The Development of the Refractive Status and Ocular Growth in C57BL/6 Mice

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PURPOSE. To investigate refraction, corneal curvature, axial components, and the correlations between the refraction and ocular growth during the emmetropization in the C57BL/6 mouse.

METHODS. Ten groups of 10 mice underwent ocular measurements at 22 to 102 days after birth. Refraction was measured by photorefractometry and corneal radius of curvature (CRC) was measured by keratometry. Corneal thickness (CT), anterior chamber depth (ACD), lens thickness (LT), vitreous chamber depth (VCD), retinal thickness (RT), and axial length (AL) were measured by optical coherence tomography (OCT) with focal plane advancement.

RESULTS. Refraction was $-1.49 \pm 3.17$ diopters (D; mean $\pm$ SD) at day 22 and the highest myopia was at day 25 ($-4.61 \pm 2.96$ D). The refractive error then increased and reached a hyperopic peak ($+9.43 \pm 3.33$ D) on day 47. The overall change in refraction was significant from 22 to 102 days ($P < 0.05$). All measured ocular components changed significantly during the study period except for CT and RT ($P > 0.05$ for CT and RT; $P < 0.05$ for others). The CRC, ACD, LT, and AL increased from 22 to 47 days. The increase in ACD, LT, and AL continued after 47 days; however, the CRC increased slowly after this age. The ACD became stable around 67 days and LT and AL at 81 days.

CONCLUSIONS. In C57BL/6 mouse eyes, myopia developed early and then the refractive error increased rapidly in the hyperopic direction to reach a peak at around 47 days with the major contributing changes being in axial length and corneal curvature. ( Invest Ophthalmol Vis Sci. 2008;49:5208–5214) DOI:10.1167/iovs.07-1545

Myopia is one of the most common ocular disorders in humans. The use of animal models has contributed greatly to our understanding of myopia.1-4 Even though a wide range of species, including chicken, tree shrew, marmoset, macaque, and guinea pig, have been established as models of myopia, incomplete information on each species’ genome, transcriptome, and proteome limits their utility in further understanding the condition. The mouse genome, however, has been largely sequenced. A mouse model of myopia was described, and many knockout strains have been made available for research, thus providing resources to study the genetic influences on the mechanism of myopia development (Beuerman RW, et al. IOVS 2003;44:ARVO E-Abstract 4338; Fernandes A, et al. IOVS 2004;45:ARVO E-Abstract 4280; Schaeffel F, et al. IOVS 2002;43:ARVO E-Abstract 182).6-12

Animal models of normal and experimental ocular development provide opportunities to study intersubject differences in biometric and refractive variables. In some animals such as chickens,13,14 tree shrews,15 marmosets,16 and guinea pigs,17 the normal development of ocular components and refractive states are available in the literature. Few studies of mouse eyes have been reported. In a study by Schmucker and Schaeffel,18 a paraxial schematic eye model for the growing mouse was developed. However, the dimensional data obtained in vitro in that study have limited reliability, in part because of the loss of intraocular pressure, which serves as an important source of turgidity for mammalian eyes that lack scleral rigidity. This makes it difficult to resolve the tiny differences in axial lengths by the histologic or video measurements of excised eyes. In another study using optical low coherence interferometry (OLCI) with single axial scanning, only axial length, corneal thickness, and anterior chamber depth were measured in mice in vivo.19 Further, these studies did not analyze the associations between the development of refraction and growth of ocular components that are very important in determining the main factors that influence the refraction state. Recently, we have developed a high-resolution biometric measurement of the mouse eye in vivo, using real-time optical coherence tomography (OCT) with focal plane advancement by a stepper motor.20 Thus, the goal of the present study was to investigate the changes in refraction associated with the development of corneal curvature and axial components in mouse eyes in vivo using OCT with a stepper motor.

MATERIALS AND METHODS

Animals and Experimental Design

The animal research in this study was approved by the Animal Care and Ethics Committee at Wenzhou Medical College (Wenzhou, China). All experiments were conducted according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. C57BL/6 wild-type mice were obtained from the Animal Breeding Unit at Wenzhou Medical College. All animals were raised in groups of four in standard mouse cages ($24 \times 18 \times 13$ cm$^3$) in a 12-hour light/dark cycle.

