Increased Iris Stiffness in Patients With a History of Angle-Closure Glaucoma: An Image-Based Inverse Modeling Analysis

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Glaucoma is a leading cause of irreversible blindness, affecting more than 63 million people worldwide.1 By 2040, 111.8 million people will be affected by glaucoma.1 In the United States, glaucoma accounts for 9% to 12% (i.e., approximately 120,000) of all cases of blindness.2,3 Two major types of glaucoma, that is, primary open angle glaucoma (POAG) and primary angle-closure glaucoma (PACG), are categorized based on the angle between the iris and the cornea, known as the anterior chamber angle (ACA; Fig. 1A). In PACG, the iris is abnormally bowed toward the anterior, thereby blocking the aqueous humor outflow pathway (Fig. 1B). Although not as prevalent among Caucasian men, PACG primarily affects women and non-Caucasian ethno-racial groups, especially Eskimos and Asians.2,4–6 In fact, because of its high prevalence in East Asia, PACG affects at least as many people as does POAG.7

Pupillary block is considered an underlying mechanism of angle closure. As shown in our previous computational models, due to a narrow gap between the iris tip and the lens, aqueous humor pressure in the posterior chamber is generally slightly higher than that in the anterior chamber.8,9 The higher pressure in the posterior chamber generates a net force that pushes the iris periphery toward the anterior, leading to a significant narrowing or complete closure of the ACA and consequently blockage of the outflow pathway.10–12 Laser peripheral iridotomy (LPI) remains the most common method for the treatment of pupillary block.13 During this procedure, a laser is used to make holes through the iris to reduce the pressure difference between the anterior and posterior chambers and to restore the normal iris configuration. The LPI procedure, however, may not necessarily widen the ACA in all cases.14,15 In addition, although in many cases the angle opens immediately following the LPI procedure,16 the long-term outcomes may not be as promising, and further surgical procedures may still be necessary to reduce the intraocular pressure.17 Recent studies have identified the success rate of LPI even as low as 24%.10,19

The existence of occludable ACAs following LPI indicates that other mechanisms in addition to pupillary block must be involved in the pathophysiology of PACG. Recent studies have shown the importance of structural components comprising the iris extracellular matrix and the mechanical properties of the tissue such as stiffness and compressibility in the context of PACG20–27 (Narayanaswamy AK, et al. IOVS 2015;56:ARVO E-Abstract 6139). Briefly,

- Histological characterizations of excised iris tissues have shown a higher density of collagen I, a major component
of the iris extracellular matrix, in patients suffering from acute angle closure.\(^2\)

- Real-time quantitative polymerase chain reaction examinations of iris samples obtained from PACG and POAG patients have shown higher level of collagen type alpha 1 chain (\(\text{COL}1\alpha1\)) mRNA expression in PACG patients.\(^2\) It is noteworthy that the amount of \(\text{COL}1\alpha1\) expressed in tissues directly scales with the mechanical stiffness across all types of tissues.\(^2\)

- Genomewide studies of more than 10,000 PACG patients in 24 countries have shown an association between the locus of the collagen type XI alpha 1 chain (\(\text{COL}11\alpha1\)) gene and PACG.\(^2\)

- In vivo imaging of the iris cross-sectional area have shown that, in PACG patients, the iris volume does not change significantly after dilation.\(^2\) Such a phenomenon indicates that although the iris is spongy and compressible in healthy and POAG eyes, in PACG and angle closure suspects, the iris exhibits an incompressible behavior.\(^2\)

- Our in silico models developed based on typical anterior segment geometric parameters have shown that following dilation, the ACA narrows much more if the iris is incompressible and less spongy.\(^2\)

- Ex vivo mechanical testing on the iris samples have shown a larger stiffness value in the irides of patients suffering from PACG when compared with those with POAG (Narayanaswamy AK, et al. IOVS 2015;56:ARVO E-Abstract 6139).

These studies collectively indicate a relationship between the iris stiffness and PACG. However, most of them refer to an indirect correlation with tissue stiffness and the only direct measurement of the iris stiffness in relation to PACG has been conducted ex vivo. By nature, ex vivo studies are limited due to a number of reasons. First, there are concerns regarding tissue availability and ethical issues involved with such invasive procedures. Second, the tissue properties have been shown to be different in ex vivo experiments when compared with the in vivo physiological condition.\(^2\) Finally, noninvasive in vivo procedures are more suitable for the development of potential new diagnostic methods and/or treatment strategies. As such, we have used clinical anterior segment optical coherence tomography (AS-OCT) images and computer simulation to estimate the mechanical properties of the irides in vivo. With this noninvasive method, we quantified the in vivo iris mechanical properties in patients who had occludable ACA after undergoing LPI and compared them with those of the healthy volunteers.

