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ORIGINAL ARTICLE

Dynamic Accommodative Changes in Rhesus Monkey Eyes Assessed with A-Scan Ultrasound Biometry

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ABSTRACT: *Purpose.* Prior studies in humans measured time constants of biometric accommodative changes as a function of amplitude, and prior studies in monkeys used slit lamp videography to analyze dynamic lenticular accommodative movements. Neither of these studies related biometric changes to refractive changes. We wished to develop and test methodology to begin to test the hypothesis that ocular biometric changes are well correlated with accommodative refractive changes in rhesus monkeys. *Methods.* Methodology is described to dynamically measure biometric accommodative changes with A-scan ultrasonography. Lens thickness, anterior chamber depth, and anterior segment length (anterior chamber depth plus lens thickness) were measured dynamically during Edinger-Westphal-stimulated accommodation in two eyes of one rhesus monkey. In addition, dynamic accommodative refractive changes were measured with infrared photorefraction. Functions were fit to the accommodative and disaccommodative responses to obtain time constants. Derivatives of these functions allow peak velocities to be determined for each amplitude. Dynamic changes in lens thickness and anterior chamber depth measured with A-scan biometry were compared with dynamic measures of accommodation using infrared photorefraction. *Results.* Lens thickness and anterior segment length increase and anterior chamber depth decreases during accommodation. The biometric changes are well correlated with the accommodative optical changes. Peak velocities of accommodative changes in lens thickness and anterior chamber depth increase with amplitude and peak velocities for disaccommodation were higher than those for accommodation. *Conclusions.* Dynamic A-scan provides a method for dynamic analysis of the accommodative biometric changes during Edinger-Westphal-stimulated accommodation in monkeys, although the measurement resolution of this approach is limited. (Optom Vis Sci 2003;80:383–394)

Key Words: accommodation, A-scan ultrasound, lens thickness, anterior chamber depth, anterior segment length

In primates, accommodation occurs through a change in form of the crystalline lens.[1, 2] During accommodation, the crystalline lens anterior and posterior radii of curvature[3–6] and equatorial diameter[7–9] decrease, and lens thickness increases.[4, 6, 9–11] A combination of changes in lens surface curvatures and ocular biometric distances bring about the accommodative change in optical power of the eye. If refraction and ocular biometry can be measured dynamically with sufficient accuracy during accommodation, the relationship between biometric distances and refraction and their dynamic interactions can be described as a function of amplitude to understand how the lens undergoes dynamic accommodative changes.

Accommodative changes in lens thickness have been studied previously in humans. Static A-scan biometry has been used to measure lens thickness and anterior and vitreous chamber depths with accommodation.[12, 13] Drexler et al.[11] used partial coherence interferometry to measure changes in lens thickness with accommodation in humans at different static levels of accommodation. Ocular biometry has been measured dynamically, and response times of accommodation and disaccommodation have been compared.[14] Beers and van der Heijde[10, 15] used continuous high-resolution A-scan ultrasonography to study dynamic changes in lens thickness in humans. However, in all these studies, although the accommodative stimulus demand was known, the actual accommodative refractive changes were not measured. If accommodative refractive and biometric changes could be simultaneously measured dynamically, it would be possible to show how lens thickness and anterior chamber depth change to produce the dioptric changes. Methodology does not currently exist to allow the simultaneous, dynamic measurement of refraction and biometry in

the same eye. It would be possible to measure accommodative refractive changes in one eye and simultaneously measure the consensual accommodative biometric changes in the other eye. However, these two measurements should ideally be done in the same eye for the appropriate comparisons to be made. In humans, another approach might be to elicit a voluntary accommodative response and measure the refractive changes dynamically and then to elicit another "similar" accommodative response and measure the biometric changes. However, there is considerable variability in the voluntarily accommodative response. It is unlikely that the two accommodative responses would be "identical" (i.e., of the same amplitude, time course, and duration) to allow meaningful comparisons and correlations.

Edinger-Westphal (EW)-stimulated accommodation in anesthetized monkeys via a permanent indwelling electrode affords a method for reliably producing accommodative responses of regulated amplitudes and durations.[16] The EW nucleus of the brain provides preganglionic, parasympathetic innervation to the ciliary muscle of the eye. Presenting a regulated current to the EW neurons produces an accommodative response via the normal neuronal pathways. Ideally, the electrode would be centered between the two lobes of the EW nucleus such that a stimulus produced an equal accommodative response in the two eyes. In practice, slight decentration of the electrodes causes different accommodative amplitudes in the two eyes for the same stimulus current. However, the full range of accommodation can be achieved independently in each eye by regulating the stimulus current as necessary. Central stimulation of accommodation also produces convergent eye movements and pupil constriction. These are undesirable for many of the different experimental protocols for which these monkeys are used. Prior complete iridectomies avoid the undesirable consequences of pupillary constriction and allow unimpeded study of the accommodative refractive changes and videographic imaging of the lens equator and ciliary processes.[8, 17, 18] Convergent eye movements are largely eliminated by applying light tension on sutures placed beneath the medial and lateral rectus muscles in the anesthetized monkeys. EW-stimulated accommodation in anesthetized monkeys provides a way to reliably elicit accommodative responses of the same amplitude, time course, and duration in which it is possible to first measure refraction dynamically and then subsequently measure biometry dynamically to allow direct comparisons and correlations.

Static accommodative changes in lens thickness, anterior chamber depth, and anterior segment length (anterior chamber depth plus lens thickness) have been measured in monkeys using both pharmacological (carbachol iontophoresis) and EW-stimulated accommodation.[4] These studies show a decrease in anterior chamber depth and an increase in lens thickness but no change in anterior segment length (i.e., no movement of the posterior lens surface) with accommodation. Only static measurements were made, so no dynamic information is available. Dynamic changes in lens thickness have been measured with Scheimpflug slit lamp videography during EW-stimulated accommodation in rhesus monkeys with known maximal accommodative amplitudes.[19] However, the Scheimpflug measurements were not optically corrected,[6, 20] and the refractive changes were not measured dynamically, so accurate information of change in lens thickness per diopter is not available.

We have previously shown from dynamic analysis that the peak velocity of accommodative refractive changes increases linearly with amplitude during EW-stimulated accommodation in rhesus monkeys.[21] The ocular accommodative refractive change is mediated by changes in lens surface curvatures and thickness. Therefore, it is likely that accommodative biometric changes are well correlated with accommodative refractive changes. If the biometric accommodative changes in the eye can be measured dynamically, a dynamic analysis can be undertaken to understand how peak velocities and time constants relate to amplitude. Furthermore, if both A-scan biometry and refraction are measured dynamically during accommodation, the dynamic relationship between biometric and optical changes can be determined. Our prior study in monkeys used refractive measurements, but not biometric measurements.[21] As a first step toward understanding whether refractive and biometric changes can be measured dynamically in monkeys, we have developed techniques and undertaken a preliminary experiment to test the hypothesis that dynamic biometric changes of the crystalline lens are well correlated with the dynamic accommodative optical changes.

