Assessing the biomechanical properties of the porcine crystalline lens as a function of intraocular pressure with optical coherence elastography

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Abstract: In this study, we investigated the relationship between the biomechanical properties of the crystalline lens and intraocular pressure (IOP) using a confocal acoustic radiation force (ARF) and phase-sensitive optical coherence elastography (OCE) system. ARF induced a small displacement at the apex of porcine lenses in situ at various artificially controlled IOPs. Maximum displacement, relaxation rate, and Young’s modulus were utilized to assess the stiffness of the crystalline lens. The results showed that the stiffness of the crystalline increased as IOP increased, but the lens stiffening was not as significant as the stiffening of other ocular tissues such as the cornea and the sclera. A mechanical hysteresis in the lens was also observed while cycling IOP, indicating that the viscoelastic response of the lens is crucial to fully understanding its biomechanical properties.

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1. Introduction

Presbyopia is a progressive, age-related loss of accommodation ability of the eye. There is strong evidence to suggest that the change in biomechanical properties of the crystalline lens plays a major role in the development of presbyopia [1–3]. Therefore, information about elastic properties of the lens is extremely important to better understand how age-related changes in elasticity of the lens contribute to presbyopia and to help evaluate and optimize approaches for presbyopia treatment that rely on lens softening [4]. The intraocular environment strongly influences the biomechanical properties and their measurements of the crystalline lens. Intraocular pressure (IOP) is critical for maintaining normal eye-globe geometry and healthy function of ocular tissues. Moreover, IOP has a profound influence on the biomechanical properties of ocular tissues. Many ocular diseases, such as glaucoma and uveitis, are well-correlated with an elevation of IOP [5,6]. The effect of IOP elevation on the biomechanical properties of the cornea and sclera has been extensively investigated, and a dramatic increase in the stiffness of both tissues has been demonstrated [7–11]. In addition, it is critical to understand the influence of IOP on the results of the elasticity measurements in the lens. However, the location of the crystalline lens inside the eye-globe makes it very challenging to measure lens biomechanical properties in vivo or even in situ. Therefore, the influence of IOP on the biomechanical properties of the crystalline lens has rarely been investigated, and their relationship has yet to be established.
Different techniques have been proposed to characterize the mechanical properties of the crystalline lens [1,2,12,13]. A majority of these approaches utilize mechanical testing on the bare lens. The crystalline lens was dissected out of the eye-globe to perform such measurements, which does not truly replicate the lens environment within the eye-globe. Therefore, these techniques are not suitable for assessing the IOP-related changes in the biomechanical properties of the crystalline lens. Acoustic radiation force (ARF) in combination with laser-induced optical breakdown inside the lens and high-pulse repetition frequency ultrasound was used to assess the mechanical properties of crystalline lens [14–17]. Although the elasticity of the lenses was mapped, the lenses were removed from the eye-globe during the measurements. Ultrasound shear wave elastography is a well-established technique to measure tissue elasticity and has been recently successfully applied to study the crystalline lens [18–20]. However, the image resolution and sensitivity of shear wave velocity measurements need to be further improved for future clinical application.

Optical elastography is an emerging noninvasive technique for characterizing and mapping tissue elasticity with nanometer-scale sensitivity and micrometer-scale spatial resolution. Various optical elastography methods have been proposed based on several optical imaging techniques, such as laser speckle imaging [21], multiphoton microscopy [22], digital holography [23,24], Brillouin microscopy [25], and optical coherence elastography (OCE) [26]. Among all the optical elastography techniques, Brillouin microscopy and OCE show particular promise for ophthalmological applications. Scarcelli et al. investigated age-related stiffening in the murine lenses with Brillouin microscopy [27], and then further employed this technique to perform the ocular measurements on a human volunteer [28]. Although there is still uncertainty about the relationship between the Brillouin shift to material parameters such as Young’s modulus, Brillouin microscopy still shows great potential for clinical assessments of lenticular biomechanical properties.

