The Effect of Acute Intraocular Pressure Elevation on Peripapillary Retinal Thickness, Retinal Nerve Fiber Layer Thickness, and Retardance

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PURPOSE. To determine whether acutely elevated intraocular pressure (IOP) alters peripapillary retinal thickness, retinal nerve fiber layer thickness (RNFLT), or retardance.

METHODS. Nine adult nonhuman primates were studied while under isoflurane anesthesia. Retinal and RNFLTs were measured by spectral domain optical coherence tomography 30 minutes after IOP was set to 10 mm Hg and 60 minutes after IOP was set to 45 mm Hg. RNFL retardance was measured by scanning laser polarimetry in 10-minute intervals for 30 minutes while IOP was 10 mm Hg, then for 60 minutes while IOP was 45 mm Hg, then for another 30 minutes after IOP was returned to 10 mm Hg.

RESULTS. RNFLT measured 1120 μm from the ONH center decreased from 118.1 ± 9.3 μm at an IOP of 10 mm Hg to 116.5 ± 8.4 μm at 45 mm Hg, or by 1.4% ± 1.8% (P < 0.0001). There was a significant interaction between IOP and eccentricity (P = 0.0006). Within 800 μm of the ONH center, the RNFL was 4.9% ± 3.4% thinner 60 minutes after IOP elevation to 45 mm Hg (P < 0.001), but unchanged for larger eccentricities. The same pattern was observed for retinal thickness, with 1.1% ± 0.8% thinning overall at 45 mm Hg (P < 0.0001), and a significant effect of eccentricity (P < 0.0001) whereby the retina was 4.8% ± 1.2% thinner (P < 0.001) within 800 μm, but unchanged beyond that. Retardance increased by a maximum of 2.2% ± 1.1% 60 minutes after IOP was increased to 45 mm Hg (P < 0.0051).

CONCLUSIONS. The effects of acute IOP elevation on retinal thickness, RNFL thickness and retardance were minor, limited to the immediate ONH surround and unlikely to have meaningful clinical impact. (Invest Ophthalmol Vis Sci. 2009;50:4719–4726) DOI:10.1167/iovs.08-3289

Retinal nerve fiber layer (RNFL) defects are a structural manifestation of the damage caused by glaucoma1 and frequently precede the development of glaucomatous vision loss.2–6 Therefore, assessment of the RNFL is recognized as an important procedure in the routine clinical care of all patients with glaucoma or suspected glaucoma. However, detecting such defects during their earliest stages by clinical fundoscopy or photography can be difficult,7 and so it is hoped that advancements in imaging techniques, such as optical coherence tomography (OCT)8–11 and scanning laser polarimetry (SLP),12,13 will improve clinical capabilities for detecting early glaucomatous RNFL damage and its progression.14–18

RNFL assessment by OCT8–11 capitalizes on the fact that the RNFL produces relatively strong reflectance of the imaging source, whereas SLP12,13 detects the relative-phase retardance of a polarized light source induced by the birefringent properties of the RNFL. Both of these optical characteristics are thought to be due to the long, thin, cylindrical shape and parallel orientation of the internal cytoskeleton—principally the microtubules—within the retinal ganglion cell (RGC) axons of the RNFL.20–26

Acute and chronic intraocular pressure (IOP) elevation are known to cause cytoskeletal abnormalities and disrupt axonal transport, resulting in organelle accumulation and axon swelling near the lamina cribrosa.27–34 It is therefore possible that RNFL measurements such as those obtained with OCT or SLP are affected by elevated IOP through such secondary effects, or perhaps directly by compression. It is important to differentiate the relatively acute IOP-related effects from chronic disease-related changes to arrive at accurate clinical diagnoses and reveal potentially meaningful pathophysiologic information or susceptibility. Currently, it is unknown whether peripapillary RNFL thickness and retardance are affected by the acute level of IOP. Therefore, the purpose of this study was to determine whether acute IOP elevation affects peripapillary RNFL thickness or retardance measurements in normal nonhuman primate (NHP) eyes.

METHODS

Subjects

In total, nine adult female rhesus macaque monkeys (Macaca mulatta) were included in the study: five in the retinal and RNFL thickness experiment and four in the retardance experiment. Table 1 lists the age and weight of each animal, as well as the experiment to which each was assigned. All experimental methods and animal care procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the local Institutional Animal Care and Use Committee (IACUC).