**METHODS**

**Imaging and Segmentation**

AS-OCT (Carl Zeiss Meditec, Inc., Dublin, CA, USA) images were acquired from two groups of an Indian population at the LV Prasad Eye Institute in Hyderabad, India. The first group (\(n = 8\)) was randomly selected from a pool of 30 patients who had undergone LPI for angle closure, but their ACA remained occluded following the procedure. Patients who exhibited abnormal pupillary light reflex response (i.e., their pupils did not dilate) were not included in the study. The second group (\(n = 8\)) was randomly selected from a group of healthy volunteers using the same exclusion criteria. The demographic and clinical factors of the enrolled population are shown in Table 1. For each group, images were taken under standard light conditions and following dim light–induced dilation (Fig. 2). For the standard light conditions, a standard light intensity of 350 lux was maintained. No light was targeted toward the eye, and standard room illumination was used in all cases to stimulate the eye. For the dim light conditions, the 350-lux light source was turned off. For all scans, the images were aligned and rotated correctly in such a way that the corneal axis is in the center axis. The tenets of the Declaration of Helsinki were followed for this study.

**Meshing**

Finite element meshes of the iris were constructed based on the dim light–induced dilated images of the eye (Fig. 2B) using the following steps as described in our previous publication\(^3\):

i. AS-OCT images were imported into Solidworks (Dassault Systemes, Velizy-Villacoublay, France) and appropriately scaled. The boundary of the iris was then manually tracked and a solid two-dimensional model of the iris was created (Fig. 3A).

ii. The Solidworks output file was then imported into Abaqus (Dassault Systemes). The two-dimensional iris model was then meshed using a paving approach (Fig. 3B). The corresponding sphincter region was also identified for each iris model (Fig. 3C).

iii. Because Abaqus is limited in that it can generate only up to 8-node quadrilateral element mesh, the output of the
Abaqus mesh was subsequently imported into an internally developed program in C, in which an extra node was added to each element to generate a 9-node biquadratic finite element mesh.

Governing Equation

A two-dimensional axisymmetric model of the iris was constructed that was similar to the one in our previous study and was based on OCT scans. The OCT scans were chosen randomly from either of the nasal, temporal, inferior, or superior configurations, and only one side of the iris was modeled. The iris was modeled as a neo-Hookean solid material governed by the following stress balance equation:

$$\nabla \cdot \sigma = 0$$  \hspace{1cm} (1)

where $\sigma$ represents the Cauchy stress tensor. The Cauchy stress tensor was defined by the neo-Hookean ($\sigma_{\text{NH}}$) and active sphincter ($\sigma_{\text{s}}$) stress tensors:

$$\sigma = \sigma_{\text{NH}} + \sigma_{\text{s}}$$  \hspace{1cm} (2)

The neo-Hookean stress is defined by

$$\sigma_{\text{NH}} = \frac{G}{J} (B - I) + \frac{2Gv}{(1 - 2v)J} \ln(J)I$$  \hspace{1cm} (3)

where $G$ is the shear modulus; $v$ is the Poisson's ratio; $I$ is the identity tensor; $J$ is the determinant of $F$ where $F$ is the deformation gradient tensor; and $B$ is the left Cauchy-Green deformation tensor. The tensors $F$ and $B$ are defined as

$$F = \frac{dx}{dX}$$  \hspace{1cm} (4)

$$B = FF^T$$  \hspace{1cm} (5)

where $x$ is the current position of a material point and $X$ is its resting position. The sphincter stress $\sigma_{\text{s}}$, which was applied in the sphincter region, is defined by the scalar active muscle contraction stress ($\sigma_{\text{Ac}}$) and the unit vector representing the direction of the sphincter muscle ($e_{\text{h}}$).

$$\sigma_{\text{s}} = \sigma_{\text{Ac}} e_{\text{h}} \otimes e_{\text{h}}$$  \hspace{1cm} (6)

For all of the simulated cases, 40 kPa was chosen as the magnitude of the active muscle concentration stress $\sigma_{\text{Ac}}$, based on the estimated values in our previous models.