OCE is an Optical Coherence Tomography (OCT)-based technology for characterizing tissue mechanical properties [26]. Like traditional elastography techniques, such as ultrasound elastography [29] and magnetic resonance elastography [30], OCE is based on measurements of induced deformations in tissues, and reconstruction of the target tissue biomechanical property by linking the measurements with appropriate mechanical models. Compared to ultrasound elastography and magnetic resonance elastography, OCE has advantages of higher resolution (micrometer-scale) and superior displacement sensitivity (sub-nanometer scale), which enable OCE to map variations in mechanical properties with greater sensitivity. Typical loading methods in OCE include mechanical actuators [31], air-pulse excitation [32], ARF [33–35], photo-thermal excitation [36], magnetic excitation [37], and Lorentz force excitation [38]. For a more in-depth understanding of OCE, the reader is directed towards recent reviews [26,39]. Due to the location of the lens inside the eye-globe, ARF is the well-suited noninvasive excitation method that can be combined with OCE to study its biomechanical properties [33,40].

In our previous studies we used ultrasound shear wave imaging to measure elastic wave velocities in the bovine lens and cornea at different IOPs [20]. In contrast to the cornea, the IOP increase did not result in significant changes in the elastic wave velocity in the lens. However, since a low-frequency ultrasound probe was used in this study, the results were still slightly ambiguous about the relationship between lens stiffness and IOP. More detailed investigations, with the aid of high resolution imaging techniques, of the IOP on lens stiffness are still required. Previously, we have employed a phase-sensitive OCE system to assess the age-related changes in the biomechanical properties of the crystalline lens in situ [33]. In this study, we investigated the changes in the biomechanical properties of porcine lenses as a function of IOP using the previously developed OCE approach based on ARF excitation and a phase-sensitive OCE system. The OCE measurements were performed with the eye-globe intact, and the IOP was cycled from 5 to 30 mmHg at 5 mmHg steps. The ARF remotely induced small amplitude (< 10 µm) localized displacements at the apex of the porcine lenses.
The maximum displacement (MD) and relaxation rate (RR) were calculated to assess the stiffness of the crystalline lens while cycling IOP. The temporal displacement profiles were further used in a model-based reconstructive approach to quantify the lenticular elasticity, in which additional liquid-tissue interaction has been taken into account for describing the presence of the aqueous humor between the cornea and the lens. For all three considered parameters, the results demonstrated that the stiffness of the crystalline lens increased along with IOP, however, this effect was less pronounced in comparison with other ocular tissues, such as the sclera and cornea.

2. Materials and methods

2.1 Experimental setup

The schematic of the system setup is shown in Fig. 1. The phase-sensitive OCE system consisted of a spectral domain optical coherence tomography (SD-OCT) and ARF delivery sub-systems. The SD-OCT system was based on a superluminescent light diode (SLD) with a central wavelength of 840 nm and a bandwidth of 49 nm. The power of the light source was 18 mW. The acquisition speed of the line-scan camera was set to 25 kHz. The displacement stability of the system was measured as 7 nm in air. The axial resolution of the OCT system was ~9 μm, and the lateral resolution was ~8 μm, both in air. The ARF excitation system consisted of single element ultrasound transducer (CTS Valpey Corporation, MA), arbitrary waveform generator (RIGOL Tech, China), and RF power amplifier (Electronics & Innovation Ltd., NY). The transducer operated at a 3.5 MHz central frequency with a focal length of 19 mm. The arbitrary waveform generator produced a sinusoidal signal that was further amplified by the 50-dB power amplifier and drove the ultrasound transducer to produce 1.06 ms ARF pulse. The waveform generator and OCT frame trigger were synchronized by a computer-generated TTL signal [33,41].

A homemade closed-loop IOP control system was used to set the pressure inside the eye-globe. The IOP control system was composed of a pressure transducer (Keller AG, Switzerland) and micro-infusion syringe pump (New Era Pump System Inc., NY). The eye-globe was placed in a custom holder, and two needles were used to cannulate the eye-globe through holes in the holder. The needles were inserted to the eye-globe through the vitreous chamber. One needle was connected via tubing to a saline-filled syringe placed in the micro-infusion pump. The other needle was connected via tubing to the pressure transducer. A Matlab (MathWorks, Natick, MA) GUI program was developed to control the closed-loop IOP system. The IOP was changed by infusion or extraction of saline from the syringe.
Fig. 1. (a) Schematic of experimental setup; (b) Typical OCT image of porcine eye schematically showing co-focused OCT and ARF beams. (cornea is shown as negative image as the lens surface was brought to the focus to maximize SNR).

