

# Effect of Anterior Zonule Transection on the Change in Lens Diameter and Power in Cynomolgus Monkeys during Simulated Accommodation

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**PURPOSE.** To quantify the role of anterior zonular tension on the optomechanical lens response during simulation of accommodation in primates.

**METHODS.** Postmortem cynomolgus monkey eyes ( $n = 14$ ; age range, 3.0–11.5 years) were dissected leaving intact the lens, zonules, ciliary body, hyaloid membrane, anterior vitreous, and a scleral rim. The lens was mounted in a lens-stretching system and stretched radially in step-wise fashion. The load, and the lens diameter and power were measured at each step and the diameter- and power-load relationships were quantified. The anterior zonular fibers were then transected, and the experiment was repeated. The equatorial lens diameter and lens optical power before and after zonular transection were compared.

**RESULTS.** Stretching increased the lens diameter by  $0.25 \pm 0.09$  mm (median  $\pm$  interquartile range) before and  $0.25 \pm 0.19$  mm after zonular transection. Stretching decreased the lens power by  $13.0 \pm 6.5$  D before and  $10.6 \pm 8.0$  D after zonular transection. The load required to change the diameter of the lens by 1 mm decreased from  $18.8 \pm 10.7$  g before to  $15.0 \pm 7.8$  g after zonular transection. The absolute change in power per gram of loading decreased from  $2.5 \pm 1.1$  before to  $2.0 \pm 1.2$  D after zonular transection.

**CONCLUSIONS.** The cynomolgus monkey lens retains a significant fraction of its accommodative ability after transection of the anterior zonules in simulated accommodation experiments. (*Invest Ophthalmol Vis Sci.* 2009;50:4017–4021) DOI:10.1167/iovs.08-2638

Accommodation is a process that relies on the change in curvature of the crystalline lens to focus on objects at different distances.<sup>1–5</sup> According to the Helmholtz theory of accommodation,<sup>3</sup> these changes in curvature are induced as the zonules apply or release a load on the lens equator.<sup>6–9</sup> When the ciliary muscle is relaxed, the zonules are taut, imparting a load on the lens capsule, causing it to flatten. This flatter curvature lowers the optical power of the eye, allowing the observer to focus on distant objects. When the ciliary muscle is contracted, the tension on the zonules is diminished, reducing the load on the lens capsule, allowing the lens to assume a more curved form. This increase in curvature increases the optical power of the eye, allowing the observer to focus on nearby objects. Since the zonules are responsible for transferring the force from the ciliary body to the lens, they serve as a critical component of the accommodative apparatus.

Rohen<sup>10</sup> described the zonular apparatus of both humans and monkeys as consisting of two functionally different sets of zonular fibers, the main fibers and the tension fibers, where the main fibers, which Rohen also refers to as anterior zonules, consist of all fibers connecting to the anterior, posterior, and equatorial region of the lens. Other anatomic investigations have led to a description of the structural arrangement of Rohen's main fibers as consisting of two primary groups with zonules inserting into the anterior and posterior lens surface and a secondary group with zonules inserting into the equatorial lens surface.<sup>8,11,12</sup> In the remainder of this article, we will refer to these three groups as the anterior, posterior, and equatorial zonules, as illustrated in Figure 1. Using scanning electron microscopy to investigate the architecture of the accommodative apparatus, Streeten and Pulaski<sup>13</sup> showed that there is a connection between the posterior zonules and the hyaloid membrane, and recently, using environmental scanning electron microscopy (eSEM), Bernal et al.<sup>14</sup> verified this connection. The posterior zonular fibers are connected directly to the hyaloid membrane, as they run through and along the hyaloid membrane before they connect to the posterior capsule.<sup>13,14</sup> In contrast, the anterior zonules are directly connected from the anterior capsule to the ciliary body.

