Effect of age and cycloplegia on the morphology of the human crystalline lens: swept-source OCT study

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Purpose: To evaluate the effect of age and cycloplegia on the morphology of the crystalline lens using a swept-source optical coherence tomography (SS-OCT) system.

Setting: Hospital.

Design: Prospective cross-sectional study.

Methods: The parameters including anterior chamber depth (ACD), the radii of curvature of the anterior and posterior surface of the crystalline lens (ALR and PLR), lens thickness (LT), lens equatorial diameter (LED), and lens vault (LV) were quantified by the SS-OCT before and after cycloplegia. The paired t test was used to compare the parameters before and after cycloplegia. A multivariate linear regression model was built to analyze the association between the parameters/cycloplegia-induced changes and age, while adjusting for the effect of axial length, refractive status, and sex.

Results: 76 individuals (age range, 18 to 86 years) were recruited. The ALR and ACD were negatively correlated with age ($P \leq .002$), and the LT, LV, and LED were positively correlated with age ($P \leq .004$). In participants younger than 60 years, the ALR and ACD significantly increased, whereas the LV and LT significantly decreased after cycloplegia (all $P < .001$). With aging, cycloplegia-induced differences of ALR ($P = .001$) and ACD ($P = .014$) significantly decreased, and of LT ($P < .001$), LT ($P < .001$), and LV ($P = .001$) significantly increased.

Conclusions: The crystalline lens morphology measured by the SS-OCT revealed steepening anterior surface and increasing equatorial diameter with age. Cycloplegia caused a significant change of anterior surface morphology in participants younger than 60 years, and this effect diminished with age.

U nderstanding the morphologic changes of the human crystalline lens with age is crucial for the understanding and treatment of presbyopia and cataracts. Accurate measurement of the human crystalline lens geometry is useful to design the intraocular lenses (IOLs) and accommodative IOLs. Further, the lens is one of the main sources of ocular aberration, and the study of ocular aberration requires accurate description and measurement of the morphology of the lens.

There are several techniques for collecting the crystalline lens data in vivo; these include slitlamp photography, Purkinje, Scheimpflug, ultrasound biomicroscopy, magnetic resonance imaging (MRI), and optical coherence tomography (OCT). However, these methods all have their drawbacks. The consistency of the slitlamp method is poor because of subjective bias. Purkinje reflex is a fast, systematic, and reliable method, but it does not provide a complete geometry of the lens surface or allow direct observation of the lens. In contrast, Scheimpflug imaging produces high-resolution images of the anterior segment and of the lens in cross-section, but the lens images obtained are distorted and must be corrected for geometric and optical distortions by a computer program. Ultrasound biomicroscopy is contact and can alter the anterior segment structure. MRI technology provides...
low-resolution information of the anterior segment of eyes; besides, it is expensive and difficult to be accepted by patients. In vitro, the storage time and environment of the crystalline lens can change its characteristics. Moreover, the scarcity of young human eyes is also a problem.12

Recently, OCT has been broadly recognized as a rapid, noncontact, and precise technology for the anterior and posterior segment biometric measurements in vivo and in real time. Anterior segment OCT (AS-OCT) is an imaging technique for measuring the anterior segment structure. However, AS-OCT imaging of the crystalline lens is imperfect, including a limited axial range, compromised by the resolution of the spectrometer or a limited instantaneous linewidth of tunable light source, which may be insufficient to image the entire anterior segment of the eye.13 The CASIA2 scanner (Tomey Corp.) is a second generation of swept-source (SS) OCT, which provides a whole image of the anterior segment ranging from the cornea to the posterior surface of the crystalline lens, with an axial scan depth of 13 mm.14,15 The built-in software in the CASIA2 successfully obtained a 3D analysis of the whole crystalline lens images in vivo, automatically drew the anterior and posterior lens boundaries, and calculated the anterior chamber depth (ACD), the radii of curvature of the anterior and posterior lens (ALR and PLR), lens thickness (LT), lens vault (LV), and lens equatorial diameter (LED).