2.2 Phantoms and tissue samples

Initial measurements were performed on tissue-mimicking agar phantoms of various concentrations (1%, 1.5%, and 2%). The phantom fabrication process has been covered in our previous work [41]. Uniaxial mechanical testing (Model 5943, Instron Corp., Norwood, MA) was performed on the agar phantoms for validation purposes. Porcine eyes (n = 8) were obtained fresh (Sioux-Preme Packing Co., Sioux City, IA) from 4 to 6 months old animals and shipped overnight on ice. The experiments were conducted when the samples were received, which was within 24 hours of enucleation. To measure the dependence of lenticular mechanical properties on IOP, the IOP was increased from 5 to 30 mmHg in 5 mmHg steps, and then decreased back to 5 mmHg using the same steps to investigate biomechanical hysteresis. Before the OCE measurements were taken, each eye was preconditioned by cycling the IOP between 5 and 30 mmHg twice. The parameters of ARF excitation were the same for all IOPs, and 40 measurements were taken at each IOP level. Each measurement took 0.5 s, and the time interval between measurements at different pressure levels was about 4 minutes with consideration of data recording, changing IOP and its stabilization, and experimental operations. M-mode imaging was performed with the ARF and OCT probe...
beams co-focused at the apex of the lens [33]. When the IOP was increased or decreased, the imaging probes were adjusted both axially and laterally to ensure the lens apex was measured.

2.3 Mechanical characterization

The temporal displacement profiles at lens apex position could be extracted from OCT M-mode images. The temporal phase profiles $\phi(t)$ were unwrapped and then converted to displacement, $d(t)$, by:

$$d(t) = \frac{\lambda_0}{4\pi n} \phi(t),$$

(1)

where $\lambda_0$ is the central wavelength of OCE system and $n$ is refractive index. A refractive index of 1.333 for water and 1.336 for aqueous humor were used to calculate the displacement of agar phantom and lens surface, respectively. To estimate the relaxation rate of the deformation, the recovery process was fitted by an exponential function:

$$d(t) = Ae^{-bt},$$

(2)

where $A$ is the maximum displacement, and $b$ is the relaxation rate as determined by fitting with the Matlab curve fitting toolbox. Figure 2(a) shows a typical displacement temporal profile and the fitting process for the relaxation process. In our previous studies we have shown that both these parameters can be used as a parameter of sample stiffness, where the maximum displacement and relaxation rate are inversely and positively related to the stiffness, respectively [33].

2.4 Model-based estimation of Young’s modulus

To quantitatively evaluate Young’s modulus of the porcine lenses, we utilized a model based reconstructive approach similar to the approach described in our previous work [33]. A complete description of this model-based approach has been detailed by Aglyamov et al. [42]. In this work, the fluid-solid interaction was also considered in the model to describe the presence of the aqueous humor between the cornea and the lens. The lens was modeled as a viscoelastic (Voigt body) half-space with a fluid-solid boundary condition at the surface. The solution was obtained in the frequency domain, assuming a harmonically applied force at angular frequency $\omega$. Thus, the inverse Fourier transform was used to obtain temporal displacement profiles [42]. An ARF impulse was considered to be an axisymmetric force applied to the surface of the tissue at depth $z = 0$ in cylindrical system ($r, \theta, z$), where $z$ was along the optical axis. Since the problem is axisymmetric, no dependences on the angle $\theta$ were considered, and the $\theta$-component of the displacement vector was set to zero.