Anesthesia

All experimental procedures began with induction of general anesthesia with ketamine (15 mg/kg IM) and midazolam (0.2 mg/kg IM). A single subcutaneous injection of atropine sulfate (0.05 mg/kg) was also administered. The animals were then intubated and a mixture of 100% oxygen and isoflurane gas (1%–2%) maintained anesthesia for the duration of the imaging session. Heart rate and arterial oxyhemoglobin saturation were monitored continuously (Propan Encore model 206EL;
Table 1. Age, Weight, and Experimental Group Assignment for Each Animal

<table>
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<th>Animal</th>
<th>Age (y)</th>
<th>Weight (kg)</th>
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<td>9</td>
<td>1</td>
<td>2.7</td>
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* The number of radial B-scans acquired during the experiment.

Protocol Systems, Inc., Beaverton, OR) and maintained above 75 minutes 1 and 95%, respectively. Body temperature was maintained with a warm-water heating pad set at 37°C. Blood pressure was measured approximately every 10 minutes (NIBP system 7100; Advanced Medical Instruments, Inc., Broken Arrow, OK) and maintained above a mean arterial pressure of 75 mm Hg with intravenous lactated Ringer’s delivered if necessary (10 mL/kg/h). If mean arterial pressure decreased below 75 mm Hg despite administration of intravenous fluid, the imaging session was discontinued, and the animal recovered immediately.

Manometric IOP Control

After application of topical anesthesia (0.5% proparacaine), the anterior chamber was cannulated by insertion of a 27-gauge needle through the peripheral cornea. The needle was connected via polyethylene tubing to an adjustable-height reservoir of sterile balanced salt solution (BSS; Alcon Laboratories, Inc., Fort Worth, TX). The height of the reservoir was precalibrated in 5-mm Hg increments with a pressure transducer (MX860; Medex, Inc., Carlsbad CA). OCT and SLP scans were obtained with IOP set to 10 mm Hg (baseline) and 45 mm Hg (acutely elevated IOP), as outlined in the following sections for each experiment. At the end of the imaging session, the needle was removed and a broad-spectrum topical antibiotic ointment was applied.

Experiment 1: Data Acquisition

RNFL and total retinal thickness measurements were obtained by spectral-domain optical coherence tomography (SD-OCT; Spectralis OCT; Heidelberg Engineering, GmbH, Heidelberg, Germany). The optical resolution of the instrument is 7 μm axially and 14 μm transversely. The depth of each A-scan is 1.8 mm and consists of 512 pixels, providing a digital depth sampling of 3.5 μm per pixel. Each B-scan spans 15° and consists of 768 A-scans providing a digital transverse sampling of 5 μm per pixel (in an emmetropic human eye with average axial length). For this experiment, B-scans were arranged in a radial pattern centered on the optic disc. The radial pattern consisted of 48 B-scans for one subset of animals (n = 2) and 80 B-scans for the others (n = 3, see Table 1). In the latter cases, only 40 of the 80 B-scans were used (i.e., manual segmentation was performed on every second scan beginning with the vertical scan). A real-time eye-tracking system measures eye movements and provides feedback to the SD-OCT scanning system to stabilize the retinal position of each B-scan. This feature allows sweep averaging to be used for reduction of speckle noise. For this experiment, nine sweeps were averaged for each B-scan.

OCT raw data were then exported from the SD-OCT instrument to complete manual segmentation of B-scan features using custom-built, multiview, 3-D visualization and delineation software (based on the Visualization Toolkit; VTK, Clifton Park, NY). Figure 1 provides an example of the segmentation process. The B-scan shown in Figure 1A represents the vertical section from a radial pattern with 80 B-scans total. It was acquired 30 minutes after IOP was set to 10 mm Hg. The colored marks were positioned manually within the software to begin segmentation of the various B-scan features delineated in this study. As the marks of each feature class are placed, the Bézier curves shown in corresponding colors are fitted in real time. The position of each mark is adjusted manually so that the Bézier curve accurately delineates the intended feature. The initial segmentations were performed by a trained technician, then one of the investigators (NS) reviewed the segmentations for fine tuning and/or corrections. Both technician and investigator were masked to the IOP condition of all scans.

In Figure 1A, the green marks and green Bézier curve represent the border between the vitreous and the inner limiting membrane (ILM), which was defined as the anterior RNFL border for the purposes of this study. The blue marks and blue Bézier curves on either side of the ONH represent the posterior RNFL border. The orange marks and orange Bézier curves represent the posterior aspect of the retinal pigment epithelium–Bruch’s membrane (RPE–BM) complex. The pair of red marks on each side of the ONH defines the neural canal opening (NCO). Figure 1B shows the full set of marks, for these four segmentation classes and all 40 radial B-scans, overlaid onto the infrared reflectance image acquired for this eye at the time of the OCT scan. In Figure 1D, the corresponding Bézier curves are shown above the reflectance image from a different perspective.