In vitro experiments on monkey crystalline lenses show linear relationships between lens geometric properties (anterior surface curvature and lens equatorial diameter) and lens optical properties (focal length) with mechanical stretching.[22] Whereas changes in surface curvature, for example, directly impact the lens optical power, it is less clear why nonoptical, geometric changes (such as lens equatorial diameter) are well correlated with optical changes (e.g., lens focal length). *In vivo*, the relationships between geometric changes in the lens and refractive changes of the eye are complicated by the many optical interactions that occur with accommodation. An increase in lens thickness and a decrease in anterior chamber depth both have optical consequences irrespective of changes in lens surface curvature. An increase in lens thickness alone without other changes would decrease lens optical power, whereas a decrease in anterior chamber depth without other changes (due to a forward translation of the lens) would increase ocular optical power. The ocular accommodative biometric changes are obviously coupled, thus it may be impossible to precisely quantify each individual optical contribution. However, if the biometric ocular accommodative changes and the refractive changes can be measured dynamically and correlated, then it may be possible to begin to understand each individual contribution to the accommodative change in power of the eye. To date, no studies have made dynamic comparisons of accommodative changes in lens thickness, anterior chamber depth, and anterior segment length with refraction for different amplitudes of accommodation. Such comparisons may prove valuable to understand how changes in the physical form of the lens produce accommodation. We have used a clinical A-scan ultrasound with a video output in an attempt to undertake a dynamic analysis of accommodative biometric changes and to understand the limitations and advantages.

The purpose of this study was (1) to determine whether the video output signal from a standard clinical A-scan ultrasound instrument can be used to measure dynamic changes in lens thickness, anterior chamber depth, and anterior segment length during EW-stimulated accommodation in rhesus monkeys; (2) to determine whether peak velocities and time constants of accommodative and disaccommodative phases of these ocular biometric distances can be measured; and (3) to determine whether dynamic

accommodative refractive changes measured using infrared photorefraction can be correlated with the dynamic changes in ocular biometric distances measured with A-scan ultrasonography.

METHODS

All experiments conducted conformed to the Association for Research in Vision and Ophthalmology statement for the use of animals in ophthalmic and vision research and were in accordance with institutionally approved animal protocols. One rhesus monkey (*Macaca mulatta*) aged 8 years was used. Under surgical-depth anesthesia, the monkey had previously undergone bilateral complete iridectomies[17] and surgical implantation of a stimulating electrode into the EW nucleus of the brain.[16, 21] The surgical iridectomy,[17] the justification for it,[23] and the impact on accommodation[24] have been described. Iridectomy does not alter the accommodative mechanism or the centrally stimulated accommodative amplitude. Electrode placement and confirmation was done with stereotaxic X-ray ventriculography[16, 21] and by comparing the EW-stimulated accommodative amplitude with that previously determined from pharmacological stimulation.[4, 21]

EW-Stimulated Accommodation

This procedure has been described in detail.[21] The monkey was anesthetized (10 mg/kg ketamine and 0.5 mg/kg acepromazine im followed by 15 mg/kg sodium pentobarbital iv with hourly supplements as required) and placed prone in a head holder, upright and facing forward. To perform dynamic A-scan measurements during accommodation, either the A-scan probe must be fixed to the eye[10, 15] or the convergence eye movement must be prevented. If neither is achieved, continuous A-scan measurements cannot be accomplished because the A-scan peaks will be lost as the eyes move. Accommodative eye movements were minimized by applying light tension to sutures tied beneath the medial and lateral rectus muscles.[8] This effectively reduced the convergent eye movements to the extent that continuous A-scan measurements could be made.

Before the A-scan biometry was performed, refraction measurements were made. The eye lids were held open with lid speculums. Plano, rigid, gas-permeable contact lenses were placed on the corneas to prevent corneal dehydration and the ensuing loss of optical clarity. Baseline resting refractions to the nearest 0.25 D were measured with a Hartinger coincidence refractometer (Zeiss, aus JENA). Accommodation was stimulated with increasing stimulus amplitudes in steps of 40 μA, and the maximum, static accommodative amplitudes were measured three times at each stimulus amplitude with the Hartinger. EW-stimulated accommodative stimulus response functions were generated for both the eyes of the monkey.[21]

Infrared Photorefraction

Infrared photorefraction was used to measure dynamic changes in accommodation.[21] The photorefractor consists of a bank of 20 infrared light-illuminated diodes placed at increasing eccentricity from a knife-edge aperture in front of a 55-mm lens on a CCD camera. The video camera was placed 0.3 m from the eye, and the video signal was recorded to videotape for off-line analysis. This customized arrangement provides flexibility in adjusting luminance of the infrared light-illuminated diodes and the camera working distance that is not possible in a commercially available photorefractor (PowerRefractor, Plusoptix). The technique of photorefraction measures the slope of the pupillary luminance profile and relates this to the refractive state of the eye. A calibration curve relating the slope of the pupillary luminance profile to the refractive state was generated for each eye at the start of the experiment.[21, 25] The luminance profile is measured in the central 50% of the iridectomized eye entrance pupil diameter. Three 2-second-long stimulus trains were delivered to the EW nucleus at eight increasing stimulus amplitudes for each eye. At each stimulus amplitude, refraction was first measured statically with the Hartinger as described above, and the accommodative responses were subsequently recorded dynamically with the photorefractor. The slope of the pupil luminance profile was measured in one video frame toward the end of each stimulus train when near-maximum accommodation was expected. For each of the eight stimulus amplitudes, the averages of three slope measurements were used to determine a mean pupil luminance slope vs. refraction calibration function. Subsequently, to measure the dynamic accommodative responses, the slope of the vertical pupil luminance profile was measured with a frame-by-frame analysis of the videotape. The calibration function was then used to convert the measured slope values to refraction. Thus, whereas the Hartinger provides only a static refraction measurement, photorefraction allows dynamic measurement of accommodation.[21]

A-Scan Ultrasound Biometry

A Sonomed ophthalmic ultrasound (A-5500 A-scan system, Sonomed, Lake Success, NY) was used to measure lens thickness and anterior chamber depth dynamically during accommodation. A 1-cm-long rubber tubing stand-off sleeve was pushed over the tip of the transducer and filled with ultrasound transmission gel (Liquasonic Ultrasound Gel, Chester Labs, Cincinnati, OH). Care was taken not to introduce air bubbles into the gel. The ultrasound transducer was mounted horizontally in a micromanipulator allowing movement in three dimensions (D-10 positioner, Research Instruments, London, U.K.). This allowed the transducer to be precisely positioned in front of the eye to maximize the A-scan peaks and get a stable recording. The Sonomed video output signal was recorded to videotape together with a signal from a VSI-Pro (TransAmerican International) to register a 11 or a 00 on each video frame to indicate whether the EW stimulus was on or off, respectively.[21] Subsequently, off-line, a PC-based image analysis system (Optimas image analysis software, Media Cybernetics, Silver Springs, MD, and ITI-PCI frame grabber, Imaging Technology) was used to control the VCR, capture, and analyze image sequences. A custom-written Optimas image analysis macro was used to find each A-scan peak to measure lens thickness, anterior chamber depth, and anterior segment length in each video frame. The macro drew a horizontal line near the base of the of A-scan trace in the video image extending from anterior corneal peak to the retinal peak (Fig. 1a). The pixel luminances along this line were extracted, and a pair of luminance peaks was located corresponding

to the leading and trailing edge of each A-scan peak (cornea, anterior, and posterior lens surfaces) (Fig. 1b).