The spectral components of the vertical displacement, $u_z$, in a half-space, $z \geq 0$, can be obtained using the Hankel transform, as [42]:

$$u_z = -\int_0^\infty \alpha J_0(\alpha r)(\nu_1 B_1 e^{\alpha z} + \nu_2 B_2 e^{\alpha z})d\alpha,$$

(3)

where

$$\nu_1 = \sqrt{\alpha^2 - k_1^2}, \quad k_1 = \frac{\alpha \omega}{c}, \quad \nu_2 = \sqrt{\alpha^2 - k_2^2}, \quad k_2 = \rho \omega^2 / (\mu_1 + i\omega \mu_2).$$

Here, $J_0$ is the Bessel function of order zero; $\mu_1$ and $\mu_2$ are shear elastic and viscous moduli, respectively; $c$ is the speed of sound; and $\rho$ is the density of medium. The two unknown constants, $B_1$, and $B_2$, are defined using the fluid-solid boundary conditions at the half-space surface $z = 0$. On the boundary we require the continuity of the vertical...
displacements in solid and in liquid, as well as zero shear stress. We also assumed a Gaussian distribution of acoustic pressure \( p(r) \) with a width \( R \) on the half-space surface:

\[
p(r) = P_0 e^{-r^2/R^2},
\]

where \( P_0 \) is the pressure amplitude. The ultrasound field in the focal point of the transducer was measured using a needle hydrophone with a sensor diameter of 0.2 mm (Precision Acoustics Ltd, Dorchester, UK). The normalized distribution of the time-averaged pressure in the focal zone of the 3.5 MHz transducer was fitted by the Gaussian profile in Eq. (4). The best obtained value of \( R = 0.37 \text{ mm} \) was corrected considering an angle of \( 45^\circ \) between the transducer and the lens surface in the experiment. We used an “effective” value of \( R \), such that the area of excitation was equal to the elliptical area of the ultrasound field in the experiment, so that \( R = 0.37 \sqrt{2} = 0.44 \text{ mm} \).

The inverse Fourier transform was applied to Eq. (3) to obtain the temporal displacement profile, \( d(t) \). Estimation of the Young’s modulus of the lens was posed as an error minimization problem, where the error was defined as the difference between the measured vertical displacement and the theoretically calculated displacement at the focal point of the ultrasound transducer \( z = 0, r = 0 \), as described previously [33]. Since it is difficult to evaluate the magnitude of the acoustic radiation pressure \( P_0 \) on the lens surface, normalized experimental displacement profiles were used, such that the amplitude of the displacement was not taken into account during minimization procedure. Each displacement profile was normalized to the maximum displacement in the profile. As a result, obtained values of Young’s modulus were independent of MD values. We assume incompressibility of the lens tissue, such that \( k_2 = 0 \) and \( E = 3\mu_1 \). For all IOPs we assume a constant shear viscosity \( \mu_2 = 1 \text{ Pa}s \) based on the results of our previous studies on porcine lenses [16], while no viscosity was assumed for agar phantoms. The speed of sound in fluid was set as 1500 m/s, the lens density as 1185 kg/m³, and density of liquid and agar phantoms as 1000 kg/m³ [43].

3. Results

Fig. 2. (a) The OCE-measured displacement at the apex of the lens in response to the acoustic radiation force excitation. The maximum displacement is indicated in green, and the fitted exponential curve for relaxation rate analysis is plotted in red. (b) Maximum displacement of agar phantoms at various concentrations. (c) Relaxation rate obtained for tissue-mimicking agar phantoms at various concentrations. (d) Comparison of the Young’s modulus values obtained from mechanical compression testing and model-based reconstruction.
Figure 2(a) shows a typical temporal displacement profile recorded at the apex of a crystalline lens. The MD is indicated in green, and the fitted exponential curve to quantify the RR is plotted in red. Figures 2(b-c) show the results of MD and RR for the agar phantoms at various concentrations. The MDs were 2.28 ± 0.04 μm, 1.56 ± 0.02 μm, and 1.12 ± 0.03 μm in the 1%, 1.5%, and 2% phantoms, respectively. For the 1%, 1.5%, and 2% agar phantoms, the RRs were 1.53 ± 0.04 ms⁻¹, 2.40 ± 0.06 ms⁻¹ and 2.90 ± 0.14 ms⁻¹, respectively. As expected, the MD decreased, and the RR increased as the agar phantom concentration and stiffness increased. Figure 2(d) presents the Young’s modulus of phantoms calculated from the model-based elastic reconstruction method as compared to the Young’s modulus measured by uniaxial mechanical compression testing. For the 1%, 1.5%, and 2% agar phantoms, the results of the uniaxial mechanical test were 3.9 ± 0.76 kPa, 7.9 ± 0.77 kPa, and 15.1 ± 1.1 kPa, respectively, while the results of the model-based estimation using OCE measurements were 3.0 ± 0.27 kPa, 11.2 ± 1.54 kPa, and 13.5 ± 2.51 kPa, respectively.