The purpose of this study was to assess the accuracy of measuring the geometry of the human crystalline lens using a second generation of SS-OCT, to investigate the relationship between the geometric parameters and age, and to examine whether the parameters are affected by cycloplegia.

METHODS

Participants
This cross-sectional study was approved by the Ethics Committee of Wenzhou Medical University and adhered to the Declaration of Helsinki. Written informed consent was obtained from patients and/or parents. This trial was registered at the NIH (ClinicalTrials.gov, NCT04576884). Healthy individuals aged between 18 and 86 years, without a history of ocular disease (except age-related cataract and/or myopia), were consecutively included from October to November 2020. All participants underwent visual acuity, cycloplegic and non-cycloplegic refraction, anterior and posterior segment examination, intraocular pressure (IOP), and axial length (AL) using the IOLMaster 700 (Carl Zeiss Meditec AG). All participants were prescribed compound tropicamide eyedrops (Mydrin-P; Santen Pharmaceutical Co., Ltd.), consisting of 0.5% tropicamide mixed with 0.5% phenylephrine hydrochloride, for pupillary dilation to perform fundus examination at an outpatient clinic. Exclusion criteria were as follows: (1) ocular diseases other than cataracts and/or myopia; (2) history of eye surgeries or injuries; and (3) shallow anterior chamber with a risk for angle closure. The data from the right eye were selected for analysis.

SS-OCT Tomography Examinations
The crystalline lens was imaged by a newly commercially available SS-OCT system (CASIA2), which provides 3D crystalline lens biometry automatically and quantitatively. The CASIA2 uses a 1310 nm swept-source laser wavelength at a frequency of 0.3 seconds, producing 16 radial 2-dimensional images from 16 different meridians, and the built-in software automatically runs a 3D analysis of the results. ACD (mm) was the distance from the posterior surface of the cornea to the anterior surface of the crystalline lens. ALR (in millimeters) was the mean value of radii at the steep and flat meridian of the anterior crystalline lens surface from 3D images. PLR (mm) was the mean value of radii at the steep and flat meridian of the posterior crystalline lens surface from 3D images. LED (mm) was calculated on the basis of a circular approximation from the measured anterior and posterior surface of the lens. LT (mm) was the thickness on the axis of the crystalline lens. LV (mm) was the straight line distance between the anterior surface of the crystalline lens and the center of the vertical bisector of the straight line connecting between the scleral spurs. Figure 1 shows a screenshot of the CASIA2 software of a representative case. Before using the CASIA2 device for the first time, the optical calibration was performed using model eyes. Three calibration model eyes provided by the manufacturer had judgment criteria for corneal curvatures of 5.50 mm ± 0.02 mm, 8.00 mm ± 0.02 mm, and 10.00 mm ± 0.02 mm, corneal thickness of 0.721 mm ± 0.02 mm, ACD of 0.09 mm ± 0.05 mm, and lens thickness of 5.09 mm ± 0.05 mm. All the measurement values of each parameter from the machine used in this study met the judgment criteria and passed all the optical calibration tests. All the SS-OCT measurements

Figure 1. A representative case showing 3D lens analysis results (A: precycloplegic, B: postcycloplegic). Front R (radii of curvature of the anterior lens), the mean value of the steep and flat meridian of the anterior crystalline lens surface (mm). Back R (radii of curvature of the posterior lens), the mean value of the steep and flat meridian of the posterior crystalline lens surface (mm). Diameter of the lens equator (LE-Dia) was calculated based on a circular approximation from the measured anterior and posterior surface of the lens (mm); LT = lens thickness on the axis of the crystalline lens (mm).
Table 1. Patient Demographics and Clinical Characteristics.

