The Zonules Selectively Alter the Shape of the Lens During Accommodation Based on the Location of Their Anchorage Points

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PURPOSE. To determine the role of anterior and posterior zonular tension on the optomechanical lens response during accommodation simulation.

METHODS. Ten eyes from nine hamadryas baboons (4.9 ± 0.7 years) and 20 eyes from 18 cynomolgus monkeys (5.4 ± 0.3 years) were dissected, leaving the lens, zonules, ciliary body, hyaloid membrane, anterior vitreous, and a segmented scleral rim intact. The lens preparation was mounted in a lens stretcher, and the outer scleral shell was displaced radially in a stepwise fashion. The load, lens, and ciliary body diameters, lens power, lens thickness, and the anterior and posterior radius of curvature were measured during stretching. The zonular fibers attached to either the posterior or anterior lens surface were then carefully transected and the experiment was repeated. Zonular transection was confirmed in four eyes via laser scanning confocal microscopy after immunostaining. The effect of zonular transection on the tissue response to stretching was quantified.

RESULTS. Without anterior zonules, 48% and 97% of the changes in anterior and posterior radii are retained. Without posterior zonules, 81% and 67% of the changes in anterior and posterior radii are retained. The changes in lens shape were reduced after transecting either the anterior or posterior zonules; however, both surfaces still changed shape.

CONCLUSIONS. While either the anterior or posterior zonules alone are capable of changing the shape of both lens surfaces, the anterior zonules have a greater effect on the anterior lens surface, and the posterior zonules have a greater effect on the posterior lens surface.

Keywords: zonule, accommodation, zonular apparatus, crystalline lens, lens shape

Accommodation is a dioptric change in the optical power of the eye brought about by ciliary muscle contraction and a concomitant steepening of the crystalline lens surface.¹⁻³ The changes in curvature of the lens are accomplished via the zonules, which apply or release a load at or near the lens equator.³⁻⁸

The anatomy of the zonular apparatus and its structural arrangement have been described previously using ultrasound biomicroscopy^{6,9} and scanning electron microscopy.¹⁰⁻¹³ To establish a convenient nomenclature, we define the zonules that attach to the anterior surface of the lens capsule as the anterior zonules and the zonules that attach to the posterior surface of the lens capsule as the posterior zonules. It was shown that there is a connection between the posterior zonules and the hyaloid membrane.¹³⁻¹⁵ The asymmetry in the anchorage points and orientation of the anterior and posterior zonular fibers has been investigated in primates using a lens stretcher and a selective anterior zonular transection procedure.¹⁶ We found that a significant amount of accommodative ability remained after transection of the anterior zonular fibers, but the change in power for a given load was reduced. The changes in curvature of one or both of the anterior and posterior surfaces must therefore

also have been altered following transection; but without curvature measurements, we could only speculate about the details. Was the anterior surface curvature still changing in response to radial stretching forces even in the absence of the anterior zonules, or was the posterior surface changing more to compensate for a relatively inactive anterior surface?

The goal of the present study was to determine the effects of the difference in anterior and posterior zonule anchorage points and orientation by quantifying the role of anterior or posterior zonular transection on the ciliary body diameter, the lens diameter, power, thickness, and anterior and posterior radius of curvature of nonhuman primate eyes during simulated accommodation in a computer-controlled lens stretcher.

METHODS

Tissue Preparation

Ten eyes from nine hamadryas baboons (*Papio hamadryas*) ($n = 10$, 1–48 hours postmortem time [PMT], 4.9 ± 0.7 years old) and

FIGURE 1. EVASII dissection procedure. First, all extraocular muscles were removed (1); then the equatorial globe diameter was measured with calipers and the best-fitting shoes were selected. Cyanoacrylate glue was applied to the spherical surface of each shoe, and the eye was aligned and mounted onto the shoes (2). After the glue dried, the posterior pole was removed (3), and a posterior retaining ring was added to the assembly (4). The tissue was then transferred to a Petri dish atop a custom-made portable dissection stage with diffuse retroillumination, and Dulbecco's modified Eagle's medium was added to preserve the tissue (5). Next, the cornea was removed (6) and scleral segments were excised, with great care being taken not to sever the ciliary body (7). Finally, the iris was removed, leaving the tissue ready for transfer into EVASII (8).