Figure 3 is box and whisker plot of the results of MDs, RRs, and Young’s moduli of the porcine lenses from one representative sample while cycling IOP. The box is the inter-quartile range, the center horizontal line is the median, the whiskers are the standard deviation, and the small inscribed box is the mean. While increasing IOP, the MD at 5 mmHg IOP (1.55 ± 0.02 μm) decreased to 1.30 ± 0.03 μm at 20 mmHg, and then gradually decreased to 1.29 ± 0.02 μm at 30 mmHg. The RR at 5 mmHg IOP (0.95 ± 0.03 ms⁻¹) rapidly increased as the IOP increased up to 20 mmHg (1.31 ± 0.05 ms⁻¹), and then stabilized to 1.34 ± 0.03 ms⁻¹ at 30 mmHg. The model-based estimated Young’s modulus increased from 3.92 ± 0.52 kPa at 5 mmHg, to 5.51 ± 0.48 kPa at 20 mmHg, and 5.79 ± 0.50 kPa at 30 mmHg. It is worth noting there was a noticeable hysteresis while the IOP was cycled.
Figure 4 includes the summarized results of MDs, RRs and Young’s moduli for all eight samples. The raw data is plotted alongside the respective inter-sample (N = 8) box and whisker plots, where the box is the inter-quartile range, the central horizontal line is the median, the small inscribed box is the mean, and the whiskers are the standard deviation. Figure 4(a) shows that the MD decreased rapidly from an IOP of 5 mmHg (1.61 ± 0.10 µm) until the IOP was 20 mmHg (1.26 ± 0.11 µm), and then the MD slowly decreased until the IOP was 30 mmHg (1.20 ± 0.12 µm). The MD decreased 21.7% while increasing IOP from 5 mmHg to 20 mmHg but only decreased 4.7% when the IOP was further increased from 20 mmHg to 30 mmHg. A noticeable hysteresis was observed while decreasing IOP, where the MD at 5 mmHg was 1.49 ± 0.07 µm compared to 1.61 ± 0.10 µm while increasing IOP. A similar, but opposite, trend was seen in the RRs as plotted in Fig. 4(b). The RR at 5 mmHg IOP (1.14 ± 0.23 ms$^{-1}$) rapidly increased by 28.9% as the IOP increased up to 20 mmHg (1.47 ± 0.31 ms$^{-1}$). However, once the IOP was further increased to 30 mmHg, there was no noticeable change in the RR (1.47 ± 0.34 ms$^{-1}$). Similar to the MD, we observed hysteresis while cycling IOP, where the RR at 5 mmHg IOP while decreasing IOP (1.20 ± 0.25 ms$^{-1}$) was different than when increasing IOP (1.14 ± 0.23 ms$^{-1}$). The dependence of Young’s modulus on IOPs from 5 mmHg to 30 mmHg are summarized in Fig. 4(c). Young’s modulus increased from 4.98 kPa to 6.67 kPa for IOP changed from 5 mmHg to 20 mmHg, and up to 7.09 kPa for 30 mmHg. The trend in Young’s modulus as a function of IOP was similar to the RR trend, where elasticity increased by 33.9% from 5 to 20 mmHg IOP. To further test the effect of the IOP and direction of IOP change on the lens stiffness, two-way repeated measures ANOVA tests were performed on the data shown in Fig. 4. The MDs, RRs, and Young’s modulus all showed a statistically significant difference as a function of IOP (P<0.01 for each of the three parameters). There was a noticeable hysteresis in the lens stiffness while cycling IOP as measured by (a) MD (P<0.01) and (b) RR (P<0.01), but not by (c) Young’s Modulus (P>0.05). In Table 1, the results for relaxation rates, displacement amplitudes, and Young’s moduli are summarized for all samples.
Table 1. Summary of the maximum displacements (MD), relaxation rates (RR), and Young’s moduli (E) from all samples (N = 8). The data are presented as the inter-sample mean ± standard deviation.

