Compromised Optic Nerve Blood Flow and Autoregulation Secondary to Neural Degeneration

Grant Cull,¹ Reinhard Told,² Claude F. Burgoyne,¹ Simon Thompson,¹ Brad Fortune,¹ and Lin Wang¹

¹Devers Eye Institute, Legacy Research Institute, Portland, Oregon, United States

²Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Vienna, Austria

Correspondence: Lin Wang, Devers Eye Institute, Legacy Health, 1225 NE 2nd Avenue, Portland, OR 97232, USA; lwang@deverseye.org.

Submitted: August 4, 2015
Accepted: October 12, 2015

Citation: Cull G, Told R, Burgoyne CF, Thompson S, Fortune B, Wang L. Compromised optic nerve blood flow and autoregulation secondary to neural degeneration. *Invest Ophthalmol Vis Sci*. 2015;56:7286–7292. DOI:10.1167/iovs.15-17879

PURPOSE. To test the hypothesis that optic nerve head (ONH) blood flow (BF) and autoregulation compromise are consequences of optic nerve degeneration induced by surgical optic nerve transection (ONT).

METHODS. In both eyes of five nonhuman primates, peripapillary retinal nerve fiber layer thickness (RNFLT) was measured by spectral-domain optical coherence tomography. Optic nerve head BF and dynamic autoregulation responses to a rapid manometric IOP increase (from 10–40 mm Hg) were measured by Laser Speckle Flowgraphy. The measurements were conducted every 10 to 15 days before and after unilateral ONT. Post-ONT measurements were repeated until RNFLT in the ONT eye was reduced by more than 40% of baseline value.

RESULTS. After ONT, RNFLT, and ONH BF progressively declined over time ($P < 0.0001$ and $P = 0.02$, respectively). Longitudinal changes between the two were highly correlated ($P < 0.0001$). When data was grouped by test session, the first significant decreases for RNFLT and BF were found 13 ± 0.8 and 24 ± 3.2 days post ONT, respectively ($P < 0.05$, both). At the final time point (55 ± 0.5 days post ONT), RNFLT, and BF were reduced by $44\% \pm 2.0\%$ and $38 \pm 5.0\%$ from baseline, respectively. Dynamic autoregulation analysis showed marginal increased response time in post-ONT eyes ($P = 0.05$). Control eyes showed no longitudinal changes for any parameter.

CONCLUSIONS. The close association between RNFLT loss and ONH BF decrease following optic nerve degeneration demonstrated a clear cause and effect relationship. Increased BF response time appears to be a sign of dynamic autoregulation dysfunction in this ONT model.

Keywords: optic nerve head, blood flow, neural degeneration

While there is a general agreement that compromised blood flow (BF) is one of the key factors in the pathophysiology of glaucoma, it remains unclear whether it is causative or secondary to neural degeneration. Debate about this “chicken or egg first” question has persisted for decades without direct, unequivocal evidence to support one or the other conclusion. A common approach in clinical studies to address this question is to assess the correlation between BF and measures of glaucomatous structural or functional damage. However, the results derived from such studies are constrained by cross-sectional design and often inconclusive. Thus, the importance of compromised BF as a potential pathogenic factor in glaucoma and as a target for diagnostics and clinical management remains unsettled.

In our recent studies designed to address the causality question, we longitudinally measured optic nerve head (ONH) BF¹ and ONH BF autoregulation^{2,3} in a nonhuman primate (NHP) model of experimental glaucoma (EG) based on chronic unilateral elevation of IOP. The ONH BF results of these studies demonstrated that ONH BF increased during the earliest stages of glaucomatous optic neuropathy, and then progressively declined as the neuropathy progressed. Because ONH BF was completely normal before induction of IOP elevation, these findings supported the conclusions that the initial BF increase may have been a manifestation of a

primary IOP effect on the ONH, and the later phase of progressive ONH BF decrease was a manifestation of reduced metabolic demand related to glaucomatous neurodegeneration. The ONH BF autoregulation analysis used in these studies demonstrated a delayed and attenuated dynamic BF response to a rapid manometric IOP elevation challenge early after the onset of chronic IOP elevation. These findings separately suggested that altered dynamic BF autoregulation contributed to (and was perhaps responsible for) the early and transient ONH BF increase in early-stage NHP EG.

The purpose of the current study was to test a hypothesis that in a model of ONH neurodegeneration induced by ONT that does not include chronic IOP elevation, there would be no early phase of ONH BF increase, only progressive ONH BF decrease. The second purpose of this study was to evaluate longitudinal changes in parameters of ONH BF dynamic autoregulation in the same model so as to test the hypothesis that there would be no early disruption of the ONH BF response to a rapid manometric IOP elevation challenge. As such, ONH BF and ONH BF autoregulation were monitored longitudinally and compared before and after unilateral surgical transection of the orbital optic nerve in five rhesus macaque.