Inverse Finite Element Modeling

An inverse approach that was combined with our finite element model of the iris relaxation during pupil constriction was used to calculate the iris shear modulus $G$. We have used a similar approach in our previous study for the estimation of $G$ in the trabecular meshwork. A basic overview of the inverse approach is shown in Figure 4. The objective function was defined as the absolute difference between distance from the root to the tip of the iris measured in the experiments ($d_{\text{exp}}$) and the simulated distance between the root and tip of the iris ($d_{\text{sim}}$).

$$\text{Error} = |d_{\text{exp}} - d_{\text{sim}}|$$  \hspace{1cm} (7)

To capture the effect of compressibility on the estimated shear modulus $G$ for each set of the iris images, two cases using different values for the Poisson’s ratio $v$ were considered: (1) discretization of the mathematical model along the meshes generated using the abovementioned procedure. The Newton-Raphson iteration and the direct linear solver Multi-frontal Massively Parallel sparse direct Solver (MUMPS; University of Bordeaux, Bordeaux, Nouvelle-Aquitaine, France) were employed in solving the nonlinear algebraic equations. To examine the effect of the mesh size on the problem outcomes, a mesh convergence study was performed similar to the one employed in our previous study.

Numerical Solution

An internally developed computer code using C was employed to apply the Galerkin finite element method for spatial discretization of the mathematical model along the meshes.
the iris was considered as a compressible material with a Poisson’s ratio of \(v = 0.35\) and (2) the iris was considered as a nearly incompressible material; hence, a Poisson’s ratio of \(v = 0.49\) was used.

The initial guess for \(G\) was chosen between 1 and 91 kPa. All simulations were performed using an HP Intel Xeon machine (Intel, Santa Clara, CA, USA) at the Ohio Supercomputing Center (Columbus, OH, USA). The maximum number of generations (i.e., the iteration levels for the inverse algorithm) was set to 50 to allow for the solution to converge. At the end of 50 iterations, convergence on a solution for a particular objective function was achieved when the individual values of \(G\) in the population set came satisfyingly close to each other, meaning that the maximum difference between each population and the best population was below a threshold value at the end of 50 iterations; otherwise, the population was defined not to have converged to a unique solution.

After obtaining the shear modulus, \(G\) for a particular Poisson’s ratio, \(v\), the corresponding elastic modulus, \(E\), was calculated as:

\[
E = 2G(1 + v)
\]

Because the calculated elastic modulus was dependent on the magnitude of the sphincter muscular stress, the calculated elastic modulus was normalized using the magnitude of the sphincter stress (\(\sigma_{Act}\)) to obtain a normalized elastic modulus value (\(E'\)).

\[
E' = \frac{E}{\sigma_{Act}}
\]

To examine whether the choice of \(\sigma_{Act}\) has any influence on the calculated normalized elastic modules, the value of \(\sigma_{Act}\) was perturbed in two cases.

### Statistical Analysis

The normalized elastic moduli for healthy eyes and post-LPI occludable ACA eyes in both the compressible and the nearly incompressible cases were used in the following null hypotheses: (1) no significant difference existed between the mean normalized elastic modulus of the compressible and nearly incompressible cases both for healthy irides and for post-LPI irides, and a one-tailed Student’s \(t\)-test was performed to compute the statistical significance \((P < 0.05)\); (2) no significant difference was found to exist between the mean normalized elastic moduli of the compressible/nearly incompressible healthy and compressible/nearly incompressible post-LPI irides, and a one-tailed Student’s \(t\)-test was performed to compute the statistical significance \((P < 0.05)\).

### Results

The average iris length (\(d\)) in normal patients under standard light and following dim light dilation was found to be 4.48 ± 0.15 mm (average ± standard error) and 4.00 ± 0.14 mm, respectively. The average iris length in PACG patients under standard light and following dim light dilation was found to be 4.28 ± 0.09 mm and 4.09 ± 0.07 mm, respectively. No significant difference was found in the iris length of normal and PACG patients during both standard light and following dim light-induced dilation \((P > 0.05\), using the Student’s \(t\)-test).

