Deformation of the Rodent Optic Nerve Head and Peripapillary Structures during Acute Intraocular Pressure Elevation

Brad Fortune, Tiffany E. Choe, Juan Reynaud, Christine Hardin, Grant A. Cull, Claude F. Burgoyne, and Lin Wang

PURPOSE. To evaluate the effect of acutely elevated intraocular pressure (IOP) on retinal thickness and optic nerve head (ONH) structure in the rat eye by spectral domain–optical coherence tomography (SD-OCT).

METHODS. Fourteen adult male Brown-Norway rats were studied under anesthesia (ketamine/xylazine/acepromazine, 55:5:1 mg/kg intramuscularly). Both eyes were imaged by SD-OCT on two baseline occasions several weeks before and again 2 and 4 weeks after the acute IOP imaging session. During the acute IOP session, SD-OCT imaging was performed 10 minutes after IOP was manometrically set at 15 mm Hg and then at 10, 30, and 60 minutes after IOP had been elevated to 50 mm Hg (n = 8) and again 10 and 30 minutes after IOP had been lowered back to 15 mm Hg (recovery). In two additional groups, IOP elevation was set to 70 mm Hg (n = 4) or 40 mm Hg (n = 2). Acute IOP results are reported for a pattern of 49 horizontal B-scans spanning a 20° square and follow-up results for peripapillary circular B-scans. Retinal and retinal nerve fiber layer (RNFL) thicknesses were measured with custom software by manual image segmentation. Friedman and Dunn’s tests were used to assess acute and longer-term effects of acute IOP elevation.

RESULTS. Acute IOP elevation to 50 mm Hg caused rapid (within seconds) deformation of the ONH and peripapillary structures, including posterior displacement of the ONH surface and outward bowing of peripapillary tissue; retinal thickness decreased progressively from 10 to 30 to 60 minutes by 16%, 18%, and 20% within the area of Bruch’s membrane opening (BMO; P < 0.0001) by 8%, 9%, and 11% within the central 10° (excluding the BMO; P < 0.0001) but only by 1%, 2%, and 2.4% beyond the central 10° (P < 0.0001). Recovery was progressive and nearly complete by 30 minutes. Acute IOP elevation to 40 and 70 mm Hg produced similar structural changes, but 70 mm Hg also interfered with retinal blood flow. There were no changes in peripapillary retinal or RNFL thickness (P = 0.08 and P = 0.16, respectively) measured 2 and 4 weeks after acute elevation to 50 mm Hg.

CONCLUSIONS. Acute IOP elevation in the rodent eye causes rapid, reversible posterior deformation of the ONH and thinning of the peripapillary retina, with only minimal retinal thinning beyond 5° of the ONH. No permanent changes in peripapillary retinal or RNFL thickness (for up to 1 month of follow-up) were caused by 60 minutes of IOP elevation to 50 mm Hg. (Invest Ophthalmol Vis Sci. 2011;52:6651–6661) DOI: 10.1167/iovs.11-7578

Rodent models are essential for elucidating the complex molecular mechanisms that lead to the development and progression of glaucomatous optic nerve degeneration and are increasingly used for such purposes.1–4 Early reports about one such rodent glaucoma model based on chronic elevation of intraocular pressure (IOP) suggested that the site of injury initiating the optic neuropathy and retinal ganglion cell death was within the optic nerve head (ONH).5 More recent evidence from other rodent models lends further support to this premise.5–12

Continued advancement of optical coherence tomography (OCT)13 has enabled fast, noninvasive imaging of tissue microstructure, including individual retinal layers and the ONH of rodents.14–16 Recent reports have also demonstrated that OCT can be applied to observe changes in optic nerve injury models in rodents, such as thinning of the retinal nerve fiber layer (RNFL) after optic nerve crush17 and changes in retinal thicknesses during the course of experimental glaucoma.18 We have also shown that longitudinal changes of deep ONH structures are detectable by spectral domain–optical coherence tomography (SD-OCT) in a non–human primate experimental model of glaucoma19 and that specific structural changes caused by the chronic disease model can be differentiated from those that occur as a result of acute IOP elevation alone20 (Strouthidis NG, et al. IOVS 2011;52: ARVO E-Abstract 4811). Most notably, the anterior lamina cribrosa surface becomes posteriorly displaced as a result of progressive plastic deformation caused by chronic IOP elevation,19 whereas its position relative to other ONH landmarks appears to change very little as a result of acute IOP elevation. (Strouthidis NG, et al. IOVS 2011;52: ARVO E-Abstract 4811).21