20 eyes from 18 cynomolgus monkeys (*Macaca fascicularis*) ($n = 20$, 2–26 hours PMT, 5.4 ± 0.3 years old) were obtained after euthanasia for experiments unrelated to this study from the University of Miami Division of Veterinary Resources following institutional animal care guidelines through an approved tissue-sharing protocol. All experiments adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. After enucleation, all eyes were placed in sealed containers with gauze soaked in balanced salt solution (BSS; Alcon, Fort Worth, TX, USA) to prevent dehydration of the globe. All eyes not used immediately after enucleation were stored at 5°C and returned to room temperature before they were dissected. As in previous studies,^{16–18} the posterior pole, cornea, and iris were removed, leaving the lens, zonules, ciliary body, hyaloid membrane, anterior vitreous, and scleral rim intact. The tissue was bonded to eight custom-made scleral shoes, each with a curvature sized to match the globe. The scleral rim was sectioned radially between the shoes to eliminate interference during stretching, and special care was taken to ensure that the ciliary body was not severed. Experiments were performed no more than 48 hours post mortem (Fig. 1).

Lens Stretching Experiments

The tissue section containing the crystalline lens was transferred to and mounted in (Fig. 2) a second-generation optomechanical lens stretching system (ex vivo accommodation simulator, EVASII)¹⁷ (Fig. 3). The outer scleral shell was displaced 2 mm radially in a stepwise fashion with a step size of 0.25 mm while eight individual load cells (FORT-100; World Precision Instruments, Sarasota, FL, USA) monitored the force on each arm. The tissue was first taken through a preconditioning stretch cycle to check that there were no problems with the tissue preparation or with the attachment of the shoes and to ensure that the stretching was symmetrical. After preconditioning, the stretch cycle was repeated three times. The average of these three cycles was taken as one measurement.

Lens and Ciliary Ring Diameter Measurement

During the stretching experiment, a top view of the lens and ciliary body was captured with a resolution of $10\ \mu\text{m}$ in the plane of the crystalline lens using a custom-built diffuse retroillumination system and a digital camera (Model PL-A776;

FIGURE 2. EVASII tissue transfer and mounting procedure. First the Petri dish containing the tissue was removed from the portable dissection stage and transferred near EVASII (1). Then, with the posterior surface up, the posterior retaining ring was affixed (2); the tissue was flipped, and the anterior retaining ring was removed (3, 4). The transfer ring was then fixed to the anterior surface of the shoes (5), the tissue was flipped, and the posterior ring was removed (6). Next, the tissue was flipped again, the alignment pins were inserted (7), and the tissue was transferred into the EVASII chamber using a custom transfer tool (8). Once the tissue was aligned, the transfer ring was removed (9), leaving the system ready for experiment (10).

FIGURE 3. Optomechanical lens stretching system. The lens, shoes, pins, and arms are labeled. The pins held the tissue in place via magnetic force generated between a small neodymium magnet mounted behind each arm and ferritic stainless steel dowels located within each pin. The linear translation stages controlled the position of each arm while a force transducer measured the load.

Pixelink, Ottawa, ON, Canada). The crystalline lens and inner ciliary ring diameters were measured in the horizontal and vertical directions using custom-designed image analysis software. The averages of these values were taken as the lens equatorial and inner ciliary ring diameters.

Lens Thickness and Radius Measurement

Cross-sectional images of the entire crystalline lens were acquired using a custom-built time-domain optical coherence tomography (TDOCT) system,¹⁹ which has an axial resolution of 12 μm in air (9 μm in tissue) and an object-side telecentric beam delivery system providing a lateral resolution of 43 μm . Images were acquired with 500 A-scans/B-scan over a 10- $\times$ 10-mm (lateral $\times$ axial) scan length. The OCT images were processed using a semiautomatic edge-detection program (MATLAB; MathWorks, Natick, MA, USA) to detect the anterior and posterior lens boundaries. Refractive distortions of the posterior lens surface were corrected assuming a uniform refractive index equivalent to the average index of the lens.²⁰ The cross-sectional profiles of the anterior and posterior lens surfaces were characterized with a spherical fit over the central 3-mm zone to calculate the radius of curvatures of both lens surfaces along with the central lens thickness.^{21–24}

Optical Power

Lens power was measured based on the Scheiner principle using the OCT beam delivery system to project a 3-mm-diameter circular ring onto the crystalline lens (825 nm), which was then detected by a charge-coupled device (CCD, Model GPCX261V; Panasonic, Osaka, Japan). The position of the CCD was adjusted until the ring converged to a single spot. This position was measured by a linear positioner so that the focal length and therefore the refractive power of the crystalline lens could be determined.