One hundred mice were assigned to 10 groups ($n = 10$ mice, 20 eyes). Each group underwent a series of ocular measurements at one of the following 10 postnatal time points: 22, 25, 29, 35, 47, 53, 68, 95, and 102 days. Refraction, corneal radius of curvature (CRC), corneal thickness (CT), anterior chamber depth (ACD), lens thickness...
(LT), vitreous chamber depth (VCD), retinal thickness (RT), and axial length (AL) were all measured.

**Refraction**

The refractive state of each eye was measured with an eccentric infrared photoretinoscope, which was calibrated according to a published procedure. Briefly, a series of lenses (−10 to +10 D) was placed in front of the mouse eyes (n = 13, 6 weeks old), and measured refractive errors were recorded. Measurements were made on one randomly selected eye of each mouse. A linear regression relation between measured refractive errors and added lens powers was obtained (r = 0.93, P < 0.001, Fig. 1).

Testing on each mouse began with an approximately 30-minute period of dark adaptation. The unanesthetized animal was gently restrained by grabbing its tail. During the measurement in the dark, the period of dark adaptation. The unanesthetized animal was gently re-

tissue was approximately 10
wavelength with a bandwidth of 60-nm. The axial resolution in the light source of the OCT instrument emitted a beam of 1310-nm center

**Corneal Radius of Curvature**

CRC was measured in unanesthetized mice with a keratometer (OM-4, Topcon, Japan). A +20.0-diopter (D) aspherical lens was mounted behind the cover of the keratometer and calibrated by using a group of stainless-steel ball bearings. The calibration regression coefficient was 0.99 (P < 0.001). The CRC for each mouse was measured in triplicate.

**Axial Components**

A custom real-time OCT instrument with focal plane advancement driven by a stepper motor was used to measure the AL and other ocular components as reported in our recent study. Details, uses, and limitations of this OCT instrument have been reported. Briefly, the light source of the OCT instrument emitted a beam of 1310-nm center wavelength with a bandwidth of 60-nm. The axial resolution in the tissue was approximately 10 μm. With a similar OCT instrument, the repeatability of measuring CT was 2 to 3 μm. A light-delivery system (probe) with a telecentric optical design was mounted on a stepper motor (T25X; Thorlabs, Newton, NJ), which was controlled by a driver (ODCC01; Thorlabs, Newton, NJ) connected with a computer.

Each mouse was anesthetized with a SC injection of 0.08 to 0.20 mL of 2.4 mL 15% ketamine hydrochloride (70 mg/kg body weight) and 0.8 mL 2% xylazine hydrochloride (10 mg/kg body weight), dissolved in 6.8 mL sterile saline. The mouse was then placed in a cylindrical holder and mounted on a positioning stage in front of the optical scanning probe. A video viewing system for observing the eye was used for final orientation and positioning. After the last imaging experiment, each mouse was returned to its cage.

Ocular dimensions were determined by moving the focal plane with the stepper motor, which had a resolution of 1 μm according to the manufacturer. The precision of stepper motor was within 2 μm, as verified by measuring a travel distance of 5000 μm 10 times using a dial caliper with 1-μm resolution. The distances traveled between two ocular interfaces were calculated to represent the dimensional variables. The measured variables included CT, ACD, LT, VCD, and RT. The VCD was defined as the distance from the back of the lens to the nerve fiber layer of the retina. The RT was defined as the distance between the nerve fiber layer and the retinal pigment epithelium (RPE). The AL was determined as the distance between the anterior cornea surface and the nerve fiber layer along the optical axis of the eye. To convert optical path length into geometrical path length, refractive indexes of the inhomogeneous lens index versus age (γ = 0.0005x + 1.557; R² = 0.98). The precision of in vivo measurements of mouse eyes derived by OCT were reported previously. In that study, each eye was imaged along the entire AL three times on two consecutive days. Measurements from those three images were averaged to determine the mean and SD. The standard deviations of three repeated measurements in the same eyes for ocular dimensions ranged from 0 to 27.5 μm. The average SD for the AL on two consecutive days was 13.0 ± 8.8 μm. The average standard deviations for anterior chamber depth, lens thickness, and VCD on two consecutive days were 4.4 ± 3.5, 5.1 ± 3.9, and 10.8 ± 8.2 μm, respectively. The average SD for RT on two consecutive days was 9.4 ± 5.7 μm.

