Laminar and Prelaminar Tissue Displacement During Intraocular Pressure Elevation in Glaucoma Patients and Healthy Controls

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Objective: To determine the response of the anterior lamina cribrosa and prelaminar tissue to acute elevation of intraocular pressure (IOP) in glaucoma patients and healthy subjects.

Design: Prospective case-control series.

Participants and Controls: Patients with open-angle glaucoma (n=12; mean age±standard deviation [SD], 66.8±6.0 years), age-matched healthy controls (n=12; mean age±SD, 67.1±6.2 years), and young controls (n=12; mean age±SD, 36.1±11.7 years).

Methods: One eye was imaged with spectral-domain optical coherence tomography to obtain 12 high-resolution radial scans centered on the optic disc. Imaging was repeated at precisely the same locations with an ophthalmodynamometer held perpendicular to the globe via the inferior lid to raise the IOP. A line joining Bruch’s membrane opening in 4 radial scans was used as reference in the baseline and elevated IOP images. The vertical distance from the reference line to the anterior prelaminar tissue surface and anterior laminar surface was measured at equidistant points along the reference line in the 2 sets of images. The difference between the 2 sets of corresponding measurements were used to determine laminar displacement (LD) and prelaminar tissue displacement (PTD).

Main Outcome Measures: Laminar displacement and PTD.

Results: Intraocular pressure elevation among patients, age-matched controls, and young controls was similar (mean±SD, 12.4±3.2 mmHg). The mean±SD LD and PTD were 0.5±3.3 μm and 15.7±15.5 μm, respectively. The LD was not statistically different from 0 (P=0.366), but PTD was (P<0.001). The mean±SD LD was similar among the groups (–0.5±3.7 μm, 0.2±2.0 μm, and 2.0±3.6 μm, respectively; P=0.366), whereas the mean±SD PTD was different (6.8±13.7 μm, 20.8±17.5 μm, and 19.6±11.8 μm, respectively; P=0.045). In all subjects, the PTD was greater than LD. In multivariate regression analyses, LD was negatively associated with optic disc size (P=0.007), whereas PTD was positively associated with the degree of IOP elevation (P=0.013).

Conclusions: In glaucoma patients and controls, the anterior laminar surface is noncompliant to acute IOP elevation. Acute optic disc surface changes represent compression of prelaminar tissue and not laminar displacement.

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The lamina cribrosa of the optic nerve head is thought to be the primary site of axonal damage in glaucoma based on findings from histopathologic studies of human eyes and from animal studies.1–4 The lamina is composed of fenestrated plates of connective tissue and elastic fibers lined by astrocytes.5 The connective tissue plates contain pores that form channels through which bundles of retinal ganglion cell (RGC) axons pass. The lamina provides structural and functional support to the RGC axons and divides the intraocular and intraorbital portions of the optic nerve. Intraocular pressure (IOP) is the primary known risk factor for the development7 and progression8,9 of open-angle glaucoma; however, the precise mechanism of how IOP alone or in combination with other factors results in glaucomatous optic nerve damage is still not understood fully.10–12 Considerable research has been directed at understanding how the lamina responds to IOP, leading to axonal damage.13–18

Displacement of the lamina in response to IOP elevation may cause the pores to deform and pinch the traversing axons.19 It is hypothesized that extension, compression, or shearing of axons within the lamina contributes to the loss of neural function in glaucoma.20,21 Optic nerve head astrocytes also may respond to changes in IOP, altering their physiologic relationship with axon bundles, connective tissues, and vasculature, leading to axonal loss and tissue remodeling.22,23
The biomechanics of the lamina have been studied widely.\textsuperscript{11,14,24–29} Experimental studies on acute IOP-related laminar deformation thus far have used 3 different approaches: (1) ex vivo models,\textsuperscript{15,18} (2) animal models where the lamina was studied after histomorphometry,\textsuperscript{13} and (3) theoretical models of the human eye.\textsuperscript{21,24,30} None of these experiments were based on a direct visualization of the lamina in vivo. With advances in optical coherence tomography (OCT) technology using spectral-domain (SD) or Fourier-domain signal detection,\textsuperscript{31–34} which allows high-speed image acquisition with high axial resolution, it is now possible clinically to visualize the lamina directly.\textsuperscript{33,34} Recent research shows accurate concordance between SD OCT and serial histologic sections, including identification of the anterior laminar surface.\textsuperscript{35}