| IOP (mmHg) | Increasing IOP | Decreasing IOP |
|------------|----------------|----------------|
|            | MD (μm) | RR (ms⁻¹) | E (kPa) | MD (μm) | RR (ms⁻¹) | E (kPa) |
| 5          | 1.61 ± 0.10 | 1.14 ± 0.23 | 4.98 ± 1.16 | 1.49 ± 0.07 | 1.20 ± 0.25 | 5.11 ± 0.86 |
| 10         | 1.44 ± 0.09 | 1.21 ± 0.21 | 5.49 ± 1.15 | 1.33 ± 0.08 | 1.32 ± 0.21 | 5.72 ± 0.91 |
| 15         | 1.32 ± 0.10 | 1.39 ± 0.27 | 6.29 ± 1.54 | 1.27 ± 0.11 | 1.45 ± 0.26 | 6.50 ± 1.32 |
| 20         | 1.26 ± 0.11 | 1.47 ± 0.31 | 6.67 ± 1.48 | 1.23 ± 0.12 | 1.48 ± 0.31 | 6.85 ± 1.20 |
| 25         | 1.23 ± 0.13 | 1.47 ± 0.30 | 7.00 ± 1.37 | 1.22 ± 0.13 | 1.47 ± 0.32 | 7.12 ± 1.44 |
| 30         | 1.20 ± 0.12 | 1.47 ± 0.34 | 7.09 ± 1.33 | 1.20 ± 0.12 | 1.47 ± 0.34 | 7.09 ± 1.33 |

4. Discussion

In this study, we investigated the dependence of the biomechanical properties of the porcine crystalline lens on IOP. The maximum displacement and relaxation rate of the ARF-induced deformation at the lenticular apex were quantified to evaluate the change in stiffness of the lens as IOP was cycled. Both parameters have a simple physical sense. The MD reflects how much the tissue is deformed under an external force, and RR describes how fast tissue returns to its initial position after excitation. Although both these parameters are directly connected with lens stiffness, they also depend on the characteristics of the applied force. While in our experiments we assume that ARF is the same for every measurement, changes in the force characteristics result in changes in MD and RR values. Thus, a model-based reconstructive approach was applied to quantify the Young’s modulus of the lens. The reconstructed values of Young’s modulus were obtained using normalized displacement profiles and can be considered as a parameter independent of MD.

The phantom experiments confirmed that the RR, MD, and estimated Young’s modulus correlate well with Young’s modulus as measured by the gold standard of uniaxial mechanical testing. Our previous work has shown that these parameters could be used to evaluate the age-related stiffness change of the crystalline lens [33]. However, we note that the phantom studies do not simulate the changes in crystalline lens elasticity or geometry under IOP. The phantom experiments only provide a validation for the OCE technique to characterize material stiffness in controlled conditions. While the change in phantom stiffness was controlled by the agar concentration, the lens stiffening is a result of the lens deformation due to IOP and the elastic nonlinearity of the lens.

The results in this study demonstrate that the stiffness of the lens increases as the IOP increases, and this trend was observed in all eight samples and for all mechanical characteristics utilized in this work. All OCE measurements were made immediately after the target IOP was reached. Our future work will investigate whether more time is required to reduce the observed biomechanical hysteresis and if a prolonged elevated IOP affects the degree of changes in the biomechanical properties of the lens. Nevertheless, the hysteresis was small and the overall changes in lenticular biomechanical properties due to an elevated IOP were generally reversed by lowering the IOP.

It is worthwhile to note that the inter-sample standard deviations of the MD and RR, and Young’s modulus were relatively large. The large inter-sample standard deviations can be caused by age difference of the samples, and our previous work has shown that age has a drastic influence on the biomechanical properties of the crystalline lens, albeit in rabbits [33]. Such a significant inter-subject variability could be due to age differences. Nevertheless, the trend is clear that, regardless of age, the IOP has a noticeable influence on the lens stiffness.