METHODS

Animals and Anesthesia

Five male adult rhesus monkeys (*Macaca mulatta*) were the subjects of this study. Their average age ($\pm$ SD) was 6.4 ± 1.2 years. In all cases, anesthesia was induced with intramuscular ketamine (15 mg/kg; Henry Schein Animal Health, Dublin, OH, USA) and xylazine (1.5 mg/kg; Akorn, Inc., Decatur, IL, USA), along with a single subcutaneous injection of atropine sulfate (0.05 mg/kg; Butler Schein Animal Health, Dublin, OH, USA). The animals were intubated and breathed air plus 10% oxygen spontaneously. Heart rate, end tidal CO_2 , and arterial oxygenation saturation were monitored continuously. Body temperature was maintained at 37°C using a warming blanket. For the ONH BF and retinal nerve fiber layer thickness (RNFLT) imaging procedures, pupils were fully dilated with 1.0% tropicamide (Alcon Laboratories, Inc., Fort Worth, TX, USA). One of the superficial branches of a tibial artery was cannulated with a 27-G needle, which was connected to a pressure transducer (BLPR2; World Precision Instruments, Sarasota, FL, USA) and a four-channel amplifier system (Lab-Trax-4/24T; World Precision Instruments) for continuous arterial blood pressure (BP) recording throughout each entire experiment. Anesthesia was maintained by continuous administration of pentobarbital (8–12 mg/kg/h, intravenous) using an infusion pump (Aladdin; World Science Instruments, Inc., Sarasota, FL, USA). During the ONT surgical procedure, anesthesia was maintained by 1.5% to 3% isoflurane in oxygen. All procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and were approved and monitored by the Institutional Animal Care and Use Committee at Legacy Research Institute (Portland, OR, USA).

Experimental Design

For each animal, five pre-ONT imaging sessions were included to establish baseline values of RNFLT and ONH hemodynamic parameters in each eye. Then in one eye of each animal, ONT was performed. The contralateral eye served as control. Thereafter, the same measurements were repeated in both eyes approximately once every 10 days during post-ONT follow-ups until RNFLT in the ONT eye was reduced by more than 40% of its baseline value. Fundus photographs were taken during the first baseline imaging session and the last imaging session post ONT.

Induction of Optic Nerve Degeneration by Surgical Transection

Under general anesthesia (isoflurane, see above), a lateral orbitotomy was performed followed by a dissection of the lateral rectus muscle to expose the orbital optic nerve. Using limbal 6-0 vicryl stay sutures the eye was rotated so that the central retinal artery entrance into the sheath could be identified, the sheath above the entrance was cut open, and the nerve was severed behind the central retinal artery approximately 6 to 8 mm behind the globe. While a full transection was attempted in each eye, where visibility made complete transection uncertain, partial transections of at least two-thirds of the optic nerve diameter were accepted. Without closing the optic nerve sheath, the lateral rectus muscle and skin incisions were closed with sutures. Digital video fundus fluorescein angiography was performed 7 to 10 days after the procedure to verify the patency of central retinal vasculature in all eyes.^{4,5}

RNFLT Measurements

At baseline and during the progression of structural damage after ONT, peripapillary RNFLT was monitored longitudinally in both eyes of each animal by spectral-domain optical coherence tomography (SDOCT; Spectralis, Heidelberg Engineering GmbH, Heidelberg, Germany). For each time point and each eye, a single circular B-scan (12° -diameter) was recorded. Nine to 16 individual sweeps were averaged to comprise the final B-scan stored for each session. The automated layer segmentations generated by the instrument were manually corrected when the algorithm had obviously erred during delineation of the RNFL inner and outer borders. Spectral-domain OCT data were exported for extraction of RNFLT values.

IOP Measurement and Control

Intraocular pressure was measured at each imaging session by rebound tonometry (Tonopen XL; Reichert, Inc., Depew, NY, USA) in both eyes of each animal (a mean of 3 measurements per eye) within 30 minutes of general anesthesia induction. After the initial measurements, two 27-G needles were inserted into the anterior chamber of each eye. One needle was connected to a manometer set at 10 mm Hg, the other needle was connected to a pressure transducer to record actual eye pressure, as previously described.¹

ONH Blood Flow Measurement With Laser Speckle Flowgraphy (LSFG)

The LSFG technique was used to measure BF in the NHP ONH as has been described in detail in previous studies.^{6–8} In brief, a fundus camera equipped within the LSFG device was focused on a 3.8×3 -mm (width $\times$ height) area centered on the ONH. After the laser is switched on ($\lambda = 830$ nm), a speckle pattern is generated due to random interference of scattered light from the illuminated tissue area. The speckle pattern is continuously imaged by a charge coupled device (700×480 pixels) at a frequency of 30 frames per second.

