Regional Deformation of the Optic Nerve Head and Peripapillary Sclera During IOP Elevation

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PURPOSE. To measure the deformation of the porcine optic nerve head (ONH) and peripapillary sclera (PPS) in response to intraocular pressure (IOP) elevation.

METHODS. High-frequency ultrasound was used to image the ONH and PPS of 12 porcine eyes during ex vivo inflation testing from 5 to 30 mm Hg. A speckle tracking algorithm was used to compute tissue displacements in the anterior-posterior direction and expansion of the scleral canal. Through-thickness, in-plane, and shear strains were calculated within the ONH. Regional displacements and strains were analyzed and compared.

RESULTS. The ONH and PPS showed overall posterior displacement in response to IOP elevation. Posterior displacement of the ONH was larger than and strongly correlated with the posterior displacement of the PPS throughout inflation testing. Scleral canal expansion was much smaller and leveled off quicker than ONH posterior displacement as IOP increased. Through-thickness compression was concentrated in the anterior ONH, which also experienced larger in-plane and shear strains than the posterior ONH. Within the anterior ONH, all three strains were significantly higher in the periphery compared with the center, with the shear strain exhibiting the greatest difference between the two regions.

CONCLUSIONS. High-resolution ultrasound speckle tracking revealed the full-thickness mechanical response of the posterior eye to IOP elevation. A mismatch in posterior displacement was found between the ONH and PPS, and regional analyses showed a concentration of strains within the periphery of the anterior porcine ONH. These deformation patterns may help in understanding IOP-associated optic nerve damage and glaucoma susceptibility.

Keywords: glaucoma, biomechanics, ultrasound, optic nerve head, peripapillary sclera

The axons of retinal ganglion cells are damaged in glaucoma, resulting in progressive and irreversible vision loss. The optic nerve head (ONH) has been a focus in glaucoma research, as this region was shown to exhibit the earliest signs of axonal damage. At the ONH, the axons converge into bundles to pass through the pores of the cribriform lamina cribrosa (LC), which spans the scleral canal and is continuous with the adjacent peripapillary sclera (PPS). The LC and PPS are the primary load-bearing structures of the posterior eye that provide structural and functional support to the axons.

One of the main risk factors for developing glaucoma is elevated intraocular pressure (IOP). Increases in IOP cause increased stresses and strains within the ONH and PPS and these mechanical insults may directly impact axonal function or impair blood flow by compressing, stretching, or shearing the blood vessels or LC beams. Evidence has also shown that astrocytes within the ONH are mechanosensitive and their responses to increased mechanical loading may result in reduced trophic support to axons or detrimental extracellular matrix remodeling. Determining the role of these potential mechanisms requires a thorough understanding of the mechanical environment experienced by the cells and tissues in this region. It is therefore important to measure the deformation of the tissues within and around the ONH in response to IOP elevation.

A number of imaging methods have been used to study the IOP-related deformation of the ONH. Optical coherence tomography (OCT) and second harmonic-generated imaging have emerged as valuable tools for high-resolution measurement of ONH deformation, but penetration depth is limited to the superficial couple hundreds of microns. Phase-contrast microcomputed tomography (PC-μCT) can achieve better penetration depth but may not be cost effective for a large sample size. These previous studies have also focused primarily on the ONH and have not analyzed the relationship between the ONH and the surrounding PPS in the same eye, which will likely provide a more complete understanding of ONH biomechanics.

Our laboratory has developed 2D and 3D high-frequency ultrasound speckle tracking methods and used them to measure IOP-induced deformation of the cornea and posterior sclera. High-frequency ultrasound achieves a balance between resolution and penetration depth, enabling accurate measurement of small deformations and full-thickness imaging of both the ONH and PPS simultaneously. In this study, we used our ultrasound speckle tracking technique to measure displace-
ments and strains within the porcine ONH and PPS during controlled IOP elevation. The goals of the study were to quantify and map the mechanical behavior of the ONH and PPS throughout the entire thickness of the tissues and to evaluate regional variability in their responses to elevated IOP.

