Qualitative Effect of Zonular Tension on Freshly Extracted Intact Human Crystalline Lenses: Implications for the Mechanism of Accommodation

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PURPOSE. To determine the topographic effects of zonular tension on the anterior surface of the human crystalline lens.

METHODS. Real-time topography of the anterior surface of seven fully relaxed, freshly extracted intact, clear, human crystalline lenses aged 3, 17, 45, 54, 54, 56, and 56 years was qualitatively obtained before, during, and after the application of zonular traction. Zonular traction was applied manually either by grasping a group of zonules 180° apart with tying forceps (three lenses), or with micrometers by clamping four portions of the ciliary body that were 90° apart (four lenses).

RESULTS. Zonular tension began with the lenses in the fully relaxed, baseline state. As zonular tension was increased across one meridian of all seven lenses, the center of the anterior surface steepened while the periphery of the anterior surface flattened across that meridian of traction. When the tension was reduced across that meridian of traction, the center of the lens flattened while the periphery steepened in that meridian. Four-point zonular traction applied 90° apart produced symmetrical central steepening (four lenses). Reduction of zonular tension across both orthogonal meridians caused symmetrical central flattening.

CONCLUSIONS. These observations reveal that when zonular tension is applied to the fully relaxed lens, the center steepens and its periphery flattens in the meridian (or meridians) in which zonular tension is applied. The reverse of this process demonstrates that as tension is reduced, the center of the lens flattens while the periphery steepens either in the meridian of relaxation or symmetrically when zonular tension is released from two orthogonal meridians. These results are *opposite* to what would have been predicted on the basis of Helmholtz's theory of accommodation. (*Invest Ophthalmol Vis Sci.* 2004;45: 2691–2695) DOI:10.1167/iovs.03-1267

The Helmholtz[1] theory of accommodation states that the lens is under increased zonular traction when viewing in the distance and decreased zonular traction when viewing near objects. The accommodative process for focusing on near objects occurs as a result of contraction of the circular ciliary muscle fibers, decreasing the ciliary muscle diameter, which simultaneously reduces tension on the anterior, equatorial, and posterior zonules. This allows the crystalline lens to become more spherical, decreasing its equatorial diameter and increasing its central thickness and central optical power. According to this theory, any increase in zonular tension will result in central flattening of the crystalline lens, independent of the baseline zonular tension.

This study was undertaken to observe qualitatively the real-time changes in topography of the anterior surface of freshly extracted intact relaxed human crystalline lenses as zonular tension was increased from the baseline, relaxed state. This study provided dynamic visual evidence of the effect of zonular tension on the topography of the central anterior surface of the crystalline lens. The observations can have important implications in understanding the mechanism associated with the accommodative process.

METHODS

As part of a larger study,[2] clear, fresh, intact 3-, 17-, 45-, 54-, 54-, 56-, and 56-year-old human crystalline lenses were obtained with large zonular skirts. Both of the two 54- and two 56-year old lenses also had their ciliary bodies attached to their zonules. The lenses had been placed in Optisol-GS (corneal storage media; Bausch & Lomb, Tampa, FL) and refrigerated within a mean of 7.7 ± 4.8 hours after death at a federally certified eye bank in the United States. Topography of their central anterior surfaces at varying levels of zonular tension was performed within a mean of 17.3 ± 12.5 hours of death. The lenses were obtained and managed in accordance with the provisions of the Declaration of Helsinki for research involving human tissue.

A topographer (Keratron Scout; Eyequip, Ponte Vedra Beach, FL) was rigidly mounted on an optical bench (Fig. 1). A group of zonules were grasped with curved, fine, nontoothed, smooth-tying forceps (model 8-0115; Rhein Medical Inc., Tampa, FL) on each side of the 3-, 17-, and 45-year-old crystalline lenses (180° apart). From this baseline, fully relaxed state, traction was slowly manually applied while topography was dynamically monitored. A video camera (model 4815-2000; Cohu, San Diego, CA, with a Macro-Cinegon 1:1, 8/10-mm lens; Leica Microsystems, Bannockburn, IL) was attached to a DVD recorder (DMR-HS2; Panasonic, Osaka Japan) for continuous recording of the image screen of the topographer. Increasing tension was slowly applied while the change in mires was monitored. Once the pattern was noted on the monitor to be elliptical, the tension was slowly relaxed.