Offline analysis software (LSFG Analysis; Software, Iizuka, Japan) computed mean blur rate (MBR) of the speckle images. Mean blur rate is a squared ratio of mean intensity to the SD of light intensity of the speckle image, which varies temporally and spatially according to the velocity of blood cell movement and correlates well with capillary BF within the ONH as validated by the microsphere method⁸ and by the hydrogen clearance method.^{9,10} Thus, a composite MBR map of ONH disc represents the BF distribution within the tissue. After eliminating the area corresponding to all large blood vessels within all images (accounting for $\sim 50\%$ of total disc area) by using an internal function of the software, MBR within the entire remaining ONH disc area was averaged as described previously.^{11,12} Based on our previous study, this LSFG device measures BF through approximately 1 mm of depth from the surface of ONH, which includes the lamina cribrosa in healthy eyes and the retrolaminar optic nerve tissue in EG eyes in which there has been significant thinning of prelaminar optic disc tissue.¹¹

For each measurement of ONH BF, the IOP in the test eye was manometrically set at 10 mm Hg and left to equilibrate for at least 5 minutes. With all vital signs (BP, oxygen saturation, heart rate, and end tidal CO_2) stabilized, BF (MBR) was measured (4 seconds for each) per eye with the LSFG.

IOP-Evoked ONH Dynamic Autoregulation Response

This test was to quantify the ONH BF autoregulation capacity.² During the test, both IOP and BP were registered

continuously. While the mean BP was stabilized between 80 and 105 mm Hg, a rapid manometer pressure change from 10 to 40 mm Hg was completed by switching a solenoid valve (Valcor Engineering, Springfield, NJ, USA) with a controller. A corresponding IOP increase evoked a BF response in the ONH. The BF measurement started 10 seconds before the onset of the IOP step increase and lasted for 60 seconds. The IOP increase was repeated three times. The first increase lasted for 5 minutes, the subsequent increases lasted for at least 70 seconds. At the end of each increase, IOP was returned to 10 mm Hg for at least 3 minutes before the next increase. The BP, IOP, and dynamic autoregulation recordings were exported for offline extraction of dynamic autoregulation parameters as described below.

Analysis of Dynamic Autoregulation

A modified time-domain dynamic autoregulation analysis was performed based on a method previously established in our lab using a custom program (Microsoft Visual Basic VBA 7.0; Microsoft Corporation, Redmond, WA, USA).¹² In brief, the cardiac pulsatile fluctuation during each 1-minute BF recording was low-pass filtered. From each smoothed response, the following parameters were extracted: BF_{BL} = baseline BF, the average BF during the 10 seconds before the rapid IOP elevation from 10 to 40 mm Hg; $BF_{\Delta MAX}$ = the maximum BF change after the IOP elevation as a percentage of BF_{BL} (%); T = BF descending time from the onset of IOP elevation to $BF_{\Delta MAX}$; K = the BF descending slope during a period from the onset of IOP elevation to $BF_{\Delta MAX}$ derived from a linear regression fitting. Because the BF descent often occurs in two distinct phases: an initial fast phase followed by a slower phase, segmental linear regression was performed to further characterize these limbs objectively. If a two-segment model provided a statistically superior fit than a single linear model (evaluated by Akaike's Information Criteria), then the second slope determined by the segmental linear regression model was chosen for analysis, otherwise, the slope derived from single segment linear regression was used.

Retrobulbar Axon Counts

Animals were euthanized under deep anesthesia (Euthasol, IV; Diamond Animal Health, Inc., Des Moines, IA, USA) and tissues were preserved by perfusion fixation with 4% paraformaldehyde. A 2- to 3-mm sample of each optic nerve, beginning 2 mm posterior to the globe, was cut with a vibratome into 0.5-mm-thick transverse sections. Each section was postfixed in 4% osmium tetroxide and embedded in epoxy resin. Optic nerve cross-sections (1- μ m thick) then were cut and stained with p-phenylenediamine for axon counting. Axon counts for 100% of the optic nerve cross-sectional area were obtained by methods described in detail previously.¹³

Data Analysis

Longitudinal change for RNFLT measurements and each BF parameter were analyzed using a General Linear Model (GLM; Statistica V12; StatSoft, Inc., Tulsa, OK, USA) to account for partial correlations between the two eyes of each animal. All raw values recorded during baseline and all post-ONT time points were included in the longitudinal analysis. To examine the strength of the relationship between BF change and RNFLT loss, Pearson correlation coefficients were determined using post-ONT data normalized to the baseline average value for each eye. Values of

RNFLT and ONH BF at each post-ONT test session were compared against the corresponding baseline values using Bonferroni's multiple comparison test.