Anterior Zonules-Cut Stretching Experiments

Anterior zonules-cut stretching experiments were performed on five baboon eyes and 10 monkey eyes. After stretching the natural lens, the tissue section was transferred from EVASII to an operation microscope (OMS300; Topcon, Tokyo, Japan) and then stretched using a miniature manual lens stretcher

FIGURE 4. Close-up images of the circumlental space in a hamadryas baboon under retroillumination before (*top*) and after (*bottom*) transection of the posterior zonules were acquired with a magnification of $\times 7$. The cut zonules can be seen hanging from the ciliary body (*arrows*).

(mMLS)¹⁵ to better expose the zonules. At $\times 20$ magnification, using an oblique illuminator and a custom-built retroillumination stage, the anterior zonular fibers were then carefully transected using a ruby knife (RK 2883 M; Meyco, Bienne, Switzerland). The tissue was returned to its initial unstretched state and mounted back into EVASII, and the stretching experiment was repeated.

Posterior Zonules-Cut Stretching Experiments

Posterior zonules-cut stretching experiments were performed on five baboon eyes and 10 monkey eyes. The posterior zonules-cut experiments were performed with the same protocol as the anterior zonules-cut experiments, except that after transfer to the operation microscope, the anterior vitreous, vitreous zonules, hyaloid membrane, and posterior zonular fibers were carefully transected (Fig. 4). When transecting the posterior zonules, the vitreous zonules¹³ and the anterior hyaloid¹⁵ were also cut; thus, the effects of these are summed together with the posterior zonules-cut experiments.

Imaging of Cut Zonules

To show that only specific zonules were cut by the surgeon, the zonular architecture of four tissue samples was imaged

FIGURE 5. Mosaic images at $\times 5$ magnification of tissues from anterior (A) and posterior (B) zonules-cut experiments. Images were acquired with fluorescent confocal microscopy of tissue with zonules intact (control eye where the zonules were not cut) and after cutting experiments to verify that the zonules were accurately transected. The *green signal* is from the zonules and the *purple signal* is from nuclear material of cells in the lens and ciliary processes. P, posterior; A, anterior; cp, ciliary process.

after the procedure using a laser scanning confocal microscope according to the following protocol. After completion of the stretching experiments, the tissue was transferred from EVASII to the mMLS¹⁵ and radially stretched 2 mm. Immediately afterward, the tissue was fixed in a 10% formalin solution for a minimum of 72 hours. Immunostaining was performed targeting the microfibril-associated glycoprotein-1 (MAGP-1) molecules of the zonules²⁵ using a goat-derived primary antibody (SC-50083; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and a complimentary secondary antibody with a bound fluorescent probe excited at a wavelength of 488 nm (A11055; Invitrogen, Grand Island, NY, USA). Lens epithelial cells in the anterior surface of the lens and cells of the ciliary processes were visualized using a nuclear stain, DRAQ5 (4084L; Cell Signaling Technology, Danvers, MA, USA). A Leica TCS SP5 (Leica Microsystems, Wetzlar, Germany) laser scanning confocal microscope was used to image the tissue using a $\times 5$ objective lens (numerical aperture [NA] = 0.15). The images were acquired using Leica Application Suite Advanced Fluorescence Lite 2.6.0 Build 7266. Individual volumetric projection images were manually

merged in Photoshop Elements 8.0 (Adobe, San Jose, CA, USA), showing the entire anterior and posterior surfaces of the lens including the ciliary processes and zonules (Fig. 5).

Data Analysis

The load, ciliary body diameter, lens diameter, lens power, thickness, and anterior and posterior radii of curvature were measured at each stretching step, and the linear optomechanical relationships were quantified: load-lens diameter, load-lens thickness, power-load, load-anterior radius, and load-posterior radius. Linear fitting of these parameters was achieved using least squares linear regression. Often, one or two data points were omitted from the linear regression to improve the goodness of fit when the measurement fell outside of the 95% confidence interval of the fit. The changes in these parameters and the slopes of the linear regressions were compared before and after transection of the zonules.

