) and its application to measure ONH BF in nonhuman primates have been described in detail within previous publications.^{31–33} In brief, a fundus camera equipped within the LSFG device was used to define an area centered on the ONH, with dimensions of approximately 3.8×3.0 mm (width $\times$ height). After switching on the laser (λ = 830 nm, maximum output power, 1.2 mW), a speckle pattern appears due to random interference of the scattered light from the illuminated tissue area, which is continuously imaged by a charge-coupled device (700 $\times$ 480 pixels) at a frequency of 30 frames per second for 4 seconds at a time.

Offline analysis software (LSFG Analysis; Softcare) computed mean blur rate (MBR) of the speckle images. MBR is a squared ratio of mean intensity to the SD of light intensity, which varies temporally and spatially according to the velocity of blood cell movement and correlates well with capillary BF within the ONH validated by the microsphere method³¹ and the hydrogen clearance method.³⁴ Thus, the MBR has been used as a BF index. A composite MBR map representing BF distribution within the ONH disc was generated from the images of each 4-second series. After eliminating the area within the images corresponding to large blood vessels, capillary BF within the remaining ONH disc area was averaged and recorded in arbitrary units (AU) of MBR.

Basal ONH BF could be measured either at the ambient IOP or under manometric IOP control. Measurement under ambient IOP in EG eyes can be complicated by potential failures of autoregulation³⁵ when ambient IOP is high. Moreover, IOP is inevitably and progressively reduced from its initial level at the start of each recording session due to IOP-lowering effects of anesthesia.^{36,37} Thus, the basal ONH BF was measured in this study under uniform conditions whereby IOP was manometrically set to 10 mm Hg in all eyes at all sessions. This IOP level is close to that measured under general anesthesia in normal NHP eyes (11.2 ± 3.1 mm Hg).³⁸ ONH BF was measured at least 5 minutes after the IOP was set at 10 mm Hg.

For each animal, three to five prelaser baseline sessions were included to establish baseline values of IOP, RNFLT, and ONH BF in each eye. Then chronic IOP elevation was initiated by laser treatment in one eye of each animal. The contralateral eye served as control. Thereafter, IOP, RNFLT, and ONH BF measurements were repeated once every 2 weeks for the duration of the postlaser follow-up (Table 1, column 5). Most animals (9 of 15) were followed until a relatively advanced stage when RNFLT in the EG eye was reduced by more than 35% of its prelaser baseline value (Table 1, rightmost column). However, 6 of the 15 animals were followed only during an earlier stage of EG to evaluate histological and molecular changes in early EG eyes (Table 1, bottom six rows). At the end of each experiment animals were killed humanely under deep anesthesia for tissue preservation and histopathological studies.

TABLE 1. Demographics, IOP Information, and Final RNFLT of Each Animal

ID	Age, y	Sex	Weight, kg	Postlaser Follow-up Duration, mo	Mean IOP		Peak IOP		Cumulative IOP Difference, mm Hg $\times$ d	Final RNFLT EG Eye, % Change From Baseline
					Control, mm Hg	EG, mm Hg	Control, mm Hg	EG, mm Hg		
135	12	M	11.4	12.5	11	14	19	40	1404	−36.5
139	10	F	6.2	11.7	13	21	19	53	3680	−41.2
140	9	F	6.1	8.0	9	11	16	36	498	−37.2
25,340	6	F	5.5	7.1	13	30	20	45	4292	−43.9
25,904	5	F	6.4	4.0	10	30	16	43	2338	−64.1
28,497	12	F	6.3	5.0	15	24	21	58	1082	−49.9
28,506	14	F	4.1	8.2	9	15	16	41	1930	−56.0
28,849	8	F	5.7	6.0	16	23	22	52	1574	−62.0
28,853	7	F	6.7	8.1	15	26	22	41	2401	−48.8
24,253	7	F	7.0	7.6	10	11	14	18	303	−16.9
28,517	11	F	7.5	10.2	13	23	21	48	2992	−7.6
29,584	7	F	4.1	9.3	9	15	16	41	1666	−12.9
28,675	8	F	6.6	7.8	14	20	19	49	1334	−4.5
23,583	8	F	5.9	3.6	15	19	23	26	244	+3.5
28,318	11	F	6.4	6.1	15	22	21	42	1070	−17.1
Average	9.0		6.4	7.7	12.3	20.2	18.9	42.2	1787	−33.0
SD	2.6		1.7	2.6	2.6	5.9	2.8	10.2	1186	22.0