Since the anterior and posterior zonules do not attach to the lens in exactly the same manner, the respective effect of each might also be different. The asymmetry in the anchorage points and orientation of the anterior and posterior zonular fibers suggest a different mode of action of each. The goal of the present study is to determine the effects of the difference in anterior and posterior zonule anchorage points and orientation by quantifying the role of anterior zonular transection on the

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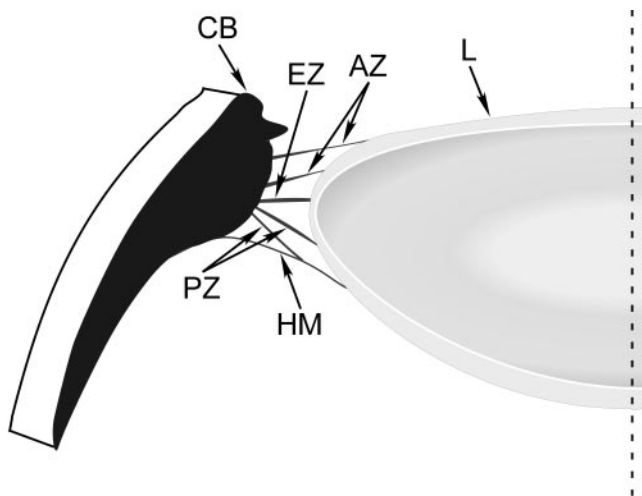
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**FIGURE 1.** Schematic showing the lens (L), ciliary body (CB), anterior zonules (AZ), equatorial zonules (EZ), posterior zonules (PZ), and hyaloid membrane (HM).

lens diameter and power of nonhuman primate eyes during simulated accommodation in a lens stretcher.

## METHODS

### Tissue Preparation

Fourteen eyes of 14 cynomolgus monkeys (*Macaca fascicularis*) (age range, 3.0–11.5 years; mean  $6.9 \pm 3.7$ ) were obtained from the University of Miami Division of Veterinary Resources after euthanization according to 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. Eyes were obtained after euthanization for experiments unrelated to this study. After enucleation, all eyes were placed in sealed containers with gauze soaked in a balanced salt solution (BSS; Alcon, Fort Worth, TX) to prevent dehydration of the globe. All eyes were stored at 5°C and returned to room temperature before they were dissected. The posterior pole, cornea, and iris were removed, leaving intact the lens, zonules, ciliary body, hyaloid membrane, anterior vitreous, and a scleral rim, which was bonded to eight custom-made scleral shoes, each with a curvature sized to match the globe. While being very careful not to sever the ciliary body, we sectioned the scleral rim radially between the shoes to eliminate interference during stretching. Experiments were performed no more than 1 day postmortem (3–24 hours). Details of the tissue preparation have been discussed elsewhere.<sup>15</sup>

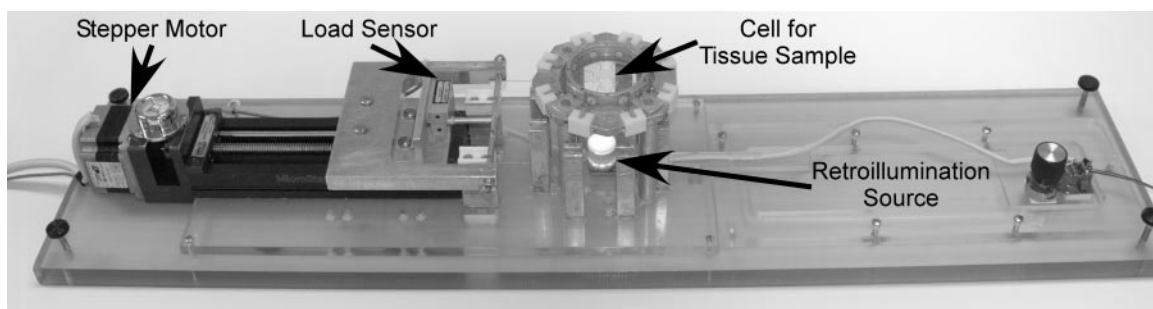
### Stretching Experiments with Natural Lens

The tissue section containing the crystalline lens was mounted in an optomechanical lens-stretching system<sup>15</sup> which simulates accommodation by simultaneous radial stretching of eight scleral segments (Fig. 2). The system uses an assembly of strings and pulleys to actuate the load on each segment, with the segment's position computer controlled by a stepper motor (L2SGQ-H3; Servo Systems Co., Montville, NJ) and a linear translation stage (MS33-LXB-L200; Servo Systems Co.). The outer scleral shell was stretched 2 mm radially in a step-wise fashion with a step size of 0.25 mm while a load cell (GSO-100; Transducer Techniques, Temecula, CA) monitored the force. A 2-mm radial stretch of the outer scleral shell produced lens diameter and power changes comparable to those in vivo during accommodation.<sup>15</sup> 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.

