Extralenticular and Lenticular Aspects of Accommodation and Presbyopia in Human Versus Monkey Eyes

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PURPOSE. To determine if the accommodative forward movements of the vitreous zonule and lens equator occur in the human eye, as they do in the rhesus monkey eye; to investigate the connection between the vitreous zonule posterior insertion zone and the posterior lens equator; and to determine which components—muscle apex width, lens thickness, lens equator position, vitreous zonule, circumlental space, and/or other intraocular dimensions, including those stated in the objectives above—are most important in predicting accommodative amplitude and presbyopia.

METHODS. Accommodation was induced pharmacologically in 12 visually normal human subjects (ages 19–65 years) and by midbrain electrical stimulation in 11 rhesus monkeys (ages 6–27 years). Ultrasound biomicroscopy imaged the entire ciliary body, anterior and posterior lens surfaces, and the zonule. Relevant distances were measured in the resting and accommodated eyes. Stepwise regression analysis determined which variables were the most important predictors.

RESULTS. The human vitreous zonule and lens equator move forward (anteriorly) during accommodation, and their movements decline with age, as in the monkey. Over all ages studied, age could explain accommodative amplitude, but not as well as accommodative lens thickening and resting muscle apex thickness did together. Accommodative change in distances between the vitreous zonule insertion zone and the posterior lens equator or muscle apex were important for predicting accommodative lens thickening.

CONCLUSIONS. Our findings quantify the movements of the zonule and ciliary muscle during accommodation, and identify their age-related changes that could impact the optical change that occurs during accommodation and IOL function.

Keywords: lens, ciliary muscle, human, accommodation, presbyopia

According to Helmholtz, the ciliary muscle moves forward and inward during accommodation, releasing tension on the zonule, and allowing the lens to thicken and increase in curvature (primarily the anterior surface curvature).¹ Rohen showed that the zonule consists of two major systems.² The posterior zonule covers the pars plana epithelium and runs forward from the region of the ora serrata toward the ciliary valleys, where it is attached and intermingles with the anterior zonule in the area of the zonular plexus. From there, the anterior zonule, comprising the posterior and anterior zonular tines, courses directly to the posterior and anterior aspects of the equatorial lens capsule.² Rohen concluded that during accommodation, there is little or no movement of the ora and zonular fiber system in the posterior part of the pars plana, while Coleman³ believed there was; concluding that the vitreous has a role in accommodation and presbyopia. Rohen theorized that during accommodative anterior-inward movement of the ciliary muscle, and thereby of the zonular plexus, the tension is taken over mainly by the posterior zonule, while the anterior zonule relaxes.² Due to the elasticity of the lens capsule, the lens can achieve a more “spherical” shape,

assuming the lens is flexible. The lens equatorial diameter decreases,⁴ and the lens anterior-posterior thickness increases.^{1,3,5–8} The shape of the lens has been described by Koretz as “two paraboloids of revolution joined along the equator”⁹ and by Coleman as a catenary.³ The refractive power of the lens, by means of its thickness and curvature, is a major contributor to the amplitude of accommodation, and has been studied extensively by other researchers.^{8,10,11}

Other studies also determined that the lens’ center of mass moves anteriorly with increasing age¹² and during accommodation.^{3,8,13,14} However, it was not possible in those studies to image the anterior/posterior position of the lens equator with the techniques used, and thus the exact amount of lens equator movements could not be quantified precisely.^{8,15} Further, Brown^{8,10} and Koretz et al.^{11–14,16–19} did not correct for the distortion of the images of the posterior lens surface by the optical surfaces in front of it; leaving the absolute value of the data in dispute.^{20,21} However, the overall findings of Koretz and Brown (age-related increased lens thickness, decreasing radius of curvature, lens paradox) were corroborated, with the

exception of whether the lens mass moves forward with age (see Discussion).^{20,21}

There are zonular attachments to the vitreous membrane that have not been investigated nor characterized fully.^{22–30} We have summarized these studies in a previous report.³¹

Previous reports of zonular architectural patterns were roughly similar^{32–34} to the more detailed descriptions reported by Lütjen-Drecoll et al.,³¹ who examined, and clearly defined the dual course of the posterior zonule and connections to the vitreous membrane, using the scanning electron microscope.³¹ In addition to the pars plana zonule, ultrasound biomicroscopic, and light and scanning electron microscopic findings demonstrated the existence of the vitreous zonule, which is separate and distinct from the pars plana zonule and is separated from it by approximately 2.5 mm.³¹ The vitreous zonule extends in a straight course from the region of the ora serrata (adjacent to the vitreous membrane) to the ciliary processes in the human and monkey.³¹ The vitreous base is attached to the ciliary epithelium in the region of the ora serrata; movements of that region toward the scleral spur during ciliary muscle contraction were posited³² or observed³⁵ to occur (as cited by Coleman³ from the original article in Japanese text); however, these connections and their movements were not characterized thoroughly nor measured in either the monkey or human eye. The movements in the region of the ora serrata and movements of the vitreous zonule during accommodation were characterized, and the association with accommodative amplitude and presbyopia were elucidated, in the live monkey eye,³¹ but not in the human eye. In the in vivo monkey eye, the vitreous zonule was associated clearly with accommodative amplitude and presbyopia: the farther forward (anteriorly) the vitreous zonule moved during accommodation, the greater was the accommodative amplitude, and the accommodative movement of the vitreous zonule declined with age.³¹ In a preliminary study, we have discovered a new connection that exists between the posterior lens equator and the posterior insertion zone of the vitreous zonule in the human eye, which also could affect lens movements and accommodation (Croft MA, et al. *IOVS* 2011;52:ARVO E-Abstract 3408).³⁶

To study human accommodation and presbyopia, the best animal model has proved to be the rhesus monkey. Its accommodative apparatus and mechanism are very similar to that of the human,^{31,37} and rhesus accommodation declines on an essentially identical relative time scale to that of humans.^{37–40} Using this model, preliminary study data showed that the lens equator moved forward during accommodation in young monkey eyes (Croft MA, et al. *IOVS* 2010;51:ARVO E-Abstract 5790). In this current study, we investigated if the same is true in the human eye.