METHODS

Sample Preparation

Twelve porcine globes from animals between 4 and 6 months old were obtained from a United States Department of Agriculture-approved abattoir under Hazard Analysis and Critical Control Points guidelines (SiouxPreme Packing Co., Sioux City, IA, USA). The globes were immersed in saline and kept cold during shipping, and all eyes were tested within 72 hours postmortem. The extracocular tissues were trimmed away, and the optic nerve was cut to be flush with the outer surface of the PPS (Fig. 1A). Eyes were mounted on a customized pressurization chamber to undergo inflation testing (Fig. 1B). A small portion of the anterior globe opposite to the ONH, consisting mostly of corneal tissue and a small amount of anterior sclera, was removed using a 7.5-mm trephine. The lens and vitreous were removed and the head of a hollow plastic mounting screw was inserted into the eye via the trephined hole. Two O-rings, one on either side of the ocular shell, were used to facilitate a fluid-tight seal. A small amount of cyanoacrylate glue was placed around the outer O-ring to ensure the integrity of the seal. The mounting screw was then wrapped with polytetrafluoroethylene tape and inserted into the pressurization chamber.

Inflation Testing

The eye and pressurization chamber were immersed in 0.9% saline. The chamber contained fluid channels that connected the end of the mounting screw to a programmable syringe pump (PHD Ultra, Harvard Apparatus, Holliston, MA, USA) and a pressure sensor (P75, Harvard Apparatus) to continuously control and monitor IOP. Each globe was preconditioned using 20 pressure cycles from 5 to 30 mm Hg, followed by an equilibration period of 30 minutes at 5 mm Hg. Inflation testing was then performed by increasing IOP from 5 to 50 mm Hg, with pressure steps of 0.5 mm Hg. A 55-MHz ultrasound probe (Vevo 660, VisualSonics Inc., Toronto, Canada) was positioned along the naso-temporal meridian of the eye (Fig. 1B). After a 15-second delay at each IOP step, a 2D image of the ONH and PPS was obtained at a frame rate of ~10 frames/sec before increasing IOP to the next pressure level. IOP control and data acquisition were implemented using a customized LabView interface (National Instruments, Austin, TX, USA).

Ultrasound Speckle Tracking

The 2D ultrasound speckle tracking technique used in this study has been validated previously. Speckle tracking is performed using the radiofrequency data from each ultrasound image. The radiofrequency data are sampled discretely to generate pixels, and the sampling rate determines the distance between pixels. The pixel height is 1.5 µm in the vertical direction (i.e., the direction of ultrasound propagation and anterior-posterior direction anatomically), and the pixel width is 20.8 µm in the horizontal direction (i.e., the direction perpendicular to the direction of ultrasound propagation and nasal-temporal direction anatomically).

The first step in the speckle tracking algorithm is to divide the tissue into a grid of rectangular kernels, each containing roughly 1500 pixels. The kernel dimensions are 76.5 µm × 644.8 µm (vertical × horizontal), and the kernels are overlapped by 50%. Previous studies have shown that overlapping kernels by 50% results in the best combination of strain spatial resolution and strain signal-to-noise ratio. Each kernel is tracked individually between images acquired at different IOP levels, and cross-correlation is used to find the new kernel location. The vertical and horizontal components of the displacement vector are calculated with respect to the kernel locations at the initial IOP level (i.e., 5 mm Hg). The displacement of kernels with a correlation coefficient below 0.8 or with a displacement that differs by more than two standard deviations from the average displacement in the 5 × 5 neighborhood of the kernel is replaced by the neighborhood average to reduce noise in the displacement field. 2D least squares strain estimation is used to calculate the vertical and horizontal strains for each kernel based on the 5 × 5 neighborhood of kernels. A coordinate transformation converts the strains from the vertical and horizontal directions to the through-thickness (perpendicular to the tissue curvature) and in-plane (parallel to the tissue curvature) directions (Fig. 2A):
where $\varepsilon_x$ and $\varepsilon_y$ are the horizontal and vertical strains, $\varepsilon_r$ and $\varepsilon_{th}$ are the through-thickness and in-plane strains, and $\theta$ is the angle between the two coordinate systems. $\theta$ is calculated from the coordinates of the kernel and those of the center of the sphere fit to the contours of the ONH and PPS. The remaining strain variables represent the shear components, and the magnitudes (absolute values) were used for quantitative analyses.

### Segmentation and Regional Analyses

After speckle tracking and displacement calculations, the kernels were manually segmented into those that belonged to the ONH and those that belonged to the PPS. The boundaries between the ONH and PPS were determined using the B-mode ultrasound images, because the sclera is much brighter than the ONH in ultrasound images. The boundaries between the ONH and PPS were selected to be the location where the bright signal from the sclera abruptly ended when moving from the edge of the image toward the ONH. The kernels between the left and right boundaries were used for calculating the average ONH displacements and strains, and the kernels outside of the boundaries were assigned to the PPS. It is noted that the subjective selection of the boundary line was performed using the B-mode image (i.e., at the pixel level). Although the interobserver difference in selecting the boundary could be up to 5 pixels according to our experiences, our results showed that the segmentation of kernels was minimally affected because the kernels were larger in size and their locations were determined prior to segmentation.