Data Analysis and Statistics

All data were reported as average $\pm$ SD unless specified. A commercial software package (Prism 5; GraphPad Software, Inc., La Jolla, CA) was used to perform ordinary least squares linear regression, ANOVA, and one-sample *t*-tests to evaluate longitudinal changes in ONH BF over time. The statistical test applied to analyze each experimental result is specifically identified within the text of the results section.

RESULTS

Demographics and IOP

Table 1 lists the demographics of all experimental animals as well as the IOP measured during the postlaser follow-up period in both eyes and the stage of EG at termination (i.e., the final RNFLT measurement relative to the baseline average of each EG eye).

There was no difference at baseline between experimental and control eyes for mean IOP (14.1 ± 2.1 vs. 13.8 ± 2.3 , $P = 0.28$). Over the total duration of postlaser follow-up, the average IOP in EG eyes was 20.2 ± 5.9 mm Hg compared with 12.3 ± 2.6 mm Hg in control eyes ($P < 0.0001$, Table 1). Over this same period, peak IOP ranged from 18 to 58 in EG eyes and from 14 to 23 in control eyes (Table 1).

RNFLT

The extent of glaucomatous damage varied across the group of EG eyes as measured by RNFLT (Table 1). Two of the 15 animals were killed before significant loss of RNFLT was observed (28,675 and 23,583; note, significant loss is defined as 7% below baseline consistent with previously published analysis of measurement variability²⁷). The rate of progressive RNFLT loss also varied across animals, as shown in Figure 1. The rate of progressive RNFLT loss was only modestly associated with mean IOP ($R = 0.45$, $P = 0.20$) or peak IOP ($R = 0.53$, $P = 0.08$). In fellow control eyes, RNFLT was consistent across all follow-up time points after laser had been initiated in the EG eyes. The average ($\pm$ SD) of all follow-up values for relative RNFLT in control eyes was $-0.4\% \pm 2.3\%$

with a range from -6.2% to $+4.3\%$ relative to the baseline average for each control eye, consistent with our previously published range of 7% measurement noise for RNFLT.²⁷

Basal ONH BF

Longitudinal changes in basal ONH BF were closely associated with stage of EG as measured by RNFLT. Figure 2 shows a scatter plot of the change in basal ONH BF from baseline against the change in RNFLT from baseline for each eye at each longitudinal measurement session. For clarity, the nine animals that progressed to a relatively more advanced stage (35% or more loss of RNFLT) are shown in panel A and the other six animals are shown in panel B. Although the data for most animals in Figure 2 appear to share a similar relationship, formal statistical testing indicates that not all 15 EG eyes can be described by a single function ($F_{[28,84]} = 5.7$, $P < 0.0001$ and Akaike's Information Criterion difference = 41.7, $P < 0.0001$). Therefore, linear regression was applied to the data for each of the EG eyes independently (Table 2). The results listed in Table 2 demonstrate that longitudinal changes in basal ONH BF were strongly associated with changes in RNFLT as EG progressed from early through moderately advanced stages of damage, with Pearson correlation coefficients ranging from 0.64 to 0.97 (average = 0.81) and an average slope of 1.0. Importantly, the Y-intercept was positive in 8 of the 12 EG eyes, ranging from 2% below to 33% above (average = 10.4% above) the basal ONH BF at baseline. This result suggests that basal ONH BF is actually higher than baseline during the earliest stage of EG.

