The Impact of Acutely Elevated Intraocular Pressure on the Porcine Optic Nerve Head

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PURPOSE. To investigate the effects of acute elevations in intraocular pressure (IOP) on the cup, prelaminar, and lamina cribrosa regions of the porcine optic nerve head (ONH).

METHODS. Ex vivo imaging of 10 porcine ONHs was performed using spectral-domain optical coherence tomography (OCT). The IOP was manipulated with a pressure head and measured with a pressure transducer. Reference scans were taken at 0 mm Hg, before further scanning was performed at 7-mm Hg steps, up to 49 mm Hg. Morphometric parameters were measured across centrally located OCT B-scans at different IOPs, and the relationship between IOP and changes in these parameters was analyzed.

RESULTS. As IOP increased from 0 to 49 mm Hg, mean cross-sectional cup area increased (28% ± 3%, P < 0.001), lamina cribrosa area decreased (18% ± 2%, P < 0.001), and prelaminar tissue area decreased (5.5% ± 0.5%, P < 0.001). Multivariate regression demonstrated that most of the change in cup area is associated with changes in both lamina cribrosa position and thickness (r = 0.89, P < 0.001).

CONCLUSIONS. Acute elevations in IOP were shown to result in posterior displacement of ONH, as well as lamina cribrosa and prelaminar tissue deformation in the porcine ONH. (Invest Ophthalmol Vis Sci. 2011;52:6192–6198) DOI:10.1167/iovs.10-7137

Elevated intraocular pressure (IOP) acting on the optic nerve head (ONH) is a major risk factor for glaucomatous optic neuropathy (GON). IOP acts to induce stress and strain on the load-bearing corneoscleral shell of the eye as well as the posterior opening in this shell, the ONH.1 This opening is traversed by neural and vascular tissues and bridged by glial and connective tissues, including the lamina cribrosa.2,3 It is the site where intraocular stress and strain are concentrated and where, over time, IOP-induced stress and strain are thought to play a central role in the progressive damage and remodelling of the ONH tissues seen in GON.4–7 Our understanding of the acute response of the load-bearing ONH to changes in IOP may facilitate better understanding of the chronic and progressive pathophysiology of GON.

Previous work has looked at the impact of acute IOP changes on the ONH. Studies using surface imaging technology to assess IOP-induced changes in the ONH have shown pressure-dependent increases in cup area, depth, volume,6–10 and, more recently, disc area.11 However, scans of the surface of the ONH provide little direct information regarding its subsurface structures.

Early mechanical studies on ex vivo human and primate eyes suggested a nonlinear posterior displacement of the lamina in response to increasing IOP, with most of this displacement occurring at the lower IOP increments.12,13 Other work comparing histologic lamina thickness between pairs of eyes fixed at different IOPs suggested no difference in lamina cross-sectional thickness.14 More recent work showed normal monkey eyes fixed at an IOP of 10 mm Hg to have significantly thinner and more anteriorly located lamina cribrosa with wider Bruch’s membrane openings, when compared with eyes fixed at 0 mm Hg.15 However, subsequent work in eyes that have been perfusion fixed at low (10 mm Hg) or high (30 or 45 mm Hg) IOP showed no significant difference in anterior scleral canal diameter or lamina cribrosa thickness and only a small posterior displacement of the lamina between the two groups.7

Taken together, these results suggest that, while acutely increased IOP may lead to increased cupping, the subsurface ONH response to changes in IOP is more complicated, with a difference in response seen at lower and higher pressure ranges, influencing the surrounding peripapillary sclera.

More recently, finite element modeling (FEM) has been used to model the likely impact of various morphometric parameters16–19 and material properties18,20,21 of the peripapillary sclera and ONH structures on the IOP-induced stress and strain seen within its different regions. Modeling has allowed for investigation of the subsurface environment of the ONH where imaging has been inadequate. However, with recent improvements in optical coherence tomography (OCT), we may be able to use this technology to directly examine the deeper tissue response of the ONH to changing pressure conditions. OCT is an imaging modality that works on the principles of Michelson interferometry to image subsurface structures with different refractive indices and orientations.22 Recent improvements in the light source and signal-capturing components of the modality have led to higher image resolution and faster acquisition, making the modality a more viable adjunct for examination of the heterogenous and complex ONH. Specifically, clear delineation of the surface of the ONH, the termination of Bruch’s membrane/RPE complex, and the anterior lamina cribrosa surface23,24 may allow us to examine the biomechanical response of the ONH to different levels of IOP.