The schematic shown in Figure 1C demonstrates the method used to derive RNFL thicknesses from the B-scan segmentation results. First, the Bézier curve defining the posterior RNFL surface is sampled in 10-μm intervals. From each of these sampled points along the posterior RNFL border, a vector is cast within the plane of the B-scan toward the segmented anterior RNFL border, to determine the minimum distance between them. In this schematic, the vector solution for each sampled point is shown as a purple-colored line. A radius is also calculated for each sampled point along the posterior RNFL border. The radius is the distance to the centroid of an ellipse, which itself is fitted to the family of segmented NCO points. Figure 1C, three of the RNFL thickness vectors are shown as black arrows pointing toward the anterior RNFL border. Each of these three sample points also has a gray arrow pointing toward the plane of the NCO ellipse, where its intersection defines the coordinate used to determine the radius. This coordinate system enabled alignment of paired SD-OCT volumes (baseline and elevated IOP time points) by translation to a commonly defined origin (the NCO centroid) and by rotation to commonly defined radial axes (the major and minor axes of the NCO ellipse). It thus helped minimize error due to minor differences in scan pattern placement between time points (e.g., Figs. 1E, 1F, show alignment) and enabled derivation of difference maps for topographic visualization of results (e.g., Fig. 1G).

Total retinal thickness measurements were derived in the same manner, except that the Bézier curve defining the posterior aspect of the RPE–BM complex was sampled rather than the posterior RNFL border. Similar topographic maps were used to visualize the results (e.g., Figs. 1H–J). These continuous maps were constructed by interpolating values between each radial B-scan.

Experiment 1 Design and Analysis: Effect of Acute IOP Elevation on RNFL Thickness and Total Retinal Thickness

The experiment was performed bilaterally during a single session for each of the five animals (see Table 1). Both anterior chambers were cannulated, and IOP was set to 10 mm Hg. OCT scans were obtained in each eye after a 30-minute stabilization period. The scan obtained after 30 minutes of IOP set at 10 mm Hg was defined as the baseline against which the scans obtained after acutely elevated IOP were compared. In addition, in six eyes of three animals (subjects 5, 6, and 7; Table 1), we also obtained a scan 10 minutes after IOP had been set to 10 mm Hg. This scan was paired with the 30-minute time point at an IOP of 10 mm Hg, to serve as a control for evaluation of interscan repeatability in the absence of any IOP change. After the scan was obtained at 30 minutes with IOP set to 10 mm Hg in all 10 eyes, IOP was then raised to 30 mm Hg for 30 minutes, at which time the OCT scans were repeated. IOP was then raised to 45 mm Hg for an addi-
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RNFL Thickness and Retardance after Acute IOP Elevation

**Experiment 2: Data Acquisition**

RNFL retardance measurements were obtained by SLP (GDxVCC; Carl Zeiss Meditec, Inc., Dublin, CA). The instrument compensates for the effects of anterior segment birefringence to more accurately determine RNFL retardance.\(^{13,36}\) Thus, the axis and magnitude of anterior segment birefringence are derived from uncompensated images before initial baseline RNFL scans and then are used to compensate all subsequent RNFL scans. A bite bar that rotated in three axes was used to properly align the head and eye, and autorefraction was used for each scan. Three RNFL scans were averaged for each eye at each time point.

The GDxVCC instrument detects the relative phase retardance of a cross-polarized laser imaging source after a double pass through the tissue sample, assumes that RNFL thickness is linearly related to retardance, and then calculates and reports an estimate of RNFL thickness by using a linear conversion factor of \(0.67 \, \text{nm/} \mu\text{m}\).\(^{12,37}\) RNFL thicknesses were exported for the small peripapillary locus (the SLP instrument’s default), divided by the conversion factor, and reported herein as the retardances. The exported data consisted of 64 samples along a peripapillary locus beginning on the temporal side of the ONH; proceeding around the superior, nasal, and inferior aspects of the ONH; and completing a circle at the temporal location, thus representing a profile commonly referred to as a TSNIT curve. Each of the values in the TSNIT curve is an average from an 8-pixel-wide band centered on the optic disc.\(^{37}\) The inner and outer limits of the band are 27 and 35 pixels from the center of the optic disc, so that the center of the band has a radius of 31 pixels.\(^{37}\) This measure corresponds to a scan angle with a radius of \(6.1^\circ\), which translates to approximately \(1120 \, \mu\text{m}\) on the macaque retina (assuming an emmetropic eye with average axial length of \(19 \, \text{mm}\)).\(^{38–40}\)