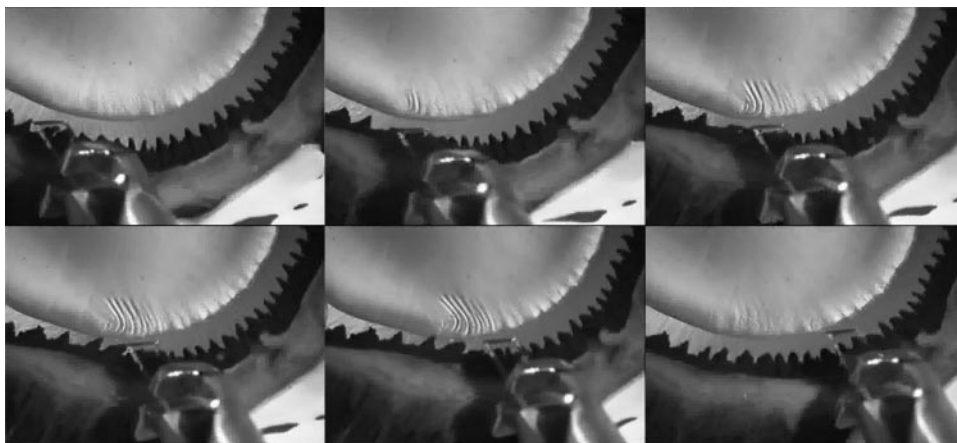
During the stretching experiment, a top view of the lens and ciliary body was captured with a resolution of 37  $\mu\text{m}$  in the plane of the crystalline lens using a diffuse retroillumination system and a digital camera (GP-KR222; Panasonic, Secaucus, NJ). The lens diameter was measured in the horizontal and vertical directions using image analysis software (Canvas 9.0; ACD Systems, Miami, FL). The average of these values was taken as the lens equatorial diameter. The lens power was measured with the Scheiner system, which uses four parallel beams (635 nm), separated by 3 mm and aimed onto the crystalline lens, which are then detected by a charge-coupled device (CCD; 15-BB13 camera; Jameco Electronics, Belmont, CA). The position of the CCD was adjusted until the four spots completely overlapped. This position was measured by a digital height gauge (570-227; Mitutoyo, Kawasaki, Japan), so that the focal length, and therefore, the refractive power of the lens could be determined. The load, lens diameter, and lens power were measured at each step and the diameter- and power-load relationships were quantified, according to a previously described protocol.<sup>15</sup>

### Stretching Experiments with Anterior Zonules Cut

After each stretching experiment on a natural lens, the tissue section was slightly stretched (<1 g) to better expose the zonules. The anterior zonular fibers were then carefully transected with a diamond blade (RK 2883 M; Meyco, Bienne, Switzerland) such that only the zonules connecting with the posterior surface of the lens capsule remained intact (Fig. 3). When transecting the anterior and equatorial zonules, the surgeon was careful to keep the blade just above the posterior zonules. The anterior zonular transection procedure was performed by the same surgeon for each eye, and after severing the zonules, using an operation microscope at 20 $\times$ , the surgeon scanned around the entire lens equator to verify that only those fibers connecting to the posterior surface of the lens remained. One eye was investigated under eSEM