Expansion of the scleral canal was calculated as the difference in the average horizontal displacement between the PPS on either side of the ONH. The strains were compared for the anterior and posterior ONH and for the central and peripheral regions of the anterior ONH (Fig. 2B). The inner and outer boundaries of the ONH were fit by two concentric circles, and the average radius of the inner and outer fits was used to split the kernels within the ONH into equal-thickness anterior and posterior halves (Fig. 3B). The kernels in the anterior ONH were further segmented into central and peripheral regions by dividing them into equal horizontal thirds. The average strain in the central region of the anterior ONH was calculated from the strain values of the kernels in the middle third, and the average strain in the periphery was calculated using the strain values of the kernels in the peripheral two thirds. Strains were not calculated for all edge kernels, which did not have a full $5 \times 5$ neighborhood of kernels.

The differences in displacement or strain between different regions were evaluated at each IOP level by using paired $t$-tests. The reported $P$ values were computed without multiple comparison corrections. The conclusion of significant difference between regions was based on all tests at different pressure levels, with the Hochberg's step-up method controlling family-wise error rate of 0.05.16 Hochberg's step-up method declares that all tests are significant if the highest $P$ value of these tests is less than 0.05, which is more powerful than the Bonferroni method or the Holms procedure for multiple comparison adjustment. As a sensitivity analysis, linear mixed models for repeated measures (at selected IOP levels and different regions for each eye) were used to confirm...
the overall differences between regions. In addition, the Pearson correlation coefficients of the measures of different regions were evaluated at each pressure level.

**Histology**

Histologic analyses were performed to identify the structures that were present in each region of the porcine ONH and PPS. Three eyes were immersed in a 10% formalin solution for 24 hours following the inflation testing. A tissue section was prepared from each eye along the nasal-temporal meridian, corresponding approximately to the cross-section imaged using ultrasound. The sections were stained using hematoxylin and eosin and compared with the ultrasound images to confirm the location of corresponding anatomic structures.

**RESULTS**

Histology images revealed the LC spanning the width of the scleral canal, with its insertion points located near the anterior boundary of the PPS (Fig. 3). This LC location is consistent with previous reports in porcine eyes, and corresponded to a region of higher echogenicity within the ONH in the ultrasound images. A division of the ONH into anterior and posterior halves showed the LC present within the anterior half of the ONH in both the ultrasound and histology images. The posterior layer appeared to be composed primarily of the retrolaminar neural tissue.

The ONH and PPS were both displaced posteriorly during IOP elevation in all 12 eyes. The relationship between IOP and posterior displacement was nonlinear. The ONH had consistently larger posterior displacement than the PPS at all IOP levels ($P < 0.001$; Figs. 4A, 5A). At the peak IOP of 30 mm Hg, displacement of the ONH was $348.9 \pm 82.2 \mu m$ (mean $\pm$ SD) ranging from 213.1 to 514.9 $\mu m$, and displacement of the PPS was $219.7 \pm 65.8 \mu m$ (mean $\pm$ SD) ranging from 131.4 to 351.5 $\mu m$. The average difference between ONH and PPS posterior displacement was $129.2 \pm 24.7 \mu m$. Although there was a substantial difference in magnitude, a strong positive correlation was found between the posterior displacement of the ONH and PPS ($R > 0.96$, for all IOP levels; Fig. 5B).