RESULTS

Fundus Appearance, RNFLT, and Axon Count in the Optic Nerve of ONT Eyes

Fifty-five days (± 0.5) after ONT surgery, all five animals developed optic disc pallor and marked thinning of RNFL in the ONT eye compared with their contralateral control eye by the final follow-up. The temporal optic disc appeared to become more pallor than the nasal in all five animals. Figure 1 illustrates the optic disc appearance and SDOCT peripapillary RNFL imaging acquired from one of the baselines and the last imaging session in one representative monkey (ID: 28107).

The average baseline RNFLT in pre-ONT eyes and contralateral control eyes was 109.2 ± 6.82 and 110.8 ± 6.75 μ m, respectively ($P = 0.08$, Wilcoxon rank sum test). After ONT, RNFLT in the ONT eyes progressively declined over time (Fig. 2, GLM, $F = 92$, $P < 0.0001$). The control eyes showed no change during the same follow-up period ($F = 0.45$, $P = 0.93$); the effect of time was significantly different between ONT and control eyes ($F = 317$, $P < 0.0001$). At the final test session, the average change of RNFLT was $-45\% \pm 4.0\%$ from the baseline in the ONT eyes and $0.1\% \pm 2.0\%$ in the control eyes. When post-ONT data were grouped by follow-up test session (time point), the first significant decrease from baseline for RNFLT was found at the second post-ONT time point (13 ± 0.82 days after ONT surgery, Bonferroni's multiple comparison test, $P < 0.05$). RNFLT was also reduced in ONT eyes at all subsequent post-ONT test sessions ($P < 0.05$).

The percent total axon count reduction in the ONT eye (compared with its contralateral control eye) ranged from 47% to 72% in the 5 ONT eyes ($57 \pm 12\%$, mean $\pm$ SD).

ONH BF

There were no significant differences between the post-ONT and baseline (pre-ONT) values of average BP or for the average IOP (before anterior chamber cannulation for manometric IOP control; Table 1).

Average BF during the baseline was 13.7 ± 1.8 for pre-ONT eyes and 14.0 ± 2.1 for contralateral control eyes ($P = 0.625$, Wilcoxon test). After ONT, BF in the ONT eyes progressively declined ($F = 3.99$, $P = 0.02$), while the control eyes had no change ($F = 0.80$, $P = 0.67$). There was a significant difference between eyes over time ($F = 82.8$, $P < 0.001$, Fig. 3). At the final test session, the ONH BF was $-38 \pm 5.0\%$ and $-3.2 \pm 5\%$ of the baseline average in the ONT and control eyes, respectively.

Compared with baseline values, the first significant BF reduction occurred at the third post-ONT test session (24 ± 3.2 days post ONT) and at all test sessions thereafter ($P < 0.05$, Bonferroni's multiple comparisons test).

One of the hypotheses in the study was that with progressive optic nerve degeneration after ONT, ONH BF would decline in proportion to reduced metabolic demand as a result of axon loss. To test this hypothesis, we examined the strength of the relationship between longitudinal change in ONH BF and longitudinal change in RNFLT (a surrogate measure of optic nerve axon loss available in vivo).⁸ Figure 4 shows that the decline in ONH BF after ONT was strongly correlated with the loss of RNFLT (Pearson's $R = 0.89$, $P < 0.0001$).

FIGURE 1. Color photographs of the optic disc (left panels) and SDOCT imaging of the peripapillary RNFL (right panels) in one representative NHP; the top pair of rows shows the ONT eye at one baseline time point (pre-ONT) and at the final follow-up time point (56 days after ONT, with 72% axonal loss); the bottom pair of rows shows the contralateral control eye at the same two time points. In the ONT eye, the optic disc developed overall pallor, which was most prominent in the temporal sector. There was overall thinning of the RNFL in the ONT eye, which was similarly most prominent in the temporal sector.