To further test whether basal ONH BF was associated with the stage of disease, the data were binned into EG severity stages according to relative loss of RNFLT as shown in Figure 3. There was a significant effect of EG severity stage on basal ONH BF ($R^2 = 0.70$, $F = 20.5$, $P < 0.0001$, ANOVA). There was no significant effect of severity stage in EG eyes on the relative basal ONH BF in control eyes measured at the corresponding time points ($R^2 = 0.14$, $F = 1.4$, $P = 0.27$, ANOVA). During the earliest EG stage when RNFLT loss was less than 10% from baseline values, basal ONH was significantly higher than baseline ($P = 0.004$, one-sample *t*-test). During the subsequent stage when RNFLT loss was between 10% and 20% below baseline, there was no significant difference in basal ONH BF as

FIGURE 1. The extent and rate of progressive loss of RNFLT varied across animals. For clarity, the group of 15 animals is split into $n = 9$ that were followed to moderate stage of damage (i.e., beyond 35% loss, [A]) and $n = 6$ that were killed at a relatively early stage of damage (B).

compared to baseline ($P = 0.74$). Basal ONH progressively declined through subsequent stages to reach levels of $8.7\% \pm 8.3\%$ below baseline ($P = 0.03$), $21.4\% \pm 11.8\%$ below baseline ($P = 0.003$), and $25.3\% \pm 9.3\%$ below baseline ($P < 0.0001$), respectively. Basal ONH BF in EG eyes was significantly elevated above fellow control eye levels during the earliest stage (RNFLT loss $< 10\%$; $P = 0.02$) and significantly reduced below the level in fellow control eyes during the latter stages (RNFLT loss 30%–40% and greater than 40%, $P = 0.03$ and $P = 0.002$, respectively), but was not significantly different from fellow control eye levels during the two intermediate stages (RNFLT loss 10%–20% and 20%–30%, $P = 0.38$ and $P = 0.74$, respectively).

BP at the Time of BF Measurement During Baseline and EG Stages

The average arterial BP at the time corresponding to baseline ONH BF measurement was 89.3 ± 4.6 mm Hg in control eyes and 89.6 ± 4.2 mm Hg in EG eyes ($P = 0.49$). During the progression of EG, BP showed no change over time (F -test, $P = 0.59$). Table 3 shows BP measured during baseline sessions and

across the five different stages of EG. The corresponding ocular perfusion pressure (OPP) can be estimated by subtracting 10 mm Hg for IOP and additional 5 mm Hg for the height difference between the eye and the femoral artery where BP was recorded. Note that the OPP levels during all stages were above the lower limit of autoregulation in the ONH of monkeys previously reported (Wang L, et al. *IOVS* 2012: ARVO E-Abstract 6842).

DISCUSSION

The results of this study show that basal ONH BF was strongly associated with the stage of EG severity as measured by loss of RNFLT and underwent a two-phase pattern of change: during the earliest stage, when RNFLT was within 10% of baseline, ONH BF exhibited mild increase, after which it progressively declined with increasing degree of EG severity. At the most severe stage evaluated in this study, when more than 40% of the RNFLT was lost, ONH BF was reduced by more than 25% below baseline. The decline of ONH BF was strongly correlated with RNFLT thinning over the range of EG studied.

In this longitudinal observation in EG, RNFLT, rather than IOP, has been used to define different stages of progression.

FIGURE 2. Longitudinal change in basal ONH BF (% from baseline) versus loss of RNFLT (% from baseline); for clarity, the group of 15 animals is split into $n = 9$ that were followed to a moderate stage of damage (A) and $n = 6$ that were killed at a relatively early stage (B). Basal ONH BF in EG eyes is greater than prelaser baseline during early-stage EG when RNFLT loss is minimal.