The main purpose of the current study was to measure the relevant intraocular dimensions, and the lenticular and extralenticular accommodative components, including the newly discovered components described above, to determine their association with accommodative amplitude, lens movements, and presbyopia. Our measurements of these accommodative components also will be useful to model the accommodative apparatus and could facilitate successful accommodating IOL design.

MATERIALS AND METHODS

Human Subjects

We recruited 12 human subjects (5 males and 7 females) ranging in age from 19 to 65 years with normal eyes, and informed consent was obtained. All subjects received a complete eye examination by an ophthalmologist as a prerequisite for enrollment. Preliminary data collection includ-

TABLE 1. Age-Matched Groups

	Age, y	
	Monkey	Human
Young, presbyopic	6–9, <i>n</i> = 5	19–23, <i>n</i> = 4
Middle, midpresbyopic	12–13, <i>n</i> = 3	27–31, <i>n</i> = 4
Older, presbyopic	19–27, <i>n</i> = 3	49–65, <i>n</i> = 4

ed refraction measurement, slit-lamp biomicroscopy, direct ophthalmoscopy, and external and ocular motility examination. Subjects with any ocular abnormalities, or a refractive correction greater than 2.0 diopters (D) from emmetropia, were excluded from the study.

This research adhered to the tenets of the Declaration of Helsinki.

Monkeys

Eleven rhesus monkeys (*Macaca mulatta*) of either sex, aged 6 to 27 years and weighing 5.7 to 14.8 kg, with normal eyes were obtained, housed, and euthanized as described previously.³¹ The monkeys were iridectomized bilaterally and a bipolar electrode was placed in the Edinger-Westphal nucleus to stimulate accommodation.^{41,42} The animals were selected randomly, and the sample size was determined based on the information from previous studies of the average ciliary muscle and lens movement amplitudes, and the variability of the data.^{31,43–45} All procedures conformed to the ARVO Animal Statement for the Use of Animals in Ophthalmic and Vision Research, and were in accordance with institutionally approved animal protocols.

Age-Matched Groups

Based on the time course of presbyopia in rhesus monkeys and humans, the monkeys and humans were grouped and age matched (2.5:1; monkeys to humans), and the age groups' classifications (e.g., "young," "middle-aged," "older") were adjusted according to the relative life spans of the two species as shown in Table 1.

Accommodation Stimulation and Response Measurements

Refractive error was measured in the resting and accommodated states to determine accommodative amplitude.

Refractometry. Total refractive power of the subjects' eyes was measured with the Hartinger coincidence refractometer during disaccommodation and accommodation in both species. The difference in refractive power between the two accommodative states was calculated to obtain accommodative amplitude.

Human Subjects. Disaccommodation was induced by 1 drop of 5% clinical grade homatropine hydrobromide in both eyes, and refractions were repeated every 15 minutes until they no longer changed (approximately 45–60 minutes) to ensure that the ciliary muscle was in the relaxed state. One week to three months later, maximum accommodation^{19,46,47} was induced by 2 drops of 4% clinical grade pilocarpine hydrochloride separated by a 5-minute interval, and refractions were repeated again every 15 minutes until they no longer changed (approximately 45–60 minutes), to ensure that the ciliary body was in the maximally accommodated state.

FIGURE 1. Ultrasound biomicroscopy images taken with E-technologies/Reichert instrument (UBM-ER) in human subjects. Measurements were taken of the various intraocular distances. (A) *Distance 1:* Spur-to-VZ insertion is the distance between the posterior insertion zone of the vitreous zonule and the scleral spur. *Distance 2:* Vitreous zonule length is the distance between the posterior insertion zone of the vitreous zonule and the muscle apex. *Distance 3:* PVZ INS-LE is the distance between the posterior insertion zone of the vitreous zonule and the posterior lens equator. (B) *Distance 4:* Width of the ciliary muscle apex. *Distance 5:* Centripetal lens equator position is the distance between the lens equator and the scleral spur. *Distance 6:* Circumferential space is the distance between the ciliary processes and the lens equator. (C) UBM-ER image of the anterior segment in a 23-year-old human subject, which included the anterior and posterior lens surfaces, and allowed measurement of lens thickness (*Distance 7*). (D) UBM-ER demonstrating how the anterior/posterior position of the lens equator with respect to the scleral spur was determined (*Distance 8*). Several points on the inner aspect of the cornea (yellow crosses) were selected, and a regression line was calculated and drawn through the points (yellow line). A line was drawn through the scleral spur (blue line) perpendicular to the corneal regression line (yellow line) and the distance of the lens equator from the perpendicular line was measured (yellow diamond; 0.143 mm anterior to the scleral spur) in the unaccommodated and accommodated states. Panel B is the same UBM image as Panel A.

Monkeys. Accommodation was induced by midbrain electrical stimulation and measured by Hartinger coincidence refractometry, as described previously.⁴² Stimulus settings were chosen that induced maximum accommodative responses (i.e., maximum forward ciliary body movement) and maximum dioptric accommodation, allowing comparisons of the accommodative responses to be made between age groups.

Ultrasound Biomicroscopy (UBM). *Instrumentation.* Several high-resolution contact imaging techniques (e.g., dynamic goniovideography and UBM) can be performed during the dynamic accommodation response in anesthetized monkeys implanted with a midbrain stimulating electrode,^{7,42,48–50} but this setup is not possible in humans. We imaged the human eye in the unaccommodated and then the maximally accommodated states (see Human Subjects section below). While magnetic resonance imaging (MRI) has proved useful in the human eye, (Strenk SA, et al. *IOVS* 1992;33:ARVO E-Abstract 1193)^{51–53} MRI resolution is limited and cannot image the vitreous zonule consistently.

The UBM-H (Model # 840; Humphrey Instruments, San Leandro, CA) has higher resolution, but its field of view is limited to 5.0 mm and, thus, it was used to image the anterior portion of the ciliary body, vitreous zonule, and lens equator (Supplementary Fig. S1). The UBM-ER (Model # MHF-1 Ultraview System Model P60; E-Technologies, Bettendorf, IA) has lower resolution than the UBM-H, but it has a wider field of view (i.e., 13 mm) and, thus, was used to image the entire sagittal extent of the ciliary body, from the region of the ora serrata to the cornea, vitreous zonule, lens equator, and anterior and posterior lens surfaces (Fig. 1).