The location of the each leading luminance peak was identified. This corresponds to the leading edge of each A-scan peak. The distances between the leading peaks were calculated to give anterior chamber depth, lens thickness, and anterior segment length in millimeters. Trailing luminance peaks were counted to identify the next leading peak and otherwise ignored. The distances between the leading peaks change during accommodation as the biometric distance in the eye changes. The changes in ocular biometric distances were measured with respect to the cornea (Fig. 1 b and c). Anterior segment length was considered as the sum of anterior chamber depth and lens thickness. The position of the center of the lens was calculated as the sum of the anterior chamber depth and half the lens thickness. The Sonomed was set to a sound velocity of 1548 m/s. The measurements were converted to actual distances using sound velocities of 1641 m/s for the lens and 1532 m/s for the aqueous and vitreous.[26–28]

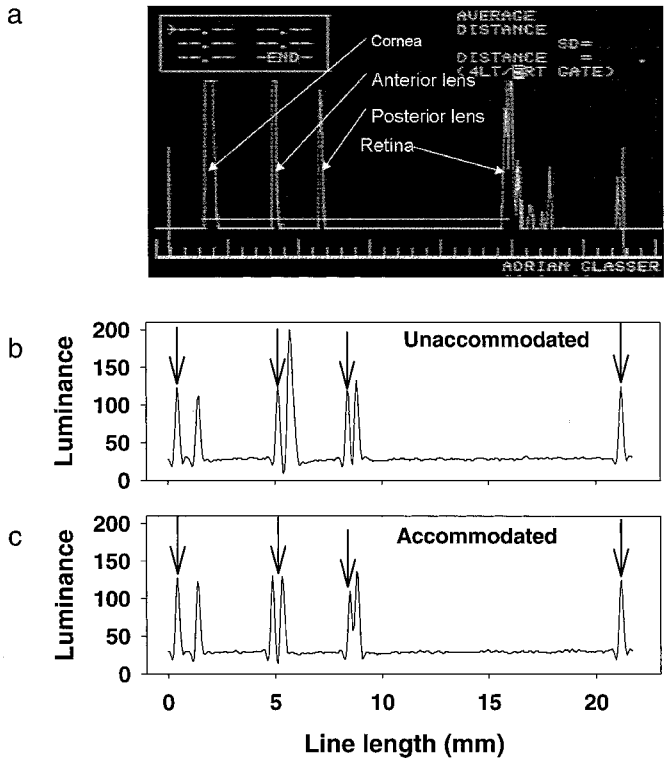

FIGURE 1.

a: A single video image captured when the eye is unaccommodated showing A-scan spikes corresponding to the positions of the cornea, lens, and retina. An image analysis macro was used to extract the image luminance along the horizontal line drawn near the base of the peaks. b: The luminance profile along this line was extracted, and the macro located each peak in the luminance profile to identify the presence and location of each A-scan spike. These positions reflect the real positions of the A-scan spikes calibrated in millimeters. c: The extracted luminance profile from an A-scan image in the accommodated eye. The vertical arrows in (b) and (c) identify the positions of the luminance peaks for the anterior corneal surface, anterior and posterior lens surfaces, and retina in the unaccommodated eye. In the accommodated eye (c), the anterior lens peak is displaced forward and the posterior lens peak is displaced backward relative to their position in the unaccommodated eye (b).

Stimulus Pulse Train Durations for Dynamic Analysis

To assess accommodative changes, A-scan biometry was performed on the left and right eyes during stimuli delivered to the EW nucleus at three different amplitudes 4 s in duration that produced responses spanning the full EW-stimulated accommodation range for each eye. To obtain static measures of maximum change in lens thickness, anterior chamber depth, and anterior segment length at each accommodative state, the last 30 frames from each dynamic A-scan response were averaged. These were plotted against the respective accommodative amplitudes (obtained from EW-stimulated accommodative stimulus response curves).

To compare dynamic accommodative changes in ocular biometric distances with the accommodative refractive changes, both A-scan biometry and infrared photorefraction were performed on the right eye only at the same four different stimulus amplitudes. Two-second-long stimulus pulse trains were used for both photorefraction and A-scan measurements. For each stimulus amplitude, three successive stimuli were delivered with 3-s-long interstimulus intervals. Both A-scan and photorefraction were analyzed from the videotape at 30 frames/s from 20 video frames preceding the stimulus onset to 40 frames after stimulus termination to yield the dynamic responses. The analyses of the three individual responses were averaged to obtain a single dynamic accommodative response starting from before accommodation commenced and ending after the eye was fully disaccommodated. Preaccommodated baseline refraction and biometry values were obtained from the first baseline video frames analyzed. The accommodative changes were determined by subtracting the baseline value from each measured value for the full duration of the accommodative response.

Function Fitting

The following functions were fitted to the dynamic accommodative responses of A-scan and photorefraction as described previously.[21]

For accommodation,

$$F = A(1 - e^{\frac{-x}{\tau}}) + bx + cx^2 \quad (1)$$

and for disaccommodation,

$$F = A(e^{\frac{-x}{\tau}}) + bx + cx^2 \quad (2)$$

where x is time, τ is the time constant, A is the amplitude attained, and b and c are constants that vary with the response characteristics. The equation for *accommodation* was fitted to data from the second video frame after the EW stimulus onset to the video frame corresponding to termination of the stimulus. The equation for *disaccommodation* was fitted to data from the second frame after stimulus termination to the last frame of the response (i.e., 39 frames total). Before fitting, anterior chamber depths were multiplied by -1 to show a rising accommodative phase and a falling disaccommodative phase. Time constants were obtained directly from the equations. Peak velocities were obtained by finding the maximum derivative of the functions fitted to the responses.[21]