**Statistical Analysis**

Data analysis was conducted with commercial software (SPSS, ver. 13.0; SPSS, Chicago, IL). The value of each parameter is reported as the mean ± SD of 20 eyes of 10 mice in each age group. One-way analysis of variance (one-way ANOVA) was used to determine overall effects for refractive status, CRC, and axial components in the different age groups for both eyes. Post hoc unpaired t-tests with Bonferroni correction was used to determine whether there were pair-wise differences between any two age groups (P < 0.05). Linear regression analysis was also performed between refraction and each of the biometric results.

**RESULTS**

**Development of Refraction**

The overall change in refraction as measured by eccentric infrared photoretinoscopy was significant from days 22 to 102 (ANOVA, P < 0.001, Table 1, Fig. 2) The refraction at day 22 was −1.49 ± 3.17 D, and the highest myopic refraction, −4.61 ± 2.96 D, was at day 25 (P < 0.05). After day 25, the refractive error increased rapidly in the hyperopic direction, reaching a hyperopic peak of +3.04 ± 3.53 D at day 47. After that age, the refractive error declined slowly and became stable at day 67 (P > 0.05 between any two adjacent days after day 67). By day 102, the refractive error reached +4.02 ± 3.77 D.

**Development of CRC**

The CRC increased rapidly from day 22 (1.316 ± 0.011 mm) to day 47, with each day being significantly greater than the preceding day (P < 0.001; Table 1, Fig. 3). After day 47 the increases were more subtle (P > 0.05 between any two adjacent days). The maximum radius of curvature was 1.540 ± 0.017 mm at day 102.
TABLE 1. Refractive and Dimensional Changes between Days 22 and 102

<table>
<thead>
<tr>
<th>Age (d)</th>
<th>Refraction (D)</th>
<th>CRC (mm)</th>
<th>CT (mm)</th>
<th>RT (mm)</th>
<th>ACD (mm)</th>
<th>LT (mm)</th>
<th>VCD (mm)</th>
<th>AL (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>−1.49 ± 3.17</td>
<td>1.316 ± 0.011</td>
<td>0.105 ± 0.006</td>
<td>0.186 ± 0.027</td>
<td>0.260 ± 0.019</td>
<td>1.470 ± 0.017</td>
<td>0.751 ± 0.037</td>
<td>2.859 ± 0.040</td>
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<td>25</td>
<td>−4.61 ± 2.96</td>
<td>1.342 ± 0.022</td>
<td>0.115 ± 0.008</td>
<td>0.186 ± 0.021</td>
<td>0.302 ± 0.013</td>
<td>1.522 ± 0.030</td>
<td>0.755 ± 0.048</td>
<td>2.968 ± 0.038</td>
</tr>
<tr>
<td>29</td>
<td>−0.10 ± 4.42</td>
<td>1.409 ± 0.015</td>
<td>0.115 ± 0.007</td>
<td>0.195 ± 0.014</td>
<td>0.315 ± 0.018</td>
<td>1.556 ± 0.020</td>
<td>0.728 ± 0.026</td>
<td>3.000 ± 0.031</td>
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<tr>
<td>35</td>
<td>1.42 ± 2.87</td>
<td>1.440 ± 0.015</td>
<td>0.188 ± 0.008</td>
<td>0.190 ± 0.021</td>
<td>0.337 ± 0.017</td>
<td>1.607 ± 0.011</td>
<td>0.721 ± 0.020</td>
<td>3.080 ± 0.023</td>
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<td>47</td>
<td>9.43 ± 3.33</td>
<td>1.494 ± 0.020</td>
<td>0.111 ± 0.012</td>
<td>0.186 ± 0.014</td>
<td>0.348 ± 0.013</td>
<td>1.706 ± 0.036</td>
<td>0.650 ± 0.014</td>
<td>3.142 ± 0.049</td>
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<tr>
<td>53</td>
<td>6.82 ± 2.52</td>
<td>1.490 ± 0.010</td>
<td>0.122 ± 0.008</td>
<td>0.186 ± 0.015</td>
<td>0.357 ± 0.034</td>
<td>1.726 ± 0.026</td>
<td>0.652 ± 0.030</td>
<td>3.186 ± 0.038</td>
</tr>
<tr>
<td>67</td>
<td>2.84 ± 4.05</td>
<td>1.503 ± 0.013</td>
<td>0.114 ± 0.007</td>
<td>0.187 ± 0.016</td>
<td>0.370 ± 0.013</td>
<td>1.734 ± 0.029</td>
<td>0.651 ± 0.024</td>
<td>3.233 ± 0.050</td>
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<tr>
<td>81</td>
<td>5.98 ± 3.20</td>
<td>1.520 ± 0.029</td>
<td>0.115 ± 0.007</td>
<td>0.186 ± 0.015</td>
<td>0.385 ± 0.013</td>
<td>1.781 ± 0.015</td>
<td>0.651 ± 0.028</td>
<td>3.284 ± 0.055</td>
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<tr>
<td>95</td>
<td>6.40 ± 3.77</td>
<td>1.546 ± 0.012</td>
<td>0.116 ± 0.007</td>
<td>0.186 ± 0.012</td>
<td>0.386 ± 0.022</td>
<td>1.817 ± 0.014</td>
<td>0.668 ± 0.027</td>
<td>3.295 ± 0.031</td>
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<tr>
<td>102</td>
<td>4.02 ± 3.77</td>
<td>1.540 ± 0.017</td>
<td>0.117 ± 0.008</td>
<td>0.182 ± 0.017</td>
<td>0.399 ± 0.020</td>
<td>1.822 ± 0.031</td>
<td>0.603 ± 0.023</td>
<td>3.355 ± 0.031</td>
</tr>
</tbody>
</table>