| Parameter   | Overall          | Group 1          | Group 2          | Group 3          | P value | Post hoc |
|-------------|------------------|------------------|------------------|------------------|---------|----------|
| n           | 76               | 28               | 24               | 24               | —       | 1 < 2 < 3 |
| Sex (F/M)   | 48/28            | 18/10            | 15/9             | 15/9             | .676a   |          |
| Age (y)     | 48.01 ± 19.34    | 26.7 ± 5.1       | 51.4 ± 4.5       | 71.4 ± 6.2       | <.001b  | 1 < 2 < 3 |
| AL (mm)     | 24.00 ± 1.44     | 25.06 ± 1.04     | 23.27 ± 1.21     | 23.51 ± 1.17     | .003b   | 2 = 3 < 1 |
| SE (D)      | −1.35 ± 3.71     | −4.27 ± 2.07     | −0.39 ± 2.81     | 1.24 ± 3.59      | .004b   | 1 < 2 = 3 |
| IOP (mm Hg)| 13.52 ± 3.23     | 13.8 ± 2.5       | 14.5 ± 3.4       | 12.2 ± 3.5       | .005b   | 3 < 1 = 2 |

AL = axial length; SE = spherical equivalent error
aP < .05: Chi-square test
bP < .05: analysis of variance and post hoc Tukey honest significant differences and Dunnett multiple comparison test

RESULTS

Seventy-six participants (76 eyes) with a mean age of 48.01 ± 19.34 years (range, 18 to 86 years) were included in the analysis, and Table 1 summarizes the demographics and clinical characteristics. To evaluate the effect of age, participants were divided into 3 groups: Group 1 represents participants aged 18 to 40 years, Group 2 represents participants aged 41 to 60 years, and Group 3 represents participants older than 60 years. A significant difference in refractive error and axial length was found among groups. Despite the IOP significantly differed, none of participants had an IOP outside normal limits. The repeatability and reproducibility were analyzed and demonstrated in Table 2.

Association Between Age and Parameters

While adjusting for the effect of axial length, refractive error, and sex, the ALR and the ACD were negatively and significantly correlated with age before and after cycloplegia (P ≤ .002). The pre cycloplegic and postcycloplegic LV, LT, and LED showed a significant and positive correlation with age (P ≤ .004). No significant correlation was found between age and the PLR either before or after cycloplegia. Table 3 shows the standardized coefficient beta, the unstandardized coefficient beta, and the 95% CI for each parameter.

Table 2. Repeatability and Reproducibility of Parameters.

| Parameter   | Intraobserver Sr (95% CI) | Interobserver Sr (95% CI) |
|-------------|---------------------------|---------------------------|
|             | Observer 1 | Observer 2 | Observer 1 | Observer 2 |
| ALR (mm)    | 0.281 (−0.498, 0.160)| 0.253 (−0.449, 0.095)| 0.622 (−1.102, 2.345)|
| PLR (mm)    | 0.186 (−0.330, 0.703)| 0.171 (−0.302, 0.644)| 0.277 (−0.492, 1.047)|
| LT (mm)     | 0.009 (−0.016, 0.034)| 0.012 (−0.022, 0.046)| 0.015 (−0.027, 0.058)|
| LV (mm)     | 0.161 (−0.286, 0.608)| 0.196 (−0.347, 0.738)| 0.180 (−0.318, 0.678)|
| LED (mm)    | 0.080 (−0.142, 0.302)| 0.063 (−0.111, 0.237)| 0.063 (−0.112, 0.237)|
| ACD (mm)    | 0.015 (−0.027, 0.058)| 0.016 (−0.028, 0.059)| 0.016 (−0.028, 0.059)|

ACD = anterior chamber depth; ALR = the radius of curvature of the anterior lens; LED = lens equatorial diameter; LT = lens thickness; LV = lens vault; PLR = the radius of curvature of the posterior lens
Paired Comparison Before and After Cycloplegia

The postcycloplegic mean values of the ALR in Groups 1 and 2 significantly increased compared with the precycloplegic values. The PLR in Group 3 showed a significantly higher value after cycloplegia compared with the value before cycloplegia. The mean value of the LT was significantly and consistently decreased, and the ACD was significantly increased in every group after cycloplegia. The mean value of the LED in Group 1 was significantly higher after cycloplegia than that before cycloplegia. Cycloplegia induced a significant declination of LV in Groups 1 and 2. Figure 2 shows the paired comparison of parameters precycloplegia and postcycloplegia.