This study was undertaken with SD OCT to investigate laminar displacement (LD) and prelaminar tissue displacement (PTD) during acute IOP elevation. The objectives were to compare LD and PTD in patients with open-angle glaucoma, age-matched healthy control subjects, and young control subjects and to determine which factors were associated with LD and PTD.

**Patients and Methods**

**Participants**

Open-angle glaucoma patients were recruited from the Eye Care Centre of the Queen Elizabeth Health Sciences Centre in Halifax, Nova Scotia. Age-matched and young healthy control subjects also were recruited. Controls were either spouses of patients or subjects who had previously taken part in research studies.

Inclusion criteria for patients were: (1) a clinical diagnosis of open-angle glaucoma with documented progressive optic disc change and visual field damage compatible with glaucoma and (2) visual field mean deviation better than $-10$ dB. Exclusion criteria for patients were: (1) a threat to fixation, (2) treated IOP of $25$ mmHg or more, and (3) previous filtering eye surgery. Inclusion criteria for controls were: (1) normal eye examination results with normal appearance of the optic disc and fundus and (2) IOP of $20$ mmHg or less. The exclusion criterion for controls was ocular surgery, with the exception of filtering surgery. Inclusion criteria for controls were: (1) a threat to fixation, (2) treated IOP of $25$ mmHg or more, and (3) previous filtering eye surgery. Inclusion criteria for controls were: (1) normal eye examination results with normal appearance of the optic disc and fundus and (2) IOP of $20$ mmHg or less. The exclusion criterion for controls was ocular surgery, with the exception of filtering surgery.

In accordance with the Declaration of Helsinki, subjects gave informed consent to participate in the study. The protocol was approved by the Capital Health Research Ethics Board.

**Confocal Scanning Laser Tomography**

Confocal scanning laser tomography\textsuperscript{36} of the optic disc was performed on the study eye with the Heidelberg Retina Tomograph II (Heidelberg Engineering GmbH, Heidelberg, Germany) to measure the optic disc size for analysis (see below).

**Spectral-Domain Optical Coherence Tomography**

Optical coherence tomography is a noninvasive imaging technology using low-coherence interferometry to generate in vivo high-resolution cross-sectional images of ocular structures.\textsuperscript{37} Currently, SD OCT is the newest generation of the optical coherence technologies to be used in ophthalmology. It has been described in detail elsewhere.\textsuperscript{31,32} Briefly, the reference beam and reflected beam from the eye are recorded in parallel by a spectrometer to generate A-scans from the Fourier-transformed time-delayed signals. Because all signals are analyzed simultaneously, SD OCT has reduced acquisition time significantly (by up to 50 times) compared with older-generation time-domain detection.\textsuperscript{2,38} Images with the device used in this study (Spectralis OCT; Heidelberg Engineering GmbH) are acquired with real-time eye tracking, and therefore, motion artifacts are eliminated.

Each subject had 1 randomly chosen study eye imaged with SD OCT. A radial scanning pattern centered on the optic disc (12 high-resolution 15° radial scans, each averaged from 30 B-scans, with 768 A-scans per B-scan) was used. The scanning speed was 40 000 A-scans per second. The software allowed serial (baseline and during IOP elevation) image acquisition at precisely the same locations.

All scans were obtained through undilated pupils. Imaging was repeated if the anterior lamina was not visualized clearly. Two sets of SD OCT images were acquired: before (baseline) and during IOP elevation.