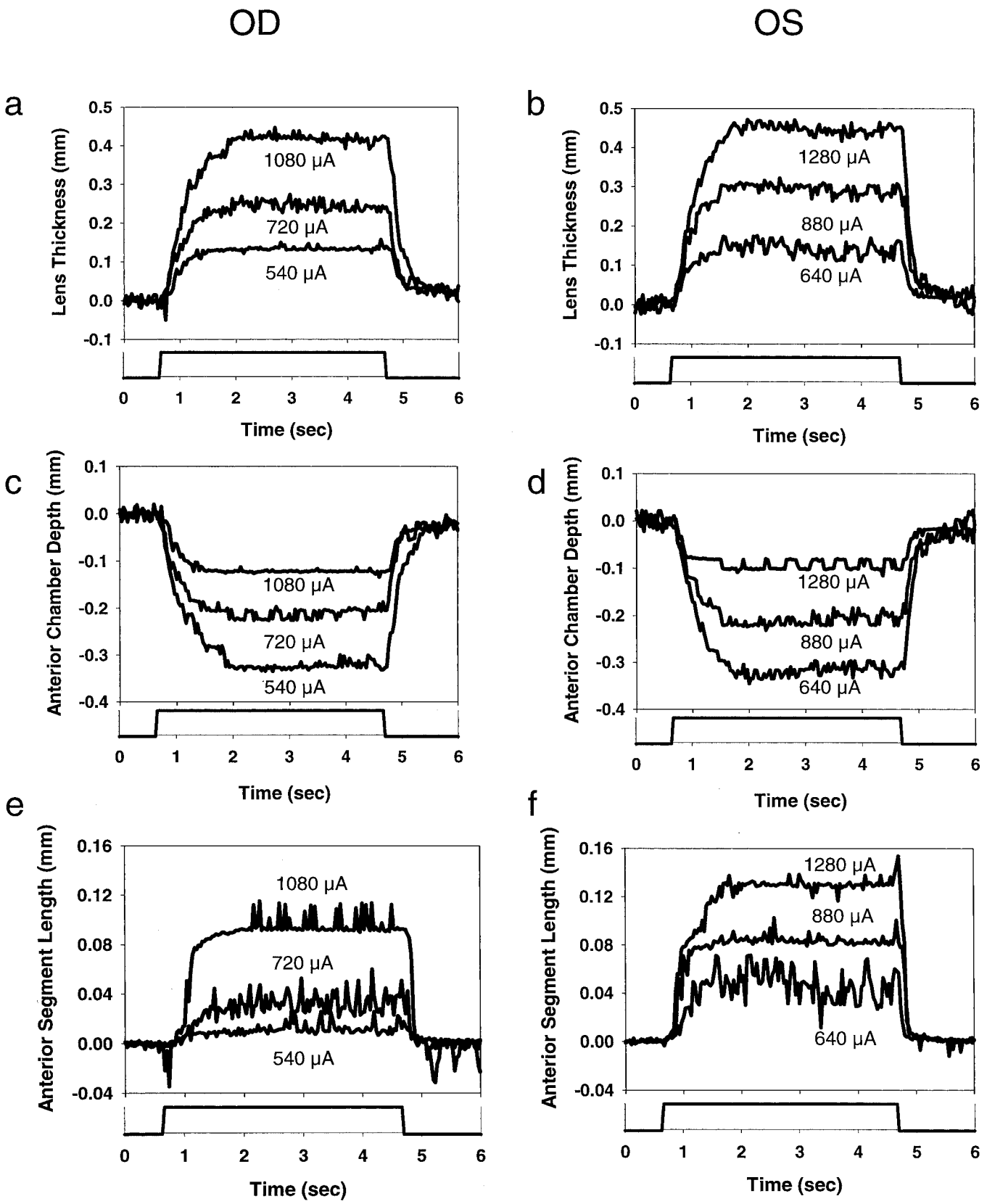

FIGURE 2.

Three dynamic responses of lens thickness (a and b), anterior chamber depth (c and d), and anterior segment length (e and f) for each eye of the monkey, spanning the entire range of EW-stimulated accommodative amplitude for 4-s-long stimulus trains. In OD (a, c, and e), stimulus amplitudes of 540, 720, 1080 μA produced accommodative responses of 5.17, 8.33, and 11.08 D; and in OS (b, d, and f), stimulus amplitudes of 640, 880, 1280 μA produced accommodative responses of 4.83, 8.00, and 10.25 D. The solid line at the bottom of each graph represents stimulus onset, duration, and termination.

Statistical analysis has previously shown that these equations in general provide excellent descriptions of the accommodative and disaccommodative responses, including any nonlinearities.[21] Often, the centrally stimulated accommodative response does not follow a pure exponential rise or decay.[21] This may be due to the physiological variability in the responses of the system to central stimulation. The equations, therefore, are a combination of an exponential and a second-order polynomial to allow accurate fits to all the accommodative responses, including those that do not follow a pure exponential.[21]

As with prior studies using dynamic A-scan analysis and methods similar to those used here, r^2 values of 0.8 or greater were considered to indicate good fits of the function to the response.[15]

RESULTS

Measurement Resolution

Because the ocular biometric distances were measured with video image analysis rather than from the Sonomed directly, the movement resolution or the smallest movement in lens surfaces that can be reliably detected was determined by the minimum unit of resolution, i.e., one pixel, converted to millimeters. This was 50 μm. Thus, a change in ocular biometric distance <50 μm can not be resolved with the methodology described.

Dynamic Changes in Biometric Distances with EW Stimulation

Dynamic changes in biometric distances, obtained with 4-s stimulus trains in the right and left eyes, are shown in Fig. 2. The stimulus current amplitudes selected produced accommodative responses of 5.17, 8.33, and 11.08 D in the right eye and 4.83, 8.00, and 10.25 D in the left eye as determined with the Hartinger coincidence refractometer. The unequal accommodative responses measured are not unexpected because a single electrode is used to stimulate both eyes and decentration of the electrode from the midline and other physiological variability can produce unequal responses.[21]

Maximum changes in biometric distances were determined by subtracting the average of the last 30 frames of the accommodative phase of the dynamic A-scan responses from the baseline values. The averages were plotted as a function of the accommodative amplitudes measured with the Hartinger in each eye (Fig. 3 a and b). A nonlinear relationship is observed with relatively larger changes at higher accommodative amplitudes. The accommodative change in lens thickness is greater than the change in anterior chamber depth and anterior segment length. The computed position of the center of the lens moves forward during accommodation. The changes in anterior segment length are close to the resolution limit of the measurement technique at low amplitudes, but exceed the resolution limits at higher amplitudes. Anterior segment length consistently increases with accommodation, demonstrating a backward movement of the posterior lens surface with accommodation.

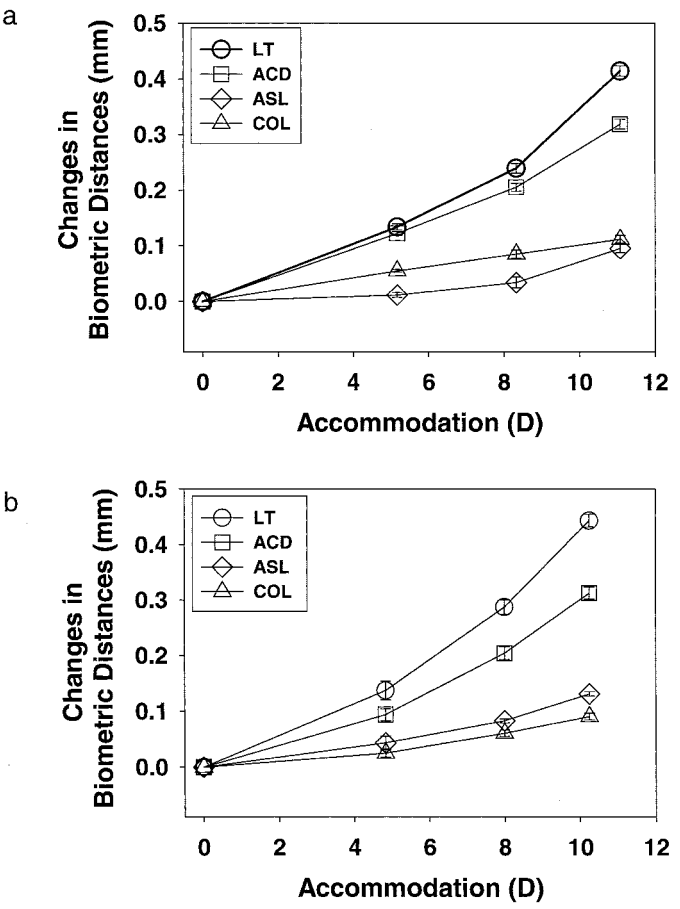

FIGURE 3.
Change in lens thickness (LT), anterior chamber depth (ACD), anterior segment length (ASL), and center of lens (COL) in the two eyes (a: OD, b: OS) plotted as a function of the accommodative amplitudes measured with the Hartinger. Refractions were measured statically with the Hartinger before dynamic A-scan biometry measurements. The changes in ocular biometric distances were obtained by subtracting the average of the last 30 frames of the accommodative phases from the baseline values of the 4-s-long dynamic responses. The distance from the cornea to the center of the lens was determined as anterior chamber depth plus half of lens thickness. Error bars represent standard deviation. With accommodation, there is an increase in lens thickness, a decrease in anterior and vitreous chamber depths, and a forward movement of the center of lens.