Images were obtained that provided the best definition of the accommodative apparatus, showing clearly defined edges of the ciliary body, lens equator, anterior and posterior lens surfaces, vitreous zonule, and cornea.

Human Subjects. UBM was done in the human subjects as described by Pavlin and Foster.⁵⁴ Each subject was seated in the examination chair and then was placed in the supine position. Obtaining UBM images requires the use of a scleral cup, which remains in contact with the eye during UBM imaging.⁵⁴ Each eye received a short-acting topical anesthetic (proparacaine hydrochloride 0.5%; Bausch & Lomb, Inc., Tampa, FL), before the scleral cup was positioned on the eye.

Goniosol (hydroxypropyl methylcellulose ophthalmic solution, USP 2.5%; HUB Pharmaceuticals, LLC, Rancho Cucamonga, CA) was used as the acoustic couplant. The subject was asked to rotate the eye and maintain it in the desired position to facilitate the proper orientation for obtaining images in the anterior segment (including the anterior and posterior lens surfaces), and in the nasal and temporal quadrants. The UBM probe was stabilized manually by the operator. Images were collected in the unaccommodated and maximally accommodated states.

Monkeys. UBM in the rhesus monkey has been described in detail previously.^{42,48,50} Briefly, each anesthetized (pentobarbital Na, 10–15 mg/kg intravenously [IV], supplemented with 0.5–10 mg/kg IV, as needed) monkey was placed supine with the head stabilized facing upward in a head holder, and a saline fluid-well was placed around the eye.^{50,54} The eye was rotated using a suture passed beneath the lateral rectus muscle.⁵⁰ During UBM imaging, the eye was stabilized with extraocular muscle sutures, so that during accommodation there was

minimal convergent eye movement, if any. The stabilizing arm of the ultrasound instrument held the transducer in place. Thus, there was very little, if any, change in angle of the transducer to the eye during accommodation. Dynamic UBM images were obtained during central stimulation of accommodation and then recorded to videotape.⁴²

For the monkeys and human subjects, analogous images were collected, with the anterior/posterior ends of the ciliary body and vitreous zonule oriented in a horizontal direction within all images.⁴² Care was taken to ensure a purely sagittal cross-section of the muscle and zonula by obtaining a UBM image in which the vitreous zonule was represented as a continuous line. This indicated that the oscillation of the UBM probe was parallel to the sagittal axis of the eye. If the vitreous zonule appeared as a dashed line in the UBM image, it implied that the UBM scan plane was not in a completely sagittal plane and was, instead, picking up several vitreous zonule strands and several 75- μ m spaces between the strands.

Measurement of Various Intraocular Structures. Measurements were taken of the various intraocular distances within the UBM images (Fig. 1), and comparisons were made between the images in the unaccommodated and accommodated states. The lens thickness and the width of the ciliary muscle apex were measured (Fig. 1), with the latter measurements analogous to those taken from histologic sections by Tamm et al.⁵⁵ The distance measured from the scleral spur to the posterior insertion zone of the vitreous zonule was designated Spur-to-VZ insertion (Fig. 1A). The distance from the muscle apex to the posterior insertion zone of the vitreous zonule was designated as the length of the vitreous zonule (Fig. 1A), because the location of the zonular plexus² was best represented by the clearly defined muscle apex in the UBM images. Additional measurements included the circumlental space (CLS, Fig. 1B), the distance between the vitreous zonule insertion zone and the peripheral lens equator (PVZ INS-LE, Fig. 1A), and the distance between the scleral spur and lens equator (centripetal lens equator position, Fig. 1B). We used the scleral spur as a reference point from which to gauge accommodative forward movement of the posterior insertion zone of the vitreous zonule. Forward movement was defined as anterior movement; in the above instance, it would be anterior movement of the insertion zone toward the scleral spur or toward the cornea. The lens equator was the point of the lens that was closest in distance to the ciliary processes (i.e., where the CLS is narrowest) in the UBM image (Fig. 1). The UBM probe was oriented into a position that allowed us to visualize and record the outermost portion of the lens equator edge and an entire ciliary process ridge, all within the same image. In all subjects, images were selected that showed the narrowest CLS (Fig. 1). The lens equator appeared at a modest angle to the sclera and not quite parallel to the axis symmetry of the eye. Nonetheless, we know this to be the lens equator as we positioned the UBM probe to detect the narrowest point between the lens and ciliary process ridge (as evidenced by the presence of anterior zonule, Fig. 1D, Supplementary Fig. S2) and then the position of the UBM probe was fine-tuned to include the vitreous zonule.

The anterior/posterior (A/P) position of the peripheral lens equator, in relation to the scleral spur, was determined from UBM images by selecting several points along the inner aspect of the cornea and generating the best curve-fit regression line. The best curve-fit regression line was fairly linear due to the fact that the inner aspect of the cornea, and thereby the selected points, were fairly linear. Nonetheless, we used a curve fit to represent most accurately the inner aspect of the cornea for each eye. We then drew a line through the scleral spur perpendicular to the corneal curve-fit regression line (Fig.

1D); the A/P distance of the lens equator from the perpendicular line through the scleral spur was ascertained in the accommodated and resting states, and the difference between the two states (pilocarpine-homatropine) was calculated. This was done to quantify the amount of forward (anterior) movement of the lens equator along a line through the A/P axis of the eye during accommodation.

Statistical Analysis

Various intraocular measurements were taken, and a 2-tailed paired *t*-test was used to detect significant differences between the resting and accommodated states (i.e., lens equator A/P position). A value of $P \leq 0.05$ was considered significant; $0.05 \leq P \leq 0.10$ was considered to indicate a trend, given the small number of monkeys or humans.

Regarding multiple *t*-tests, it generally is accepted⁵⁶ that the results showing significance in these situations do not simply occur by “chance” and we are not testing a “universal null hypothesis;” it is reasonable to consider each test on its own⁵⁶ (see Supplementary Statistical Explanatory Text online for a more complete explanation).