To test the effect of symmetrical traction on the crystalline lens, zonular traction was applied from four positions approximately 90° apart. Four nonrotating micrometers (model 262; L. S. Starrett, Co., Athol, MA) were attached 90° apart to a stainless-steel ring. The ring had the following dimensions: an inner diameter of 115.0 mm, an outer diameter of 140.0 mm, and a height of 25.4 mm. Stainless-steel clamps were attached to each shaft of the micrometers. A rectangular aluminum strip measuring $20.0 \times 4.0 \times 0.8$ mm was placed in each stainless steel clamp so that 15 mm of each strip protruded toward the center of the ring. The ring was placed on the optical bench so that it was centered under the topographer. A black firm rubber cylindrical block measuring 12.8 mm in diameter and 16.0 mm in height was placed at the center of the ring. Each of the 54- and 56-year-old crystalline lenses was placed on the rubber block with their posterior surfaces down. Four positions of the intact ciliary bodies, 90° apart, of the two 56-year-old crystalline lenses were clamped to the aluminum strips with microaneurysm stainless steel clips (part no. 610186; Harvard Apparatus, Holliston, MA; Fig. 2).
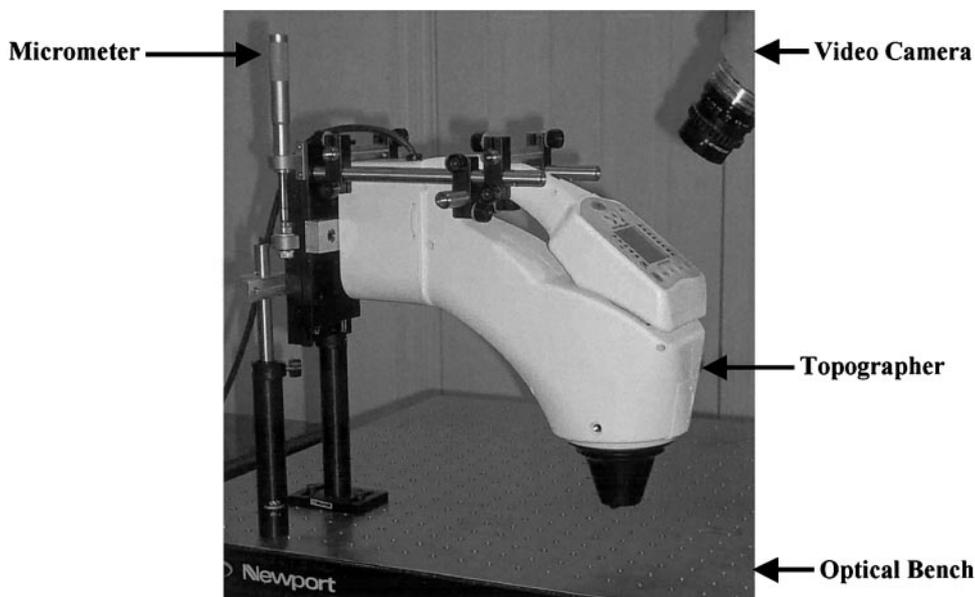
FIGURE 1. The topographer was mounted on an optical bench and the viewing screen was videographed.

While dynamically monitoring and recording the topography, traction was slowly applied by manually turning the screws of both micrometers in the 180° meridian counterclockwise until the mires appeared to be elliptical. Then, traction was applied to the 90° meridian by turning the micrometers in that meridian counterclockwise until the mires were again circular. To reduce zonular traction, the micrometer screws in the 90° meridian were turned clockwise until the mires became elliptical. Then, the screws of the micrometers in the 180° were turned clockwise until the mires again became circular. The process of symmetrically increasing and decreasing zonular tension was repeated at least three times with each lens; however, the initial application of zonular tension was alternated between the 180° and 90° meridians.