ONH BF Dynamic Autoregulation

Generally during all pre-ONT tests of dynamic autoregulation (blue curve in Fig. 5), the pattern of temporal change in BF can be characterized as follows: immediately after the onset of the IOP elevation, BF decreases rapidly reaching a trough ($BF_{\Delta MAX}$ observed at 3.67 ± 0.84 seconds), thereafter, BF starts to rebound toward the previous level recorded when IOP had been 10 mm Hg. The average values for all pre-ONT dynamic BF autoregulation parameters (baseline period) in both eyes of five animals are listed in Table 2. Each parameter was averaged

from five consecutive baselines. None of the parameters show significant difference between eyes ($P > 0.05$). Table 2 also lists corresponding post-ONT parameters averaged from all post-ONT sessions (five sessions each in two of the animals and six sessions each in the other three animals; see symbols for each animal in Figs. 2 and 3 for examples of time series).

To evaluate the effect of ONT on the dynamic autoregulation, a generalized linear model was applied including parameters acquired during each test session across the experiments in both ONT and contralateral control eyes. The result shows a significant effect of “treatment group” (i.e., the difference between ONT and control eyes) for BF_{BL} and T ($P = 0.012$ and $P = 0.05$, respectively), but not K and $BF_{\Delta MAX}$. Both BF_{BL} and T in the ONT eyes were also significantly different from their own baseline values ($P < 0.001$, and $P = 0.012$, respectively). The control eyes had no longitudinal changes ($P = 0.69$, $P = 0.57$, respectively). The red curve in Figure 5 shows a representative example of altered dynamic autoregulation response post ONT.

FIGURE 2. Longitudinal change in RNFLT after ONT (filled symbols) and in fellow control eyes (open symbols and asterisks). Data have been normalized to the baseline average of each individual eye (day zero) for clarity of presentation. There was a clear decline over time in ONT eyes but no change in fellow control eyes.

TABLE 1. Mean Arterial BP and IOP During Baseline (Pre-ONT) and Post ONT

	BP, mm Hg	IOP, Control, mm Hg	IOP, Control, mm Hg	IOP, Control, P, t-test
Pre-ONT	94.05 $\pm$ 8.21	13.50 $\pm$ 1.29	13.23 $\pm$ 0.97	0.358
Post-ONT	98.51 $\pm$ 5.79	11.74 $\pm$ 0.74	12.69 $\pm$ 1.06	0.072
P, t-test	0.49	0.072	0.539

FIGURE 3. Longitudinal change in ONH BF after ONT (filled symbols) and in fellow control eyes (open symbols and asterisks). Data have been normalized to the baseline average (day 0) of each individual eye for clarity of presentation. There was a clear decline over time in ONT eyes but no change in fellow control eyes. The data point circled with a broken line was determined to be an outlier observed in an ONT eye during the first post-ONT test session (Grubbs' test), but was not excluded from the analysis.

DISCUSSION

The first purpose of the current study was to test the hypothesis that in a model of ONH neurodegeneration that does not include chronic IOP elevation, there would be progressive ONH BF decrease due to optic nerve degeneration. Our data strongly support this hypothesis as evidenced by the close correlation between progressive RNFLT thinning and ONH BF decline in the ONT eyes. Although a similar correlation has been reported in previous studies in NHP EG¹ and in human glaucoma,^{14–17} the ability of the latter clinical studies to determine causality were constrained by their cross-sectional design while our previous study of EG was confounded by the presence of increased IOP. By eliminating the role of chronically increased IOP, the longitudinal observation in this ONT model strongly suggest that decreased ONH BF in the ONT eyes was secondary to the degeneration of its RGC axons. This observation is consistent with the concept that each tissue maintains its local BF in proportion to its metabolic needs¹⁸ and suggests that this demand-supply relationship applies also to pathologic conditions. It confirms that at least a portion of the decreased ONH BF in the EG eyes

FIGURE 4. Correlation between longitudinal change in ONH BF (% change from baseline average) and RNFLT in ONT eyes (filled black circles) and contralateral control eyes (open red circles). The data point circled with a broken line was the same outlier shown in Figure 3 and similarly not excluded from this analysis. The dashed diagonal line and equation represent the results of Deming linear regression.

FIGURE 5. A representative dynamic BF response to a rapid IOP increase (10–40 mm Hg, arrowhead) recorded during a baseline session (pre-ONT, blue) and 42 days post ONT (red). Compared with pre-ONT response, post-ONT T (red dots) is increased. Note although the absolute value of BF is smaller in post-ONT due to reduced baseline BF (BF_{BL}), the percentage BF change ($BF_{\Delta MAX}$) remains similar. The two regression lines represent K.

in our previous study¹ was due to diminished metabolic activities secondary to neurodegeneration.