Fitting the Dynamic Responses of Lens Physical Parameters

Three dynamic responses at each amplitude were averaged to obtain a single response, and functions were fitted to this mean response to obtain peak velocities and time constants. The dynamic changes in lens thickness and anterior chamber depth were, in general, well fitted with the equations described above (Fig. 4) with r^2 values >0.8. Exceptions to this were accommodative changes in lens thickness and anterior chamber depth at the lowest amplitude (640 μA; 4.83 D) in the left eye (r^2 values in these two cases were 0.76 and 0.7, respectively). r^2 Values for the dynamic changes in anterior segment length were >0.8 only for the highest amplitudes in both the eyes because only these responses were greater than the resolution limit of the methodology.

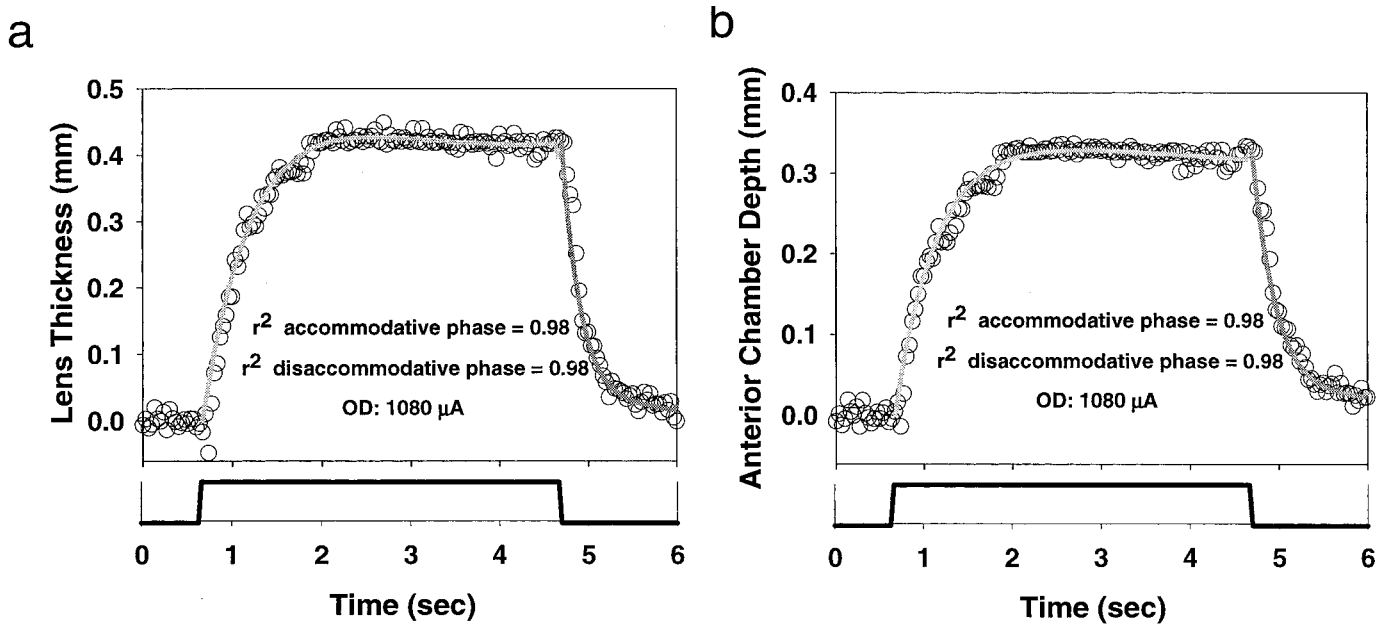

FIGURE 4.

Examples of fits (light gray line: accommodation; dark gray line: disaccommodation) using Equations 1 and 2 to dynamic changes in lens thickness (a) and anterior chamber depth (b) for one stimulus amplitude. The fits shown all have r^2 values = 0.98. Three dynamic responses at each amplitude have been averaged to obtain a single response, and functions were fit to this response to obtain peak velocities and time constants. Before fitting the functions, anterior chamber depth was multiplied by −1 to show a rising accommodative phase and a falling disaccommodative phase. The solid line at the bottom of each graph shows the stimulus onset, duration, and termination.

Peak Velocities and Time Constants of Changes in Biometric Distances

Peak velocities of accommodative and disaccommodative changes in biometric distances were plotted against amplitude of accommodation (Fig. 5). Maximum peak velocities of changes in lens thickness during accommodation and disaccommodation for this monkey were 1.04 mm/s and 3.39 mm/s, respectively, at an amplitude of 10.25 D. For anterior chamber depth, these were 0.75 mm/s and 1.82 mm/s, respectively, at 10.25 D. Although the peak velocities in the two eyes are slightly different, they generally increase with increase in amplitude of accommodation. The peak velocities of disaccommodation were consistently higher than peak velocities of accommodation (Fig. 5 a and c).

Time constants obtained from fitting Equations 1 and 2 were also plotted as a function of accommodative amplitudes. Time constants of changes in lens thickness and anterior chamber depth during accommodation show a slight tendency to increase (Fig. 5 b and d). Time constants for disaccommodative phases were lower than the time constants for accommodative phases and did not change with increasing amplitude.

Comparison of A-Scan Biometric Changes with Refraction

Four dynamic responses of increasing accommodative refractive and biometric changes were measured for 2-s-long stimulus trains. The same stimulus amplitudes and durations were used for both measurements, allowing the dynamic responses to be directly compared (Fig. 6). Lens thickness and anterior chamber depth change at different rates with increasing amplitude of accommodation. Regression lines fitted to the combined data from all the dynamic accommodative responses provides an indication of the relative changes in lens thickness and anterior chamber depth as a function of the change in dioptric power. In this eye, lens thickness changed between 21 and 23 μm/D, whereas anterior chamber depth changed between 18 and 19 μm/D, as determined from the accommodation and disaccommodation phases. There was no statistical difference between the rate of change of anterior chamber depth between accommodative and disaccommodative phases ($p = 0.391$). Rate of change of lens thickness between accommodative and disaccommodative phases was statistically different ($p = 0.02$). Rate of change in lens thickness was higher than rate of change in anterior chamber depth ($p < 0.001$). Anterior chamber depth and lens thickness change roughly linearly with increasing amplitude for both accommodation and disaccommodation. Dynamic changes in vitreous chamber depth were close to the resolution limit of the A-scan methodology at the lower amplitudes and have not been compared as a function of accommodation.

DISCUSSION

Dynamic A-Scan Ultrasonography

We have previously shown that dynamic analysis of EW-stimulated accommodative refractive changes are possible in monkeys.[21] The refractive change that occurs with accommodation is due to a combination of changes in lens thickness, anterior chamber depth, and anterior segment length. Our prior study did not include biometric measurements.[21] Therefore, this study has been undertaken to develop and test ways to do dynamic biometric and refractive measurements with accommodation to begin to test the