TABLE 2. Correlation Between Relative ONH BF and Relative RNFLT in EG Eyes

ID	Longitudinal Observations	Pearson R	Y-Intercept	Slope	P Value
135	8	0.86	17.6	1.20	0.0061
139	5	0.70	13.4	0.96	0.1893
140	6	0.92	-0.2	0.90	0.0088
25,340	9	0.88	16.6	0.74	0.0017
25,904	6	0.84	4.7	0.46	0.0372
28,497	7	0.88	19.8	1.02	0.0088
28,506	9	0.91	-0.9	0.41	0.0007
28,849	9	0.97	3.0	0.72	0.0001
28,853	12	0.77	10.1	0.58	0.0037
24,253	5	0.65	8.6	1.24	0.2379
28,517	18	0.64	-2.0	0.66	0.0039
29,584	11	0.67	33.4	3.07	0.0240
28,675	3	n.a.	n.a.	n.a.	n.a.
23,583	3	n.a.	n.a.	n.a.	n.a.
28,318	3	n.a.	n.a.	n.a.	n.a.

n.a., not applicable; these three EG eyes had only three postlaser observations each.

This is because the magnitude of IOP elevation varies substantially between animals and during the course of EG development that it does not correlate with optic nerve axon loss, the most reliable landmark to define the stages of EG. In contrast, RNFLT is a more definitive marker than IOP for the stage of progression in this model with a close correlation to orbital optic nerve axon loss.³⁹

The relationship between chronically increased IOP and ONH BF has been investigated in a similar EG model in cynomolgus monkeys.²⁹ In that study, which had a cross-sectional design, the EG eyes had a slightly higher average level of IOP elevation (34 ± 8 mm Hg) and a much longer duration (more than 8 months) than in our study; however, no significant ONH BF changes were demonstrated even at very late stages of EG. Possible reasons for this discrepancy may include the limited time window observed by a single “snapshot” and/or the lower sensitivity of the iodoantipyrine tracer method used. Caprioli and Miller³⁰ demonstrated that iodoantipyrine tracer measurements of ONH BF were largely influenced by tracer diffusion from the nearby choroid, which has high BF. In the current study, a significant two-phase pattern of ONH BF change was demonstrated following chronic IOP elevation and the BF changes were also closely correlated with RNFLT loss. Although the initial, “early-stage” increase of ONH BF was not strongly correlated with IOP summary parameters, it was specific to EG eyes (i.e., did not occur in fellow control eyes), which suggests this initial increase of ONH BF is most likely a direct effect of chronic IOP elevation in the EG eye. Thereafter, the continuous decrease of ONH BF in correlation with RNFLT loss suggests that diminished metabolic demand in ONH due to progressive axon loss is likely the cause of reduced ONH BF. The significance of these changes and their potential mechanisms are discussed further in the following sections.

The observation of increased BF during early-stage EG is interesting. However, potential confounding factors that might result in a similar apparent increase need to be ruled out before further consideration of its importance. Basal ONH BF in this study was always measured at a fixed IOP of 10 mm Hg by inserting a needle into the anterior chamber for manometric IOP control. According to previous studies, this procedure may result in a temporary hyperperfusion because of sudden increase of perfusion pressure associated with decreasing IOP from a level that was otherwise chronically

FIGURE 3. Basal ONH BF is plotted as % change from baseline (average $\pm$ SEM) and binned into stages of EG severity according to loss of RNFLT (EG eyes, filled squares). Control eyes were binned according to corresponding EG eye level of RNFLT loss (open circles). Not all 15 EG eyes contributed observations to each bin; if two or more observations were available for a given eye and bin, they were averaged; thus, the sample sizes for each bin were as follows: less than 10% loss, $n = 12$; 10% to 20% loss, $n = 8$; 20% to 30% loss, $n = 6$; 30% to 40% loss, $n = 6$; more than 40% loss, $n = 8$.