A similar procedure was performed with the two 54-year-old crystalline lenses, except that the sections of ciliary body that were clamped were separated from the nonclamped ciliary body by making radial incisions through the ciliary body on each side of the aluminum strip. The amount of tension applied was not quantified.

The Keratron Scout topographer that was used in this study can obtain only quantitative topography at a fixed, very short working distance that cannot be altered or adjusted. This short fixed working distance, required to obtain quantitative measurements, was too close to permit insertion of the forceps to grasp the zonules, or to allow the ring with the attached micrometers and clamps to fit under the micrometer. Therefore, only qualitative data were obtained in this study.

RESULTS

As traction was applied to the zonules of the 3, 17, and 45 year old lenses, the first five *central* mires became elliptical, with

the short axes along the meridian of zonular traction. This indicates that the central anterior surface had become steeper in the axis of traction. Simultaneously, the *peripheral* mires became elliptical, with the long axes in the meridian of zonular traction. This indicates that the peripheral anterior surface became flatter in that same axis (Movie 1 at www.iovs.org/cgi/content/full/45/8/2691/DC1 and Fig. 3). The movement of the central and peripheral mires in opposite directions in all three lenses demonstrates that this is a consistent observation. These observations are unlikely to be due to artifact, because of the simultaneous movement of the central and peripheral mires in opposite directions, both in the axis of traction and in the axis at 90° to the axis of traction.

Applying zonular traction to the 54- and 56-year-old crystalline lenses by pulling from four positions of the ciliary bodies, approximately 90° apart resulted in central steepening. This occurred whether the ciliary bodies were intact or the pull was applied to four separate ciliary body sections. When zonular traction was applied along one meridian, the *central* mires became elliptical, with their short axes along the meridian of zonular traction. Then when zonular traction was applied at 90° to this meridian, the central mires became both circular and smaller. Four-point zonular traction 90° apart produced symmetrical central steepening (Fig. 4). The opposite occurred when zonular traction was reduced.

Because the micrometer-induced changes occurred very slowly, it was difficult to perceive the dynamic changes at normal speed. To make the dynamic changes in mire shape more readily visible, the standard video playback speed (30
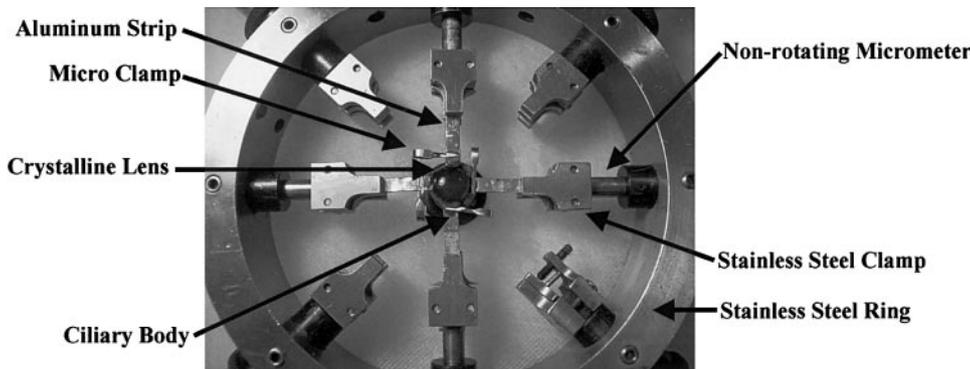