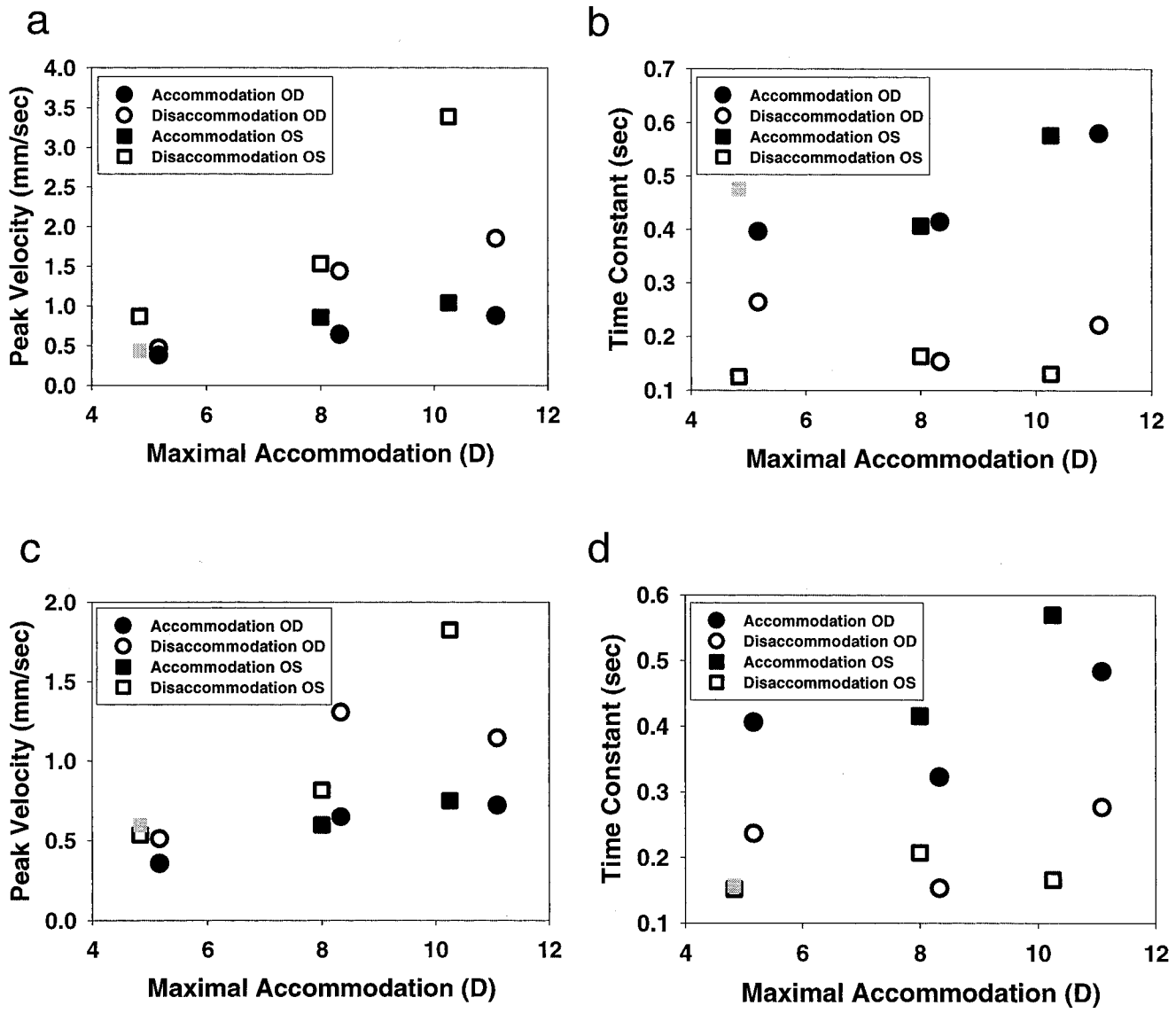

FIGURE 5.

Peak velocities (a and c) and time constants (b and d) from dynamic changes in lens thickness (a and b) and anterior chamber depth (c and d) as a function of the maximum accommodative amplitudes measured with a Hartinger coincidence refractometer from both the right and left eyes. Time constants were determined directly from the equations fitted. The gray squares represent peak velocities and time constants obtained from fits with r^2 values <0.8.

hypothesis that the accommodative refractive and biometric changes are well correlated.

EW stimulation in monkeys offers an ideal approach for this kind of study. The stimulus delivery is rigorously controlled by the stimulator. This provides rigorous control of the accommodative responses. With voluntary accommodation, such as in conscious humans, there is little likelihood that a similar accommodative response of similar amplitude and time course would be elicited for the refractive measurements and some time later for the biometric measurements. With central stimulation of accommodation in anesthetized monkeys, we can rigorously control the accommodative responses.[21] There is, to our knowledge, no methodology available for doing these experiments simultaneously on the same eye.

Care was taken to position the A-scan transducer on the eye such that peaks of maximum possible height occurred at each optical interface. This could only be achieved when the transducer was carefully oriented along the axis of the eye. Accommodative convergence eye movements, which could cause the peaks to be lost, were minimized by applying light tension to sutures placed beneath the lateral and medial rectus muscles.[8] The eye movements were minimized sufficiently in this manner to allowed continuous A-scan measurements to be recorded during accommodation and disaccommodation without having to move the transducer. Suctioning a custom made transducer to the eye with negative pressure has been used previously to maintain alignment,[10] but this would not have been possible with the Sonomed transducer due its large size.

Off-line image analysis of the A-scan video signal allows dynamic analysis, but with limitations. The positions of the A-scan peaks were determined by finding the maximum pixel luminance for each luminance peak in the video image (Fig. 1b). The A-scan video image is pixellated, each peak is several pixels wide, and the video image has inherent noise. As a consequence, the position of maximum pixel luminance of a stationary spike is not always found at the same pixel location. The step changes seen in the dynamic