Cycloplegia-Induced Differences of Parameters

Cycloplegia-induced differences of parameters were defined as the postcycloplegic value minus the precycloplegic value. The differences of the ALR and ACD were negatively correlated with age ($P = .001, P = .014$). A significantly positive correlation between the difference of LT, LED, LV, and age ($P < .001, P < .001, P = .001$) and were noted. There was no significant correlation between the differences in the PLR and age ($P = .630$). Table 4 shows the standardized coefficient beta, the unstandardized coefficient beta, and the 95% CI of the differences of parameters induced by cycloplegia.

DISCUSSION

In this study, we investigated the impact of age and cycloplegia on the morphology of the crystalline lens in a population with an extended range of age (18 to 86 years). According to our results, the CASIA2 showed generally favorable repeatability and reproducibility for each parameter, and the reproducibility was improved after cycloplegia. Generally, the effect of age and cycloplegia was presented on the anterior surface of the crystalline lens.

Several studies have reported that the CASIA2 produced reliable results of in vivo crystalline lens measurement with good repeatability and reproducibility.14,15 Shoji et al.

### Table 3. Correlation of the Morphologic Parameters of the Human Crystalline Lens With Age: Adjusted for Axial Length, Refractive Status, and Sex (Multivariate Analysis).

| Parameter | Precycloplegia | Postcycloplegia |
|-----------|----------------|-----------------|
|           | $P$ value      | Standardized coefficient $\beta$ | Unstandardized coefficient $\beta$ | 95% CI for coefficient $\beta$ | $P$ value | Standardized coefficient $\beta$ | Unstandardized coefficient $\beta$ | 95% CI for coefficient $\beta$ |
| ALR       | .002           | -0.397          | -0.032          | -0.051, -0.012 |
| PLR       | .738           | -0.086          | -0.001          | -0.008, 0.006 |
| LT        | <.001          | 0.847           | 0.021           | 0.017, 0.025 |
| LV        | <.001          | 0.523           | 0.019           | 0.006, 0.013 |
| LED       | <.001          | 0.557           | 0.013           | 0.007, 0.019 |
| ACD       | <.001          | -0.535          | -0.101          | -0.014, -0.006 |

ACD = anterior chamber depth; ALR = the radius of curvature of the anterior lens; LED = lens equatorial diameter; LT = lens thickness; LV = lens vault; PLR = the radius of curvature of the posterior lens.
reported good repeatability and reproducibility of the measurement of the crystalline lens obtained by the CASIA2 regardless of accommodation stress (the mean age in the study was 35.6 ± 11.7 years). Shoji et al. and we found that the noncycloplegic PLR and the other parameters tended to have better repeatability and reproducibility than the ALR, although the signal strength in OCT generally decreases with deeper penetration in the axial direction. The relatively wide variability of ALR may be due to the fact that accommodative microfluctuations occurred primarily on the front surface rather than the back surface. In addition, we found that the reproducibility after cycloplegia was higher than that before cycloplegia. One reason could be that dilation of the pupil resulted in more complete crystalline lens imaging, making the automatic fitting more accurate. Although CASIA2 has shown good reproducibility in measuring the parameters of the crystalline lens, little is known about the measurement accuracy for special population such as ectopia lentis and spherical lens patients. Therefore, the scope of research objects should be expanded.