FIGURE 2. The setup for applying zonular traction to two orthogonal meridians.
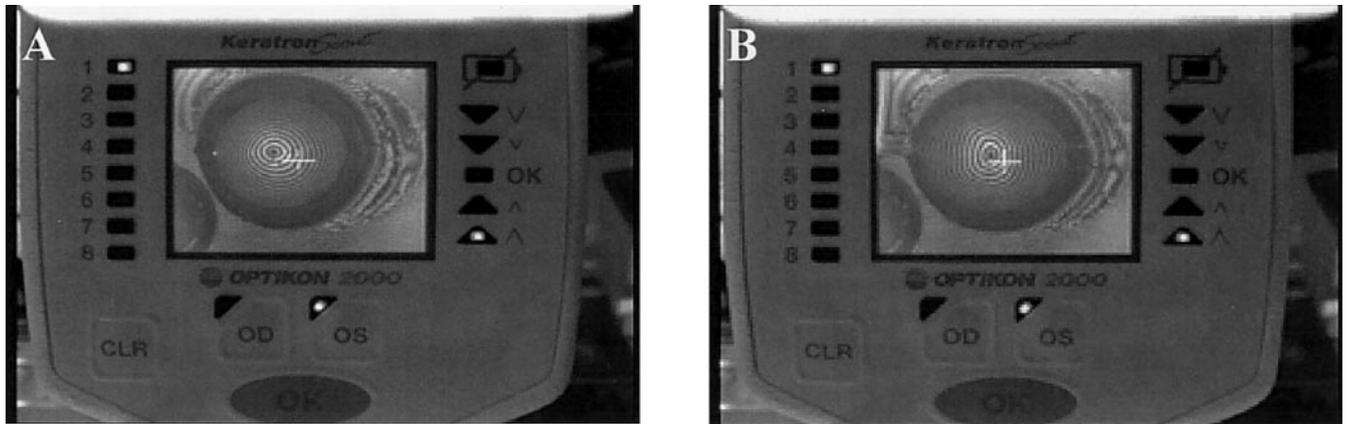
FIGURE 3. Real-time topography of a 17-year-old, freshly extracted intact human crystalline lens, (**A**) without and (**B**) with zonular traction in one meridian.

frames/sec) was increased by 40 times (Movie 2 at www.iovs.org/cgi/content/full/45/8/2691/DC1).

DISCUSSION

The Helmholtz theory[1] predicts that an increase in zonular tension will result in additional central flattening of the crystalline lens, regardless of the initial level of zonular tension. Our observations, commencing *at a baseline of no zonular tension*, demonstrated the opposite. Mathematical models[3,4] confirm that as zonular tension increases from zero, the center of the crystalline lens steepens. However, these models also predict that, when zonular tension exceeds physiologic levels, the center of the crystalline lens flattens.

The present observations are opposite to those reported by Glasser and Campbell[5] and Koopmans et al.,[6] who demonstrated a decrease in central optical power with increased zonular tension using laser scanning optical power measurements of extracted human crystalline lenses. The differences observed could result from having commenced the measurements with the lenses at higher than physiologic levels of baseline tension. Glasser and Campbell[5] specifically state that: "the zonular fibers were not flaccid but were extended without actually being stretched." In addition, the crystalline lenses used by Glasser and Campbell[5] and Koopman et al.[6] may have been subject to post mortem changes. The applicability of in vitro observations to the in vivo changes associated with zonular traction depends, in part, on the functional equivalence of

the lenses used to actual living tissue. The fresh lenses of the present study maintained in a physiologic solution, offered tissue that was very similar in quality to its in vivo state. Optisol-GS (Bausch & Lomb) has been demonstrated to maintain the corneal epithelium with minimal damage for 5 days.[7] The storage of human crystalline lenses in Optisol-GS has been observed in our laboratory to maintain central thickness and lenticular clarity for 4 days.[2]

In 1896, Stadfeldt[8] applied zonular tension to extracted human crystalline lenses and measured the change in central radius of curvature with a Javal ophthalmometer. With zonular tension he found that the central anterior radius of curvature steepened. He observed that the peripheral anterior surface flattened, but did not quantify this aspect of the accommodative process. The present study, using real-time topography of the intact extracted human crystalline lens, confirms Stadfeldt's observations. The dynamic changes provide additional support to his earlier observation—that is, the central anterior radius of curvature steepens and the peripheral anterior surface flattens with zonular tension.