$P < 0.05$ <0.05 >0.05 >0.05 <0.05 <0.05 <0.05 <0.05

Data are the mean ± SD of measurements in 20 eyes of 10 mice in each parameter in each age group. $P$ is by ANOVA.

Growth of Ocular Dimensions

The CT (Fig. 4A, Table 1) and RT (Fig. 4B) did not change significantly between 22 and 102 days of age (ANOVA, $P < 0.05$, Table 1). For that period, the average CT was $1.14 ± 0.005$ mm, and the average RT was $0.187 ± 0.003$ mm. However, ACD, LT, VCD, and AL did change significantly with age (ANOVA; $P < 0.05$; Figs. 4C, 4D, 4E, 4F; Table 1). The ACD increased from days 22 to 67 ($P < 0.05$, Fig. 4C), but changed slowly after that time point ($P > 0.05$ between any two adjacent time points after day 67, whereas $P < 0.05$ between days 67 and 102). It reached $0.399 ± 0.020$ mm by 102 days (Table 1). The LT and AL increased significantly from day 22 to 81 ($P < 0.05$; Figs. 4D, 4F; Table 1). Thereafter, each gradually increased but with very minimal changes between adjacent days ($P > 0.05$ between days 81, 95, and 102). In contrast to the increases in other ocular dimensions, the VCD decreased from 22 to 47 days ($P < 0.05$ between any two adjacent days; Fig. 4E, Table 1), followed by smaller declines after that time point ($P > 0.05$ between any two adjacent days). In addition, LT ($R^2 = 0.918$, $P < 0.05$) had the highest correlation with AL, followed by ACD ($R^2 = 0.806$, $P < 0.05$) and VCD ($R^2 = 0.514$, $P < 0.05$).

Correlation between the Development of Refraction and Growth of Ocular Components

The refractive error correlated best with CRC ($R^2 = 0.26$, $P < 0.001$, Fig. 5A) and LT ($R^2 = 0.24$, $P < 0.001$, Fig. 5B). Refractive error also correlated significantly with the ACD ($R^2 = 0.15$, $P = 0.000$, Fig. 5C), VCD ($R^2 = 0.15$, $P < 0.001$, Fig. 5D), and AL ($R^2 = 0.14$, $P < 0.001$, Fig. 5E). The refractive error did not correlate with either CT or RT ($P > 0.05$, Figs. 5F, 5G).