elevated.^{40,41} It is possible that the magnitude of this transient hyperperfusion was higher in EG eyes than in control eyes because the IOP in EG eyes was generally higher than normal and/or because BF autoregulation might be abnormal in EG eyes. To rule out such a possibility, we compared ONH BF measured before the needle insertion with that measured 5 minutes after the needle insertion in the same eyes. In total, 226 pairs of such measurements from 24 eyes (12 EG eyes and 12 control eyes) were available and show that ONH BF in both the EG and control eyes was increased after manometric IOP lowering to 10 mm Hg. However, the magnitude of transient ONH hyperperfusion was not significantly different between EG and control eyes, nor was there any significant difference in the experimental eyes at baseline versus after induction of EG. Thus, it is unlikely that the ONH hyperperfusion observed during early-stage EG was due to a higher transient hyperemic response following manometric IOP lowering in EG eyes as compared with control eyes. It is rather more likely to reflect a chronic difference in basal ONH BF during this early EG stage.

The mechanisms underlying ONH BF increase during early EG are not clear. One possible reason is that the initial IOP elevation might cause a functional disturbance of ONH BF autoregulation by affecting either vascular smooth muscles directly or via a signal released from cells that modulate the BF autoregulatory system, such as perivascular astrocytes.⁴² Speculatively, these changes might have reset both the basal vascular tone that determines the basic BF level (to be higher) and/or reduced the sensitivity of BF autoregulatory mechanisms to respond to altered perfusion pressure. Alternatively, regional metabolism might be increased during early-stage EG due to increased energy demands of ONH gliotic changes or lamina cribrosa remodeling.⁴³⁻⁴⁵ In contrast, during the later stages of EG, basal ONH BF was reduced, which may reflect reduced metabolic demand associated with neuronal loss and connective tissue changes and/or a possible disruption of the BF autoregulatory system. Thus, there exists a temporal

TABLE 3. Average BP Measured During Baseline and Across Different Stages of EG (RNFLT Loss)

	Baseline	<10% Loss	10%–20% Loss	20%–30% Loss	30%–40% Loss	>40% Loss
Control	89.3 ± 4.7	92.1 ± 3.6	89.6 ± 6.7	89.6 ± 6.7	89.2 ± 6.3	90.3 ± 6.0
Glaucoma	89.6 ± 4.2	92.2 ± 5.0	90.9 ± 6.8	90.9 ± 6.8	88.2 ± 6.4	88.9 ± 6.7
P, t-test	0.49	0.35	0.35	0.77	0.18	0.94

transition from increased ONH BF to a decrease in EG eyes: during the early stage, mild increase was initially observed within the anterior ONH; subsequently ONH BF declined gradually below the normal level in association with the progression of structural damage measured by thinning of the peripapillary RNFL. In a parallel study in which a microsphere technique was used to measure ONH BF at various depths through the ONH and anterior orbital ON, we noted that some eyes with early EG exhibited decreased BF in the anterior ONH but an increased BF in the posterior ONH, relative to their contralateral control eyes.³¹

Our observation of a high correlation between ONH BF and RNFLT during the progression of EG agrees with a number of clinical cross-sectional studies, which have shown similar correlation between ONH BF and RNFLT,⁴ or between ONH BF and visual field deficit.^{17–21} In a separate study on NHPs with idiopathic bilateral optic atrophy, a disease in monkeys that causes nonglaucomatous optic nerve degeneration without apparent IOP elevation, also found that decreased ONH BF was highly correlated with RNFLT.³⁵ Together, the results from this and previous studies suggest that the compromised ONH BF in human glaucoma is at least in part secondary to structural damage and reduced neuronal activities. This compromised ONH BF, in turn, may increase the vulnerability of the ONH to additional neural and connective tissue damage at any level of IOP. Although the evidence is strongly suggestive based on the two-phase pattern of ONH BF results observed here, this study's limitation requires an important caveat to be considered, which is that it is unable to establish a direct causative relationship per se; moreover, the two factors may interact with each other in a complicated manner.