Using spectral-domain OCT (SD-OCT) we wanted to examine the impact of increasing IOP on a sample of 10 porcine...
ONHs, to better understand the role that subsurface load-bearing structures within the ONH play in response to raised IOP. Elevated IOP may cause SD-OCT detectable changes in prelaminar and lamina cribrosa thickness and lamina cribrosa position. Porcine eyes were selected for this study because of their similar size to human eyes, similar scleral thickness, and prominent collagenous lamina cribrosa. The porcine lamina cribrosa is also located more anteriorly within the ONH, and this allows relatively accurate imaging of prelaminar and lamina tissues. This study was conducted ex vivo to remove the influence of cerebral spinal fluid pressure on the position of the lamina cribrosa.

METHODS

Animals

Ten porcine eyes, received from an abattoir within 6 hours of the animal’s death were used. All pigs were aged between 18 to 24 months. The eyes were placed in an eye holder at room temperature and kept moist with normal saline. The anterior chamber was cannulated with two 25-gauge needles connected to a pressure head and a pressure transducer, respectively. The IOP was initially set to 0 mm Hg and increased by 7-mm Hg increments every 5 minutes, to a maximum of 49 mm Hg. A hard contact lens was placed on the cornea of each eye to minimize astigmatism.

Optical Coherence Tomography

A spectral-domain OCT system (Spectralis SD-OCT; Heidelberg Engineering, Heidelberg, Germany) was used to image the optic nerve. The technical specifics of this machine are described elsewhere. For this study the reflectance image was used to align the ONH with the OCT. With the automatic real-time (ART) function set to 9 and a B-scan angle of 15°, 25 horizontal B-scans were acquired, to generate a volume cube centered on the optic nerve. A first volume scan at an IOP of 0 mm Hg was taken with the eye-tracking function of the device turned on and set to reference. Subsequent aligned volume scans were acquired at increasing IOP for each eye. Only scans registering signal quality of ≥20 dB were used for analysis. In addition, volume scans of the retina 2 disc diameters from the porcine ONH and with clearly defined retinal vascular landmarks were also acquired at increasing IOPs for six of the eyes, looking for IOP-induced changes to the retinal cross-sectional area. Again, a reference volume scan was taken at an IOP of 0 mm Hg, before the IOP was increased by 7-mm Hg increments and matched volume scans of the same region of retina were acquired for each of the six eyes.

Biometry

Porcine eye axial lengths were measured using electronic calipers (model CD-6 BS; ST Industries; St. James, Minnesota, MN), to correct for retinal magnification differences. The axial lengths ranged from 20.1 to 26.9 mm (mean, 22.8 ± 2.1 mm). The biometric methods used have been described elsewhere.

Selection of Analysis Locations

Based on the reflectance image, a centrally located SD-OCT B-scan through the long axis of each porcine ONH was selected from the reference volume scan and in subsequent aligned scans at higher IOPs. Figure 1 illustrates centrally located SD-OCT B-scans for one eye matched at IOP 7 mm Hg (Figs. 1a–d) and IOP 49 mm Hg (Figs. 1e, 1f). For the retinal analysis, SD-OCT B-scans with clearly defined retinal vascular landmarks were selected from the reference volume scan of each of the six eyes and from their subsequent aligned scans at increasing IOP.

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Image Processing

The B-scan sets for each eye were analyzed using image analysis software (Image); National Institute of Health, Bethesda, MD). ONH landmarks were identified (Fig. 1a) before the following morphometric parameters of the ONH were measured at increasing IOP:

1. Bruch’s membrane opening (BMO) cross-sectional diameter (Fig. 1a): in SD-OCT, the termination of the reflectance band corresponding to the RPE/Bruch’s membrane complex corresponds with the BMO. The distance between the terminal points of this reflectance band on either side of the ONH was measured as the diameter of the BMO.

2. Cup cross-sectional area (Fig. 1b): defined as the area bounded by the inner limiting membrane and a line drawn across the neuroretinal rim. This line was determined by taking vertical lines from the BMO to intersect the neuroretinal rim surface, with the line connecting these two points.

3. Lamina cribrosa cross-sectional area (Fig. 1b): the area occupied by the reflectance band corresponding to the lamina cribrosa. SD-OCT B-scans of the porcine ONH reveal a band of high reflectivity within the BMO and posterior to the ILM/vitreous interface. We’ve recently shown that the cross-sectional area of this band correlates well with the cross-sectional area of the lamina cribrosa in matched histologic sections.