These studies indicate that it is important to differentiate the effects of chronic structural alterations from acute changes with IOP level, though each may damage retinal ganglion cells and their axons by specific mechanisms. Indeed, acute IOP elevation to 50 mm Hg, or even to just 35 mm Hg, is known to alter axonal transport in the rat optic nerve.22–25 and repeated daily IOP “spikes” of 1-hour duration have been shown to cause optic neuropathy in the rat.26 Therefore, in this study, we sought to evaluate the effects of acute IOP elevation on ONH and peripapillary retinal structure in the rat eye using SD-OCT.
SUBJECTS AND METHODS

Subjects

Fourteen adult male Brown-Norway rats (*Rattus norvegicus*; Charles River Laboratories Inc., Wilmington, MA) were the subjects of this study. They were maintained under a 12-hour light/12-hour dark cycle with normal rat chow and water available ad libitum. At the time of testing they were 10 to 12 weeks old and weighed 200 to 275 g. All experimental methods and animal care procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were preapproved by the Legacy Institutional Animal Care and Use Committee.

Experimental Design and Protocol for Acute IOP Elevation

Animals were anesthetized with an intramuscular injection of a rodent cocktail containing ketamine (55 mg/kg, Ketaset; Fort Dodge Animal Health, Fort Dodge, IA), xylazine (5 mg/kg, AnaSed; Lloyd, Inc., Shenandoah, IA), and acepromazine maleate (1 mg/kg; Vedco, Inc., St. Joseph, MO) then were placed on a custom-built imaging stage and kept warm with a thermostatically controlled system (TP650; Gaymar Industries, Inc., Orchard Park, NY). Tropicamide (0.5%; Alcon Laboratories Inc., Fort Worth, TX) and phenylephrine (2.5%; Bausch and Lomb Pharmaceuticals Inc., Tampa, FL) were applied topically to dilate the pupils, and proparacaine hydrochloride (0.5%; Alcon Laboratories Inc.) was applied for topical anesthesia. The anterior chamber of the right eye was cannulated through the peripheral cornea (Fig. 1A) using a 30-gauge needle that was connected by polyethylene tubing to a reservoir filled with normal saline (0.9% NaCl). IOP was controlled manometrically by setting the reservoir to heights that were precalibrated in 5-mm Hg increments using a pressure transducer (MX860; Medex, Inc., Carlsbad CA). Custom rigid gas-permeable contact lenses (3.5-mm posterior radius of curvature, 5.0-mm optical zone diameter, +5.0-diopter back vertex power) were then placed on both eyes (Fig. 1A) to maintain corneal hydration and clarity, and the experimental protocol was begun as follows.

Baseline IOP was set to 15 mm Hg, close to the level typically measured by tonometry in anesthetized Brown Norway rats. SD-OCT scans and confocal scanning laser ophthalmoscope (CSLO) images were obtained (Spectralis HRA+OCT; Heidelberg Engineering GmbH, Heidelberg, Germany) under manometric IOP control 10 minutes after IOP was set to 15 mm Hg (baseline); 10, 30, and 60 minutes after IOP was raised to 50 mm Hg; and 10 and 30 minutes after IOP was set back to 15 mm Hg (recovery). At the end of the imaging session, the needle and contact lenses were removed, and a broad-spectrum topical antibiotic ointment was applied. The main study group included animals (*n* = 8) whose IOP was elevated to 50 mm Hg, as described. A

---

**Figure 1.** Anterior chamber cannulation by 30-gauge needle through superotemporal peripheral cornea for manometric IOP control; custom rigid gas-permeable contact lens fit over cornea and pupil dilated for imaging by CSLO and SD-OCT (A). Infrared CSLO image of the fundus, 30° × 30° (B). SD-OCT raster scan pattern (red lines) consisting of 49 horizontal B-scans, 20 × 20°. B-scan 15 of 49 highlighted by green line in (B) is shown in (C). Sectors for analysis included the peripheral quadrants and a central, circular 10° area. Example of B-scan without segmentation (C); inset within red box shows magnified view. Same B-scan with segmentations (D). The green line marks the border between the vitreous and the ILM. The pink line marks the original native segmentation result for the RPE/BM. The yellow line marks the manually corrected segmentation for the posterior aspect of the RPE/BM. Colored squares represent control points on the spline that define each segmentation.
second group (n = 4) had IOP elevated to 70 mm Hg under the same temporal protocol as the 50-mm Hg group; two other animals had IOP elevated to 40 mm Hg with the same protocol as well as additional SD-OCT data collected in rapid sequence for time lapse video of acute elevation to either 50 or 70 mm Hg.