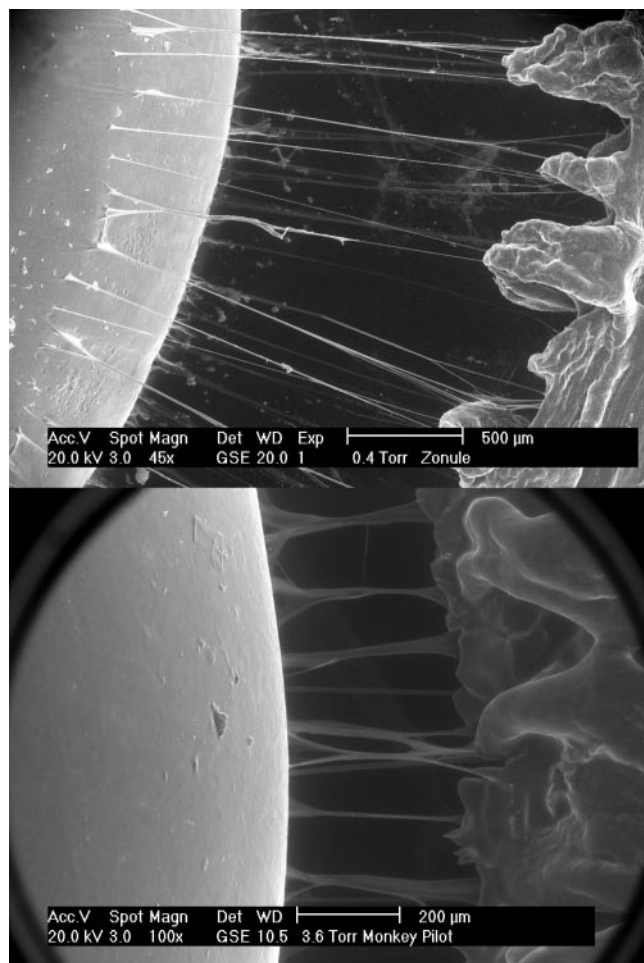
**FIGURE 2.** Optomechanical lens stretching system. The stepper motor, load sensor, tissue holder, and retroillumination source are labeled. The tissue is placed inside the cell and then mounted on eight individual hooks.



**FIGURE 3.** Transection of the anterior zonules was accomplished with a diamond blade, with the surgeon observing through an operation microscope at 20 $\times$  and employing retroillumination to increase contrast and verify that all the anterior zonules were cut in each eye studied.

after anterior zonular transection to ensure that all the anterior zonules were severed (Fig. 4). The tissue was returned to its initial unstretched state, and the stretching experiment was repeated. In this process, care was taken not to damage the posterior zonules, hyaloid membrane, and anterior vitreous. The changes in the equatorial lens diameter and lens optical power before and after transection of the zonules were compared, and the diameter-and power-load relationships were also quan-

tified and compared. Typically, a two-tailed, paired *t*-test is used to test for significance in this type of study, but this parametric description of the data is only accurate when it is normally distributed. Since not all the measured parameters were normally distributed, nonparametric statistical techniques (Wilcoxon's signed-rank test and Spearman rank correlation) were used in the analyses. Numerical results are presented as the median and interquartile range (median  $\pm$  interquartile range), where the interquartile range is the difference between the third (75th percentile) and first (25th percentile) quartiles of the distribution.



**FIGURE 4.** Environmental scanning electron micrograph of a human lens (*top*) before and a cynomolgus monkey lens (*bottom*) after anterior zonular transection. The posterior zonules are seen, and only stubs of the anterior zonules remain on the anterior lens surface in the anterior zonule cut image (*bottom*).

## RESULTS

Results for six parameters of the pretransection and post-transection tissue preparation and the differences between the two conditions are shown in Tables 1, 2, and 3, respectively.

Based on Spearman's rank correlation coefficient, the data show no significant age dependence for anterior zonules, intact or cut ( $P > 0.069$ ). This provided the basis for the pooling of all data from lenses of different age in subsequent analyses. Anterior zonular transection had no significant effect on the initial unstretched lens diameter, the initial unstretched lens power, and the change in lens diameter. Stretching increased the lens diameter by  $0.25 \pm 0.09$  mm before and  $0.25 \pm 0.19$  mm after zonular transection, which were not significantly different ( $P = 0.695$ ). Stretching decreased the lens power by  $13.0 \pm 6.5$  D before and  $10.6 \pm 8.0$  D after zonular transection, a significant difference ( $P = 0.001$ ) giving a median reduction in accommodation of 31%.