However, the fact that ONH BF decline is secondary to ONH neurodegeneration does not mean that reduced ONH BF is not a direct contributor to the susceptibility of the remaining optic nerve axons to damage in NHP ONT, EG, and/or human glaucoma. Even if reduced ONH BF is a secondary effect of reduced demand, it is possible that such changes in ONH BF become detrimental to what axons remain at any point in the disease course.

Interestingly, both RNFLT loss ($-45\% \pm 4.0\%$) and ONH BF decrease ($-37\% \pm 2.0\%$) were less than average axon loss ($-57\% \pm 12\%$) in the ONT eyes compared with contralateral healthy eyes. In addition to a possible underestimate of ONH BF due to inherited limitations of LSFG technique (see later discussion), the smaller amount of change in RNFLT is in agreement with previous reports in human glaucoma, in which the relative dynamic range of in vivo RNFLT parameters may be limited for a “floor effects” compared with axon counts, which can achieve complete (100%) loss.^{19,20} Another possible reason may relate to the site and the degeneration rate differences after ONT (i.e., the axons are maintained longer within the ONH and peripapillary RNFL as compared with the site of axon counts in orbital optic nerve). As for the lesser BF decrease, a possible explanation may be that even if the ONH is completely devoid of axons the remaining nonfunctional tissues are still metabolically active, and thus require a constant blood supply.

It is unclear whether the reduced BF was associated with any loss of capillary density in ONT eyes. According to previous studies on both ONT²¹ and EG models^{22,23} in NHP, the ONH capillary density remains largely normal albeit some capillaries are occluded after a 1- to 4-year exposure to chronic IOP elevation.²³ In our previous study in rhesus monkeys with idiopathic bilateral optic neuropathy,²⁴ while BF in the atrophic optic nerve is significantly lower than healthy control eyes, the capillary density was actually “higher” due to the shrinkage of axonal fascicles and thickening of surrounding connective tissues where the capillaries reside. As such, it is anticipated that the optic nerve capillaries in ONT eyes most likely remain intact, particularly at the relatively early post-ONT time points studied here, which suggests that the progressive ONH BF decline appears to be a functional adjustment to adapt to diminished metabolic demand following ONT.

TABLE 2. Mean Dynamic Autoregulation Parameters Pre and Post ONT in ONT and Control Eyes

Eye	Pre/Post	BF _{BL} , AU		BF _{ΔMAX} , %		T, s		K	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
ONT	Pre	13.9	0.46	21	0.94	4.68	0.37	−0.32	0.03
	Post	10.4	0.39	21	0.80	6.59	0.32	−0.24	0.03
CTL	Pre	14.2	0.45	20	0.91	5.24	0.37	−0.33	0.03
	Post	13.7	0.40	20	0.81	5.51	0.32	−0.26	0.03

The second purpose of this study was to evaluate longitudinal changes in parameters of ONH BF dynamic autoregulation in the same model so as to test the hypothesis that there would be no early disruption of ONH autoregulation. Contrary to our hypothesis, a significantly increased BF responding time (T) was demonstrated in the ONT eyes, though the increase was marginal. Because T was also increased in the EG eyes of our previous study,² our data now suggest that not only chronic IOP elevation but also neural degeneration may disrupt monkey ONH BF autoregulation. However, this finding of altered dynamic autoregulation parameters should be further investigated with larger sample size. Another noteworthy observation for the dynamic autoregulation change is that the parameter BF_{ΔMAX} was affected differently in the two models. In the EG model, EG eye BF_{ΔMAX} was significantly less decreased compared with control eyes,² which suggests that the ONH blood vessels were more resistant to the rapid IOP elevation compared with the control eyes. In the ONT eye, BF_{ΔMAX} demonstrated no difference from control eyes.

Interestingly, the BF_{ΔMAX} change in both models, described above, does not follow general concepts of autoregulation dysfunction. Based on the theory of autoregulation dysfunction, a consistent perfusion pressure alternation should result in a greater BF change or more passively when regulatory mechanisms are malfunctioning. This concept has been applied in both the classic “static” analyses that measures the difference between the stabilized BF levels before and after the perfusion pressure challenge,³ and also in “dynamic” analysis that measures aspects of the rapid time course of BF change during the period immediately following a perfusion pressure challenge.^{2,25} However, in both the EG from previous studies^{2,3} and neural degeneration in the current ONT model, neither static autoregulation analysis nor dynamic autoregulation analysis revealed an increased BF_{ΔMAX} after perfusion pressure decrease. These data taken together suggest that the ONH autoregulation disruption may manifest differently compared with other tissues in the eye and brain. These complexities may also explain the inconclusive results of previous studies specifically designed to investigate ONH BF in response to increased BP²⁶ or IOP²⁷ in human glaucoma by static autoregulation analysis. While detailed mechanistic studies are required to fully understand the unconventional response, BF_{ΔMAX} may not be a sensitive parameter to detect static and dynamic ONH autoregulation dysfunction in these experimental models. Instead, increased T may be a more consistent manifestation of autoregulation dysfunction in the ONH.