SD-OCT scans reported here consisted of a raster pattern of 49 horizontal B-scans spanning a 20° × 20° area centered on the optic disc (Fig. 1B). The depth of each A-scan was 1.8 mm and consisted of 512 pixels providing a digital axial resolution of 3.5 μm per pixel. Each B-scan spanned 20° horizontally and consisted of 1024 A-scans. Automatic real-time eye tracking software was used to enable B-scan sweep averaging for speckle noise reduction. For this experiment, nine sweeps were averaged for each B-scan. The eye tracking software also enables repeat scans to be acquired at the same fundus location. Therefore, at each time point in the protocol, one set of recorded scans was automatically registered to the baseline location and another set was manually positioned by the operator to be as close as possible to the baseline location (centered on the optic disc). Though the results for these two data sets were essentially identical, the software-registered scans better compensated for cyclotorsional changes; therefore, the results for that set are reported here. Note also that an additional +25-diopter lens was mounted to the camera objective for this experiment.

SD-OCT raw data were then exported from the instrument (Spectralis; Heidelberg Engineering GmbH) to perform manual segmentation of B-scan features or to edit the instrument’s native segmentations using custom software. Manual segmentation was performed by trained observers who were masked to animal identification and scan conditions. The native segmentation algorithm, designed to identify the posterior aspect of the retinal pigment epithelium/Bruch’s membrane (RPE/BM) complex in the human retina, instead identifies some posterior features within the choroid (typically either the choroidal-scleral junction or the posterior aspect of large vessel walls; see e.g., Figs. 1D, 3) in B-scans of the rat retina. Thus, manual editing was required to correct the position of the BM/RPE segmentation to obtain retinal thickness values. Retinal thickness values were derived for each A-scan as the distance between the internal limiting membrane (ILM) and the corrected BM/RPE segmentations. The distance between the corrected BM/RPE segmentation and the original native segmentation was taken as the choroidal thickness (Fig. 1D). Thus the posterior choroidal segmentation was not edited unless it was not present at all in a given B-scan (−1%) in which case it was replaced with the segmentation from an adjacent B-scan. Other features identified in B-scans where present were the Bruch’s membrane opening (BMO) and the border tissue (BT) at the edge of the choroid along the neural canal. An ellipse was fit to the BMO points and another to the BT points for quantification and visualization.

Longitudinal Follow-up

We also collected a longitudinal data set with four time points to determine the longer-term consequences of acute IOP elevation. These data included baseline SD-OCT scans obtained on two occasions (1–2 weeks apart) several weeks before the date of the acute IOP elevation session and then repeated at 2 and 4 weeks after the acute IOP elevation session. The SD-OCT scans were acquired in the same manner as described, including the same anesthesia regimen, only without anterior chamber cannulation for manometric IOP control. Here we report the longitudinal results for a circular B-scan centered on the optic disc, registered to the fundus position of the first longitudinal baseline scan (Fig. 2). This single ‘peripapillary’ circular B-scan consisted of 1536 A-scans and had a diameter of 12°. The final recorded scan in each case was also a real-time average of nine individual sweeps. Segmentations (e.g., Fig. 2B) were manually corrected in the same manner described, and the values for retinal and RNFL thickness were derived for each A-scan sample as described.

Statistical Analysis

Tissue thickness values for control and elevated IOP conditions were compared using nonparametric repeated-measures analysis of variance matched for subjects (RM-ANOVA, Friedman test) with Dunn’s multiple comparison post hoc tests to compare medians across time points. The effect of location (superior vs. inferior) was assessed using two-way RM-ANOVA. The longitudinal data (change over weeks) for retinal thickness and RNFL thickness from the peripapillary circle sweeps were also assessed using two-way RM-ANOVA. Potential changes within individual eyes were assessed by comparing values obtained at the 2- and 4-week follow-up time points against the repeatability coefficients calculated from the 16 pairs of baseline values. All statistics were performed using the same program (Prism v4; GraphPad Software, Inc., San Diego, CA).

RESULTS

Figure 3 presents results of two animals (from n = 8 total). Baseline SD-OCT scans obtained at an IOP of 15 mm Hg are shown in Figures 3A and 3C, whereas SD-OCT scans obtained after 60 minutes of IOP elevation to 50 mm Hg are shown in Figures 3B and 3D, respectively. In each panel, the infrared CSLO fundus image is shown at the left, with a pseudocolor representation of retinal thickness overlaid onto the raster scan pattern (thickness values range from 190 μm, blue to 275 μm, green for all panels). In each panel, the SD-OCT B-scan shown to the right is number 29 of 49 (indicated by the green line in the CSLO image), which crosses the superior pole of the ONH near to the superior edge of the choroidal border tissue (marked in the B-scan by the blue squares). Note that the ellipse fit to the choroidal border tissue points was always the better match to the optic disc margins apparent in the infrared CSLO images of the rat fundus. The ellipse fit to the BMO was always smaller than the apparent disc margin, reflecting an ‘overhang’ of BM common to all rat eyes studied. Acute IOP elevation to 50 mm Hg resulted in a decrease of tissue thickness within the ONH (i.e., within the choroidal border tissue ellipse) and its immediate peripapillary surround (Figs. 3B, 3D, green arrows). The blue areas on the pseudocolor thickness maps indicate that retinal thinning during elevated IOP occurred between the optic disc margin and the 5° eccentricity contour. That is, thinning was most prominent within the central circular area (10° diameter). There is also the suggestion in Figures 3B and 3D that retinal thinning superior to the optic disc was greater than that inferior to the disc.