The response of the iris to changes in ambient light was captured reasonably accurately by the computational simulation (as shown in Fig. 5). The outcomes of inverse finite element modeling and the subsequent statistical analyses are summarized in Tables 2 and 3. The mean normalized iris elastic moduli for healthy eyes and post-LPI occludable ACA eyes in both the compressible and the nearly incompressible cases were used in the following null hypotheses: (1) no significant difference existed between the mean normalized elastic modulus of the compressible and nearly incompressible cases both for healthy irides and for post-LPI irides, and a one-tailed Student’s \(t\)-test was performed to compute the statistical significance \((P < 0.05)\); (2) no significant difference was found to exist between the mean normalized elastic moduli of the compressible/nearly incompressible healthy and compressible/nearly incompressible post-LPI irides, and a one-tailed Student’s \(t\)-test was performed to compute the statistical significance \((P < 0.05)\).
modulus for the healthy group was 0.89 ± 0.13 and 0.97 ± 0.14 for the compressible and nearly incompressible cases, respectively. Similarly, for post-LPI patients with occludable ACAs, the mean normalized iris elastic modulus was 2.57 ± 0.69 and 2.72 ± 0.71 for the compressible and nearly incompressible cases, respectively. Comparing the normalized elastic modulus of the compressible and nearly incompressible cases for healthy and post-LPI irides did not lead to any significant difference (P > 0.05, Fig. 6). However, when comparing the healthy and post-LPI cases for different possible combinations of compressible and incompressible cases, we found out that the post-LPI group had a significantly larger normalized elastic modulus than the healthy control group (P < 0.05, Fig. 7).

The mesh convergence study for a randomly selected case showed that changes in the iris deformation (identified by the distance between the root and tip of the iris as shown in Fig. 5) using G obtained from the inverse method did not change beyond a total number of 1161 elements (Table 4). A global size of approximately 0.05 mm generated the 1161 elements. The same global size was used for generating the mesh for all samples. As for the validation of the simulated outcomes, perturbation of the model for typical simulated cases showed that only one absolute minimum error was obtained from the inverse modeling approach (Fig. 8). Regarding the effect of the magnitude of the muscle stresses on the calculated stiffness, we have shown that irrespective of the magnitude of the muscle stress, the normalized elastic modulus remains unchanged. We have shown this concept for two different cases of a compressible healthy iris (Fig. 9).

### Table 4. Results for a Mesh Convergence Study of the Distance Between the Root and Tip of the Iris

<table>
<thead>
<tr>
<th>Total Number of Elements</th>
<th>Distance, mm</th>
</tr>
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<tbody>
<tr>
<td>1161</td>
<td>3.97</td>
</tr>
<tr>
<td>1798</td>
<td>3.97</td>
</tr>
<tr>
<td>3159</td>
<td>3.97</td>
</tr>
<tr>
<td>4533</td>
<td>3.97</td>
</tr>
</tbody>
</table>

### DISCUSSION

Quantifying the mechanical properties of the iris is expected to provide more insights into the pathophysiology of PACG. In this study, using noninvasive imaging and an inverse computer simulation approach, we quantified the stiffness of the iris in healthy and diseased eyes. We found the irides were significantly stiffer in patients with a history of PACG when compared with those of healthy volunteers. Previous studies have found a significantly higher density of type I collagen in the stromal tissues of acute angle closure irides and the eyes in general when compared with the irides in the healthy control group. However, in this study, such a difference was not observed between the chronic angle closure eyes and the healthy control group eyes. Conversely, the amount of COL1A1 mRNA expression in PACG iris samples has been shown to be larger when compared with POAG patients. Although many of these recent quantitative analyses may suffer from limitations such as small sample size, it has been clearly demonstrated that, from soft tissues such as fat and tissues in the brain to extremely stiff tissues such as those found in bones, the amount of COL1A1 mRNA expression directly scales with the mechanical stiffness. As such, the results of limited studies on the potential link between the iris stroma extracellular matrix collagen content and angle closure agrees with the outcome of our in vivo stiffness calculation.

Unlike ex vivo mechanical testing experiments, the computer simulation approach presented in this study does not require any surgical interventions to isolate the tissue.
samples. Our approach primarily relies on AS-OCT images obtained via a noninvasive procedure. Other investigators have conducted similar studies to quantify iris mechanical properties in vivo. Their models, however, did not include the active deformation of the iris. In addition, due to simplification in the form of constitutive tissue model, less complexity in the tissue behavior was considered. For example, the iris compressibility was not taken into consideration in those models.