A salient point to mention in regard to the findings of dynamic BF_{ΔMAX} changes in the two models is related to the manipulation by which the ONH autoregulation system is challenged. Studies in ours and others' laboratories have demonstrated that autoregulation functions better in response to ocular perfusion pressure dips produced by elevated IOP than when they occur by reduced BP.^{28–30} Thus, it is possible that the BF_{ΔMAX} may manifest a passive response if the ONT eyes were challenged by reducing BP. As such, when

comparing the current data with different experimental models and clinical studies, it is important to consider whether BP or IOP changes were applied to challenge the autoregulation system.

There are several limitations in this study. First, in both ONT and EG models, compromised BF or autoregulation dysfunction has been eliminated as an initial, primary insult. In human glaucoma, these hemodynamic changes may well be important pathogenic factors that contribute to glaucomatous optic nerve damage.^{31–34} In addition, our studies were conducted in anesthetized animals, so the hemodynamic responses might be altered from physiological conditions. Therefore, caution is warranted when extrapolating the results of experimental models to a clinical setting. Second, the LSFG method, like most other optically-based techniques, has limited penetration and range of linearity between actual and measured blood flow; further, the measurements may also be affected by different tissue properties.^{7,35} The limited capacity in tissue penetration of LSFG may measure BF in deeper ONH regions when the per-laminar neural tissue becomes thinner.¹¹ Thus, BF measured by LSFG at the end of an experimental injury course may include BF contributions from slightly deeper regions compared with that measured during preinjury baselines. Moreover, due to the limitations imposed by the high cost and limited availability of NHPs as experimental animals, the sample size of these experiments is relatively small. This may limit power in this study to detect effects on some of the dynamic autoregulation parameters. Finally, whether a likely increased translaminal pressure gradient due to “left open” dura cut during ONT surgery exerted a role similar to an increased IOP and contributed to the ONH BF decrease is unknown.

In summary, the current study demonstrated a clear cause-effect relationship between neural degeneration and compromised ONH BF by eliminating the impact of chronically elevated IOP through a common model of optic nerve injury (ONT). The results show that in both high IOP and nonhigh IOP induced neural degeneration, ONH BF becomes compromised. Increased BF response time appears to be a sign of dynamic autoregulation dysfunction in this ONT model.

Acknowledgments

Supported by grants from R01-EY019939, Legacy Good Samaritan Foundation, Portland, Oregon, United States.

Disclosure: **G. Cull**, None; **R. Told**, None; **C.F. Burgoyne**, None; **S. Thompson**, None; **B. Fortune**, None; **L. Wang**, None

References

1. Cull G, Burgoyne CF, Fortune B, Wang L. Longitudinal hemodynamic changes within the optic nerve head in experimental glaucoma. *Invest Ophthalmol Vis Sci*. 2013;54:4271–4277.
2. Wang L, Cull G, Burgoyne CF, Thompson S, Fortune B. Longitudinal alterations in the dynamic autoregulation of optic