Figure 4 presents the data for the entire group (n = 8) and all time points. There was a slight decrease for the global average thickness over the entire scan area during acute IOP elevation (Fig. 4A; P = 0.0001). The average thickness for the whole scan area decreased compared with baseline by 2.8%, 5.5%, and 4.3% at 10, 30, and 60 minutes, respectively, after IOP was elevated to 50 mm Hg. Average thickness did not fully recover even after 30 minutes of IOP having been lowered back to 15 mm Hg; average thickness was still 3.5% thinner than at baseline (P < 0.05).

Figure 4B shows the results for the area within the BMO. Here the tissue thinning was more prominent, with reductions of 16%, 18%, and 20% at the 10-, 30-, and 60-minute time points, respectively. Average tissue thickness within the BMO area recovered nearly to baseline values at both the 10-minute and the 30-minute recovery time points: there was no significant difference between baseline values and those at either recovery point, and tissue thickness values were significantly increased at both recovery time points compared with the 60-minute IOP elevation time point (P < 0.05 and P < 0.01, respectively). However, the group average value was still approximately 6% thinner at both recovery time points compared...
with baseline. It should be noted that the “thickness” within the BMO ellipse represents the distance from the surface of the optic disc and the spline fit to the BM/RPE segmentation, including the two BMO points in each B-scan. Thus, the thickness values within the BMO represent tissue thickness relative to a reference plane (the BMO) and not to a true anatomic thickness.

Figure 4C shows the results for the central 10° circular peripapillary area, excluding the area within the BMO. Similar to the results for the area within the BMO, peripapillary retinal thickness decreased by 8%, 9%, and 11%, respectively, 10, 30, and 60 minutes after IOP was elevated to 50 mm Hg. Recovery on lowering IOP back to the baseline value of 15 mm Hg was nearly complete at both 10 and 30 minutes; thickness had increased significantly compared with the 60-minute IOP elevation time point (P < 0.01 for both recovery time points), and neither was significantly different (though they were still thinner by approximately 6% on average) from baseline.

Figure 4D shows the average retinal thickness for four peripheral quadrants. Similar to the behavior observed for the global scan area average, retinal thickness decreased in the peripheral quadrants by 1.3%, 1.9%, and 2.4% compared with baseline but tended not to recover on IOP returning to baseline, with the peripheral average retinal thickness actually decreasing slightly further to 3% below baseline on average, though the values at the 30-minute recovery time point were not statistically different from those at baseline. In general, it can be seen that the effect of acute IOP elevation is much smaller for the peripheral quadrants of the scan area than for the area within the BMO and the peripapillary retina extending to a 5° eccentricity.

Although there was no significant difference in acute IOP effect between the four individual peripheral quadrants, there was a significantly greater effect of acute IOP elevation to 50 mm Hg on the superior peripapillary retina compared with the inferior peripapillary retina (P = 0.001, time-hemisphere interaction, two-way RM ANOVA) at all three elevated IOP time points (P < 0.001, Bonferroni multiple comparison post hoc tests for each compared with baseline). This is consistent with the results observed in the pseudocolor thickness maps, as shown, for example, in Figure 3.