DISCUSSION

Accurate measurements of ocular dimensions are prerequisites for studies on development of refractive errors in animal models and humans. Myopia is associated with increased AL in some models. In these models, changes in AL are easily measured with cryosections, ultrasound, and callipers. However, in the small mouse eye, changes in refraction of 1 D are equivalent to changes in AL of only 5 μm, according to the schematic eye model. The resolution limit of ultrasound is approximately 50 μm. Thus, it does not have the needed sensitivity to detect changes in AL in the mouse. Similarly, video morphology and cryosections produce measurement errors of 0.08 and 0.14 mm, respectively (Purdue MT, et al. IOVS 2004;45:ARVO E-Abstract 4281). Based on the model eye calculations, the mouse eye would have to shift 16 to 28 D for differences in AL to be detected with these techniques. OLCI has the accuracy to measure mouse eyes in vivo. However, the in vivo dimensions of the lens, which makes up approximately 60% of the total AL in the mouse, and the vitreous chamber could not be obtained with this technique. Evidence from other animal models of myopia (Table 2) suggests that vitreous chamber length is the major component. A novel method of measuring all dimensions of the mouse eye with high resolution by applying real-time OCT in conjunction with a moving focal plane was developed and demonstrated to have good repeatability and accuracy.
we investigated in vivo normal development of the refractive state and ocular dimensional changes as well as the associations between the refraction and growth of ocular components during the emmetropization in the mouse.

**Development of Refraction**

Guinea pigs, tree shrews, and nonhuman primates are more hyperopic at birth or eye opening (Table 2), the refractive state then decreases and becomes stable with age. For the BALB/c mice, the refractive error measured by streak retinoscopy increased from young to adult mice. The mean refractive error of untreated mice was −10 D on day 28, increased to −13 D on day 42, and reached a peak of −16 D on day 60. In our study, the C57BL/6 mice started with myopic refractive error, but then progressed to more hyperopic refractive errors with age and reached a stable refraction of hyperopia. The trend toward greater hyperopia after 4 weeks is consistent with those found in other studies. In contrast to previous studies, we noted that the measured refractive errors before 4 weeks were myopic. Measurement errors may be responsible for the results. Although the same configurations of the measurement device were used as the one used by Pardue et al., we noticed some variation in the methods. In their calibration, the refractive errors spanned 7 D for the potential 20 D of trial lens power. In our calibration, the refractive errors spanned approximately 20 for the 20 D of the trial lens power range (Fig. 1). If the calibration curve by Pardue et al. was used to predict our results (−4.61 D) at the age of 25 days, the prediction would be +5 D, which is similar to their result. Clearly, device calibrations play a role in the small discrepancy, which is approximately 1% of total refraction (≈500 D) of the mouse. Pupil size was illumination dependent during the calibration, and study measurement may also influence the results. Measurements of refraction are influenced by pupil size, as described previously, with greater myopia being reported with smaller pupils and greater hyperopia with larger pupils, especially for young mice. We examined the influence of different pupil sizes induced by changes in illumination on refractions. The refraction of 12 eyes of adult C57BL/6 wild-type mice was measured by eccentric infra-red photoretinoscopy in two different illuminated environments, which were 0 and 0.03 lx respectively. The tested results showed that the pupil diameters were smaller with the increased intensities of illumination and the measured refraction shifted to myopic state with the decreases of pupil size. From linear regression, a decrease of 0.24 mm in pupil size induced approximately 5-D myopic shifts in relative refraction. This simple experiment demonstrated the discrepancy between our results and others is most likely due to the pupil size under different illuminations. Although the comparison with a study or a single site can be made in the measurement of refraction under fixed illumination, it would be better to standardize the illumination during measurement for further comparison across studies from different sites.

Other mammalian and avian species undergo emmetropization during early development that begins with hyperopia and decreases to near-zero refractive error. However, the refractive error in mice seemed to progress to hyperopic refrac-
tive errors, as shown in the present study and others.\textsuperscript{8,18} This apparent hyperopia may be a small-eye artifact due to the retinoscopic reflection coming from the inner limiting membrane instead of the other limiting membrane.\textsuperscript{44}

Development of CRC

The CRC increased rapidly from days 22 to 47, and increased slowly thereafter. Such slow increases in corneal curvature during the study period were also observed in tree shrews\textsuperscript{15} and primates.\textsuperscript{16,41} The in guinea pigs also increases within the first 3 weeks, despite a transient decrease in the first week.\textsuperscript{17} In humans, there is also a small increase, approximately 0.3 D, from the ages of 8 to 15 years.\textsuperscript{45,46} The averaged CRC during development in the present study was $1.476 \pm 0.016$ mm, which was consistent with the data ($1.414 \pm 0.019$ mm) measured with infrared photokeratometry in mice of the same strain.\textsuperscript{18} However, the CRC in that study did not change significantly with age.\textsuperscript{18} This discrepancy is probably due to the different methodologies and/or differences in the genetic backgrounds of the strain.