Simple Linear Regression. Simple linear regression (i.e., accommodative forward [anterior] movement of the vitreous zonule insertion zone versus age) was undertaken to determine if there was a significant relationship between variables.

Stepwise Regression Analysis. To determine which variables could predict accommodation accurately, we undertook a stepwise regression analysis. A full model includes all 24 explanatory variables of interest (i.e., lens thickness, muscle apex thickness, vitreous zonule length, and so forth). A *t*-test was performed to determine the significance of each variable in the full model, at a significance level of 0.05. The final model included variables that have a significant effect on the response variable (i.e., accommodation), given the other variables in the model.

The stepwise regression analysis used was bidirectional, which combines forward selection and backward elimination, and was performed using the statistical software R (available in the public domain at www.r-project.org). The selection/elimination criterion is based on the Akaike Information Criterion (AIC) first and then the *t*-test/*F*-test. The correlations among explanatory variables in the regression model were accounted for by this analysis. For example, if two explanatory variables are highly correlated and, further, if either one could have a significant effect on the response variable, then only one of them, but not both, would remain in the final model determined by stepwise regression.

RESULTS

The data showed that the group of human subjects included in the study were ocular-normal and were a representative sample of the human population, with regard to accommodation, presbyopia, accommodative lens thickening, and age-related lens thickening (see sections entitled “Accommodation,” “Accommodative Lens Thickening,” and Supplementary Fig. S3).

Accommodation

Pilocarpine-stimulated accommodation in the 12 human subjects, aged 19 to 65 years, ranged from 0 to 13.25 D and declined by 0.32 ± 0.04 D per year ($P = 0.001$), similar to the age-related decline in voluntary accommodation reported by Duane³⁹ (Supplementary Fig. S4).

FIGURE 2. (A) Accommodative forward movement of the lens equator with respect to the scleral spur, versus accommodative amplitude (A) or age (B) in 12 human subject eyes. (C, D) A/P lens equator position with respect to the scleral spur, versus accommodative amplitude (A) or age (B) in the resting (homatropinized) or accommodated (pilocarpinized) human eye.

Where the young and middle-aged groups did not differ significantly, the measurements were averaged. The accommodative change in distance is reported below for all variables and the mean and SEM for all of the distances measured in the unaccommodated and accommodated eye for each group are reported in the Supplementary Text online.

The Lens

Accommodative Lens Thickening. Accommodative lens thickening was significantly related to accommodative amplitude ($P = 0.001$, Supplementary Fig. S3) and declined significantly with age ($P = 0.001$) in the human eyes. With increased accommodative lens thickening, there was increased accommodative amplitude, and the lens thickness in the resting eye increased with increasing age (Supplementary Fig. S3). The lens thickness data, with respect to aging and accommodative amplitude, was similar to previously reported findings,^{21,57,58} demonstrating the validity of our measurement techniques.

Lens A/P Position and Forward Movement. In the resting human eyes, the A/P position of the lens equator with respect to the scleral spur (as defined in Fig. 1) was 0.04 ± 0.003 , 0.15 ± 0.03 ($P=0.045$), and 0.18 ± 0.05 mm ($P=0.08$) more anterior to the scleral spur in the young, middle-aged, and older eyes, respectively. In the PILO-treated eyes, the A/P

position of the lens equator was 0.53 ± 0.04 , 0.59 ± 0.07 , and 0.29 ± 0.07 mm more anterior to the scleral spur in the young, middle-aged, and older eyes, respectively.

During accommodation, the lens equator moved forward (anteriorly) with respect to the scleral spur by 0.48 ± 0.044 mm (mean $\pm$ SEM, $P=0.002$) in the young human eyes, by 0.45 ± 0.08 mm ($P=0.010$) in the middle-aged group, and by 0.11 mm $\pm$ 0.08 mm ($P=0.25$) in the older eyes. Accommodative forward movement of the lens equator was significantly related to accommodative amplitude ($P = 0.001$) and declined significantly with age ($P = 0.005$; Figs. 2A, 2B). The farther forward (anteriorly) the lens equator moved during accommodation, the higher was the accommodative amplitude.

In the accommodated (pilocarpinized) eyes, the lens equator A/P position was significantly positively related to accommodative amplitude (the more anterior-positioned the lens equator, the higher the accommodative amplitude ($P = 0.016$, Fig. 2C), and this accommodative anterior positioning was lost with age ($P = 0.021$, Fig. 2D). In addition, the accommodative forward movement (A/P) of the lens equator (pilocarpine minus homatropine) with respect to the scleral spur was significantly related to accommodative lens thickening ($P = 0.019$, Fig. 3).

The A/P lens equator position in the resting human eyes tended to be more anteriorly positioned with age ($P = 0.069$,

FIGURE 3. Accommodative forward movement of the lens equator with respect to the scleral spur, versus accommodative lens thickening in 12 human subject eyes.

Fig. 2D), and this A/P position in the resting eyes was inversely related to accommodative amplitude ($P = 0.02$, Fig. 2C).

Centripetal Lens Equator Movement. In the 12 human subjects, the lens equator moved away from the sclera (centripetally) in the accommodated versus the resting eyes by 0.15 ± 0.03 mm ($P = 0.02$) in the young and middle aged eyes grouped together, and by 0.06 ± 0.04 mm in the older age group ($P = 0.17$). The accommodative centripetal lens equator movement tended to increase with increasing accommodative amplitude ($P = 0.058$, Fig. 4A) and tended to decline with age ($P = 0.063$, Fig. 4B). In the resting eyes, the distance between the lens equator and the scleral spur tended to decline with age ($P < 0.091$, Fig. 4D), and in the accommodated eyes the decline with age was significant ($P = 0.034$, Fig. 4D, see Discussion). The centripetal lens equator position regression analysis showed one outlier at age 31; the centripetal lens equator position was 0.4 mm farther inward (centripetally) from the scleral spur than all other subjects of this age group (Fig. 4D, See Supplementary Text online for further details).