Our study has the following limitations. First, the NHP EG model may differ from human glaucoma in that the model insult is presumed to exclude any preexisting ONH ischemia. Second, this study does not take into account potential ONH BF autoregulation deficiencies that might have developed during the pathological processes of EG. Our ongoing studies are designed to address this interesting question and details regarding static and dynamic autoregulation changes in the ONH will be reported separately in a series of subsequent reports. Third, although the relative ONH BF changes measured by the LSFG device have been recently validated using microsphere techniques, the scale for absolute BF values measured by LSFG (in AU) appears to be compressed.³¹ This is likely a result of an error in the LSFG MBR calibration, which was derived from in vitro studies.³³ As such, the percentage BF change in EG eyes observed in this study (e.g., Fig. 3) is likely an underestimate of the actual BF deficit. In addition, the penetration depth of laser into the ONH has not been well defined.³¹ Fourth, as previously mentioned, this EG model is based on chronic IOP elevation as an initial insult, so the role of ischemia as a primary risk factor independent of IOP remains unclear, although a detrimental effect of reduced ONH BF is expected for the ONH.^{46,47} Finally, because the magnitude and duration of IOP elevation and rate of progression of RNFLT loss may differ from clinical glaucoma, the ONH BF changes may not share the same pathophysiological processes. Thus, caution should be taken before extrapolating these results to human patients, which deserves further investigation.

In summary, this longitudinal study demonstrates that basal ONH BF exhibited a two-phase change from early-stage mild BF increase to eventual significant decrease, with the latter phase showing a high correlation to the extent of RNFL loss. These findings provide strong experimental evidence suggesting that compromised ONH BF observed in glaucomatous patients with high IOP (or without) is at least in part the result of neural loss (and presumed reduced metabolic demand).

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References

- Jia YL, Morrison JC, Tokayer J, et al. Quantitative OCT angiography of optic nerve head blood flow. *Biomedical Optics Express*. 2012;3:3127–3137.
- Yokoyama Y, Aizawa N, Chiba N, et al. Significant correlations between optic nerve head microcirculation and visual field defects and nerve fiber layer loss in glaucoma patients with myopic glaucomatous disk. *Clin Ophthalmol*. 2011;5:1721–1727.
- Sugiyama T, Shibata M, Kojima S, Ikeda T. Optic nerve head blood flow in glaucoma. In: Kubena T, ed. *Mystery of Glaucoma*. New York: InTech; 2011:207–218.
- Chiba N, Omodaka K, Yokoyama Y, et al. Association between optic nerve blood flow and objective examinations in glaucoma patients with generalized enlargement disc type. *Clin Ophthalmol*. 2011;5:1549–1556.
- Sato EA, Ohtake Y, Shinoda K, Mashima Y, Kimura I. Decreased blood flow at neuroretinal rim of optic nerve head corresponds with visual field deficit in eyes with normal tension glaucoma. *Graefes Arch Clin Exp Ophthalmol*. 2005;244:1–7.
- 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.
- 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.
- Findl O, Rainer G, Dallinger S, et al. Assessment of optic disk blood flow in patients with open-angle glaucoma. *Am J Ophthalmol*. 2000;130:589–596.
- Kerr J, Nelson P, O'Brien C. A comparison of ocular blood flow in untreated primary open-angle glaucoma and ocular hypertension. *Am J Ophthalmol*. 1998;126:42–51.
- Michelson G, Schmauss B, Langhans MJ, Harazny J, Groh MJ. Principle, validity, and reliability of scanning laser Doppler flowmetry. *J Glaucoma*. 1996;5:99–105.