4. Total prelaminar tissue cross-sectional area (Fig. 1b): defined as the area within the confines of the BMO, bounded anteriorly by the signal from the ONH/vitreous interface and posteriorly by the anterior surface of the lamina cribrosa reflectance band.

5. Post-BMO cross-sectional area (Fig. 1c): a measure of the location of the anterior surface of the collagenous lamina cribrosa relative to the BMO plane. This was defined as an area bounded by a plane running through the opening in the Bruch’s/RPE reflectance band, through the anterior margin of the lamina cribrosa reflectance band, and within the BMO.

The prelaminar tissue was examined more closely, by dividing it into five equal regions for each eye, with the width of each region being equal to one fifth of the BMO cross-sectional diameter for that eye:

6. Central prelaminar cross-sectional area (Fig. 1d): the central one-fifth portion of the total prelaminar area.

7. Midperipheral prelaminar cross-sectional area (Fig. 1d): the two one-fifth portions of the total prelaminar tissue adjacent to the central one-fifth portion.

8. Peripheral prelaminar cross-sectional area (Fig. 1d): the two one-fifth portions of the total prelaminar tissue, within and adjacent to the BMO.

9. Peripapillary retinal thickness (Fig. 1d): the cross-sectional area of retina adjacent to and outside the BMO. On either side of the ONH, the cross-sectional area of retina 250 μm wide along the x-axis was measured.

10. Peripheral retina cross-sectional area: for the six eyes where retinal scans were acquired, the cross-sectional area of the retina adjacent to a clearly defined vascular landmark and 500 μm in width along the x-axis was measured for each eye at increasing IOP.

Statistical Analysis

All measurements were analyzed using statistical analysis software (Sigma-plot 11.0; Systat, Chicago, IL). Each measured parameter was normalized by subtracting its value at IOP 0 mm Hg from subsequent measurements at higher IOPs, to give the change induced with increasing IOP. Spearman rank correlation was performed to test the strength of the relationship between changing IOP and each of the normalized parameters, before a two-way analysis of variance (ANOVA) was performed using IOP and eye as factors to assess whether differences in the normalized data occurred at various IOPs while accounting for variation between eyes. Where signif-
icant differences did occur, the Holm-Sidak method was used in the post hoc analysis to identify the lowest IOP at which a change in the parameter was significantly different from 0.

We then performed univariate and multivariate linear regression analyses between cup area and post-BMO, lamina cribrosa, and total prelaminar areas to assess how much a change in cup area is explained by each of these three parameters.

RESULTS

The relationship between IOP and each of the normalized parameters is presented in Figures 2 and 3, together with a summary of the statistical analysis (Table 1). The multivariate regression analysis was performed with the cup cross-sectional area as the dependent variable and the post-BMO cross-sectional area, lamina cribrosa cross-sectional area, and total prelaminar cross-sectional area all included as independent variables.

Across the 10 eyes, as IOP increased, cup cross-sectional area increased (Fig. 2a). The change was equal to a mean increase of 28% ± 3% (P < 0.001) at an IOP of 49 mm Hg (Table 1). This increase was mirrored by a posterior displacement of the anterior lamina cribrosa surface, with an increasing post-BMO cross-sectional area (Fig. 2d). The change in the post-BMO area was equal to a mean increase of 267% at an IOP of 49 mm Hg (Table 1).

The change in cross-sectional area for both cup and post-BMO first reached statistical significance at an IOP of 14 mm Hg, and graphs of both parameters suggest a nonlinear relationship with IOP. Univariate linear regression showed a strong relationship between the two parameters (r = 0.881, P < 0.001) and this relationship remained significant within the multivariate analysis (coefficient = 0.43, P < 0.001).

The change in lamina cribrosa cross-sectional area (Fig. 2b) first reached statistical significance at an IOP of 21 mm Hg, with the dataset demonstrating a negative relationship with IOP. Univariate linear regression showed a strong relationship between the two parameters (r = −0.881, P < 0.001) and this relationship remained significant within the multivariate analysis (coefficient = −0.43, P < 0.001).

The change in lamina cribrosa cross-sectional area (Fig. 2b) first reached statistical significance at an IOP of 21 mm Hg, with the dataset demonstrating a negative relationship with IOP. Univariate linear regression showed a strong relationship between the two parameters (r = −0.881, P < 0.001) and this relationship remained significant within the multivariate analysis (coefficient = −0.43, P < 0.001).