The Ciliary Muscle

The PILO-induced human ciliary muscle apex thickening in young eyes was 0.34 ± 0.05 mm, and was significantly reduced to 0.15 ± 0.07 mm in middle-aged eyes ($P = 0.025$) and to 0.08 ± 0.01 mm in older eyes ($P = 0.012$), compared to the young eyes.

In the human eyes, accommodative ciliary muscle apex thickening was significantly related to accommodative amplitude (Fig. 5A, $P = 0.005$), and declined significantly with age (Fig. 5B, $P = 0.003$), and did so roughly in parallel to the loss in accommodative amplitude with age (Fig. 5B).

The muscle apex width increased significantly with age in the unaccommodated human eyes ($P = 0.017$), but not in the accommodated eyes ($P = 0.226$, Fig. 5D), due to the ability of the young apex to thicken (Fig. 5D) in response to pilocarpine. Resting muscle apex width was related inversely to accommodative amplitude (Fig. 5C, $P = 0.013$), due to the age-related apex thickness increase (Fig. 5D). Thus, the thicker the resting apex width, the lower the accommodative ability. As a result, the contracted muscle apex width was unrelated to accommodative amplitude because the young muscle apex thickened substantially in response to pilocarpine (Fig. 5), while the older muscle apex did not.

The CLS and the Anterior Zonule

The CLS in the human eyes was significantly related to accommodative amplitude and declined significantly with age (Fig. 6), similar to the monkey eyes.⁴⁸ In the young human subjects, the unaccommodated CLS was very similar to the accommodated state (mean CLS difference = 0.003 ± 0.009 mm, unaccommodated minus accommodated). Therefore, it was no surprise that the anterior zonule, which extend from the region of the ciliary processes to the lens equator, were observed to be taut in the unaccommodated and accommodated states (Supplementary Fig. S2), similar to the monkey.⁴⁸ In the four older human eyes, the CLS in the accommodated state was 0.15 ± 0.03 mm narrower ($P = 0.015$, $n = 4$) than in the unaccommodated state. The older anterior zonule always was taut in the unaccommodated state, but at times was slack in the accommodated state (Supplementary Fig. S2).

The greater the narrowing of the CLS from the unaccommodated to the accommodated state, the lower the accommodative amplitude ($P = 0.01$, Fig. 6C). The accommodative CLS narrowing (CLS narrowing = unaccommodated CLS minus the accommodated CLS) increased with increased age ($P = 0.01$, Fig. 6D, see Discussion for further explanation).

The Vitreous Zonule

The entire extent of the ciliary muscle and vitreous zonule can be visualized by UBM, in the human and the monkey eyes. In the human eyes, greater care was required to image the vitreous zonule than in the monkey eyes. The movement of the vitreous zonule insertion zone was measured as the change in distance between the scleral spur and the vitreous zonule insertion zone (Spur-to-VZ insertion distance). In the human eyes, the posterior insertion zone of the vitreous zonule clearly moved forward in a sagittal plane along the curvilinear boundary of the globe (anteriorly, toward the scleral spur) during accommodation by 1.01 ± 0.05 , 0.46 ± 0.08 , and 0.15 ± 0.03 mm in the young, middle-aged, and older subjects, respectively (see Table 2). Across the age range, this forward movement was related significantly to accommodative amplitude ($P = 0.001$, Fig. 7); the greater the forward movement of the insertion zone, the higher the accommodative amplitude (Fig. 7). The forward movement of the vitreous zonule posterior insertion zone declined significantly with age ($P = 0.002$, human, Fig. 7).

In the resting human eyes, the Spur-to-VZ insertion distance did not change with age (Fig. 7D). However, in the accommodated older eyes the Spur-to-VZ insertion distance tended to be longer, in comparison with the young eyes, due to the accommodative shortening of this distance in the younger eyes, but not the older eyes (Fig. 7D). Thus, neither the resting nor the accommodated Spur-to-VZ insertion distance was related to accommodative amplitude in these 12 subjects (Fig. 7C).

Accommodative Relationship of the Lens, Ciliary Muscle, and the Zonule

Accommodative ciliary muscle apex thickening was related significantly to accommodative lens thickening ($P = 0.021$) and to lens centripetal movement ($P = 0.045$) in the 12 human subjects, but the association with lens equator forward (anterior) movement was of borderline significance ($P = 0.058$; Figs. 8A, 8C, 8E). The more the muscle apex thickened, the more the lens thickened, and the lens equator tended to move forward (anteriorly) and internally away from the sclera toward the optical axis of the eye, so that the overall movement was antero-inwardly during accommodation. Ac-

FIGURE 4. In 12 human subjects: (A) Accommodative amplitude versus centripetal lens equator movement (defined as the change in distance between the scleral spur and the lens equator in the unaccommodated [homatropinized] and the accommodated [pilocarpinized] state [pilocarpine-homatropine]). (B) Centripetal lens equator movement versus age. (C) Accommodative amplitude versus the centripetal lens equator position (distance between the lens equator and the scleral spur) in the resting (homatropinized) and accommodated (pilocarpinized) state. (D) Centripetal lens equator position versus age in the resting and accommodated state. Circled data points signify an outlier (age 31) whose lens equator position was 0.4 mm farther from the scleral spur than any other subject. Analysis without this outlier is included in Supplementary Figure S6.

commodative forward movement of the vitreous zonule posterior insertion zone was related significantly to lens equator forward movement ($P = 0.023$) and to lens thickening ($P = 0.006$), but was not related to lens centripetal movement ($P = 0.28$, Figs. 8B, 8D, 8F). The more the vitreous zonule insertion zone moved forward, the more the lens equator moved forward, and the more the lens thickened during accommodation.

The Resting Eye: Lens Thickness Versus Apex Thickness

Although the resting ciliary muscle apex width and lens thickness increased significantly with age, these two variables were not correlated with each other in these 12 human subjects (Supplementary Fig. S5). However, if the outlier described in Figures 4C and 4D were excluded, a regression analysis showed correlation of borderline significance between the two variables: resting muscle apex width tended to increase as the lens thickness increased ($P = 0.057$, $r = 0.59$, $n = 11$; Supplementary Fig. S5).