The ratio of the performance parameter was calculated, where the ratio was defined as the parameter after the

FIGURE 6. Typical stretch-response curves for one anterior and one posterior zonules-cut cynomolgus monkey eye, showing the results before (black) and after (red) anterior zonular transection (top) and posterior zonular transection (bottom).

procedure divided by the parameter before the procedure (unless stated otherwise, “procedure” indicates anterior and/or posterior zonular transection). Since the distributions of the ratios were right-tailed, the ratios were log-transformed to normalize the data, and the logarithm of the ratio was evaluated for statistical purposes. Initially, the data were divided by species and procedure, and box plots of the logarithm of the ratio were made for all 12 parameters to check for asymmetry and screen for technical outliers. Further, we proceeded with the assumption that the observed variation was representative of the variation in the species; thus, statistical outliers were not excluded from subsequent analysis. In this study, only a narrow age range was evaluated in order to

minimize the age dependence of the effect of the procedure, so simple regressions were performed to ensure that age and weight were not significant confounders. Also, in this repeated-measures study, missing data were Winsorized to avoid omitting samples from statistical tests where one or another repeated measure was missing for a particular sample. Finally, a two-factor identity multivariate analysis of variance (MANOVA) was performed on all of the parameters, providing support for subdivision by procedure and generalization across species. Once divided by procedure, Fisher’s protected least significant difference (PLSD) post hoc testing was used to evaluate the significance of the procedure on each parameter. The same analytical procedure was repeated without the statistical

FIGURE 7. Typical optomechanical response for the same two anterior and posterior zonules-cut cynomolgus monkey eyes as in Figure 6, showing the results before (*black*) and after (*red*) anterior zonular transection (*top*) and posterior zonular transection (*bottom*). The *green data points* were omitted from the regression.

outliers to ensure that these observations did not alter the conclusions of the study.

RESULTS

Imaging of Cut Zonules

Magnified views (Fig. 4) of the circumlental space, acquired after zonular transection, showed that zonules were severed all the way around the lens. Confocal microscopy images of the lens demonstrated the surgeon's ability to selectively cut specific sets of zonules. However, due to the very small scale

of the zonular architecture and the difficulty in visualizing the zonules during the procedure, the zonular transection procedure is not perfect. In particular, not every anterior zonule was severed in each of the anterior zonule transection procedures. Likewise, not every posterior zonule was severed in each of the posterior zonule transection procedures. Despite this limitation, the confocal microscopy images indicated that the eyes that underwent the anterior zonular transection procedure had most of the anterior zonules severed, with the posterior zonules remaining largely intact, and analogously for the posterior zonular transection procedure.

FIGURE 8. Time-domain OCT images of the natural lens of a cynomolgus monkey (*top row*) in the unstretched (*left*) and stretched (*right*) states along with the anterior zonules-cut lens (*bottom row*) for comparison.

Potential Confounding Factors

Simple regressions of the effect of procedure versus age and weight were performed on data that were divided by species and procedure. In total, 120 simple regressions were performed, and only eight of them were found to be significant below the 0.05 level. Cumulatively, the finding of eight significant regressions out of 120 regressions was not statistically significant ($P=0.105$). The results of the regression analysis suggest that the effect of the procedure is independent of age and weight for most of the parameters measured in this study. This finding serves as the basis for proceeding with a two-factor MANOVA, neglecting age and

weight, and considering only species and procedure as pertinent factors.

MANOVA

The results of the two-factor MANOVA indicate a primary effect of procedure ($P = 0.013$). The effect of procedure was not significantly different between species ($P = 0.691$). This provides justification for generalization across species and further subdivision of the data by procedure (anterior zonular transection versus posterior zonular transection).

FIGURE 9. Time-domain OCT images of the natural lens of a cynomolgus monkey (*top row*) in the unstretched (*left*) and stretched (*right*) states along with the posterior zonules-cut lens (*bottom row*) for comparison.

