THE EFFECT OF INTRAOCULAR PRESSURE ON THE COLLAGEN CRIMP PERIOD IN THE LAMINA CRIBROSA

Natalie Rutkowski
Ian Sigal, Ning-Jiun Jan, UPMC Laboratory of Ocular Biomechanics, University of Pittsburgh, Department of Bioengineering

INTRODUCTION

Glaucoma is a disease that causes irreversible damage to the optic nerve fibers in the optic nerve head (ONH). It progressively leads to blindness and is the second most common cause of blindness worldwide. The main risk factor for glaucoma is increased intraocular pressure (IOP). Currently, treatments are limited to reducing this intraocular pressure, most commonly by in eye drops. Diagnosis of the disease is also limited to simply testing for increased IOP. The reason IOP leads to damage of the optic nerve head is unknown. Currently, in order to better understand why IOP causes damage, how to better diagnose the disease, and how to better treat it, researchers have been looking at the biomechanics of the optic nerve head, specifically the biomechanical properties of the ONH as a whole. Along with the lamina cribrosa, and has seen significant changes over small ranges of IOP. These measurements were used to study the biomechanical properties of the ONH as a whole. Specifically, I characterized the properties of crimp period and waviness of collagen in the lamina cribrosa, and examined the crimp period dependence on IOP.

HYPOTHESIS/SUCCESS CRITERIA

I hypothesized that an increase in intraocular pressure would stretch the collagen fibers, making the fibers less wavy and the crimp period increase. Prior to conducting experiments and collecting data, a repeatability and reproducibility study among the experimenters was evaluated to determine a common experimental method in order to ensure success of the experiment. As a group, we marked the same images for repeatability within 2.5 pixels and reproducibility within 6.0 pixels. The preliminary study allowed for us to learn the software, anatomical knowledge of the ONH, and terminology associated with the experiment.

METHOD

To test my hypothesis, 21 sheep eyes were studied. The eyes were exposed to individual pressures ranging from low to high IOPs, including: 0 mmHg, 10 mmHg, 15 mmHg, 20 mmHg, 25 mmHg, and 50 mmHg, with at least one eye at each IOP. Normal physiological pressure is 10-15 mmHg, and would be considered standard for both a sheep and human. Pressures above 21 mmHg are considered high, and pressures below 10 mmHg are considered low. Once the eyes were exposed to the desired IOP, they were fixed and sectioned. Following this, images of the sections were taken through the lamina cribrosa using polarized light microscopy (Olympus, 10x, 0.3 NA, 0.73 µm/pixel).

After the eyes were imaged, the images were stitched and aligned using the computer program Fiji. The images were marked manually in Fiji for both crimp period and waviness. To mark for crimp period, the image was analyzed by a custom program to locate the crimp. As shown in Figure 1 on the left, the image is purple and yellow in color, and crimp is distinguishable by its purple and yellow banding. One purple and one yellow band is exactly one period on the crimp. Each bright beam was marked with a short ROI in the direction of the beam. The red lines on the images are ROIs.
Figure 1. Examples of lamina beam marking. The image on the left is an example of marking for crimp period. The image on the right is an example of marking for waviness.

Following marking the image, the ROIs were analyzed using Fiji and custom programs to take measurements and examine collagen crimp and waviness. Linear mixed effect models with alpha values of 0.05 were used to analyze the measurements taken for crimp period.

RESULTS

Figure 2. Crimp Period vs. Pressure in the Lamina Cribrosa. This is a box plot of collagen crimp period at different IOPs.

Figure 2 is a box plot of the resulting measurements of crimp period in micrometers at each IOP. Overall, the plot shows that median crimp period is generally shorter as pressure increases. For example, at 0 mmHg, the median crimp period is 13 μm, and at 50 mmHg, the median crimp period is 6 μm. At pressures 12, 20, & 25 mmHg, which are the middle-middle high pressures, there is a large range of crimp periods seen. Whereas, at the lower pressures, 0 & 10 mmHg, and high pressure, 50 mmHg, there is a small range of crimp periods seen.

DISCUSSION

Collagen crimp period showed a general decrease with an increase in IOP. As shown in Figure 2, median crimp period at lower pressures was higher than median crimp period at higher pressures. This disproves my initial hypothesis that crimp period would increase with an increase in IOP. One reason for this is that crimp period may become less visible as it becomes longer or shorter. Decreased crimp period at higher IOPs may indicate that larger crimp periods disappear at lower IOP and smaller crimp periods at higher IOP. For this reason, we may only be marking the crimp that is easy to see at these pressures rather than the crimp that would be more characteristic at these pressures. For this reason, more data is needed to better understand how collagen crimp period will vary with varying IOP.

Waviness was quantified by taking the standard deviation of individual fiber angles over each given ROI. Waviness was highest at low pressures and decreased from 4-5° to 1-2° between 10 and 15 mmHg. As shown in Figure 3, median waviness remains relatively constant from 15 to 50 mmHg. This indicates that collagen fibers in the lamina cribrosa tend to have a higher waviness at low pressures and a constant, lower waviness at normal to high IOPs. This was consistent with my hypothesis that waviness will decrease as pressure increases. In the future, more measurements will be taken to get a more comprehensive understanding of waviness and how it varies with IOP.

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REFERENCES