**Experiment 2 Design and Analysis: Effect of Acute IOP Elevation on RNFL Retardance**

Only one eye was imaged by SLP during a given session, to maintain optimal alignment and increase temporal resolution. Initially, a set of three SLP scans was obtained before anterior chamber cannulation; thereafter, another set of three SLP scans was obtained at each time point. Time points were separated by 10-minute intervals. There were three time points during the baseline stabilization period after cannulation during which IOP was set to 10 mm Hg. IOP was then raised to 45 mm Hg and a set of three SLP scans was obtained every 10 minutes for 1 hour (six time points). IOP was then lowered to 10 mm Hg, and recovery was observed for 30 minutes, again with a set of three SLP scans averaged at each of three recovery time points.
RM-ANOVA was applied to assess the main effect of acute IOP elevation using the raw retardance samples from the peripapillary (TSNIT) profile for each eye and time point. Two-way RM-ANOVA was also applied to determine whether there was significant interaction between subject and acute IOP elevation.

**RESULTS**

After 60 minutes of acute IOP elevation from 10 to 45 mm Hg, the most readily apparent changes revealed by SD-OCT were in the architecture of the ONH, as previously reported (Burgoyne CF, et al. IOVS 2008;49:ARVO EAbstract 3655). Figure 2 shows a horizontal section through the center of the ONH of one eye. The B-scan in the top panel was acquired 30 minutes after IOP was set to 10 mm Hg. The B-scan in the bottom panel was acquired after 60 minutes of IOP set to 45 mm Hg. Changes in the shape of the peripapillary connective tissues were also consistently observed, such as posterior bowing of the RPE-BM complex immediately adjacent to the ONH (arrows).

The effect of acute IOP elevation on RNFL thickness was analyzed first for this particular eccentricity, because it matches the standard SLP peripapillary measurement locus. RNFL thickness at this eccentricity was 118.1 ± 9.3 μm 30 minutes after IOP was set to 10 mm Hg (n = 10 eyes, five animals). Then 60 minutes after IOP was increased to 45 mm Hg, the average RNFL thickness at this eccentricity was 116.5 ± 8.4 μm, or 1.4% (1.8%) thinner (P < 0.0001, RM-ANOVA). The effect of acute IOP elevation was thus small in magnitude but statistically significant, because it was consistent across eyes and peripapillary (TSNIT) locations. The latter two variables accounted for nearly all the RNFL thickness variability (15%, P < 0.0001 and 83%, P < 0.0001, respectively). There was no significant interaction between acute IOP elevation and radial peripapillary (TSNIT) location (i.e., polar angle, P = 0.80); however, it is possible that the 1.4% thinning represents constriction of the retinal blood vessels within the RNFL, as Figure 3 shows that the effect on average manifested predominantly at the superior and inferior positions where retinal vessels make a substantial contribution to the cross-sectional area of the RNFL.

When the RNFL thickness analysis included data from all eccentricities, a similarly small degree of thinning was found overall (2.7% ± 3.6%, P < 0.0001, two-way RM-ANOVA). However, there was a significant interaction between elevated IOP and retinal eccentricity (P = 0.0006) such that the effect was larger closest to the ONH and diminished with increasing distance from the NCO (Fig. 4). Within 800 μm of the NCO, the RNFL was 4.9% ± 3.4% thinner after 60 minutes of IOP elevation to 45 mm Hg (P < 0.001), whereas there was no significant change for eccentricities larger than 800 μm (analyzed in eccentricity bins of 250 μm; Fig. 4). Note that the eccentricities represent the linear dimension converted from
The retina was 4.8% thinner at an eccentricity of 800 μm from the center of the ONH (Fig. 6). In the six eyes in which control data were available, total retinal thickness was 1.0% thinner 30 minutes after IOP was set to 10 mm Hg, compared with 10 minutes of IOP set to 10 mm Hg (P = 0.01). Thus, the effect of acute IOP elevation on retinal thickness was small and approximately equal to the magnitude of interscan variability of the control condition. In contrast to the effect of acute IOP elevation; however, there was no significant interaction between eccentricity and time for the control comparison (P = 0.78), suggesting that the small degree of retinal thinning near the ONH after acute IOP elevation is a real effect.