FIGURE 10. The log of the ratio (after: before) of all 12 accommodation metrics. A value of zero indicates no change; a negative value is a reduction; and a positive value is an enhancement after the procedure. Anterior zonules cut (AZC) is shown in blue, and posterior zonules cut (PZC) is shown in cyan. The black circles indicate a primary effect of procedure; the bracket indicates a significant difference between procedures; and an asterisk indicates a significant difference after further subdivision by procedure (AZC or PZC). The error bars are the standard error of the mean. CBD, ciliary body diameter; LD, lens diameter; LT, lens thickness; AR, anterior radius; PR, posterior radius; A, accommodation; ML, maximum load; L-D, load-diameter slope; P-L, power-load slope; L-AR, load-anterior radius slope; L-PR, load-posterior radius slope; L-T, load-thickness slope.

Response to Stretching

Typical responses are shown in Figures 6 and 7, and typical TDOCT images are shown in Figures 8 and 9. The results are summarized in Figure 10, where significance of Fisher's PLSD post hoc testing is indicated with a black circle for a primary effect of procedure and with a bracket for an effect that was different for anterior and posterior zonular transection. For these parameters, significance after considering only anterior or posterior zonular transection alone is indicated with an asterisk. In general, most parameters were significantly altered by both procedures, with the majority of the measured changes reduced by the procedures. In particular, the changes in lens diameter, lens thickness, anterior radius, and posterior radius were reduced after either procedure. Also, the amplitude of accommodation, the maximum load, the load-diameter slope, the load-posterior radius slope, and the load-thickness slope all decreased after either procedure.

Most interestingly, the effect differed by procedure for four of the parameters evaluated: change in anterior radius, change in posterior radius, load-anterior radius slope, and load-posterior radius slope (Table). Transecting the anterior zonules

reduced the change in anterior radius by 52% (0.48 ± 0.07 , $P < 0.001$), while transecting the posterior zonules reduced the change in anterior radius by only 19% (0.81 ± 0.06 , $P < 0.002$). Conversely, transecting the anterior zonules resulted in a rather small, statistically insignificant, reduction in the change in posterior radius of 3% (0.97 ± 0.08 , $P = 0.444$), while transecting the posterior zonules reduced the change in posterior radius by 33% (0.67 ± 0.07 , $P = 0.005$). This shows that, although each zonule acts to change both surfaces, there is a degree of spatial selectivity of that action such that the anterior zonule preferentially alters the anterior surface of the lens and the posterior zonule preferentially alters the posterior surface of the lens. However, the degree of spatial selectivity is particularly evident for the anterior zonule, while the posterior zonule seems to significantly influence both surfaces.

Furthermore, anterior zonular transection resulted in a decrease in the load-posterior radius slope of 30% (0.70 ± 0.04 , $P < 0.001$), while posterior zonular transection resulted in a more modest, statistically insignificant, decrease in load-posterior radius slope of 3% (0.97 ± 0.11 , $P = 0.347$). Finally, anterior zonular transection resulted in an increase in the load-anterior radius slope of 42% (1.42 ± 0.10 , $P < 0.001$), while posterior zonular transection resulted in a decrease in load-anterior radius slope of 18% (0.82 ± 0.09 , $P < 0.001$); these results demonstrate the greatest differences between the procedures. Together, these results indicate that without the anterior zonules, the accommodative mechanism becomes more efficient (i.e., requires less load) at changing the posterior surface of the lens and less efficient at changing the anterior surface. Without the posterior zonules, the accommodative mechanism becomes more efficient at changing the anterior surface of the lens.

DISCUSSION

The anterior or posterior zonules alone are capable of changing the shape of both lens surfaces. The anterior zonules have a greater effect on the anterior lens surface, and the posterior zonules have a greater effect on the posterior lens surface. The changes in both the anterior and posterior surfaces of the lens were significantly reduced after transection of the posterior zonules, while anterior zonular transection resulted in a significant reduction in only the change in anterior radius, showing that the posterior zonules contribute significantly to the changes in both the anterior and posterior surface of the lens while the anterior zonules are more specialized for changes in the anterior lens surface. This may be due to the thickness difference between the anterior and posterior capsule²⁶ and, potentially, the role of the hyaloid membrane and its connections with the posterior zonules, which could act together to augment the effect of posterior zonular tension.¹⁵ Furthermore, the fact that the posterior surface of the lens still changes shape even after posterior zonular transection and that the anterior surface of the lens still changes shape after anterior zonular transection, provides