However, in interpreting our results, one should be cautious as our study was not without limitations. An iris consists of the following two active muscular components: the circumferentially aligned sphincter muscles and the radially oriented dilator muscles. Pupil diameter is controlled via relaxation and constriction of these two smooth muscles. In this study, however, only the sphincter muscular component was considered, and dilation was primarily modeled by relaxation of the sphincter muscle to a flaccid iris state. The reason for the lack of a dilator muscle component in the model is based on our previous porcine study, where we observed a pronounced bowing of the iris toward the posterior, a phenomenon that was not present in the AS-OCT images of the human iris after switching from standard light conditions to a dim light level. Other limitations of our study pertained to the identification of the sphincter region in the irides and the magnitude of the sphincter stress. Although a sphincter region was chosen in proportion to the iris size based on previously quantified histological measurements, more accurate patient-

Figure 7. Comparison of normalized elastic modulus between (A) compressible healthy and post-LPI, (B) compressible healthy and nearly incompressible post-LPI iris (C) nearly incompressible healthy and compressible post-LPI, and (D) nearly incompressible healthy and post-LPI irides.

Figure 8. Error plot shows there exists only one solution for a particular compressible healthy iris.
specific quantifications of the sphincter region could offer more accurate stiffness measurements. We also made the assumption that the magnitude of the sphincter stress was similar in all simulated cases and defined the normalized elastic modulus (normalized stiffness) as a parameter for comparison. Although the significant difference between the normalized values of the stiffness is most likely due to the extracellular collagen content of the stromal layer of the irides, theoretically, same stromal stiffness and different sphincter stresses could produce similar outcomes. Calculating the in vivo patient-specific sphincter muscular stress remains a challenge.

Another limitation of this study was the assumption of the isotropic behavior of the iris. The iris consists of a number of different segments, namely anterior border layer, stroma, radially oriented dilator smooth muscle, and posterior pigment epithelium, with each segment differing from the others. Therefore, in addition, a sphincter muscle, which consists of circumferentially aligned smooth muscle fibers, is located close to the pupillary edge of the iris. As such, the isotropic assumption of this model can be improved upon by incorporating more advanced anisotropic material models.

In addition, due to the small sample size, a normal distribution of the data could not be reliably assumed despite the outcomes of the normality tests indicating a normal distribution. Therefore, an additional nonparametric, one-tailed Mann-Whitney U test was also performed to evaluate the significant difference between the healthy control iris and the post-LPI occludable iris. The outcomes were similar to that of the Student’s t-test with the exception of one case, where no significant difference was found between the nearly incompressible healthy control iris and the compressible post-LPI occludable iris. This particular case could be considered as a nonrealistic comparison, as our previous study has shown that the normal healthy iris behaves in a compressible manner, whereas a post-LPI occludable iris displays incompressible behavior (Wojcik M, et al. IOVS 2017;58:ARVO E-Abstract 2087). Nonethess, some level of caution is required in interpreting the data presented in this study.

One could also identify the type of the light source used to stimulate dilation as a potential variable that could influence the calculation of iris stiffness as studies have shown that different colors of light induce different pupillary responses. For example, blue light has been shown to evoke greater pupillary responses when compared with red light. However, for our study, this color dependency can be disregarded because we used the same source of illumination for examining the eyes of all individuals who participated in our study.

To determine whether the choice of a standalone distance-based objective function was reliable for the estimation of shear modulus, we performed an additional optimization study similar to the one conducted in our previous study; in the additional study, we fixed the Poisson’s ratio and only optimized the shear modulus. We found that using the standalone chord length as the objective function was sufficient to accurately calculate the shear modulus of 9 kPa, which was initially used for the generation of numerically simulated displacement data.

In this study, we limited the fitting parameters only to the shear modulus G. Perturbation of our results showed that the error was confidently minimized at the value obtained from the model. We have shown that simultaneous optimization of more than one parameter required more detailed scans of the anterior segment cross-section and more complex objective functions. With more accurate deformation measurements, for example, using digital image correlation, one would expect to improve the current methods. Digital image correlation has been used in ocular tissues alone, along with ultrasound, OCT, or microscopy for measurement of scleral and corneal strain. Nonetheless, with appropriate region and stress quantification, this method can be more confidently used to quantify the mechanical properties of the human iris in vivo. Further studies are necessary to find a mechanistic link between the stiffening of the iris and onset and progression of PACG. In addition, this method can also be used to determine the effect of LPI on the iris by comparing the iris stiffness for the AGG patients who did and who did not undergo the LPI procedure to examine whether the LPI itself contributes to the stiffening of the iris. If iris stiffening is indeed omnipresent in PACG, especially in those patients who continue to suffer from occludable ACAs after the LPI procedure, alternative/additional therapeutic procedures may need to be developed. For example, new pharmacological agents that prevent iris stiffening or soften stiff iris may become a treatment option for these patients.

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References

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