Figure 7 shows the result of acute IOP elevation on RNFL retardance. During the 30-minute baseline period when IOP was 10 mm Hg, the average peripapillary RNFL retardance was 36.1 nm (n = 8 eyes, four monkeys). This increased to an average of 36.6 nm during the 60-minute period when IOP was elevated to 45 mm Hg (P = 0.0031, RM-ANOVA). This small but statistically significant increase is more clearly visualized in Figure 7B, where retardances are normalized relative to the average baseline for each eye. The average increase across the 60-minute period of IOP elevation was 1.5%; the peak effect, which occurred at the final 60-minute time point, was 2.2% ± 1.1%. RNFL retardance varied across subjects as expected (P < 0.0001), and there was a high correlation between the eyes of each subject (P < 0.0001); however, there was no significant interaction between the effects of acute IOP elevation and subject (P = 0.61, with eyes matched for each subject).

**DISCUSSION**

The results of this study show that acute IOP elevation from 10 to 45 mm Hg in healthy NHP eyes had only minor effects on...
peripapillary retinal thickness, RNFL thickness, and retardance. Although the effects on ONH architecture and immediately adjacent structures could be relevant to glaucoma pathophysiology, the minor effects observed here for the peripapillary retina and RNFL are not likely to have meaningful impact on clinical practice. Indeed, the effects were so small that one could interpret the results to mean that acute IOP level is not important in clinical measurements of the peripapillary retina and RNFL. That is, when patients present with elevated IOP at the time of clinical imaging, the outcome is likely to represent the chronic status of the peripapillary retina and RNFL rather than any direct effect of acutely elevated IOP.

The small decrease observed in both retinal and RNFL thickness 60 minutes after IOP was elevated to 45 mm Hg occurred only in the immediate peripapillary region, where the connective tissue architecture was also observed to change (Fig. 2). Thus, although the results for larger distances from the center of the ONH suggest that neither the retina nor the RNFL thickness after surgical or medical IOP reduction in glaucoma patients. Although the results of the present study are based on acute IOP elevation in healthy NHP eyes rather than long-term follow-up after IOP-lowering treatment in patients with glaucoma, they suggest one possibility for the discrepancy among the results in those other studies. In the initial study, Aydin et al. used a prototype OCT system, whereas the investigators in the other two recent studies used a commercial Stratus OCT instrument. It is possible that the average eccentricity was closer to the ONH in Aydin et al. than in the other studies, even though all three reported using an approximately 1.7-mm radius for the circumpapillary scan. For example, the two studies reporting no change in RNFL thickness may have included a larger number of subjects with axial myopia, for whom the circumpapillary scan radius would have been effectively larger. In any case, the results of the present study suggest that analysis of more than a single peripapillary eccentricity could be beneficial to the study of both acute and chronic effects of IOP. Further, the present results suggest that if a single peripapillary circular sample is used to estimate RNFL thickness, it should be sufficiently distant from the ONH center to minimize effects of ambient IOP level. Most current systems sampling at a single eccentricity meet this criterion.

In contrast to the minor degree of retinal and RNFL thinning observed after acute IOP elevation, RNFL retardance actually increased, though by a similarly small percentage. Two minutes after a 45-second period of acute IOP elevation to 100 mm Hg in human eyes, Lester et al. observed a 0.3% increase in retardance, a similarly small change that was not statistically significant in their study. Although acute IOP elevation is known to alter axonal cytoskeletal components, an alternative explanation may be that the conformational changes of the peripapillary retina noted during acute IOP elevation (e.g., Fig. 2) cause the SLP scan path through the RNFL (and retina) to increase slightly and perhaps thus encounter a proportionally greater number of cytoskeletal elements. In the absence of any change in RNFL thickness, the increased path length would vary inversely with the cosine of the increased angle of incidence at the ILM. By this model, the observed 1.4% retardance increase would require an approximately 10° change to have occurred in the angle of incidence between the scanning beam and RNFL. This angle is more than a factor of two larger than that observed in most eyes, including the example shown in Figure 2, which is consistent with the average posterior displacement of the NCO being only 41 μm. Thus, it is unlikely that this simple conformational model offers a complete explanation of the small change in retardance observed during acutely elevated IOP.

Although we did not measure axial length in this study, previous studies have shown that axial length measured by conventional A-scan ultrasonography (thus presumably close to or along the optical axis of the eye) does not change with acute IOP elevation to either 30 or 45 mm Hg. The results of
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