- nerve head blood flow revealed in experimental glaucoma. *Invest Ophthalmol Vis Sci.* 2014;55:3509–3516.
3. Wang L, Burgoyne CF, Cull G, Thompson S, Fortune B. Static blood flow autoregulation in the optic nerve head in normal and experimental glaucoma. *Invest Ophthalmol Vis Sci.* 2014; 55:873–880.
 4. Fortune B, Cull GA, Burgoyne CF. Relative course of retinal nerve fiber layer birefringence and thickness and retinal function changes after optic nerve transection. *Invest Ophthalmol Vis Sci.* 2008;49:4444–4452.
 5. Stowell C, Arbogast B, Cioffi G, Burgoyne C, Zhou A. Retinal proteomic changes following unilateral optic nerve transection and early experimental glaucoma in non-human primate eyes. *Exp Eye Res.* 2011;93:13–28.
 6. 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.
 7. 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.
 8. 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.
 9. 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.
 10. Aizawa N, Nitta F, Kunikata H, et al. Laser speckle and hydrogen gas clearance measurements of optic nerve circulation in albino and pigmented rabbits with or without optic disc atrophy. *Invest Ophthalmol Vis Sci.* 2014;55:7991–7996.
 11. 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.
 12. Liang Y, Fortune B, Cull G, Cioffi GA, Wang L. Quantification of dynamic blood flow autoregulation in optic nerve head of rhesus monkeys. *Exp Eye Res.* 2010;90:203–209.
 13. Reynaud J, Cull G, Wang L, et al. Automated quantification of optic nerve axons in primate glaucomatous and normal eyes—method and comparison to semi-automated manual quantification. *Invest Ophthalmol Vis Sci.* 2012;53:2951–2959.
 14. Aizawa N, Kunikata H, Shiga Y, Yokoyama Y, Omodaka K, Nakazawa T. Correlation between structure/function and optic disc microcirculation in myopic glaucoma, measured with laser speckle flowgraphy. *BMC Ophthalmol.* 2014;14: 113.
 15. 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.
 16. 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.
 17. 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.
 18. Guyton AC, Hall JE. *Textbook of Medical Physiology*. 11th ed. Philadelphia: Elsevier, Inc.; 2006:161–257.
 19. Sihota R, Sony P, Gupta V, Dada T, Singh R. Diagnostic capability of optical coherence tomography in evaluating the degree of glaucomatous retinal nerve fiber damage. *Invest Ophthalmol Vis Sci.* 2006;47:2006–2010.
 20. Chan CK, Miller NR. Peripapillary nerve fiber layer thickness measured by optical coherence tomography in patients with no light perception from long-standing nonglaucomatous optic neuropathies. *J Neuroophthalmol.* 2007;27:176–179.
 21. Quigley HA, Anderson DR. The histologic basis of optic disk pallor in experimental optic atrophy. *Am J Ophthalmol.* 1977; 83:709–717.
 22. Quigley HA, Hohman RM, Addicks EM, Green WR. Blood vessels of the glaucomatous optic disc in experimental primate and human eyes. *Invest Ophthalmol Vis Sci.* 1984; 25:918–931.
 23. Furuyoshi N, Furuyoshi M, May CA, Hayreh SS, Alm A, Lutjen-Drecoll E. Vascular and glial changes in the retrolaminar optic nerve in glaucomatous monkey eyes. *Ophthalmologica.* 2000; 214:24–32.
 24. 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.
 25. Tiecks FP, Lam AM, Aaslid R, Newell DW. Comparison of static and dynamic cerebral autoregulation measurements. *Stroke.* 1995;26:1014–1019.
 26. Pournaras CJ, Riva CE, Bresson-Dumont H, De Gottrau P, Bechettille A. Regulation of optic nerve head blood flow in normal tension glaucoma patients. *Eur J Ophthalmol.* 2004; 14:226–235.
 27. Weigert G, Findl O, Luksch A, et al. Effects of moderate changes in intraocular pressure on ocular hemodynamics in patients with primary open-angle glaucoma and healthy controls. *Ophthalmology.* 2005;112:1337–1342.
 28. 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 nonhuman primates. *Invest Ophthalmol Vis Sci.* 2009;50:2154–2160.
 29. Wang L, Cull GA, Fortune B. Optic nerve head blood flow response to reduced ocular perfusion pressure by alteration of either the blood pressure or intraocular pressure. *Curr Eye Res.* 2015;40:359–367.
 30. Tani T, Nagaoka T, Nakabayashi S, Yoshioka T, Yoshida A. Autoregulation of retinal blood flow in response to decreased ocular perfusion pressure in cats: comparison of the effects of increased intraocular pressure and systemic hypotension. *Invest Ophthalmol Vis Sci.* 2014;55:360–367.
 31. Cherecheanu AP, Garhofer G, Schmidl D, Werkmeister R, Schmetterer L. Ocular perfusion pressure and ocular blood flow in glaucoma. *Curr Opin Pharmacol.* 2013;13:36–42.
 32. Flammer J, Mozaffarieh M. What is the present pathogenetic concept of glaucomatous optic neuropathy? *Surv Ophthalmol.* 2007;52(suppl 2):S162–S173.
 33. Harris A, Ciulla TA, Chung HS, Martin B. Regulation of retinal and optic nerve blood flow. *Arch Ophthalmol.* 1998;116: 1491–1495.
 34. Pournaras CJ, Rungger-Brandt E, Riva CE, Hardarson SH, Stefansson E. Regulation of retinal blood flow in health and disease. *Prog Retin Eye Res.* 2008;27:284–330.
 35. Harris A, Cantor L, Kagemann L. Imaging of blood flow in glaucoma. In: Schuman JS, ed. *Imaging in Glaucoma*. Thorofare, New Jersey: SLACK, Inc.; 1997:135–154.