Although the effects of acute IOP elevation to 50 mm Hg on choroidal thickness were significant for the global average scan area (P = 0.0078) and for the central peripapillary area (excluding the area within the choroidal border tissues; P = 0.0008) and the peripheral average (of all four quadrants; P = 0.0078), the effects were more homogeneous throughout the scan area and generally limited to the 10- or 30-minute elevation time points. For example, the global scan area average choroidal thickness decreased from baseline by 9.4%, 12.7%, and 8.1% at 10, 30, and 60 minutes of IOP elevation to 50 mm Hg, but only the 30-minute time point was significantly different from baseline (P < 0.01). Similarly, choroidal thickness decreased by 9.2%, 11.9%, and 9.4% within the peripapillary central area (excluding the area within the border tissues) and by 9.9%, 13.3%, and 8.6% within the four peripheral quadrants, but again only the 30-minute elevation time points were significantly different (P < 0.01) from baseline.
Acute IOP elevation to 70 mm Hg resulted in structural changes similar to those observed at 50 mm Hg. For example, at the 10-, 30-, and 60-minute elevation time points, retinal thickness decreased by only 1.6%, 3.0%, and 1.9%, respectively, compared with baseline for the overall scan area and by only 0.8%, 0.4%, and 1.0% across the four peripheral quadrants; neither regional analysis was statistically significant ($P = 0.12$ and $P = 0.71$, respectively). In contrast, tissue thickness within the BMO area decreased by 18%, 22%, and 21% after 10, 30, and 60 minutes of IOP elevation to 70 mm Hg ($P = 0.005$). Peripapillary retinal thickness decreased by 5.7%, 6.3%, and 6.0% at these time points; however, the overall effect for this region was not statistically significant ($P = 0.15$) because of the smaller sample size of this group ($n = 4$). IOP elevation to 70 mm Hg also caused an asymmetrical effect similar to 50 mm Hg, whereby thinning of the superior peripapillary retina was approximately double the magnitude in the inferior region at 10 minutes ($P = 0.01$) and 50% greater at 60 minutes ($P < 0.01$). Choroidal thickness changes were again more homogenous across the scan area, for example, decreasing by 15% for the overall scan area after 10 minutes of IOP elevation ($P = 0.07$), by 18% for the peripheral quadrants ($P = 0.08$), and by 10% in the peripapillary central area (excluding the area within the border tissues; $P = 0.02$). The pattern of change was also similar in the two animals whose IOP was elevated to 40 mm Hg. Tissue thinning was greatest within the BMO area and the peripapillary ring; the latter was greater superiorly than inferiorly, with relatively small changes in the peripheral quadrants. Image quality had degraded slightly by the 60-minute time point in 2 of the 4 eyes whose IOP was elevated to 70 mm Hg, presumably because of transient corneal edema, which had recovered by the 10-minute recovery time point but was still sufficient to complete the B-scan segmentation/editing process without alteration of any methodology. The most remarkable difference after IOP was raised to 70 mm Hg, compared with 50 mm Hg, was the dramatic reduction of retinal vessel caliber and blood flow at 70 mm Hg. In all four animals whose IOP was raised to 70 mm Hg, the retinal veins

![Figure 3](image-url)

**Figure 3.** Results of acute IOP elevation. Baseline SD-OCT scans obtained at an IOP of 15 mm Hg are shown for two subjects (A, C). The infrared CSLO fundus image is shown at the left, with a pseudocolor representation of retinal thickness overlaid onto the raster scan pattern (range, 190 μm [blue] to 275 μm [green] for all). As in Figure 1, the sector borders are shown for the four peripheral quadrants and the central area. Also indicated are the BMO (white ellipse) and the choroidal border tissues (blue ellipse). In each panel, the B-scan shown to the right is number 29 of 49 (indicated by the green line in the CSLO image), which crosses the superior pole of the ONH near to the superior edge of the choroidal border tissue (marked in the B-scan by the blue squares). SD-OCT scans obtained 60 minutes after IOP elevation to 50 mm Hg are shown for each eye in (B) and (D), respectively. The most salient structural change was a decrease of tissue thickness within the ONH and its immediate peripapillary surround (green arrows). The blue areas on the pseudocolor thickness maps indicate that retinal thinning during elevated IOP occurred between the optic disc margin (blue ellipse) and the 10° eccentricity contour (i.e., within the central circular area).
collapsed and the major arterial branches exhibited labored pulsation with the cardiac cycle. Figure 5A shows a single frame from a movie obtained using the CSLO approximately 2 minutes after IOP was raised to 70 mm Hg (Supplementary Movie S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7578/-/DCSupplemental). It is clear even in this single frame obtained during the diastolic phase of the cardiac cycle that the retinal veins are largely collapsed and the peripapillary arterial caliber is narrowed. The features of the optic disc are blurred because of posterior deformation relative to the peripheral portions of the fundus image. This pattern persisted beyond the 30-minute time point in all four animals and began to exhibit some recovery by the 60-minute time point (presumably as systolic blood pressure began to increase with increasing duration after anesthesia induction). After IOP was lowered back to the baseline level of 15 mm Hg, the vascular pattern and apparent flow also returned to baseline characteristics.