11. Michelson G, Langhans MJ, Groh MJ. Perfusion of the juxtapapillary retina and the neuroretinal rim area in primary open angle glaucoma. *J Glaucoma*. 1996;5:91-98.
12. Hamard P, Hamard H, Dufaux J, Quesnot S. Optic nerve head blood flow using a laser Doppler velocimeter and haemorrheology in primary open angle glaucoma and normal pressure glaucoma. *Br J Ophthalmol*. 1994;78:449-453.
13. Maumenee AE. Causes of optic nerve damage in glaucoma. Robert N. Shaffer lecture. *Ophthalmology*. 1983;90:741-752.
14. Flammer J, Orgul S, Costa VP, et al. The impact of ocular blood flow in glaucoma. *Prog Retin Eye Res*. 2002;21:359-393.
15. Hayreh SS. The 1994 Von Sallman Lecture. The optic nerve head circulation in health and disease. *Exp Eye Res*. 1995;61:259-272.
16. Anderson DR. Introductory comments on blood flow autoregulation in the optic nerve head and vascular risk factors in glaucoma. *Surv Ophthalmol*. 1999;43:S5-S9.
17. Zink JM, Grunwald JE, Piltz-Seymour J, Staii A, Dupont J. Association between lower optic nerve laser Doppler blood volume measurements and glaucomatous visual field progression. *Br J Ophthalmol*. 2003;87:1487-1491.
18. Yaoeda K, Shirakashi M, Fukushima A, et al. Relationship between optic nerve head microcirculation and visual field loss in glaucoma. *Acta Ophthalmol Scand*. 2003;81:253-259.
19. Ciancaglini M, Carpineto P, Costagliola C, Matropasqua L. Perfusion of the optic nerve head and visual field damage in glaucomatous patients. *Graefes Arch Clin Exp Ophthalmol*. 2001;239:549-555.
20. Hwang JC, Konduru R, Zhang X, et al. Relationship among visual field, blood flow, and neural structure measurements in glaucoma. *Invest Ophthalmol Vis Sci*. 2012;53:3020-3026.
21. Resch H, Schmidl D, Hommer A, et al. Correlation of optic disc morphology and ocular perfusion parameters in patients with primary open angle glaucoma. *Acta Ophthalmol*. 2011;89:544-549.
22. Januleviciene I, Ehrlich R, Siesky B, Nedzelskiene I, Harris A. Visual function, optic nerve structure, and ocular blood flow parameters after 1 year of glaucoma treatment with fixed combinations. *Eur J Ophthalmol*. 2009;19:790-797.
23. Schwartz B. Circulatory defects of the optic disk and retina in ocular hypertension and high pressure open-angle glaucoma. *Surv Ophthalmol*. 1994;38:S23-S34.
24. Shoshani Y, Harris A, Shoja MM, et al. Impaired ocular blood flow regulation in patients with open-angle glaucoma and diabetes. *Clin Exp Ophthalmol*. 2012;40:697-705.
25. Hollo G, van den Berg TJ, Greve EL. Scanning laser Doppler flowmetry in glaucoma. *Int Ophthalmol*. 1996;20:63-70.
26. Burgoyne CF, Downs JC, Bellezza AJ, Suh JK, Hart RT. The optic nerve head as a biomechanical structure: a new paradigm for understanding the role of IOP-related stress and strain in the pathophysiology of glaucomatous optic nerve head damage. *Prog Retin Eye Res*. 2005;24:39-73.
27. Fortune B, Burgoyne CF, Cull GA, Reynaud J, Wang L. Structural and functional abnormalities of retinal ganglion cells measured in vivo at the onset of optic nerve head surface change in experimental glaucoma. *Invest Ophthalmol Vis Sci*. 2012;53:3939-3950.
28. Alm A, Lambrou GN, Maepea O, Nilsson SF, Percicot C. Ocular blood flow in experimental glaucoma: a study in cynomolgus monkeys. *Ophthalmologica*. 1997;211:178-182.
29. Quigley HA, Hohman RM, Sanchez R, Addicks EM. Optic nerve head blood flow in chronic experimental glaucoma. *Arch Ophthalmol*. 1985;103:956-962.