**FIGURE 4.** (A) Displacement maps for three porcine eyes at 30 mm Hg. Positive displacements correspond to posterior vertical movement or rightward horizontal movement (the color bar is adjusted for each direction). The ONH had a larger vertical (posterior) displacement than PPS. The horizontal displacement was negative (blue) in the left PPS and positive (red) in the right PPS, indicating canal expansion. The horizontal displacements of the ONH were not included in the calculation of canal expansion and are not shown. (B) Strain maps for the same three eyes at 30 mm Hg. Large compressive through-thickness strains were mostly in the anterior ONH, whereas the in-plane and shear strains were largely concentrated in the periphery of the anterior ONH. The shear strains were opposite in sign for the left and right sides of the ONH, indicating posterior bending. The yellow dashed lines in the ultrasound images show the separation of the ONH and PPS. The strains within the PPS were not calculated. The maps were generated after interpolation and smoothing of the kernel displacements and strains.
Expansion of the scleral canal also exhibited a nonlinear increase during inflation, leveling off quickly after IOP reached about 10 mm Hg (Fig. 6A). The average expansion was $127.7 \pm 31.3 \mu m$ (min, 76.6 \mu m; max, 163.5 \mu m) at 30 mm Hg. The posterior displacement of the ONH was greater in magnitude than the expansion of the scleral canal throughout the inflation test ($P < 0.001$ for all IOP levels; Figs. 4A, 6A). The ONH posterior displacement and canal expansion were correlated at lower levels of IOP, with a larger posterior displacement associated with more canal expansion. However, the correlation weakened with increasing IOP (Figs. 6B–D).

The strains within the ONH showed nonlinear increases during IOP elevation (Fig. 7A). In all eyes, the strains exhibited substantial depth-dependent variability (Fig. 4B). The magnitudes of all three strains were significantly higher in the anterior ONH compared with the posterior ONH ($P < 0.001$ at all IOP levels for each type of strain; Fig. 7A). The largest measured deformation at 50 mm Hg was through-thickness compression of the anterior ONH (Table 1). In the posterior ONH, some eyes showed through-thickness compression, whereas others exhibited through-thickness expansion, causing the average across the 12 eyes to be close to zero. In both the anterior and posterior ONH, the through-thickness strain was significantly larger than the in-plane and shear strains ($P \leq 0.005$ for both comparisons at all IOP levels). The in-plane strains in the anterior and posterior ONH were positively correlated at every IOP level (all but one $R > 0.75$; Fig. 7B). The anterior and posterior shear strain magnitudes were correlated with $R > 0.6$ at levels up to 19 mm Hg, but the correlation steadily decreased at higher IOP levels (Fig. 7B). The through-thickness strains for the two regions were correlated with $R > 0.6$ only at a few IOP levels below 9 mm Hg.

In the anterior ONH, the largest deformations were concentrated at the periphery (Fig. 4B). Both the in-plane stretch and shear strain were significantly higher in the periphery than the center throughout the inflation ($P < 0.02$ for all IOP steps; Fig. 8A). At 30 mm Hg, the in-plane stretch and shear strains were approximately 2 times higher in the periphery compared with the center, whereas the through-thickness compression was about 1.2 times higher (Table 2). The through-thickness compression was consistently larger than the in-plane stretch and shear strain in both the center and periphery ($P < 0.002$ for all IOP steps). A correlation between the central and peripheral regions was found at all IOP levels for the through-thickness compression and at all IOP levels except 5.5 mm Hg for the in-plane stretch (all $R > 0.8$; Fig. 8B). The shear strains were also correlated between the two regions for the majority of the IOP steps (all $R > 0.6$).

**DISCUSSION**

To the best of our knowledge, this is the first study to simultaneously measure the mechanical behavior of both the ONH and PPS in the porcine eye, which has a collagenous LC structure similar to that of the human eye. The primary findings include the following:

- During an acute IOP increase, both the ONH and PPS were displaced posteriorly, the ONH exhibited a significantly larger posterior displacement than the PPS, and the displacements of the two regions were strongly correlated.
- The expansion of the scleral canal was much smaller in magnitude than the posterior displacement of the ONH, and the correlation between canal expansion and ONH posterior displacement decreased as IOP increased from...
10 to 30 mm Hg. During IOP increase, the canal expansion leveled off much faster than the ONH posterior displacement.

- Within the ONH, larger strains were found in the anterior region, where the LC is located in porcine eyes. Through-thickness compression was the dominant mode of deformation, but the in-plane stretch and shear strain were also higher in the anterior ONH.

- The largest strains overall were concentrated in the periphery of the anterior ONH in the vicinity of the peripheral LC. In-plane stretch and shear strain were larger in the periphery than the center, and the shear strain showed the greatest difference between the two regions.

**FIGURE 6.** (A) Average ONH posterior displacement and scleral canal expansion during inflation ($n = 12$). (B) Pearson correlation between ONH posterior displacement and scleral canal expansion at all IOP levels. Scatterplots of ONH posterior displacement and canal expansion are shown in (C) and (D) at 10 and 30 mm Hg, respectively.