Figure 6B shows the SD-OCT scan obtained 10 minutes after IOP was raised to 70 mm Hg. The bulk of the structural changes are evident within 10 seconds of the manometer being set back to 15 mm Hg. One movie (Supplementary Movie S3, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7578/-/DCSupplemental) contains the horizontally oriented B-scans through the ONH (Figs. 7B, 7D, 7F), and the other movie (Supplementary Movie S4, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7578/-/DCSupplemental) contains the vertically oriented B-scans through the ONH (Figs. 7A, 7C, 7E), and the other movie (Supplementary Movie S4, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7578/-/DCSupplemental) contains the horizontally oriented B-scans through the ONH (Figs. 7B, 7D, 7F). Figures 7A and 7B, the first frame of each movie, were obtained with IOP set to 15 mm Hg. Figures 7C and 7D were obtained within 10 seconds of IOP elevation to 50 mm Hg; Figures 7E and 7F were obtained within seconds, on lowering IOP back to 15 mm Hg. One movie conditions of IOP elevation and then returned toward baseline, again within seconds, on lowering IOP back to 15 mm Hg. One movie (Supplementary Movie S3, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7578/-/DCSupplemental) contains the vertically oriented B-scans through the ONH (Figs. 7A, 7C, 7E), and the other movie (Supplementary Movie S4, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7578/-/DCSupplemental) contains the horizontally oriented B-scans through the ONH (Figs. 7B, 7D, 7F). Figures 7A and 7B, the first frame of each movie, were obtained with IOP set to 15 mm Hg. Figures 7C and 7D were obtained within 10 seconds of IOP elevation to 50 mm Hg, and Figures 7E and 7F were obtained within 10 seconds of the manometer being set back to 15 mm Hg. The bulk of the structural changes are evident within 10 seconds of the IOP change in either direction. This rapid
imaging sequence experiment was repeated in two other subjects, producing the same results.

Because IOP elevation to 70 mm Hg had such a dramatic effect on retinal blood vessel caliber, an analysis was performed to assess whether similar effects occurred when IOP was elevated to 50 mm Hg. Using peripapillary circular B-scans such as those shown in Figure 6, obtained during the acute baseline with IOP set at 15 mm Hg and at the 10-minute acute elevation time point in the eight eyes elevated to 50 mm Hg, we measured the diameter along the axial dimension of each major blood vessel crossing. There were five to seven major branch vein crossings and four to seven major arterial branch crossings in this group of eight eyes at this eccentricity from the optic disc. At baseline, with IOP set to 15 mm Hg, veins were slightly thicker than arteries (67.6 ± 4.3 vs. 58.7 ± 3.7 μm; P = 0.0007, paired t-test). Acute IOP elevation to 50 mm Hg had a significant effect on blood vessel diameter (P < 0.0001, two-way RM-ANOVA); however, the effect varied, depending on vessel type (P = 0.0374), such that there was a significant decrease in the diameters of veins (6.9% ± 3.5% thinner; P < 0.001) but no significant change for arteries (3.1% ± 3.9% thinner; P > 0.05).

To determine whether acute IOP elevation for 1 hour would result in long-term damage to retinal ganglion cell axons, or in changes to the peripapillary retina, longitudinal evaluation was carried out by SD-OCT, as described in Subjects and Methods. Table 1 shows the results of longitudinal follow-up. There was no significant effect of time or eye (experimental vs. control) for either peripapillary RNFL thickness (P = 0.13 and P = 0.08, respectively, RM-ANOVA) or peripapillary total retinal thickness (P = 0.82 and P = 0.16, respectively) in the group with unilateral acute IOP elevation to 50 mm Hg. The experiment had 80% power to detect a 3.25-μm decrease from baseline and 97% power to detect a 10% decrease (4.6 μm). None of the individual eyes (n = 8 experimental or n = 8 control) had a decrease of peripapillary RNFL thickness at either follow-up time point that was beyond the lower limit of test-retest repeatability (5th percentile, −13.5%) calculated from the 16 pairs of longitudinal baseline values. One of the experimental eyes and two of the control eyes had peripapillary total retinal thickness values during follow-up that were below the lower limit of test-retest repeatability (−3.0%).

Similarly, there was no significant effect of time or eye for peripapillary total retinal thickness (P = 0.20 and P = 0.21, respectively) in the group with unilateral acute IOP elevation to 70 mm Hg. However, there was a small increase in the peripapillary RNFL thickness in the experimental eyes at 2 and 4 weeks after acute IOP elevation to 70 mm Hg; the effect of eye (P = 0.0141) and eye-time interaction (P = 0.0166) both had a relatively small chance of being caused by random selection. Nevertheless, there was no evidence of RNFL loss in the eyes with acute IOP elevation to 70 mm Hg after 2 and 4 weeks of longitudinal follow-up.

**DISCUSSION**

The results of this study demonstrate that acute IOP elevation causes rapid and reversible conformational changes in the rat ONH and peripapillary tissues. Tissue thickness changes are greatest within 5° of the center of the optic disc and are more pronounced for the superior peripapillary hemisphere than for the inferior peripapillary hemisphere. Tissue thickness measurements tended to become progressively thinner over the course of the 1-hour period of IOP elevation, without evidence of recovery until IOP was lowered back to the baseline level. These observations add further evidence to suggest that the site of original injury leading to damage and death of retinal ganglion cells in IOP-related experimental rodent models of glaucoma lies within or around the ONH.5–12 Interestingly, Morrison et al.5 noted that the superior portion of the optic nerve is more susceptible to damage from both chronic and acute IOP elevation than the inferior portion of the optic nerve. Mabuchi et al.29 reported a similar susceptibility of the superior portion of the optic nerve in a mouse model of glaucoma, and WoldeMussie et al.30 observed a greater susceptibility of retinal ganglion cells in the superior retina in a different rat model. These findings are consistent with the
hemispheric asymmetry of structural changes observed here during acute IOP elevation.