**Experimental Elevation of Intraocular Pressure**

An ophthalmodynamometer (Inami, Tokyo, Japan) was used to elevate IOP. The ophthalmodynamometer was used to apply a fixed external force through the inferior lid with the device held perpendicular to the globe (Fig 1). A force of 30 to 40 Pa was necessary to obtain an IOP increase of approximately 10 mmHg, a level at which SD OCT image quality was not compromised.

The IOP was measured with a Tono-Pen (Innova Medical Ophthalmics, Toronto, ON, Canada) at baseline and then again during IOP elevation with the ophthalmodynamometer in place. The ophthalmodynamometer was kept in place during the second set of SD OCT imaging. The IOP was elevated until imaging was completed, usually within 2 minutes.

**Analysis**

After examining all 12 radial scans, the 4 best corresponding scans at baseline and during IOP elevation in which the anterior laminar surface and termination of Bruch’s membrane–retinal pigment epithelium were visible clearly were chosen for analysis (Photoshop CS3; Adobe Corporation, San Jose, CA; and Image J; National Institutes of Health, Bethesda, MD).

A line joining the Bruch’s membrane–retinal pigment epithelium opening\textsuperscript{35,36} was used as a reference line for the baseline and elevated IOP images. The software allowed the 2 sets of registered
images (baseline and elevated IOP images) to be viewed in quick succession to verify that the Bruch’s membrane–retinal pigment epithelium border remained stable. The anterior laminar surface was delineated as the highly reflective region beneath the prelamellar tissue33,35 (Fig 2). The vertical distance from the reference line to the anterior prelamellar tissue surface and anterior laminar surface was measured at equidistant points along the reference line for the 2 sets of images. Measurements were converted to micrometers using the scale bar in the imported images. Prelamellar tissue thickness was defined as the difference between the prelamellar tissue surface to the anterior laminar surface. The LD and PTD resulting from IOP elevation were computed as the mean difference between the corresponding vertical distances from the reference line in the baseline and elevated IOP conditions, with positive values indicating posterior displacement and negative values indicating anterior displacement.

All delineations and measurements were performed by 1 observer (YA). The reproducibility of the measurement technique was assessed from duplicate measurements of LD and PTD in 4 radial sections each of 3 randomly selected patients and 3 age-matched control subjects. Absolute differences between the 2 sets of LD and PTD measurements were computed. The order in which sections were analyzed was randomized and masked for subject and set (baseline or elevated IOP).

An analysis of variance was used for evaluating group differences and for the reproducibility study. Post hoc paired group comparisons were made with the Fisher least significant difference test. Multiple regression analysis was performed to establish which independent variables determined LD and PTD. Post hoc power calculations were performed for single and 2-sample t tests. Statistical significance was assumed when $P<0.05$.

**Results**

Twelve patients with glaucoma and 24 healthy control subjects were tested. Subjects were divided into 3 groups of 12 subjects each: (1) glaucoma patients with average visual field mean deviation of $-2.8$ dB (range, $-0.3$ to $-9.0$ dB), (2) age-matched healthy controls, and (3) younger healthy controls. The demographic and ocular variables are shown in Table 1. Baseline IOP was significantly different among the groups ($P = 0.011$), with higher values in the glaucoma group compared with either control group ($P<0.037$). The mean±standard deviation (SD) IOP elevation was $12.4±3.2$ mmHg. There were no significant differences in IOP elevation, optic disc size, or central corneal thickness among the groups ($P>0.230$). The anterior laminar surface could not be delineated adequately in 1 young control subject, and therefore LD measurements were not computed for this subject.