Comparisons to the Monkey

The vitreous zonule was more difficult to image in the human eyes than in the monkey eyes. The accommodative forward movement of the vitreous zonule posterior insertion zone also

declined with age in the monkey eyes as reported previously³¹ (see Table 2), but the decline was more pronounced in the human eyes (reported here) than reported previously in the monkey eyes. The accommodative forward movement of the lens equator also occurred in the young monkey eyes and is observed best in the dynamic UBM video clip taken during central electrically-induced accommodation (see Supplementary Video Clip S1, left panel). Further, in the monkey eyes, the forward movement of the lens equator was lost with age (see Supplementary Video Clip S1, right panel), as in the human eyes.

The accommodative lens thickening was greater and, thus, accommodative amplitude was higher in the monkeys, compared to the human eyes (Supplementary Table S1). In the monkey eyes accommodative amplitude from young to middle-age and from young to older age declined by 42% and 48%, respectively, and in the human eyes by 30% and 92%, respectively (see Discussion). Correspondingly, in the monkey eyes, lens thickening from young to middle age and from young to older age declined by 46% and 71%, respectively, and in the human eyes by 35% and 99%, respectively (Supplementary Table S1, see Discussion).

During accommodation, the nasal and temporal quadrants behaved similarly in the monkey eyes^{42,48} and likewise, by qualitative assessment from this study, in the human eyes. The effect of aging could be discerned regardless of the quadrant used, as long as the images were clear. In the human eyes, the

FIGURE 5. Accommodative apex thickening versus accommodative amplitude (A) or age (B) in the human eye. Right y axis in (B) shows that the decline in accommodative amplitude versus age is roughly in parallel with the age-related loss in accommodative apex thickening. Thin solid and dashed lines represent a quadratic fit regression equation for accommodative apex thickening (solid) and accommodative amplitude (dashed) versus age; Thick shaded line represents least squares linear regression of accommodative apex thickening versus age. (C, D) Human muscle apex width versus accommodative amplitude (C) or age (D) in the unaccommodated (homatropinized) and accommodated (pilocarpinized) eye.

nasal quadrant provided the best and most distinct images of the relevant accommodative structures. In the monkey eyes, the clearest images came from the temporal quadrant, in which the relevant structures were measured, analyzed, and used for comparison with the human data. These results were very similar between the species, lending further credibility to the idea that aging changes can be discerned from either quadrant.

Averaged across the entire age range, the length of the vitreous zonule did not change significantly during the accommodative response in either the monkey eyes or the human eyes. The measured accommodative change in vitreous zonule length (homatropine minus pilocarpine) was small (ranging from +0.5 to -0.4 mm) but, given the age, it was important to predict accommodative lens thickening (see Accommodative Lens Thickening in the Stepwise Regression section below).

Stepwise Regression (Human)

The issue of the measurements being related to each other due to the geometry of the eye can be found in the Supplementary Statistical Explanatory Text online.

Accommodation. In the stepwise regression analysis, all variables were incorporated in the model to determine which variables were most important to predict accommodation. Two variables were important for predicting accommodative amplitude in the human eyes: accommodative lens thickening and the resting muscle apex thickness. The accommodated

apex thickness was not important for predicting accommodative amplitude, due to the fact that the young apex thickened in response to pilocarpine, while the older muscle already was thick and did not thicken much further in the presence of pilocarpine.

The final model from the stepwise regression was:

$$\text{Accommodation} = 17.62 + 20.75*(\text{Lens thickening}) - 20.39*(\text{ATR apex thickness})$$

Note that age is not included in the final model (i.e., based on the Akaike Information Criterion [AIC]⁵⁹ and P value). This means that age did not explain accommodation over and above what accommodative lens thickening and resting apex thickness could do together. In the final model, the P value is slightly smaller (0.000002 vs. 0.000002), but the R^2 value is much higher (94.52% vs. 84.81%) and the residual SE also is smaller (1.332 vs. 2.104) than the model that includes age alone in these 12 human eyes.

In another stepwise regression analysis, we included only those human subjects ranging in age from 19 to 31 years. In this analysis, four variables were important to predict accommodative amplitude: accommodative lens thickening, resting apex thickness, the accommodative change in vitreous zonule length (homatropine minus pilocarpine), and accommodative change in distance between the lens equator and the scleral spur (pilocarpine minus homatropine). Again, note that age is not included in this model (i.e., age could explain

FIGURE 6. CLS plotted versus accommodative amplitude (A) or age (B) in 12 human subjects. The greater the CLS, the greater the accommodative amplitude. Difference between the CLS in the unaccommodated versus the accommodated state plotted versus accommodative amplitude (C) or age (D). The difference between the CLS in the unaccommodated minus the accommodated state represents CLS narrowing.

accommodation [$P = 0.050$] but did not explain accommodation as well as these four variables could do together). This is based mainly on AIC and P values; however, we have included the R^2 and residual SE to provide extra information useful to compare models. In the final model that included the four variables, the P value is smaller (0.00066 vs. 0.05035), the R^2 value is much higher (99.59% vs. 49.84%), and the residual SE is smaller (0.274 vs. 2.154) than the model that included age alone. The final model from the stepwise regression was:

$Accommodation = 19.75 + 10.68 \times (accommodative\ lens\ thickening) - 22.07 \times (resting\ apex\ width) + 14.29 \times (accommodative\ change\ in\ distance\ between\ the\ lens\ equator\ and\ the\ scleral\ spur\ [pilocarpine\ minus\ homatropine]) + 4.91 \times (accommodative\ change\ in\ vitreous\ zonule\ length\ [homatropine\ minus\ pilocarpine])$.

Accommodative Lens Thickening. In the stepwise regression analysis, three variables were important to accommodative lens thickening: age, the accommodative change in vitreous zonule length (homatropine minus pilocarpine), and accommodative change in PVZ INS-LE distance (homatropine minus pilocarpine; PVZ INS-LE distance = the distance between the lens equator and the posterior insertion zone of the vitreous zonule, Fig. 1). Given the age of the subject, the accommodative change in vitreous zonule length and the accommodative change in PVZ INS-LE distance were important to predict accommodative lens thickening, even though the differences were small. However, the accommodative change

in lens thickness also was small and, therefore, the idea that the distance changes had significant impact on lens thickening is understandable. The calculation of the accommodative change in both distances is dependent upon the position of the posterior insertion zone of the vitreous zonule, and the posterior restriction of the insertion zone's movement during accommodation may dampen accommodative lens thickening.