**FIGURE 7.** (A) Average strains in the anterior and posterior ONH during inflation ($n = 12$). (B) Pearson correlation between the strains in the anterior and posterior ONH at all IOP levels.
In this study, we quantified the posterior displacement of the porcine ONH and PPS, which showed a nonlinear relationship to IOP (Fig. 5A). Posterior displacement of the porcine LC and/or PPS has been reported by others using alternative imaging techniques. Coudrillier et al. 9 observed posterior displacements over nearly the same IOP range (6–30 mm Hg) by using phase-contrast μCT imaging, although quantitative analyses of the displacements were not reported. Spectral-domain OCT has been used to image the porcine ONH during increases in IOP, and a similar finding of posterior LC displacement was reported. 20 The OCT technique, however, could not measure the displacements in the sclera and the tissues posterior to the LC due to limited tissue penetration. Our ultrasound speckle tracking approach is applicable to the full thickness of both the sclera and the ONH. In addition, by performing speckle tracking on the densely sampled radiofrequency signal, we are able to achieve high sensitivity and accuracy in measuring displacements. 12,21

With the advantages of high-frequency ultrasound speckle tracking, we were able to compare the posterior displacement of the ONH and the PPS. Although there was a strong correlation between PPS and ONH posterior displacement, the ONH consistently displaced more posteriorly than the PPS in response to acute IOP increase. To ensure that the larger ONH displacement was not simply a result of the ONH being positioned at the apex of the imaged area of the globe, we calculated the displacements in the through-thickness (i.e., radial) direction by using coordinate transformation and found a similar difference between those two regions. For example, at 30 mm Hg, the outward through-thickness displacement of the ONH was significantly larger than that of the PPS (343.7 ± 79.0 μm vs. 202.8 ± 56.8 μm; P < 0.001). This suggests that the larger posterior/outward displacement of the ONH may be explained by the ONH being a “weaker” discontinuity in the ocular shell surrounded by a tougher collagenous sclera. The difference in posterior movement between the ONH and PPS increased as IOP increased, likely due to IOP-related stiffening of the PPS. 22 This mismatch in posterior displacement also leads to bending deformations within ONH, especially in the peripheral ONH (see more detailed discussion later), which could potentially contribute to glaucomatous damage.

Our results showed that the porcine ONH experienced canal expansion that was smaller in magnitude than the posterior displacement (Fig. 6A). Canal expansion has been observed during porcine eye inflation, 23 and studies using digital image correlation to track the outer surface of the bovine and murine posterior sclera have also reported canal expansion that was minimal relative to posterior movement of the ONH. 24,25 This response, and the decrease in correlation between canal expansion and posterior displacement of the ONH at higher IOP, are likely attributed to the collagen annular ring within the PPS 26,27 that limits canal expansion but is less

### Table 1. Average Strains in the Anterior and Posterior ONH of All Eyes at 30 mm Hg (n = 12)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Minimum, %</th>
<th>Maximum, %</th>
<th>Mean ± SD, %</th>
</tr>
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<tr>
<td>Anterior ONH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Through-Thickness</td>
<td>−14.55</td>
<td>−5.09</td>
<td>−9.62 ± 2.83</td>
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<tr>
<td>In-Plane</td>
<td>1.56</td>
<td>7.25</td>
<td>4.62 ± 1.89</td>
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<tr>
<td>Shear (magnitude)</td>
<td>2.25</td>
<td>8.09</td>
<td>5.30 ± 1.71</td>
</tr>
<tr>
<td>Posterior ONH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Through-Thickness</td>
<td>−4.70</td>
<td>3.51</td>
<td>−0.05 ± 2.48</td>
</tr>
<tr>
<td>In-Plane</td>
<td>0.30</td>
<td>4.18</td>
<td>2.59 ± 1.02</td>
</tr>
<tr>
<td>Shear (magnitude)</td>
<td>1.43</td>
<td>4.43</td>
<td>2.63 ± 0.97</td>
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</tbody>
</table>