The load required to change the diameter of the lens by 1 mm decreased from  $18.83 \pm 10.67$  g before to  $15.04 \pm 7.82$  g after zonular transection ( $P = 0.371$ ). The absolute change in power per gram of loading decreased from  $2.52 \pm 1.12$  D before to  $2.02 \pm 1.23$  D after zonular transection ( $P = 0.099$ ). Neither of the foregoing was significantly different. There was a 20% decrease in the change in power per gram of load after transecting the anterior zonules.

No statistically significant age dependence was found for any of the measured parameters, even though power and accommodation amplitude are expected to decrease with age.<sup>15</sup> The lack of age dependence in the present study is probably due to the comparatively small sample size and age range explored relative to the variability between animals.

## DISCUSSION

Our results show that the lens, in the stretcher, retains a significant fraction of its accommodative ability after transection of the anterior zonular fibers. In the absence of the anterior zonules, 69% of the change in power is retained during



TABLE 1. Six Performance Parameters for All 14 Eyes with the Anterior Zonules Intact

Lens No.	Age (y)	Initial Lens Diameter (mm)	Initial Power (D)	Change in Lens Diameter (mm)	Change in Lens Power (D)	Load–Lens Diameter Slope (g/mm)	Power–Load Slope (D/g)
1	2.96	7.26	51.67	0.26	15.85	29.79	–2.16
2	3.67	7.50	50.60	0.34	19.06	14.94	–3.34
3	3.73	7.05	59.34	0.40	16.24	10.90	–3.08
4	5.30	7.45	39.94	0.22	8.19	18.88	–2.07
5	6.00	7.54	32.81	0.24	9.57	26.48	–1.20
6	6.04	7.46	53.97	0.23	12.14	22.97	–2.49
7	6.47	7.48	45.45	0.19	9.80	21.71	–2.55
8	7.30	7.60	50.88	0.25	13.28	19.50	–2.53
9	8.29	7.79	33.64	0.37	6.03	11.88	–1.40
10	8.32	7.51	56.46	0.23	12.71	12.31	–4.58
11	9.42	7.43	53.20	0.26	20.29	9.31	–6.85
12	9.50	7.56	53.22	0.25	13.98	18.78	–2.50
13	9.74	7.50	49.92	0.19	7.83	11.57	–5.73
14	11.52	7.43	50.43	0.35	19.18	24.50	–2.15
Median	6.89	7.49	50.74	0.25	13.00	18.83	–2.52
IQR	3.67	0.10	6.65	0.09	6.52	10.67	1.12

simulated accommodation. Since the change in power for a given load is reduced, the flattening in curvature of one or both of the anterior and posterior surfaces must therefore also have been reduced after transection. Cutting the anterior zonular fibers results in a significant alteration in the balance of zonular forces that normally exist at and near the lens equator. This alteration certainly has an effect on the anterior and posterior lens surfaces, and therefore, could result in a reduction in the change in anterior lens surface and an increase in the change in the posterior lens surface with stretching. In either case, it shows that the posterior zonules alone can produce significant accommodative changes.

The change in lens diameter remains constant after anterior zonular transection. No significant changes were observed in the load required to produce a 1-mm change in lens diameter after anterior zonular transection, although the median trend was a reduction in required load. Based on the altered geometry of the accommodative apparatus after anterior zonular transection, the retention in the amplitude of diameter changes suggests that there is a compensatory loading from the posterior zonules.

It is important to note that we used an ex vivo accommodation simulator (EVAS), which produces forces that may not be identical with in vivo conditions. First, the entire posterior

portion of the globe was removed. Although the anterior vitreous and hyaloid membrane remained intact, any contribution from the posterior portion of the eye was not present in the EVAS. The excision of the posterior pole disturbed Bruch's membrane insertion, and this may also have an effect on the movement of the tissue preparation during the EVAS experiments. In addition, the positioning of the lens in the EVAS was such that the gravitational loading vector corresponded to a supine position. Despite the differences between the EVAS and in vivo conditions, a previous study has shown that lens performance in the EVAS is similar to other in vivo measurements, which indicates that the zonular loading (although not directly measured) is comparable to in vivo conditions.<sup>15</sup>

These findings could provide an alternate explanation for one of the observations that led Coleman and Fish<sup>16,17</sup> to propose the hydraulic suspension theory of accommodation, which postulates that during accommodation, ciliary muscle contraction initiates a change in aqueous and vitreous pressure. A key point of this theory is the supporting role of the vitreous. An alternative is that the posterior zonules and hyaloid membrane provide this supporting role. It has been shown<sup>14</sup> that the posterior zonules do not connect directly to the posterior lens capsule, but they run through and along the hyaloid membrane before connecting to the posterior capsule.