The final model from this stepwise regression is:

Lens Thickening

$$= 0.56 - 0.01 \times Age \\ - 1.29 \times (accommodative\ change\ in\ PVZ\ INS-LE\ distance) \\ + 0.3 \times (accommodative\ change\ in\ vitreous\ zonule\ length)$$

The results were the same if we included only those subjects ranging in age from 19 to 31 years old.

DISCUSSION

Optically, it is the lens shape change by which the refractive power of the eye is increased and accommodation occurs, and, ultimately, the lens is the prime component/indicator for the loss of accommodation with age in human and monkey eyes. The vast majority of statistical analyses, from various studies, have reported that age also has been one of the main factors predicting accommodative amplitude.^{19,38–40,60} Some have

TABLE 2. Accommodative Forward Movement of the Vitreous Zonule Insertion Zone

Monkey	Mean $\pm$ SEM, mm	n	P Value
Young, 6–9 y	1.05 $\pm$ 0.07*	4	
Middle, 12–13 y	0.59 $\pm$ 0.18	3	0.084
Older, 19–21 y	0.40 $\pm$ 0.12*	4	0.028
Human			
Young, 19–23 y	1.01 $\pm$ 0.05	4	
Middle, 27–31 y	0.46 $\pm$ 0.08	4	0.005
Older, 49–65 y	0.15 $\pm$ 0.03	4	0.01

Data are mean $\pm$ SEM accommodative forward movement of the vitreous zonule insertion zone for the respective age groups in the monkey eye and the human eye.

* Data for young and older monkey eyes adopted with permission from Lütjen-Drecoll E, Kaufman PL, Wasielewski R, Ting-Li L, Croft MA. Morphology and accommodative function of the vitreous zonule in human and monkey eyes. *Invest Ophthalmol Vis Sci*. 2010;51:1554–1564.³¹ Copyright 2010 ARVO.

postulated that this is because it is the single best biomarker, taking into account all aging changes that occur in the eye. However, in our study, stepwise regression analysis showed, for the first time to our knowledge, that accommodative lens thickening and the resting ciliary muscle apex thickness explained accommodative amplitude slightly better than age alone (as explained in the Results) in the 12 human eyes. It is striking that in this model, age was not necessary, that is, age did not explain accommodative amplitude over and above what accommodative lens thickening and age-related resting muscle apex thickening could do. This demonstrates an associative relationship suggesting that an extralenticular component, that is, age-related changes in the muscle, in addition to age-related changes in the lens, may have a role in the pathophysiology of presbyopia.

We chose subjects that were within 2 D of emmetropia and that had pharmacologically-induced maximum accommodative amplitudes ranging from 0 to 15 D, the full range of accommodative amplitude. Further, in all subjects, the images of the sagittal cross-sections of the ciliary muscle were collected systematically, aligned with a known landmark (the vitreous zonule, which lies in the sagittal plane of the eye); and the eyes were imaged at a known level of maximum accommodative amplitude. These techniques substantially reduced variability, and allowed the study of the full range of

FIGURE 7. (A, B) Accommodative forward movement of the vitreous zonule insertion zone versus accommodative amplitude (A) or age (B) in human subjects (ages 19–65 years). These data are very similar to rhesus monkey data reported previously.³¹ (C, D) Spur-to-VZ insertion length versus accommodative amplitude (C) or age (D) following homatropine or pilocarpine treatment in the human eye.

FIGURE 8. For 12 human subjects: Accommodative muscle apex thickening (*left*) or accommodative forward movement of the vitreous zonule posterior insertion zone (*right*) plotted versus accommodative lens thickening (**A**, **B**), accommodative lens equator forward movement (**C**, **D**), or lens equator centripetal movement (**E**, **F**).

accommodative amplitude and its decline with age. This comprehensive approach may explain why our study found that the accommodative muscle movement was reduced with age, while other studies did not.⁶¹

Brown in 1973⁸ and 1974¹⁰ reported the phenomenon referred to as the “lens paradox”^{17,18} in the human eye: the lens becomes thicker and more sharply curved with age (appearing to be in an “accommodated state”) and yet the ability of the eye to accommodate is lost almost completely. Although the absolute numbers collected by Brown and Koretz are in dispute (due to the distortion of the posterior lens surface), the overall findings are not.^{20,21} Our data, collected using UBM, also showed that the lens thickens with increasing age in the resting eye and that this is related inversely to accommodative ability, as reported previously.^{10,20,21} These particular associations are not new, but they

do demonstrate that these are normal human subjects and they add additional validity to our measurement techniques as reported previously.^{7,31,43,44}

By direct imaging of the lens equator, we actually measured the accommodative lens equator movements, rather than inferring them from forward translation of the lens mass. As stated previously in the Introduction, the lens shape, while biconvex, is not equiconvex; its anterior surface is flatter than its posterior surface.^{3,9} Therefore, the forward translation of the lens equator may not necessarily be the same numerically as the forward translation of the lens mass. Accommodative forward movement of the lens equator in our study averaged 0.48 mm in the four young subject eyes accommodating an average of 12.1 D. This is somewhat greater than what Coleman³ reported for accommodative forward movement of the lens center of mass, which ranged from 0.03 to 0.21 mm in

human subjects (ages 22–29 years) during voluntary accommodation to a visual stimulus of 6 D. Using Scheimpflug imaging, Brown estimated that, based on extrapolation in one 30-year-old subject eye that accommodated 10 D, the lens equator moved forward by 0.26 mm, but he did not directly image the lens equator,⁸ and did not correct for distortion of the posterior lens surface.^{20,21} The differences in forward translation of the lens mass measured by others, versus the forward movement of the lens equator measured in this study, could be due to the amplitude of accommodation induced, or it may be that forward lens equator movement may not be the same as forward translation of the lens mass, because of how the lens is reshaped (i.e., capsular forces, lens geometry, internal lens cells).^{62–64}

According to Duane's curve, the middle age group (27–31 years old; accommodating an average of 8.5 D) was mid-presbyopic (Supplementary Fig. S4) even if not yet symptomatic, and the results from this group might have differed more from the young age group (accommodating an average of 12.1 D) if younger subjects had been available (i.e., <18 years). Nonetheless, there were significant differences between these two age groups in regard to accommodative forward movement of the insertion zone and resting A/P position of the lens equator.