Although in this study zonular traction was applied in either one meridian or two orthogonal meridians, we can generalize that, if zonular traction were applied over 360°, the central crystalline lens surface would similarly steepen. According to the principle of Saint-Venant,[9] the influence of a distributing force over 360° versus four symmetrically placed forces should have a negligible effect on the results, because the center of the surface of the lens is far away from the equator where the
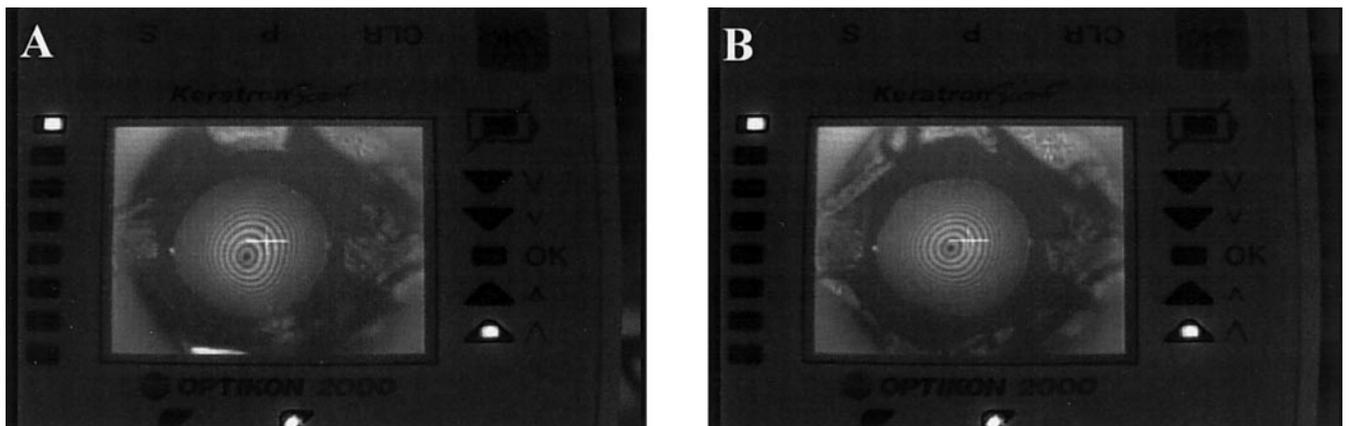

FIGURE 4. Real-time topography of a 54-year-old freshly extracted intact human crystalline lens with zonular traction along (**A**) the 180° meridian and (**B**) both the 180° and 90° meridians.

pulling force is applied. In agreement with Saint-Venant's principle, a nonlinear, finite element model of human crystalline lens accommodation has demonstrated that zonular traction from eight symmetrical positions around the lens results in the same surface changes as a 360° zonular tractional force.[10]

The present experiment involved simultaneous traction on all zonular elements—that is, the anterior, equatorial, and posterior zonules. The well-documented relaxation of the anterior and posterior zonules during accommodation,[11,12] with simultaneous and continued stability of the position of the crystalline lens during accommodation[13–15] (Sokolowska A, et al. *IOVS* 2002;43:ARVO E-Abstract 2015) is consistent with the theory in Schachar and Bax[3] and Schachar[16–18] that lenticular accommodation occurs exclusively because of increased equatorial zonular tension. Nonlinear finite element analysis of accommodation has demonstrated that when tension is applied to all three sets of zonules of the relaxed human crystalline lens, there is steepening of the central surfaces, flattening of the peripheral surfaces, and increases in central thickness and central optical power.[3] However, this same analysis predicts that traction applied specifically only to the equatorial zonules is more efficient than applying traction to all the zonules simultaneously; that is, for a given amount of zonular traction to the equatorial zonules alone, there is a greater increase in central optical power. Demonstrating this difference experimentally would be extremely difficult, because the equatorial zonules are very thin, 5 to 15 μm in diameter,[19] and difficult to segregate from the other zonules.

During pharmacologically induced accommodation, there is an increase in equatorial diameter and an increase in the central optical power, as demonstrated by in vivo ultrasound biomicroscopy of human[20] and primate[21] crystalline lenses. Our findings on freshly preserved lenses support and are consistent with these in vivo studies.