Growth of Ocular Dimensions

By this novel technique, we did not detect any age-related changes in the development of mouse CT and RT from 22 to 102 days of age. In contrast, Schmucker and Schaeffel,\textsuperscript{18} using frozen sections, determined corneal and retinal thicknesses in the growing C57BL/6 mouse and found a small increase between days 22 and 100. The data determined by the OLCI technique also showed that CT slightly increased between the ages of 25 (84.0 \textmu m) and 53 (95.2 \textmu m) days.\textsuperscript{19} The differences of change observed in ocular dimensional developments may be caused by the measurement errors due to the manual measurement. In addition, subjective judgment of the interface position and animal movements during our in vivo OCT measurements made it difficult to detect slight dimensional changes with age.

The mouse ACD increased significantly from days 22 to 67 and changed slowly afterward. Similar findings were reported in eyes from the same mouse strain measured in frozen sections and by OLCI, though the anterior chamber depth included CT.\textsuperscript{19} Similar growth change also occurs in other animals such as macaques,\textsuperscript{37} marmosets,\textsuperscript{16} tree shrews,\textsuperscript{15} and humans.\textsuperscript{37} The mouse LT increased significantly from 22 to 81 days followed by considerably slower change thereafter. This is similar to kittens\textsuperscript{68} and tree shrews\textsuperscript{15} where lens growth is continuous over the lifespan. In humans, the lens also grows continuously\textsuperscript{58} except between the ages of 6 and 12 years, where it actually becomes thinner.\textsuperscript{58}
There was a decline in the mouse VCD from 22 to 47 days, after which there was little change. The same result was found in frozen sections. However, in the tree shrew eye, the VCD initially increased rapidly within 15 days of eye opening and then decreased due to prominent growth of the lens. Our OCT measurements of the mouse eye in vivo showed that the AL increased with age from days 22 to 81. For the BALB/cJ mice, AL (measured by AC-Master; Carl Zeiss Meditec, Jena, Germany) increased linearly between postnatal days 1 and 56, but grew slowly beyond 60 days. Similar in vivo changes between 25 and 53 days were also found using OLCI.

Our absolute values of ocular dimensions at each measured time point did not match very well with those calculated from the schematic eye model. This discrepancy may have three explanations. First, there were different definitions of ocular dimensions adopted by two methods. Second, measurements were performed under different conditions, in vitro versus in vivo. Third, measurement error may be introduced by using the refractive indices from the literature, which may not be correct for the group of mice in the present study. In the future, we will further refine the methods of measurement to implement automated determination of the interfaces. This adjustment will improve the precision of the estimated variables.

The growth in AL correlated most strongly with the changes in LT, followed by ACD. Thus, changes in these two dimensions contribute most to the increases in AL. In animals such as tree shrews and guinea pigs, the increase in AL is also mainly determined by the lens thickness and secondarily influenced by the VCD during the early developmental stage. However, in nonhuman primates and chickens, the lengthening of the vitreous chamber contributes most to the axial growth of the eye. The next most important factor was the deepening of the anterior chamber and the thickening of the lens. In early human childhood, changes in AL are mainly determined by the deepening of the anterior chamber, followed by lengthening of the vitreous chamber and thickening of the lens.

**Correlation between the Development of Refraction and Growth of Ocular Components**

The refraction in mice correlated significantly with the CRC, ACD, LT, VCD, and AL. Among these, the change of refraction correlated highly with the growth of CRC, followed by LT. However, in other myopic models, such as guinea pigs, the increase in AL is also mainly determined by the lens thickness and secondarily influenced by the VCD during the early developmental stage. However, in nonhuman primates and chickens, the lengthening of the vitreous chamber contributes most to the axial growth of the eye. The next most important factor was the deepening of the anterior chamber and the thickening of the lens. In early human childhood, changes in AL are mainly determined by the deepening of the anterior chamber, followed by lengthening of the vitreous chamber and thickening of the lens.

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