Our current study showed for the first time to our knowledge, in the rhesus monkey eye and in the human eye, that the young lens equator moves forward during accommodation and that, with age in the resting human eye, the lens equator tends to be in a more anterior (accommodated) position. This may add another aspect to the lens paradox. With respect to the forward movement of the lens mass, it is interesting to note that some carnivores (e.g., raccoons, dogs, and cats) accommodate essentially solely through anterior movement of the lens.⁶⁵

In the resting human eyes, the CLS declined with age, as we found in the monkey eye.⁴⁸ Since others have reported that there is no change in lens equatorial diameter with age,^{52,66} the age-related decline in CLS that we report is likely due to the age-related increase in ciliary muscle apex thickness and corresponds with the age-related decline in the ciliary ring diameter reported by others.⁵² The decline with age of the distance between the lens equator and the scleral spur that we found suggests that the sclera and muscle apex, as a whole, are closer to the lens equator, possibly due to a change in scleral contour ("bowing inward") reported in our companion study³⁶ and/or to other age-related changes in the geometry of the eye. The CLS that we measured likely encompassed Hannover's canal,^{67,68} which lies between the anterior and posterior tines of the anterior zonula. The loss in CLS in the aging eye may limit the space available for Hannover's canal,^{67,69} and/or affect the physiologic processes that putatively occur there, and it has been speculated that this may lead to cataracts.^{67,68} However, we are reluctant to speculate further as we did not measure or visualize Hannover's canal (see our companion paper³⁶). These observations may warrant further investigation, but are beyond the scope of this report.

The accommodative narrowing of the CLS was greater in the oldest eyes because the centripetal lens equator movement was nearly abolished, while some centripetal muscle movement, although reduced, still was present. Thus, the greater the narrowing of the CLS, the lower the accommodative amplitude.

The geometric theory of accommodation and presbyopia posits that the increasing lens thickness with age pulls the ciliary muscle via zonular tension into a more anterior and inward position.⁷⁰ Our human data show a correlation of borderline significance between lens thickness and muscle apex thickness (with one outlier excluded), but since our

findings are equivocal, more subjects are needed to make a definitive conclusion.

These results are in line with the Helmholtz theory of accommodation and Rohen's theory of the role of the zonula in accommodation, with the exception that our data demonstrated that the region of the ora serrata does move forward during accommodation, as theorized by Coleman. However, none of these theories included, nor could they address, the role of the new structures that have been discovered^{31,36} using different tissue preparation techniques and more current technology. Nonetheless, these earlier pioneers made some amazingly accurate predictions of the accommodative mechanism that still are being discussed in the literature today.

In our study, we demonstrated that the vitreous zonule and its posterior insertion zone have a role in accommodation and presbyopia in the human eye. During accommodation, the lens equator moves forward and inward, and this movement is reduced with age, possibly due to the age-related loss in accommodative forward movement of the vitreous zonule posterior insertion zone, previously demonstrated in the monkey eye³¹ and now in the human eye. This loss in the insertion zone's forward movement may not only dampen forward muscle movement, but also may dampen lens movements, given its direct attachment to the posterior lens equator (see our companion paper³⁶) and to the tensile fibers that spread to the walls in the valleys between the ciliary processes. Likewise, this reduced movement of the insertion zone during accommodation could inhibit forward movement of the muscle apex, and cause a posterior "drag" on the ability of the lens equator to move forward and of the lens to thicken.

We have imaged and characterized the vitreous zonule, but further study of the vitreous is needed; other reports in the literature indicate the existence of a network of fibers contained within the vitreous.^{33,69,71} The study of all these would require specific contrast agents to enhance their visualization, well beyond the scope of this report. We were able to discover one such agent to visualize the anterior hyaloid membrane and those results are reported in the companion article.³⁶

The data in Figure 8 suggested that the forward movement of the vitreous zonule/ciliary muscle has more effect on lens equator forward movement and lens thickening than on centripetal movement of the lens equator during accommodation—perhaps due to the direction of the muscle/vitreous zonule movement and muscle/vitreous zonule/lens architecture. The muscle/vitreous zonule accommodative forward movement is in an A/P direction, as is the accommodative forward lens equator movement, and of course the lens thickens in the A/P direction during accommodation. The data in Figure 8 also suggested that the centripetal movement of the muscle (as measured by apex thickening) releasing tension on the anterior zonule has more to do with centripetal rather than forward movement of the lens equator, again perhaps due to the direction of the muscle/zonule movement and muscle/anterior zonule/lens architecture. These findings supported observations discussed by Helmholtz (p. 405) regarding zonular attachments to the anterior and posterior lens surfaces.¹

UBM (see Table 2) in the human eye confirms previous UBM findings⁴² and earlier histologic findings that forward ciliary body movement diminishes significantly with age, but is never lost completely, even in the oldest eyes.⁵⁵

It is not surprising to find additional similarities between the accommodative apparatus of the monkey eye and human eye. The muscle moves forward and inward in both species, and the lens thickens and becomes more sharply curved during accommodation. The accommodative amplitude in the monkey eye is higher than in the human eye, and our data showed

that this likely is due to greater accommodative lens thickening and muscle apex thickening in the monkey eye versus the human eye. In the monkey, the eyes are placed closer together than in the human, and during accommodative convergence there is a need for higher accommodative amplitudes in the monkey. It is unknown why there was a difference in age-related accommodative amplitude decline between monkeys and humans regarding middle-aged versus older age groups (Supplementary Table S1). It may relate to the thickness of the vitreous zonule posterior insertion zone, which is far thicker in human eyes than in monkey eyes.³¹ This may dampen accommodative amplitude differently between these two age groups in the humans. These differences merit further study, but are beyond the scope of this report.

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