Table 5 shows a summary of changes in lens central geometric parameters with age of previous in vivo studies using various imaging technologies. Although the measurement principles vary in different methods, our results were in good agreement with previous studies. The ALR ranged from means of −0.0438 to −0.11 mm/y, and our result neared the bottom (−0.032 mm/y before cycloplegia, −0.064 mm/y after cycloplegia). The LT ranged from means of +0.0180 to +0.031 mm/y, whereas our outcome also neared the bottom (+0.021 mm/y before cycloplegia, +0.022 mm/y after cycloplegia). The ACD significantly decreased with age at a rate of −0.011 to −0.031 mm/y, and our result neared the bottom (−0.010 mm/y before cycloplegia, −0.010 mm/y after cycloplegia). At present, the age-related changes of LED are inconsistent with previous in vivo studies. We found that the LED increases annually slightly (+0.013 mm/y before cycloplegia, +0.009 mm/y after cycloplegia), and its annual growth was smaller than that reported by Kasthurirangan et al. (+0.028 mm/y in 2011, +0.56 mm/y in 2008) and Atchison et al. (an increasing rate of 0.3 ± 0.6 mm from the youngest to the oldest group, P < .02). On the contrary, Richdale et al., Jones et al., and Strenk et al. did not observe a significant correlation between the LED and age. Despite Chang et al. and Kasthurirangan et al. suggested that the PLR increases with age, whereas most previous studies and our study agreed that the PLR is not related with age. Most studies did not find age-dependent changes of LV, but our study noted that the LV increases with age (+0.009 mm/y before and after cycloplegia). Römken et al. and Gavin et al. also found the positive correlation between the LV and age; however, they only included subjects older than 40 years. We herein complemented the LV data in the younger group (age 18 to 40 years), which had a significant value for the implantable collamer lens design and surgical planning. Taken together, we found that the LT, LED, and LV increase with age, and the ACD and ALR decrease with age, and that the PLR does not change with age.

Ex vivo studies reported an increase in lens diameter with age, ranging from +0.01 mm/y to +0.02 mm/y. Removing the crystalline lens from the eyeball will cause morphologic changes; therefore, the results may differ from those in vivo. In vivo LED was usually measured by MRI, possibly due to the low resolution of the MRI to the eye, resulting in inconsistent results. We used SS-OCT to study the LED in vivo and found an age-related increase of approximately 0.009 mm/y to 0.013 mm/y. Based on our data, it is useful for planning phakic IOL implantation, accommodating IOL implantation. However, it should be known that our LED data were obtained from fitting the curve automatically, which cannot represent the true LED. Thus, in future studies, caution is needed when interpreting the LED using the CASIA2, and more work is needed to be done to get more accurate data.

The studies about the effects of cycloplegia on the anterior segment were limited to central corneal thickness, ACD, pupil diameter, and LT, and few studied the effect on geometric lens parameters. Previous and our study showed that the ACD significantly increased and the LT significantly decreased after cycloplegia, including the elderly. Our results of an increase in ALR (<60 years) after cycloplegia corresponded with the results shown by Zhang et al. (31.05 ± 5.84 years, +0.609 mm). Changes in ALR in subjects older than 60 years were not statistically significant. We found that no studies had reported the changes in LED and LV before and after cycloplegia. In the
Present study, the cycloplegia caused a significant increase of LED in subjects younger than 40 years. With respect to LV, cycloplegia induced a significant reduction in Groups 1 \((/C0_{0.058 \text{ mm}})\) and 2 \((/C0_{0.098 \text{ mm}})\). In general, cycloplegia had a greater impact on the measurements of ACD, ALR, LT, and LV. Attention should be paid to the changes of the parameters after cycloplegia in determining IOL power and planning refractive surgeries. Furthermore, cycloplegia-induced differences in ALR \((P = .001)\) and ACD \((P = .014)\) decreased with age, those in LT \((P < .001)\), LED \((P < .001)\), and LV \((P = .001)\) increased with age, and those in