### Table 2. Average Strains in the Center and Periphery of the Anterior ONH of All Eyes at 30 mm Hg (n = 12)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Minimum, %</th>
<th>Maximum, %</th>
<th>Mean ± SD, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Anterior ONH</td>
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<td></td>
</tr>
<tr>
<td>Through-Thickness</td>
<td>−14.17</td>
<td>−3.97</td>
<td>−8.70 ± 3.07</td>
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<tr>
<td>In-Plane</td>
<td>0.19</td>
<td>4.36</td>
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<tr>
<td>Shear (magnitude)</td>
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<td>5.53</td>
<td>3.09 ± 1.16</td>
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<tr>
<td>Peripheral Anterior ONH</td>
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<td></td>
<td></td>
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<tr>
<td>Through-Thickness</td>
<td>−14.78</td>
<td>−5.89</td>
<td>−10.21 ± 2.74</td>
</tr>
<tr>
<td>In-Plane</td>
<td>1.94</td>
<td>9.14</td>
<td>5.76 ± 2.44</td>
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<tr>
<td>Shear (magnitude)</td>
<td>2.61</td>
<td>10.64</td>
<td>6.69 ± 2.34</td>
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</table>

**Figure 8.** (A) Average strains in the central and peripheral regions of the anterior ONH during inflation (n = 12). (B) Pearson correlation between the central and peripheral strains at all IOP levels.
effective in preventing posterior displacement of the ONH. This displacement pattern suggests that at higher IOP levels, the ONH bows more posteriorly with respect to the PPS, consistent with the clinical observation of ONH “cupping”. It has been postulated that a low sclera modulus may cause large canal expansion and anterior movement of the LC. If it is pulled taut by the sclera, and several computational models have predicted this behavior. Our experimental data showed only posterior displacement of the entire ONH in response to acute IOP increase, despite the level of canal expansion. This may be explained by the high compliance of both the porcine LC and sclera, which could result in both canal expansion and posterior LC displacement. Future studies will investigate this behavior in human donor eyes.

Compression, stretch, and shear strains within the ONH increased nonlinearly with IOP increase. The nonlinear behavior is expected for collagenous tissues such as the sclera and cornea, but the ONH has a complex structure with a significant presence of neuroglial tissue and its nonlinear response has not been well established. Regional analyses revealed that the largest deformations occurred in the anterior ONH (Fig. 7A), and the through-thickness compression was nearly twice as large as the in-plane stretch and shear strain in this region. The LC was located within the anterior region, and, thus, the deformation measured in the anterior ONH was likely the combined response of the LC beams and intralamellar neural tissue. Our results suggest that the porcine LC experiences more through-thickness compression than in-plane stretch or shear when IOP is elevated. This is consistent with phase-contrast μCT measurements. Large compressive strains have also been predicted from computational models of the human eye. Interestingly, a recent study using scanning laser multiphoton microscopy showed essentially no anterior-posterior strain within the murine ONH. This discrepancy is possibly related to differences in LC composition and structure (e.g., cellular vs. collagenous). Excessive compression of the ONH has been suggested to precede visual field defects and likely contributes to axonal damage and abnormal extracellular matrix remodeling within the LC. The compressive strains measured in this study were found almost exclusively within the anterior ONH where the porcine LC resides, suggesting a potential role for the LC to shield the retrolaminar tissue from compression.

Our experimental data support computational modeling predictions by Grytz et al. that the ONH is largely shielded from in-plane stretch by the collagen annulus in the PPS. However, the collagen annulus is not effective in preventing compression or bending within the ONH. Significant shear was measured in the porcine ONH, especially in the periphery of the anterior ONH where the LC meets the PPS (Fig. 8A). These deformations may be a result of the mismatch in the mechanical stiffness of the PPS and ONH, because the stiffer PPS can better resist deformation while the ONH is pushed posteriorly by opposing IOP from the posterior side of the LC. Future studies are needed to evaluate the effect of these pressures on ONH displacements and strains. Postmortem tissue changes may also have had some effects on our measurements. For example, possible through-thickness strains were detected in the posterior ONH in some eyes, which might be due to swelling or changes in tissue permeability. Lower-frequency ultrasound may be used to evaluate the ONH and PPS in vivo, but future studies are needed to optimize tissue penetration and resolution. Another limitation was that scleral strain was not computed due to there being an insufficient number of kernels for least squares strain estimation with current data acquisition methods. The optic nerve sheath may have created acoustic shadowing in the transition zone between the ONH and PPS. Strains calculations were omitted for this region and thus were not included in the ONH strain analyses (Fig. 4B).

In summary, high-frequency ultrasound speckle tracking is a unique and powerful tool for measuring the mechanical behavior of both the ONH and PPS through the entire thickness of the tissues. The regional patterns and differences in displacements and strains observed in this study may provide important insights into the role of ONH and PPS biomechanics in the disease process of glaucoma.

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