The change in lamina cribrosa cross-sectional area (Fig. 2b) first reached statistical significance at an IOP of 21 mm Hg, with the dataset demonstrating a negative relationship with IOP. Univariate linear regression showed a strong relationship between the two parameters (r = −0.881, P < 0.001) and this relationship remained significant within the multivariate analysis (coefficient = −0.43, P < 0.001).
The total prelaminar cross-sectional area (Fig. 2c) remained stable at lower IOPs, with the first statistically significant difference in area found at an IOP of 35 mm Hg. Overall, the change in total prelaminar cross-sectional area was equal to a mean reduction (axial compression) of $5.5\% \pm 0.5\%$ ($P < 0.001$) across the IOP range of this experiment (Table 1) and, while statistically significant, the univariate linear relationship between total prelaminar and cup area changes was relatively weak ($r = -0.275$, $P = 0.020$) and was not significant within the multivariate analysis ($P = 0.573$). Results of the multivariate linear regression suggest that changes in

**FIGURE 2.** Scatterplots (mean ± SE) demonstrating the relationship between IOP and the normalized measurements of cup (a), lamina cribrosa (b), total prelaminar (c), post-BMO (d), and peripheral retina (f) cross-sectional areas, as well as BMO diameter (e).
The cup area can mostly be explained by changes in the position and cross-sectional area of the lamina cribrosa:

$$\Delta \text{Cup area} = 0.03 + 0.43(\Delta \text{post-BMO}) - 0.32(\Delta \text{lamina cribrosa})$$

where multivariate linear regression $r = 0.89$, $P < 0.001$.

The mean BMO cross-sectional diameter for the 10 eyes at an IOP 0 mm Hg was $3370 \pm 220 \, \mu m$ and showed no significant relationship with IOP (Fig 2e, Table 1: $r = 0.101$, $P = 0.373$). The peripheral retina cross-sectional area (mean area, $0.127 \pm 0.01 \, mm^2$ at IOP 0 mm Hg) also showed no significant relationship with IOP (Fig. 2f, Table 1: $r = -0.145$, $P = 0.44$).

Examining the prelaminar and peripapillary retina in more detail (Fig. 3) revealed that the central and midperipheral prelaminar regions showed a reduction in cross-sectional area.
at lower IOP as IOP increased (first significant change in cross-sectional area at IOP 21 mm Hg for both regions) when compared with the peripheral prelaminar and peripapillary retina regions, where the first significant change in cross-sectional area occurred at IOP 35 mm Hg (Table 1). Table 1 shows the Spearman rank correlation co-efficient and P value between each normalized parameter and IOP. For those parameters for which a significant relationship was found, the lowest IOP significantly different from the reference value (and its P value) as well as the mean percentage change in cross-sectional area at the highest IOP is presented. No significant relationship was found between IOP and BMO diameter or peripheral retina.

**DISCUSSION**

Taken together, our results imply that in an eye with acutely elevated IOP, changes at the vitreoretinal interface are initially and mostly explained by a posterior displacement of the lamina cribrosa (Figs. 2a, 2d). As IOP increased further, compression of the lamina itself occurred, with a reduction in its axial cross-sectional area (Fig. 2b). At higher levels of IOP, the prelaminar tissue compressed. This compression was first evident centrally in the prelaminar region, before involving more peripheral regions, including the peripapillary retina (Figs. 2c, 5).

Previous histologic and modeling studies using primates have suggested that increasing IOP may be associated with scleral canal and BMO expansion and that, at the lower IOP range, this expansion may result in anterior displacement of the lamina cribrosa toward the BMO plane.\(^{15}\) More recently, in vivo observations in human subjects using ophthalmodynamometry and OCT suggest that the increased cupping seen as IOP is due to prelaminar tissue compression with no significant posterior displacement of the lamina cribrosa.\(^{31}\)

In this study, the 10 porcine eyes displayed posterior displacement of the lamina cribrosa with increasing IOP (Fig. 2d), and there was no statistically significant relationship between the change in BMO diameter and IOP (Fig. 2e, Table 1: \(r = 0.101, P = 0.373\)). In addition to IOP, various geometric factors and material properties of the peripapillary sclera and lamina cribrosa are thought to play an important role in determining how the scleral canal and lamina cribrosa respond to increasing IOP.\(^{16–21,32}\)