| Parameter | Study | Method | Cycloplegia | Age (y) and sample size (n) | Changes (mm/y) |
|-----------|-------|--------|-------------|-----------------------------|----------------|
| ACD       | Richdale et al. | Cycloplegia with 1% tropicamide | No\(^a\) | 30-50 (n = 85) | 0.036 ± 0.006 x age (P < 0.001) |
| ALR       | Chang et al. | Extended-depth OCT | No\(^a\) | 21-71 (55 eyes of 33 subjects) | 0.029 x age (P = .001) |
| LED       | Richdale et al. | Cycloplegia with 1% tropicamide | No\(^a\) | 30-50 (n = 26) | 0.003 x age (P < 0.001) |
| PLR       | Richdale et al. | Cycloplegia with 1% tropicamide | No\(^a\) | 18-50 (Scheimpflug: n = 62; MRI: n = 25) | 0.008 x age (P = .001) |
| LV        | Römikens et al. | SS-1000 OCT | No\(^a\) | 40-80 (n = 647) | 0.007 x age (P < 0.001) |

ACD = anterior chamber depth; ALR = the radius of curvature of the anterior lens; LED = lens equatorial diameter; LT = lens thickness; LV = lens vault; PLR = the radius of curvature of the posterior lens

\(^{a}\)Unaccommodated, relaxed

\(^{b}\)Univariate linear regressions were conducted to determine the relationship between each variable and age, with age centered at 30 years (designated as “age 30”).

There are some limitations in our study. First, because the CASIA2 provided 3D crystalline lens morphology by fitting the circular curves, the parameters obtained may not completely represent the most realistic state of the lens, especially in the peripheral lens, which is covered by the iris.
According to the mean LEDs measured using MRI (ranging from 9.03 to 9.42 mm), the mean LEDs measured by the CASIA2 (9.88 ± 0.44 mm and 9.93 ± 0.42 mm, pre-cycloplegic and post-cycloplegic, respectively) are overestimated. Second, subjects with age up to 18 years were cyclopleged with a different product than those older than 18 years. This is because cyclopentolate is the widely accepted drug of first choice for children undergoing cycloplegic refraction, whereas a combination of 0.5% tropicamide and 0.5% phenylephrine hydrochloride is widely used to perform dilated fundus examination in adults in China. This might have subtly affected the results.

In conclusion, CASIA2 is a reliable tool for observing the human crystalline lens morphology. Age-related changes of lens morphology mainly occurred on the anterior surface and manifested as the ALR and ACD decreased with age, whereas the LT, LV, and LED increased with age. Cycloplegia caused significant changes on the anterior surface in participants younger than 60 years and manifested as the ALR and ACD increased, whereas the LT and LV decreased after cycloplegia. In addition, the effect of cycloplegia on the anterior surface weakened with age.

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WHAT WAS KNOWN
- Second-generation anterior segment SS-OCT produced reliable results of in vivo crystalline lens measurement with good repeatability and reproducibility.
- The radius of curvature of the anterior lens and anterior chamber depth (ACD) were significantly and negatively correlated with age, and the lens thickness (LT) and lens vault (LV) were positively correlated with age.
- Studies about the effects of cycloplegia on the anterior segment were limited to central corneal thickness, ACD, pupil diameter, and LT, and few studied the effect on geometric lens parameters.

WHAT THIS PAPER ADDS
- The effect of age and cycloplegia was presented on the anterior surface of the crystalline lens.
- The lens equatorial diameter and LV increase slightly annually, and the radius of curvature of the posterior lens is unrelated with age.
- Cycloplegia caused significant changes on the anterior surface in participants younger than 60 years and manifested as the radius of curvature of the anterior lens and ACD increased, whereas the LT and LV decreased after cycloplegia. The effect of cycloplegia on the anterior surface weakened with age.

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