There was a tendency for nonlinear effects to manifest such that the structural changes increased slightly with increased duration of IOP elevation. Similarly, on return of IOP to the baseline level of 15 mm Hg, the tissue thicknesses tended to progressively return to baseline values. Recovery of tissue thickness was nearly complete by the 30-minute recovery time point. However, the term “recovery” is used here only to refer to the IOP setting and the thickness values; it is not meant to imply normalization of anatomy or physiology because the thickness values “recovering” could in part represent pathologic changes such as axonal beading and organelle accumulation, which are consequences of axonal transport disruption.8,23,25

There was no evidence of long-term damage to retinal ganglion cell axons from 1 hour of acute IOP elevation to either 50 mm Hg or 70 mm Hg based on longitudinal evaluation of peripapillary RNFL or total retinal thickness. It is possible that postmortem histopathologic evaluation of orbital optic nerve axons would more sensitively reveal damage induced by the duration and level of IOP elevation studied here. Previous studies of retinal functional changes by electroretinography27 have demonstrated long-term loss of retinal ganglion cell and distal retinal function (2 and 4 weeks) after a slightly longer duration (~90 minutes) of acute IOP elevation to 70 mm Hg but not to 50 mm Hg (Bui BV, et al. IOVS 2004;45:ARVO E-Abstract 2126). Given the observation of dramatic blood flow interruption at 70 mm Hg, it might not be surprising to find longer-term consequences of acute elevation to this level and perhaps a threshold duration. Indeed, Holcombe et al.31 have reported a similar threshold at ~70 mm Hg for retinal glutamate homeostasis and evidence of inner retinal ischemia after 1 hour of acutely elevated IOP in the rat. This observation must be considered with respect to systemic blood pressure, ocular perfusion pressure (difference between blood pressure and IOP), and autoregulation capacity, however, because the absolute level of IOP will alter blood flow depending on those variables.32 In other experiments in our laboratories in which a femoral artery was cannulated for direct readings of blood pressure in Brown Norway rats, the same anesthetic regimen used here (and in previous experiments on retinal function)27 resulted in mean arterial pressures as low as 85 mm Hg. In contrast, isoflurane gas anesthesia tends to result in higher mean arterial pressures (~95 or 100 mm Hg; Morrison J, personal communication, February 2011).33 We found arterial pulsations (such as those shown in Fig. 5 and Supplementary Movie S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7578/-/DCSupplemental) when IOP approached to within 5 to 10 mm Hg of mean arterial pressure measured directly in the femoral artery. Others25 have observed that an ocular perfusion pressure of 25 mm Hg is sufficient to avoid retinal arterial pulsation. Taken together, it appears there is a relatively limited window of IOP, in the setting of an acute elevation, that can be expected to cause damage to retinal ganglion cells without significant alteration of retinal blood flow in the rodent. The choice of anesthesia will affect the span of that window. Novel methods of measuring blood flow in vivo provide evidence that an IOP of 70 mm Hg, even under isoflurane anesthesia (with presumed better preservation of blood pressure in the rat), causes a dramatic reduction of retinal blood flow and some reduction even at 50 mm Hg.34

**FIGURE 6.** Peripapillary circular SD-OCT scans from this same eye shown in Figure 5; during baseline with an IOP of 15 mm Hg (A); 10 minutes after IOP was elevated to 70 mm Hg (B); and 30 minutes after IOP returned to the baseline level of 15 mm Hg (C). Insets indicated by the red boxes in (A) and (B) shown at higher magnification in (D) and (E), respectively. For each panel, the B-scan is shown to the right, while the green line in the CSLO image at the left shows the position and path of the B-scan. Two arteries and two veins are highlighted in (A) and in the inset in (D) and (E).
Interestingly, repeated acute elevations (or spikes) of 1-hour duration to only \( \geq 35 \text{ mm Hg} \) have been shown to cause optic neuropathy in rodents. This is an IOP level that might not significantly alter retinal or ONH blood flow in rats,\(^3^4\) depending on the systemic blood pressure and the degree of autoregulation.\(^2^5,^3^2\) Thus, it seems possible that the mechanical effects presumed to coincide with the structural changes found in this study could directly result in axonal injury caused by elevated IOP, perhaps beginning with axonal transport disruption at the ONH.\(^8,^1^1,^2^2,^2^3,^2^5,^3^5\)