TABLE. Ratio and Standard Error of the Change in Anterior Radius, Change in Posterior Radius, Load-Anterior Radius Slope, and Load-Posterior Radius Slope

	Change in Anterior Radius	Change in Posterior Radius	Load-Anterior Radius Slope	Load-Posterior Radius Slope
AZC				
Ratio, after: before	0.48 ± 0.07	0.97 ± 0.08	1.42 ± 0.10	0.70 ± 0.04
PZC				
Ratio, after: before	0.81 ± 0.06	0.67 ± 0.07	0.82 ± 0.09	0.97 ± 0.11

support for the notion that the lens capsule acts as a force distributor during accommodation.

This relationship is further explored by considering the load–radii slopes, which are indicative of the efficiency of the accommodative mechanism. In particular, our results indicate that without the anterior zonules, the accommodative mechanism becomes more efficient at changing the posterior surface of the lens and less efficient at changing the anterior surface, and that without the posterior zonules, the accommodative mechanism becomes more efficient at changing only the anterior surface of the lens. In other words, without the anterior zonules, less force is required to produce equivalent changes in the posterior surface because the entire load is applied to the posterior zonules. At the same time, the change in shape of the anterior surface of the lens decreased and the load required to produce that change increased, while the change in shape of the posterior surface remained relatively unaffected. Without the posterior zonules, less force is required to produce equivalent changes in the anterior surface because the entire load is applied to the anterior zonules. At the same time, the force required to produce equivalent changes in the posterior surface remained approximately constant. Thus, the capsule does not redistribute the load uniformly, with the location of the load determining the manner in which the lens changes shape. In particular, there is an asymmetry in the accommodative mechanisms' ability to redistribute the load, such that posterior zonular tension is more efficiently redistributed than anterior zonular tension.

Theories of accommodation that attribute either an active^{27,28} or a passive^{29,30} role to the vitreous have been proposed. In the catenary theory,^{27,28} the anterior capsule and the zonules act as a “zonular sling or hammock” that serves as a support to help mold the lens. The capsule, zonule, hyaloid membrane, and anterior vitreous compose a diaphragm between the anterior and vitreous chambers of the eye, and the accommodative process requires changes in differential pressure between the anterior and vitreous chambers. Limitations of the experimental apparatus used in the current study preclude a definitive assessment of the role of vitreous support during accommodation. In our experiments, there is no ocular chamber confinement and thus there is likely no pressure differential between the vitreous and aqueous humor; however, we still found a significant amplitude of accommodation. We also found that the posterior surface of the lens still changes shape even after posterior zonular transection and that the anterior surface of the lens still changes shape after anterior zonular transection, suggesting that changes in either surface do not require an intact catenary. These observations support the concept that the lens capsule acts as a force distributor during accommodation. In addition, the apparatus used in this study did not preserve the accommodation-induced, forward movement of the ciliary body and vitreous zonule as observed recently in ultrasound biomicroscopy studies of accommodation.^{9,31} Although the vitreous zonule¹³ system was likely transected during the posterior zonular transection procedure, the contribution of the vitreous zonule to the forward movement of the ciliary body could not be determined from this experiment since the experimental setup was incompatible with forward movement of any of these structures. Despite these limitations, the differential response of the accommodative mechanism to anterior or posterior zonular transection procedures supports recent findings that the anterior hyaloid plays a significant role in the accommodative process.³² The more distributed effect of posterior zonular tension may be facilitated by the connection between the posterior zonules, vitreous zonule, hyaloid membrane, and posterior capsule.

In conclusion, the results show that the relative effect of zonular transection is similar for hamadryas baboons and cynomolgus monkeys, and that either the anterior or posterior zonules are capable of changing the shape of both lens surfaces, suggesting that the lens capsule acts as a force distributor. In addition, the action of the zonules exhibits a degree of spatial selectivity, such that the anterior zonules have a greater effect on the anterior lens surface and the posterior zonules have a greater effect on the posterior lens surface. Finally, the effect of zonular tension is more local for the anterior zonule and more distributed for the posterior zonule.

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