30. Caprioli J, Miller JM. Measurement of optic nerve blood flow with iodoantipyrine: limitations caused by diffusion from the choroid. *Exp Eye Res*. 1988;47:641-652.
31. Wang L, Cull GA, Piper C, Burgoyne CF, Fortune B. Anterior and posterior optic nerve head blood flow in nonhuman primate experimental glaucoma model measured by laser speckle imaging technique and microsphere method. *Invest Ophthalmol Vis Sci*. 2012;53:8303-8309.
32. Fujii H, Nohira K, Yamamoto Y, Ikawa H, Hjura T. Evaluation of blood flow by laser speckle image sensing, Part 1. *Appl Opt*. 1987;26:5321-5325.
33. Sugiyama T, Araie M, Riva CE, Schmetterer L, Orgul S. Use of laser speckle flowgraphy in ocular blood flow research. *Acta Ophthalmol*. 2010;88:723-729.
34. Takahashi H, Sugiyama T, Tokushige H, et al. Comparison of CCD-equipped laser speckle flowgraphy with hydrogen gas clearance method in the measurement of optic nerve head microcirculation in rabbits. *Exp Eye Res*. 2013;108:10-15.
35. Piper C, Fortune B, Cull G, Cioffi GA, Wang L. Basal blood flow and autoregulation changes in the optic nerve of rhesus monkeys with idiopathic bilateral optic atrophy. *Invest Ophthalmol Vis Sci*. 2013;54:714-721.
36. Jia L, Cepurna WO, Johnson EC, Morrison JC. Effect of general anesthetics on IOP in rats with experimental aqueous outflow obstruction. *Invest Ophthalmol Vis Sci*. 2000;41:3415-3419.
37. Kim J, Sapp HL Jr, Plummer CE, Brooks DE, Kim D, Kim MS. IOP change undergoing anesthesia in rhesus macaques (*Macaca mulatta*) with laser-induced ocular hypertension. *J Vet Med Sci*. 2012;74:1359-1361.
38. Gardiner SK, Fortune B, Wang L, Downs JC, Burgoyne CF. Intraocular pressure magnitude and variability as predictors of rates of structural change in non-human primate experimental glaucoma. *Exp Eye Res*. 2012;103C:1-8.
39. Cull GA, Reynaud J, Wang L, Cioffi GA, Burgoyne CF, Fortune B. Relationship between orbital optic nerve axon counts and retinal nerve fiber layer thickness measured by spectral domain optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2012;53:7766-7773.
40. Riva CE, Harino S, Petrig BL, Shonat RD. Laser Doppler flowmetry in the optic nerve. *Exp Eye Res*. 1992;55:499-506.
41. Liang Y, Downs JC, Fortune B, Cull GA, Cioffi GA, Wang L. Impact of systemic blood pressure on the relationship between intraocular pressure and blood flow in the optic nerve head of non-human primates. *Invest Ophthalmol Vis Sci*. 2009;50:2164-2160.
42. Attwell D, Buchan AM, Charkpak S, Lauritzen M, Macvicar BA, Newman EA. Glial and neuronal control of brain blood flow. *Nature*. 2010;468:232-243.
43. Yang H, Downs JC, Girkin C, et al. 3-D histomorphometry of the normal and early glaucomatous monkey optic nerve head: lamina cribrosa and peripapillary scleral position and thickness. *Invest Ophthalmol Vis Sci*. 2007;48:4597-4607.
44. Yang H, Thompson H, Roberts MD, Sigal IA, Downs JC, Burgoyne CF. Deformation of the early glaucomatous monkey optic nerve head connective tissue after acute IOP elevation in 3-D histomorphometric reconstructions. *Invest Ophthalmol Vis Sci*. 2011;52:345-363.
45. Roberts MD, Grau V, Grimm J, et al. Remodeling of the connective tissue microarchitecture of the lamina cribrosa in early experimental glaucoma. *Invest Ophthalmol Vis Sci*. 2009;50:681-690.
46. Chauhan BC, LeVatte TL, Jollimore CA, et al. Model of endothelin-1-induced chronic optic neuropathy in rat. *Invest Ophthalmol Vis Sci*. 2004;45:144-152.
47. Cioffi GA. Ischemic model of optic nerve injury. *Trans Am Ophthalmol Soc*. 2005;103:592-613.