The results of this study further indicate that other measures of ONH structure, such as surface topography heights measured by confocal scanning laser tomography,\(^5^6\) are likely to be affected by the IOP level at the time of imaging. The pattern of ONH deformation and apparent tissue thinning observed here in the rodent eye during acute IOP elevation is strikingly similar to what we have observed in the non-human primate\(^2^0\) (Strouthidis NG, et al. \textit{IOVS} 2011; 52: ARVO E-Abstract 4811) and to what others have observed in humans.\(^2^1\) Some aspects are also similar to chronic disease-related structural changes determined under IOP control conditions,\(^1^9\) though the latter also include posterior displacement\(^1^9\) and remodeling\(^7\) of the connective tissue lamina cribrosa in the primate, which will influence

### Table 1. Longitudinal Results for RNFL Thickness and Retinal Thickness

<table>
<thead>
<tr>
<th></th>
<th>Baseline 1</th>
<th>Baseline 2</th>
<th>Follow-up, 2 wk</th>
<th>Follow-up, 4 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp Eyes</td>
<td>Control Eyes</td>
<td>Exp Eyes</td>
<td>Control Eyes</td>
</tr>
<tr>
<td><strong>IOP50 Group (n = 8)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNFL thickness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>45.8</td>
<td>43.6</td>
<td>46.0</td>
<td>46.8</td>
</tr>
<tr>
<td>SD</td>
<td>4.3</td>
<td>2.2</td>
<td>4.6</td>
<td>3.2</td>
</tr>
<tr>
<td>Retinal thickness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>230.2</td>
<td>228.0</td>
<td>229.4</td>
<td>227.6</td>
</tr>
<tr>
<td>SD</td>
<td>7.5</td>
<td>6.5</td>
<td>7.0</td>
<td>6.4</td>
</tr>
<tr>
<td><strong>IOP70 Group (n = 4)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNFL thickness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>43.2</td>
<td>44.8</td>
<td>44.9</td>
<td>47.6</td>
</tr>
<tr>
<td>SD</td>
<td>2.2</td>
<td>1.7</td>
<td>1.4</td>
<td>2.6</td>
</tr>
<tr>
<td>Retinal thickness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>220.0</td>
<td>222.2</td>
<td>224.8</td>
<td>224.9</td>
</tr>
<tr>
<td>SD</td>
<td>10.0</td>
<td>3.0</td>
<td>7.1</td>
<td>6.0</td>
</tr>
</tbody>
</table>

All values are in micrometers. Exp, experimental.
apparent tissue thickness estimates, depending on the reference plane and the type of imaging. These findings underscore the need to consider the acute IOP level and its effects on the specific type of imaging to discriminate those from chronic disease-related changes.

The goal of future studies will be to determine the specific nature of apparent tissue thinning within the ONH and peripapillary surround in terms of displacement (e.g., of blood from capillaries or axoplasm from axons as they enter the ONH) or of redistribution of tissues within the neural canal (e.g., if the canal is expanding during acute IOP elevation). To this end, similar to what we have found in the non-human primate eye (Strouthidis NG, et al. IOVS 2011;52: ARVO E-Abstract 4811),20 there was no evidence in this study that the anterior portion of the neural canal expanded during acute IOP elevation because there was no increase in either the BMO or the choroidal border tissue areas. Nevertheless, expansion limited to the more posterior depths of the neural canal could result in tissue redistribution and apparent thinning such as that observed in this study and in others on non-human primates (Strouthidis NG, et al. IOVS 2011;52: ARVO E-Abstract 4811)20 and human patients.21 Indeed, chronic IOP elevation in the rat has been shown to result in substantial expansion of the scleral canal (Pazos M, et al. IOVS 2010;51: ARVO E-Abstract 4806).30 Ongoing studies using “enhanced depth imaging” or longer wavelength source SD-OCT devices might help to resolve some of these questions.

Similarly, these other techniques might enable better visualization of the full choroid and the anterior scleral surface so as to provide a more accurate and reliable estimate of choroidal thickness than was available in this study. Assessment of choroidal thickness changes was not the primary purpose of the present study; rather, this study was focused on the ONH and retina. It is also important to note that the tissue thickness values inside the area of the BMO are not true thicknesses because they represent the distance from the ONH surface to a reference plane defined in this study only in two dimensions (i.e., within each individual B-scan). Nevertheless, the peripapillary changes were based on distances between actual B-scan features, and the results were continuous with the changes inside the BMO area providing some validation of the ONH changes observed during acute IOP elevation.

In summary, the results of this study demonstrate that acute IOP elevation in the rodent eye causes rapid, but reversible, posterior deformation of the ONH and thinning of the peripapillary retina, with only minimal retinal thinning beyond 5° of the ONH. These data add further evidence to suggest that the site of injury to retinal ganglion cell axons in rodent experimental models of glaucoma based on IOP elevation is within or adjacent to the ONH.

References