In magnetic resonance imaging (MRI) experiments during voluntary in vivo human accommodation, investigators have claimed to demonstrate a decrease in equatorial diameter associated with accommodation.[22,23] Calculation of the mean decrease in equatorial diameter of eight of the MRI subjects of Strenk et al.[22] aged 22 to 29.2 years (mean, 25.5 years) for an 8-D accommodative stimulus was 604 ± 167 (SD) μm. In contrast, for a 6.7-D accommodative stimulus, the eight MRI subjects of Demer et al.[23] with a mean age of 24 ± 4 (SD) years had a statistically insignificant decrease in equatorial diameter of 100 ± 200 (SD) μm—that is, in some of the subjects, the equatorial diameter actually *increased* by 100 μm. The difference in outcomes can be attributed to the variation in resolution, precision, and accuracy and the lack of accounting for eye movements,[24–26] such as excyclotorsion,[27] that occur during accommodation.

Other in vivo human and primate experiments have also demonstrated that the crystalline lens equator decreases in diameter during accommodation.[12,28,29] Our careful analysis of these studies reveals a systematic error. There appears to be movement between the imaging device and the eye. Measurement of the thickness of the cornea in the accommodated and unaccommodated state reveals a change in corneal thickness. Since neither corneal curvature nor corneal thickness changes during accommodation,[27,30] these experiments are flawed.

Storey and Rabie[31] in 1985 placed an A-scan ultrasound probe over the temporal sclera in line with the lens equator of seated human subjects. They measured the change in equatorial diameter of the lens equator during voluntary accommodation, assuming no eye rotation between measurements. Their data demonstrate that the nasal lens equator actually moves away from the temporal sclera during accommodation. This finding can be interpreted as consistent with our observation of increasing lenticular equatorial diameter associated with accommodation. Unfortunately, the position of the temporal lens equator echo was highly variable because of movements of the probe and the eye. Therefore, their use of the time between the temporal and nasal lens equatorial echoes to determine the changes in lens diameter during accommodation was unreliable.

Investigators have incorrectly used the pupil as a reference to measure lenticular changes during accommodation. In 1997, Wilson[32] used retroillumination of infrared radiation to measure the equatorial diameter of an albino patient's crystalline lens during pharmacologically controlled accommodation. He stated that, "measurement was taken in frames where the circular light was concentric with the pupil, guaranteeing no angle deformity." During pupillary constriction the pupil moves nasally and becomes eccentric.[33–35] Therefore, for Wilson to keep the circular light concentric with the pupil, the alignment between the eye and the camera would have had to be changed between the measurements made of the unaccommodated and accommodated states. The presence of rotation of the eye relative to the axis of the camera is readily verified by measuring the horizontal diameter of the cornea in the unaccommodated (Fig. 3 of Wilson[32]) and accommodated (Fig. 4 of Wilson[32]) states. The percentage change of the horizontal corneal diameter and the horizontal diameter of the crystalline lens, as determined by measuring the distance between the vertical cursor marks shown in Wilson's Figures 3 and 4, is approximately the same.

Using Scheimpflug photography, Dubbelman et al.[36] in 2003 claimed that there are internal changes in the crystalline lens during accommodation. The authors changed the position of the target, a Maltese star, "until the corneal reflex returned to the center of the pupil," in an attempt to correct for the convergence that occurs during accommodation. Their method for addressing convergence artifact only insured that their images were *not* obtained in the same plane, since the pupil had shifted during the accommodative process.

Glasser and Kaufman[37] stated that the small amount of spurious eye movement observed in their in vivo primate experiments could not account for the changes in the crystalline lens size and configuration during Edinger-Westphal nucleus or pharmacologic stimulation. They provided no controls to support this statement. When the artifactious extraocular eye movements were eliminated from their ultrasound biomicroscopic (UBM) images of the primate crystalline lens equator by using objective computer image-analysis techniques, the equator of the lens was demonstrated to move in an outward direction with accommodation (Schachar RA, Kamangar F, manuscript submitted). This is a finding consistent with our observations.

The present study demonstrates dynamically the changes in configuration of the human crystalline lens associated with zonular traction from a baseline tensionless state. With an increase in lenticular equatorial diameter due to zonular traction, there is an observed steepening of the central anterior radius of curvature and a flattening of the peripheral anterior surface.

CONCLUSION

When zonular tension is initially applied to the human crystalline lens, real-time, dynamic topography reveals that the anterior surface steepens and its periphery flattens.

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