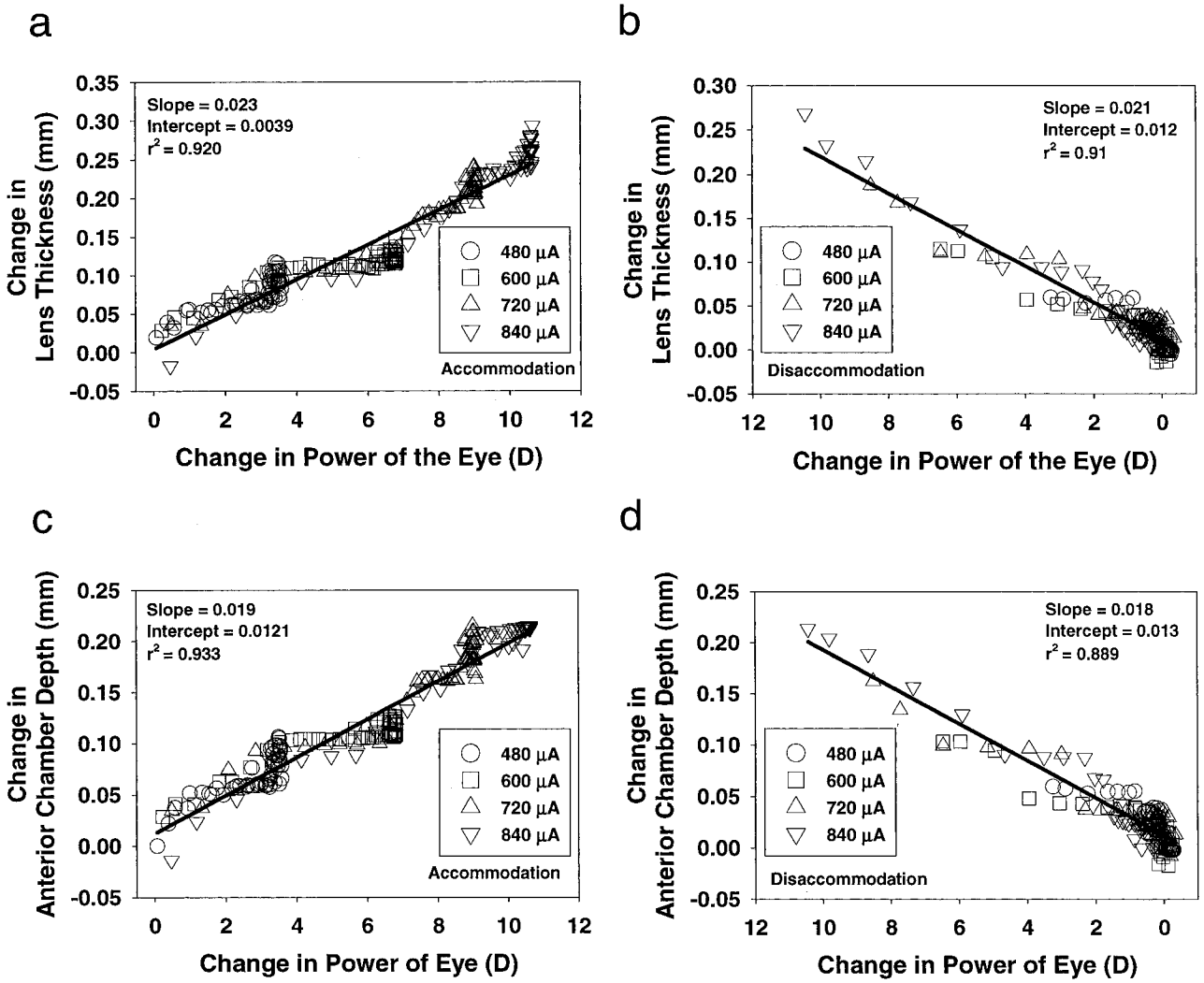

FIGURE 6.

Comparison of accommodative and disaccommodative changes in lens thickness (a and b) and anterior chamber depth (c and d) as a function of refractive changes. Refraction was first measured dynamically with infrared photorefraction, and lens thickness and anterior chamber depth were subsequently measured dynamically with the A-scan. Four responses of different amplitudes from each eye are plotted together in each figure. During accommodation, lens thickness increases and anterior chamber depth decreases (a and c). The different symbols represent the responses recorded for different stimulus amplitudes. The response from the highest stimulus amplitude spans the full range of accommodation and follows a similar path as the data for the smaller responses from the lower amplitudes. Orthogonal regression lines are plotted using all the data to estimate the per-diopter change in lens thickness and anterior chamber depth.

biometric responses are a result of these "quantal" pixel jumps. Hence, although a dynamic A-scan analysis can be done in this way, it has a lower signal-to-noise ratio than the A-scan instrument. Beers and van der Heijde[10, 15] used high-resolution continuous ultrasound biometry to measure dynamic changes in lens thickness with accommodation in humans. Their instrument has a two-point axial resolution of 2 µm and a sampling frequency of 100 Hz, which is better suited for dynamic measurement of small changes. We now have such an instrument, and it will be used for future dynamic biometric measurements.

To analyze the biometric changes, functions were fitted to the dynamic A-scan measurements as has been done previously for dynamic biometry[10, 15] and dynamic accommodative refractive changes.[21] Fitting functions to dynamic measurements allows the opportunity for additional analysis beyond what is possible from static measures because time constant and peak velocities can also be obtained. Although no general conclusions are possible from results of one monkey, we wished to explore the possibilities of dynamic A-scan and the benefits it might offer. Peak velocities, time constants, and dynamic variability in the accommodative response may provide metrics from which inferences on the mechanical properties of the lens and age changes could be quantified.

Dynamic Accommodative Biometric Changes

Koretz et al.[4] used static methods to compare accommodative changes in biometric distances with refractive changes. Refraction was measured with a Hartinger coincidence refractometer and biometry with both A-scan ultrasonography and Scheimpflug slit lamp photography. The data presented show a linear relationship between changes in biometric distances and refraction at all amplitudes in one rhesus monkey with similar results from both A-scan and Scheimpflug. Our

results in one monkey show a nonlinearity (Fig. 3). A-scan biometry and refraction cannot be measured simultaneously. If the accommodative responses were slightly higher when A-scan biometry was performed than when the refraction was measured at the same stimulus amplitude with the Hartinger, this could produce the nonlinearity. However, any study attempting to correlate biometric changes and refractive changes suffers this disadvantage.[4] Unlike Koretz et al.,[4] where linear correlations were drawn from multiple experiments using multiple techniques on a single monkey, the approach we have taken here allows a closer scrutiny of this relationship from within a single experiment on the same eye. Drexler et al.[11] observed in a 27-year-old emmetropic human subject that for about 9 D of accommodative *demand*, lens thickness increased by 322 μm, and anterior chamber depth decreased by 229 μm. In this rhesus monkey, 10 D of EW-stimulated accommodation produced an increase in lens thickness of 230 μm and a decrease in anterior chamber depth of 198 μm. Although the size of our rhesus monkey eye (axial length, 20.24; unaccommodated lens thickness, 3.42 mm) is considerably smaller than that of the human eye (approximate axial length of average human eye is about 24 mm, and the unaccommodated lens thickness given by Drexler et al.[11] was 4.007 mm), these two studies show similarity in the changes in lens thickness and anterior chamber depth for similar accommodative amplitudes in a human and a rhesus monkey.

The calculated position of the axial center of the monkey lens was found to move forward slightly with accommodation. The anterior surface of the primate lens is flatter than the posterior surface, and this center of the lens is posterior to the equatorial plane. The small anterior translation of the center of the lens is probably a consequence of a greater change in the anterior lens surface curvature than in the posterior surface curvature and not simply due to a forward translation of the entire lens with accommodation. There is a small, but consistent backward movement of the posterior lens surface with accommodation (increase in anterior segment length), but a greater forward movement of the anterior lens surface as evident from a greater decrease in anterior chamber depth with accommodation. A backward movement of the posterior lens surface is an indication that the posterior lens surface is not stationary and probably does play a role in accommodation. The relative effects of the anterior and posteriors lens surface on accommodation could be determined by measuring changes in these surface curvatures directly such as with Scheimpflug or video phakometry.[29]

Much of the existing literature on accommodation has suggested that the posterior surface of the lens does not change curvature or is stationary during accommodation.[1, 3, 4, 30] The positions of the anterior and posterior lens poles have been measured statically with respect to anterior corneal surface during accommodation in humans.[13, 11] Drexler et al.[11] showed from partial coherence interferometry in 10 eyes that there is a backward movement in the posterior pole of the lens during accommodation. Findl[31] also showed this result with voluntary accommodation in eight young human subjects. Beauchamp and Mitchell[13] showed that backward movement of the posterior lens surface accounts for about 30% of the accommodative increase in lens thickness in three human subjects. Garner and Yap[5] used ultrasonography, video phakometry, and an autorefractor to study the contribution of posterior surface curvature change during accommodation in humans. They concluded that for an accommodative demand of 8 D, the posterior surface radius of curvature decreased by 1.34 mm. In conjunction with these findings in human eyes, we believe that the backward movement of the posterior lens surface is a real component of accommodation in rhesus monkeys. Our preliminary results with higher-resolution continuous ultrasound biometry confirms this (unpublished observations).

Koretz et al.[4] showed a shallowing of the anterior chamber with accommodation in rhesus monkeys. An estimate of changes in anterior chamber depth and lens thickness extracted from their graphed data shows that change in anterior chamber depth represents only 78.1% of change in lens thickness. Therefore, although Koretz et al.[4] say that there is no systematic change in posterior lens position with accommodation, the graphed data suggests that about 22% of the change in lens thickness is due to an increase in anterior segment length. Drexler et al.[11] showed using static measures from partial coherence interferometry in humans that the absolute change in anterior chamber depth is 72% of the change in lens thickness during accommodation. We showed in one monkey that when considering the highest amplitude in the two eyes, the change in anterior chamber depth was 75% of the total change in lens thickness (76% OD and 74% OS). Thus, in accordance with other primate studies, it would seem that approximately 25% of the accommodative increase in lens thickness occurs due to a backward movement of the posterior lens surface.