Porcine eyes have a larger, more eccentric ONH shape and a relatively dense, thick, collagenous lamina cribrosa, located more anteriorly within the ONH, when compared with human and primate eyes. Primate and human fundi also have a macula and a nasally displaced ONH. In addition, these experiments were performed using enucleated eyes. These important discrepancies between our animal model and those used in previous studies may account for the differences seen at the lower IOP range.\(^{12,15}\) In vivo ophthalmodynamometric analysis of IOP may alter intraorbital pressure, which may alter optic nerve tissue pressures. This difference in experimental protocol may additionally account for differences in IOP-induced lamina displacement and consequently, changes in the prelaminar tissue thickness presented here and the in vivo human observations made recently.\(^{31}\)

The change in prelaminar and peripapillary cross-sectional area with increasing IOP was small but statistically significant (Figs. 3). This change was not seen in the peripheral retina (Fig. 2f). Although the prelaminar porcine ONH is predominantly neural in composition and neural tissue is thought to be largely incompressible,\(^{33,34}\) it does contain a central collection of veins.\(^{35,36}\) It is possible that some of the IOP-induced changes in the prelaminar cross-sectional area seen here were due to central vessel lumina collapsing under increasing stress. Previous studies on dog ONH suggest that IOP and prelaminar tissue pressure are equal and that tissue pressure decreases across the lamina cribrosa only.\(^{35}\) This translamellar pressure gradient is dependent on IOP, retrolaminar tissue pressure, and lamina cribrosa axial thickness. However, at higher IOP, the change in prelaminar cross-sectional area seen in these experiments implies that a small portion of the pressure gradient could exist across the prelaminar region of the eye at increasing IOP. This change in cross-sectional area may be due to a compression of the tissues within the prelaminar region of the ONH, but may also be the result of axoplasm redistribution, either upstream into the peripapillary retina or downstream through the lamina cribrosa.

Conversely, no significant change was seen in the cross-sectional area of the peripheral retina. We believe that this incompressibility occurs because this region is relatively avascular and also because it is not subject to a pressure gradient at any IOP.

Limitations of this study include our two-dimensional analysis of the three-dimensional ONH, the use of the BMO as a reference point from which parameters were derived, and the inability of current SD-OCT technology to image the full thickness of the peripapillary sclera and scleral canal. Also, this study was performed on enucleated porcine eyes with an effective cerebrospinal fluid pressure of 0 and no ONH blood flow. This limits the extrapolation of results presented here, which require in vivo confirmation.

A single SD-OCT B-scan through the center of each ONH was analyzed at increasing IOP and, while succinct, this method of analysis can at best be an approximation of the behavior of the whole ONH, given its structural heterogeneity and in particular, the anisotropic lamina cribrosa. As is the case in human eyes, the porcine lamina cribrosa also displays significant regional variation in thickness and pore size.\(^{37}\) If, as suggested by others,\(^{15}\) there are changes to the anterior scleral canal and BMO with increasing IOP, it may represent a confounding factor, as the morphometric parameters measured had boundaries derived from the BMO. However, our results showed no significant change in BMO diameter with IOP change (Fig. 2e, Table 1), and so the BMO is likely to have provided a relatively stable reference. The importance of the geometric and material properties of the peripapillary sclera and scleral canal in determining ONH biomechanics has been one of the most consistent findings in FEM analysis of the ONH.\(^{18–21}\) As such, the inability of current SD-OCT technology to image this region of the eye limits its utility in investigating ONH biomechanics, and our inability to measure the scleral canal is a key limitation of this study.

There are concerns regarding the ability of SD-OCT to image the posterior margin of the lamina cribrosa, with the reflectance band corresponding to the lamina cribrosa fading along its posterior boundary (Fig. 1a). The correlation between the thickness of this reflectance band and the thickness of the lamina cribrosa within matched histologic sections through the ONH has previously been found to be significant \((r = 0.64, P = 0.048)\).\(^{37}\) We believe the poorly defined posterior boundary of the reflectance band corresponding to the lamina on OCT scans may be due to a fading of the signal, but also, the end of the lamina may have been reached.

Despite these limitations, we have used SD-OCT across a range of IOPs to demonstrate morphometric changes in the ONH indicative of its biomechanical response to increasing IOP-induced stress. Changes to the prelaminar and lamina cribrosa cross-sectional areas seen here reflect the increasing axial strain in these regions as IOP was increased. We have shown that anterior lamina position and lamina cribrosa thickness significantly change with acute changes in IOP and, can
be measured with SD-OCT. These may be useful parameters to measure to assess glaucoma risk and likelihood of progression.

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References