A common cause of ocular hypertension is excessive aqueous production by the ciliary body. In this study, an IOP control device was used to artificially change the intraocular pressure by infusion and extraction of saline. Similar techniques have been commonly used
for IOP-related measurements of ocular tissues in other studies [44,45]. We have shown that the stiffness of the lens generally increases as IOP increases. The IOP change likely causes deformation of the crystalline lens, and consequent exhibition of the nonlinear elastic properties of the lens. The mechanical compression tests have confirmed such elastic nonlinearity of the crystalline lens, when the slope of the strain-stress relation grows with the strain level resulting to stiffening of the lens [33].

The relationship between IOP and biomechanical properties of crystalline lens has rarely been investigated previously due to the location of the lens within the eye-globe. Hence, the vast majority of research has focused on the influence of IOP on corneal biomechanical properties. However, many ocular diseases are correlated with an elevation IOP, most notably glaucoma [33]. The present study shows that the biomechanical properties of the lens were also affected by IOP, and the changes were reversible in the short term. Recently, we studied the impact of IOP on elastic wave propagation in the bovine crystalline lens and cornea using ultrasound elastography, which demonstrated relatively small increase in the elastic wave velocity in the lens compared to cornea These results show good agreement with previous findings [20]. Our results show a significantly less pronounced increase in lenticular stiffness when the IOP is increased, which is in contrast to the very large increases in the stiffness of cornea and sclera due to elevated IOPs that have been reported in the literatures [9–11,20]. Compared to ultrasound elastography, phase-sensitive OCE detection can achieve measurements of submicron displacement and enable more accurate elasticity analysis of the sample. High variations of measured elastic wave velocities were observed at IOP above 20 mmHg for single sample in the previous study. In this study, a more obvious trend of the elasticity change along IOP is shown due to the high sensitivity of OCE technique. We observed more than three-fold increase in Young’s modulus of the cornea, when IOP increases from 15 to 30 mmHg [11], while in the presented results for Young’s modulus of the lens increased less than 12.7% for the same IOP increasing range. The effect of stiffening with IOP for the lens is significantly less noticeable than for the cornea. One of the possible explanations of the difference in the mechanical behavior between the lens and cornea is that the lens undergoes less significant deformation during IOP elevation, such that nonlinear elastic properties of the lens do not play a significant role in the response to acoustic excitation.

The Young’s modulus of porcine lens calculated from the model was 4.98 ± 1.16 kPa at 5 mmHg, which agrees with previous studies [16,46]. One of the limitation of the model-based approach includes an assumption that the lens is considered as a homogeneous half-space medium. However, the porcine crystalline lens is an inhomogeneous object with a stiffness gradient inside, which was not investigated in this work [14,16]. The estimated Young’s moduli may mostly correspond to the superficial anterior parts of the lens, which are softer than the center. Therefore, this limitation could result in an underestimation in the Young’s modulus of the lens. In addition, if the lens is deformed inhomogenously inside the eye-globe during IOP elevation, it could result in the changes of the stiffness spatial distribution. Measurements at different locations, and more rigorous mechanical models that incorporate elastic nonlinearity and inhomogeneity of the crystalline lens are required to more accurately quantify lentricular biomechanical properties. Shear wave based OCE could be utilized to study the internal structure of lens since this method has demonstrated the capability of depth-resolved analysis of corneal stiffness. In addition, Brillouin microscopy could be also combined with OCE to investigate the spatial distribution of changes in lenticular biomechanical properties as a function of IOP, which is another possible avenue of our future work.

5. Conclusion

In this study, we investigated the influence of IOP on the biomechanical properties of *in situ* porcine crystalline lenses with OCE, and the results showed that the stiffness of the lenses
increased when the IOP was elevated. While the lens stiffness did increase as IOP increased, it was not monotonic, and the stiffening decreased once the IOP was increased above 20 mmHg. The OCE measurements were then repeated while the IOP was decreased, and the observed increase in lenticular stiffness was generally reversed once the IOP was decreased. Even though there was a small hysteresis while cycling IOP, the results demonstrate that the changes in lens elasticity is mostly reversible in the short term. Due to the noninvasive OCE measurements and ARF excitation, the presented technique may be viable for in vivo measurements of lens biomechanical properties.

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The authors declare that there are no conflicts of interest related to this article.

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