The mean LD among the glaucoma patients, age-matched controls, and young controls, respectively, was not statistically different (Table 2; $P = 0.161$). The overall mean±SD LD was $0.5±3.3$ μm and not significantly different from 0 ($P = 0.366$). The mean PTD was significantly different among the 3 groups (Table 2; $P =
0.045), with significantly less PTD in the glaucoma group compared with either control group (P<0.039). The overall mean ± SD PTD was 15.7 ± 15.5 μm and was significantly different from 0 (P<0.001). In all subjects PTD was greater than LD (Fig 3). Expressed as a percentage of prelaminar tissue thickness before IOP elevation, PTD was significantly different among the groups (Table 2: P = 0.002), with significantly less PTD in glaucoma patients and age-matched controls compared with young controls (P<0.022).

The power to detect a 2-, 3-, and 4-μm difference in LD between the glaucoma patients and age-matched controls was 34%, 64%, and 87%, respectively. The power to detect these differences between the 2 control groups was 36%, 66%, and 89%, respectively. Finally, the power to differentiate an LD of 1, 1.5, and 2 μm from 0 was 42%, 76%, and 94%, respectively.

In multivariate regression analyses, LD was related negatively to optic disc size (P = 0.007; Table 3), whereas PTD was related positively to IOP elevation (P = 0.013; Table 3). The model for LD yielded an R² value of 0.38 (P = 0.049), whereas that for PTD yielded a value of 0.44 (P = 0.017). The relationship of LD and PTD as a function of prelaminar tissue thickness and IOP is shown in Figure 4.

The difference in LD and PTD measurement reproducibility was not statistically different: mean ± SD paired absolute differences in radial sections, 3.5±4.9 μm and 4.0±2.9 μm, respectively (P = 0.931). Reproducibility of LD measurements in glaucoma patients was 1.4±0.5 μm, whereas for PTD it was 6.2±2.5 μm. In the age-matched controls, the respective figures were 6.8±3.7 μm and 4.0±2.9 μm, respectively.

### Table 1. Summary Statistics of Variables in the 3 Subject Groups*  

<table>
<thead>
<tr>
<th>Group</th>
<th>Glaucoma Patients</th>
<th>Age-Matched Controls</th>
<th>Young Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>66.8 (6.0)</td>
<td>67.1 (6.2)</td>
<td>61.6 (11.7)</td>
</tr>
<tr>
<td>Baseline IOP (mmHg)*</td>
<td>18.6 (4.5)</td>
<td>14.9 (2.8)</td>
<td>14.4 (2.8)</td>
</tr>
<tr>
<td>IOP elevation (mmHg)</td>
<td>11.5 (1.8)</td>
<td>13.7 (4.7)</td>
<td>12.2 (1.9)</td>
</tr>
<tr>
<td>Optic disc size (mm²)</td>
<td>2.17 (0.50)</td>
<td>2.06 (0.34)</td>
<td>2.07 (0.62)</td>
</tr>
<tr>
<td>CCT (μm)</td>
<td>531 (25)</td>
<td>532 (27)</td>
<td>545 (29)</td>
</tr>
</tbody>
</table>

*CCT = central corneal thickness; IOP = intraocular pressure.

*Values shown are mean (standard deviation).

†Baseline IOP was significantly higher in glaucoma patients compared with the 2 control groups (P<0.037).

### Table 2. Summary Statistics of Laminar and Prelaminar Tissue Variables*  

<table>
<thead>
<tr>
<th>Group</th>
<th>Glaucoma Patients</th>
<th>Age-Matched Controls</th>
<th>Young Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-laminar tissue thickness (μm)</td>
<td>176 (153)</td>
<td>273 (135)</td>
<td>146 (79)</td>
</tr>
<tr>
<td>Laminar displacement (μm)</td>
<td>-0.5 (3.7)</td>
<td>0.2 (2.0)</td>
<td>2.0 (3.6)</td>
</tr>
<tr>
<td>Prelaminar tissue displacement (μm)</td>
<td>6.8 (13.7)</td>
<td>20.8 (17.5)</td>
<td>19.6 (11.8)</td>
</tr>
<tr>
<td>Percent pre-laminar tissue displacement (%)†</td>
<td>3.5 (6.8)</td>
<td>8.0 (6.0)</td>
<td>15.4 (8.8)</td>
</tr>
</tbody>
</table>

*Values shown are mean (standard deviation).