TABLE 2. Six Performance Parameters for All 14 Eyes with the Anterior Zonules Cut

Lens No.	Age (y)	Initial Lens Diameter (mm)	Initial Power (D)	Change in Lens Diameter (mm)	Change in Lens Power (D)	Load–Lens Diameter Slope (g/mm)	Power–Load Slope (D/g)
1	2.96	7.31	55.03	0.34	13.29	18.74	–1.76
2	3.67	7.43	52.72	0.37	14.96	10.90	–3.50
3	3.73	7.21	55.60	0.49	13.21	7.95	–3.33
4	5.30	7.51	39.79	0.17	6.16	19.48	–1.68
5	6.00	7.27	32.26	0.17	4.92	27.05	–1.41
6	6.04	7.48	55.04	0.30	11.55	17.48	–2.45
7	6.47	7.45	44.01	0.15	3.04	13.17	–3.06
8	7.30	7.59	51.24	0.25	13.60	13.77	–2.36
9	8.29	7.24	33.61	0.19	4.55	16.31	–1.48
10	8.32	7.45	55.51	0.17	7.92	11.30	–2.08
11	9.42	7.56	55.32	0.37	14.02	12.00	–1.95
12	9.50	7.32	52.43	0.24	9.54	30.39	–0.81
13	9.74	7.71	48.02	0.16	3.98	7.65	–3.85
14	11.52	7.51	52.85	0.46	11.80	28.95	–1.68
Median	6.89	7.45	52.58	0.25	10.55	15.04	–2.02
IQR	3.67	0.20	10.03	0.19	8.04	7.82	1.23

TABLE 3. The Difference in the Six Performance Parameters with Anterior Zonules Cut versus Intact

	Age (y)	Initial Lens Diameter (mm)	Initial Power (D)	Change in Lens Diameter (mm)	Change in Lens Power (D)	Load-Lens Diameter Slope (g/mm)	Power-Load Slope (D/g)
Median	6.89	0.01	-0.09	-0.01	-3.98	-1.98	0.28
IQR	3.67	0.14	2.77	0.13	2.59	7.3	1.53
P*	N/A	0.863	0.838	0.695	0.001	0.371	0.099

The data are derived from cut minus intact zonules, showing the relative difference in performance before and after the anterior zonules were cut. The median and interquartile ranges are given. There were no statistically significant trends for age or postmortem time (data not shown).

\* Wilcoxon's signed-rank test, cut vs. intact.

We found that without the anterior zonules, accommodation is not only still possible, but that a large portion of accommodation amplitude is retained. This finding suggests that the lens, lens capsule, posterior zonules, and hyaloid membrane are sufficient to produce significant accommodation. However, our results depart from the Coleman theory of accommodation. In our experiments, there is no ocular chamber confinement, and thus there cannot be a pressure differential between the vitreous and aqueous humor; yet, we still found a significant amplitude of accommodation. Furthermore, our findings support the notion that the capsule influences the form of the lens during accommodation. The lens diameter increase with stretching after the anterior zonules were cut can only be due to the action of the remaining posterior zonules and the capsule. With stretching, it is the capsule surrounding the lens that caused the increase in lens diameter and a decrease in lens thickness. The effect of the capsule on molding the lens into the accommodated state has been demonstrated with the EVAS<sup>18</sup> and in isolated lenses, in which neither the vitreous nor the hyaloid membrane is present.<sup>6,19</sup>

In conclusion, the results show that the cynomolgus monkey lens retains the majority of its accommodative ability after transection of the anterior and equatorial zonular fibers in simulated accommodation experiments. These findings suggest that the posterior zonules and hyaloid membrane play a significant role in accommodation.

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