Time Constants of Accommodative and Disaccommodative Changes in Lens Thickness and Anterior Chamber Depth

Our results suggest that the time constants of accommodative changes in lens thickness and anterior chamber depth tend to increase with increasing amplitude (Fig. 5 b and d). Beers and van der Heijde[10] studied dynamic changes in lens thickness with accommodation in humans using high-resolution ultrasonography at far range (0 to 4 D) and near range (4 to 8 D) of accommodation and found that the time constants of accommodation increase with amplitude at both these ranges. Their time constants were graphed as a function of stimulus amplitude rather than response amplitude, and the range of stimulus amplitudes used represented only about 50% of the accommodative range available to their subjects. The data presented here for one monkey represent the full accommodative amplitude available. In accordance with the results from humans, time constants of disaccommodation are lower than the time constants of accommodation, and the time constants of disaccommodation show no tendency to increase with increasing amplitude.

Peak Velocities of Accommodation and Disaccommodation

Vilupuru and Glasser[21] have shown that peak velocities of accommodative and disaccommodative refractive changes increase linearly with amplitude and that peak velocities are higher for disaccommodation than for accommodation. Storey et al.[14] showed in humans that the speed of accommodative changes in lens thickness and anterior chamber depth are dependent on amplitude. They also observed that the response times were lower for disaccommodation than for accommodation. Similar results were found here for dynamic changes in lens thickness and anterior chamber depth, i.e., peak velocities for accommodation increase with amplitude, and peak velocities are higher for

disaccommodation than for accommodation (Fig. 5 a and c). This study shows that dynamics of the accommodative lenticular biometric changes and refractive optical changes are similar. This is not surprising because the optical changes are a consequence of the biometric changes. Differences in peak velocities of the physical changes in the lens between accommodation and disaccommodation may reflect differences in the mechanics of accommodation and disaccommodation. In accommodation, the ciliary muscle actively contracts to release zonular tension to allow the elastic capsule to passively mold the lens into an accommodated form. With disaccommodation, the ciliary muscle is passively pulled back into an unaccommodated state by the elasticity of the posterior attachments of the ciliary muscle to increase zonular tension and cause the capsule to actively mold the lens into an unaccommodated form. The fact that accommodation occurs with a lower peak velocity suggests a rate limiting step in accommodation. This might be a limitation in the peak velocity of active ciliary muscle contraction or a limitation in the ability of the capsule to mold the lens against the resistance of the lens substance. If age-dependent changes are found in peak velocities, this may provide clues to specific aspects of the accommodative system that are affected by aging.

Comparing Accommodative Refractive and Biometric Changes

In this study, we compared dynamic changes in refraction with dynamic changes in biometry for the same current amplitudes that were recorded subsequently rather than simultaneously. Variations in the EW-stimulated accommodative responses can add uncertainty to results obtained this way. Some physiological variability in the dynamic accommodative responses to the same stimulus currents have been observed, however these are nonsystematic and relatively small.[21] Although the nonsystematic variations may add some uncertainty or variability to the biometry vs. refraction comparisons, it is not likely to induce any systematic errors. Additional experiments currently underway will use more monkeys and will use more accommodative responses per session to get a clearer indication of the extent of this variability.

Koretz et al.[4] show a linear relationship between change in lens thickness and refraction during accommodation. Our dynamic data shows local nonlinearities, although an overall linear relationship (Fig. 6), but the static data comparing biometry and refraction measured with the Hartinger shows an overall nonlinearity (Fig. 3). With the static measurement, refraction is only measured at a single time point. The accommodative responses do not always reach and maintain a stable plateau. The nonlinearities observed can be accounted for by differences in the characteristics of the individual accommodative responses. These nonlinearities are evident from the dynamic measurement because the entire accommodative response is recorded.

In vitro experiments on isolated monkey crystalline lenses[4] show linear relationships between changes in focal length, anterior surface radius of curvature, and lens equatorial diameter. Also, comparisons of isolated crystalline lenses of various ages in humans[32] and in pigs[33] yielded systematic linear relationships between focal length and lens equatorial diameter, thickness, and anterior and posterior surface curvatures. The linear relationships between many of these parameters suggest that crystalline lenses show predictable geometric relationships and that optical and physical properties are codependent. Therefore, it may be expected that similar codependent relationships exist between physical changes in the lens and the refractive state of the eye during accommodation. The dynamic measurements presented here further the notion that the ocular biometric and optical accommodative changes are codependent.

Future Clinical Impact

Some details of how the primate crystalline lens undergoes accommodative changes are still unclear. Gullstrand[34] postulated that the form of the gradient refractive index of the lens is coupled to the lens anatomy and that anatomical accommodative changes in the lens suggest that the form of the lens gradient refractive index changes with accommodation. Understanding exactly how the lens anatomical changes contribute to the lens optical changes may provide clues to how the anatomy and the gradient refractive index are linked. In addition, much remains to be learned about how and why presbyopia develops. Understanding the dynamic relationships between accommodative biometric and optical changes in young monkeys will provide a benchmark against which future studies of older monkeys can be compared. Ultimately, it is of interest to repeat such studies on monkeys of increasing age to learn how these biometric and optical relationships change with aging and the progression of presbyopia. There is also tremendous interest in understanding if and how the progression of presbyopia can be slowed or prevented or if accommodation can be restored in presbyopes. Data obtained from studying the dynamic accommodative performance of the natural crystalline lens may provide important information for understanding how surgical procedures or artificial accommodative intraocular lenses can restore accommodation to presbyopes when such procedures or lenses are tested in monkey eyes.

We have developed and tested a video-based technique for dynamic accommodative A-scan biometry measurements to compare the biometric changes with dynamic refractive changes at increasing amplitudes *in vivo* in rhesus monkeys. The aim of the study was to test the hypothesis that the accommodative biometric and optical changes are well correlated. Although the results show that the technique has relatively low resolution, and although this approach was tested in only one monkey, we have been able to demonstrated well-correlated biometric and optical accommodative changes. Further studies are currently underway using a high-resolution A-scan ultrasound instrument to better understand smaller changes in lens biometric distances, especially posterior lens movement, and to better compare these biometric changes with optical changes.

CONCLUSIONS

We have explored the possibility of using standard A-scan ultrasound to measure dynamic changes in biometric distances during accommodation. Dynamic accommodative refractive changes have been compared with biometric accommodative changes in one monkey. Using videographic analysis of A-scan ultrasonography, it is possible to study the dynamic accommodative biometric changes and compare these with accommodative dioptric changes, although the resolution of this approach is relatively low. Despite this, results were qualitatively and quantitatively similar to prior studies in primate eyes.

Lens thickness and anterior segment length increase and anterior chamber depth decreases in a manner well correlated with the accommodative optical changes. Peak velocities of changes in lens thickness and anterior chamber depth are higher for disaccommodation than for accommodation, and the time constants for disaccommodation are lower than the time constants for accommodation. Although this approach demonstrates some of the benefits of dynamic analysis, it lacks sufficient resolution to accurately measure small ocular changes at low accommodative amplitudes. Future efforts to dynamically measure the small accommodative changes that occur will utilize techniques with higher resolution.

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