†Pre-laminar tissue displacement was significantly less in glaucoma patients compared with the 2 control groups (P<0.039).

‡Percent pre-laminar tissue displacement was significantly less in glaucoma patients and age-matched controls compared with young controls (P<0.022).

### Table 3. Results of Multivariate Regression Analyses of Laminar and Prelaminar Tissue Displacement*  

<table>
<thead>
<tr>
<th>Group (glaucoma = 2; age-matched controls = 1; young controls = 0)</th>
<th>Laminar Displacement</th>
<th>Prelaminar Displacement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>0.06</td>
<td>-0.10</td>
</tr>
<tr>
<td>Baseline IOP (mmHg)</td>
<td>0.25</td>
<td>0.94</td>
</tr>
<tr>
<td>IOP elevation (mmHg)</td>
<td>0.15</td>
<td>2.06‡</td>
</tr>
<tr>
<td>Optic disc size (mm²)</td>
<td>-3.90‡</td>
<td>-4.75</td>
</tr>
<tr>
<td>CCT (μm)</td>
<td>0.04</td>
<td>-0.05</td>
</tr>
<tr>
<td>Prelaminar tissue thickness (μm)</td>
<td>-0.01</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*CCT = central corneal thickness; IOP = intraocular pressure.

*Values shown are unstandardized regression coefficients.

†P = 0.007.

‡P = 0.013.
patients with a large IOP reduction after medical treatment.\textsuperscript{47} However, IOP elevation causes a posterior displacement of the disc surface with increase in cupping.\textsuperscript{48–50} Similar results have been observed in animal studies\textsuperscript{16,25} and enucleated human eyes.\textsuperscript{15,18} The presence of optic disc surface displacement seems to be related to the degree of IOP change,\textsuperscript{16} although the effects are variable\textsuperscript{51} and may not be linear.\textsuperscript{15,16} Studies using imaging techniques assumed that optic disc positional changes could be used as a surrogate for changes in laminar position,\textsuperscript{16,52,53} presumably because the prelaminar tissue was assumed to be largely noncompressible.

Experimental in vivo studies where eyes have been fixed at different IOPs and analyzed histomorphometrically permit unparalleled quantitative analyses but may be prone to effects of tissue shrinkage or swelling from fixation protocols. Studies in enucleated eyes have the disadvantage of postmortem changes in connective tissue and lack of vascular supply. Until the advent of SD OCT, it has not been possible to visualize the anterior laminar surface directly in the presence of the overlying prelaminar tissue and to quantify its displacement in a clinical setting.

The principal finding of this study was that with a mean acute IOP elevation of 12.4 mmHg, the lamina was resistant to displacement in glaucoma patients, age-matched controls, and young controls. These findings do not concur with previous studies of enucleated human eyes\textsuperscript{15,18} and experimental studies of primates.\textsuperscript{13} The large methodologic differences, including degree and duration of IOP increase and the techniques used to measure LD, may account for the discrepancy in the findings. Nonetheless, a recent study using serial 3-dimensional histomorphometric reconstructions in monkey eyes with elevated IOP showed that although there was laminar thinning resulting from stretching and expansion of the scleral canal, there was minimal posterior LD, and in some cases an anterior LD.\textsuperscript{54} Although this study used different methodologies, the results are consistent with the present findings. Additional in vivo studies in humans will be necessary to confirm these findings and those of a modeling study that also predicted lateral rather than anteroposterior LD.\textsuperscript{21} In the present study, significant PTD with elevated IOP was observed in healthy control subjects of approximately 20 μm for an average IOP elevation of approximately 13 mmHg. A statistical difference in PTD was not noted among younger and older healthy subjects; however, when corrected for prelaminar tissue thickness, the percentage of prelaminar tissue displacement was 2-fold higher in younger controls compared with older age-matched controls, which in turn was approximately 2-fold higher compared with glaucoma patients.

Previous human studies with experimental IOP elevation used either optic disc summary measurements\textsuperscript{48–50} or prob-

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**Figure 4.** Laminar displacement and prelaminar tissue displacement as a function of prelaminar tissue thickness (A and B, respectively) and intraocular pressure (IOP) elevation (C and D, respectively) in glaucoma patients, age-matched control and young control subjects. IOP = intraocular pressure.
ability maps\textsuperscript{51} and are therefore not comparable with the present study. However, in normal monkey eyes, Burgoyne et al\textsuperscript{15} reported a posterior optic disc displacement of 28 m\textmu m with IOP of 45 mmHg, whereas with the same IOP level, Coleman et al\textsuperscript{56} showed a posterior displacement of 18 m\textmu m. These values are similar to those found in normal subjects in the present study. The extent of PTD in glaucoma patients was significantly less compared with that in controls. This decreased compliance may be a result of increased stiffness in the prelaminar resulting from tissue remodeling associated with glaucomatous damage.

Although the average LD resulting from IOP elevation was close to 0 in both patients and healthy controls, it was statistically negatively related to optic disc size. An explanation for this finding is not obvious, but if a larger optic disc has a significantly less compared with that in controls. This finding is a type I statistical error, and because the average LD in this study was statistically indistinguishable from 0, the practical relevance of this finding is unclear. The degree of PTD was associated positively with IOP elevation and is consistent with previous studies in which the disc surface displaced posteriorly with increasing IOP.\textsuperscript{16}

This study has several limitations. Although mean LD is reported, measurements may not have come from the entire laminar surface in radial scans because of shadowing from large vessels obscuring visualization of the lamina. Segregation of the central from peripheral LD displacement was attempted; however, the inability always to visualize the entire anterior laminar surface from the insertion points at either end of the scleral canal made these analyses problematic. The study was adequately powered to detect a mean LD of 1.5 m\textmu m or less had it existed; however, mean differences in LD of less than 4 m\textmu m among the groups may not have been detected. Reproducibility measurements of the LD and PTD were different in patients and controls, with a higher variability in LD estimates in controls compared with patients. The lamina was easier to visualize in patients because of higher contrast with prelaminar tissue (Fig 2). The reason for this increased contrast is not obvious but could be related to tissue remodeling in the lamina with glaucomatous structural changes resulting in higher optical density. Finally, the degree of IOP elevation was maintained for a relatively short period (approximately 2 minutes). Higher IOP, a longer duration, or both, may have yielded different observations. The effects of IOP on LD are thought to be nonlinear.\textsuperscript{16} At lower translamellar pressure differences (IOP–cerebrospinal fluid pressure), LD may be predominantly in the anteroposterior plane, whereas at higher translamellar pressure differences, the displacement may be lateral\textsuperscript{16} and not readily detectable with the measurement techniques used. Lateral displacement of prelaminar tissue also may not have been detected with this technique.

In summary, this study showed that modest acute increases in IOP lead to negligible LD in both glaucoma patients and healthy subjects. In contrast to previous reports that have assumed that compliance measurements of the optic disc surface reflect laminar movement, it was determined that optic disc surface changes can be explained by displacement of prelaminar tissue and that the lamina is relatively rigid. The displacement of prelaminar tissue may result from a reduction in blood volume or axoplasmic material. This study was designed to investigate the acute effects of IOP elevation on the optic nerve head, which represent distinct phenomena to the well-characterized permanent changes from stress, strain, and eventual failure of the optic nerve head connective tissues leading to a backward bowing and compression of the lamina associated with glaucoma.\textsuperscript{57}

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Footnotes and Financial Disclosures

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