

# Lenticular accommodation in relation to ametropia: The chick model

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Our goal was to determine whether experimentally induced ametropias have an effect on lenticular accommodation and spherical aberration. Form-deprivation myopia and hyperopia were induced in one eye of hatchling chicks by application of a translucent goggle and +15 D lens, respectively. After 7 days, eyes were enucleated and lenses were optically scanned prior to accommodation, during accommodation, and after accommodation. Accommodation was induced by electrical stimulation of the ciliary nerve. Lenticular focal lengths for form-deprived eyes were significantly shorter than for their controls and accommodation-associated changes in focal length were significantly smaller in myopic eyes compared to their controls. For eyes imposed with +15 D blur, focal lengths were longer than those for their controls and accommodative changes were greater. Spherical aberration of the lens increased with accommodation in both form-deprived and lens-treated birds, but induction of ametropia had no effect on lenticular spherical aberration in general. Nonmonotonicity from lenticular spherical aberration increased during accommodation but effects of refractive error were equivocal. The crystalline lens contributes to refractive error changes of the eye both in the case of myopia and hyperopia. These changes are likely attributable to global changes in the size and shape of the eye.

**Keywords:** accommodation, ciliary nerve, optics, scanning laser, induced ametropia

## Introduction

In normal young animals, growth of the eye is modulated to ensure that the image focal plane coincides with the retina. This process, called emmetropization, is the underlying basis of an extensive body of work that shows that induction of specific visual cues can lead to the development of refractive errors. Ametropias (myopia and hyperopia) have been experimentally induced in a variety of animals, including, but not limited to, chickens (Irving, Sivak, & Callender, 1992; Schaeffel, Glasser, & Howland, 1988), tree shrews (Norton, 1999; Siegwart & Norton, 1993), and monkeys (Hung, Crawford, & Smith, 1995). Myopia, manifested as an increased axial length of the globe, is induced by form-deprivation of the eye or by imposition of a hyperopic defocus using negative (concave) spectacle lenses, whereas hyperopia, manifested as shorter axial lengths and choroidal thickening, is induced by exposure to myopic defocus by application of positive (convex) lenses (Irving et al., 1992; Schaeffel et al., 1988; Wildsoet & Wallman, 1995).

Controversy exists over the role that accommodation may play in mediating emmetropization. Several studies support the idea that accommodation may be a driving force for growth of the eye to a myopic refractive state. In humans, near-work, which includes reading, writing, or any other task requiring accommodation, has been associated with the development of myopia, and population studies indicate a high prevalence of myopia in students from some

Asian countries, which are known to have exacting educational standards (Lin et al., 1999; Saw et al., 2000; Wu et al., 2001). While there is support for the idea that myopia may be genetically inheritable, the Barrow, Alaska, study showed that school-attending grandchildren of nomadic Inuits tended to be more myopic than their ancestors, who tended to be hyperopic (Young et al., 1969), suggesting that environmental visual factors can influence growth of the eye. It has been suggested that in chicks, accommodation may be the mechanism by which emmetropization is mediated; images imposed with hyperopic defocus (diverging negative lenses) can become clear with accommodation, whereas images imposed with a myopic defocus (positive lens) cannot (Schaeffel et al., 1988). Observations that chicks imposed with different spectacle lenses accommodated and become functionally emmetropic while wearing these lenses lend support to this idea (Schaeffel et al., 1988). Studies showing that optic nerve-sectioned eyes elongate to become more myopic in response to both form-deprivation (Troilo, Gottlieb, & Wallman, 1987) and negative lenses (Wildsoet & Wallman, 1995) indicate that control of emmetropization is at the level of the retina and that connection to the brain is not necessary for emmetropization to occur. However, while optic nerve-sectioned eyes compensated correctly to their respective visual cues, in both studies, these eyes were shorter prior to lens-wear, which suggests that the brain, of which the accommodative apparatus is a part, may be required to regulate ocular growth.

The crystalline lens is a primary contributor to accommodation, imparting some, if not all, of the refractive power required during accommodation, depending on the species. However, its role in experimentally induced ametropias remains unclear. In fact, the effect on the lens itself remains somewhat controversial, with most investigations showing little or no effect in lenticular weight, focal length, or axial thickness. However, while Priolo and colleagues (2000) showed no refractive error-associated differences in lenticular focal lengths, the optics of lenses from form-deprived eyes and eyes treated with +10 D spectacle lenses were degraded relative to their controls, showing that the crystalline lens, too, is an intraocular structure that may be affected by experimentally induced ametropias. Given the potential importance of accommodation in experimentally induced ametropias and of the lens in accommodation, this study was undertaken to determine whether experimentally induced ametropias have an effect on lenticular accommodative function and on lenticular spherical aberration.

## Methods

White leghorn (*Gallus gallus domesticus*) hatchling chicks (Maple Leaf Poultry, New Hamburg, ON) were unilaterally fitted with a velcro ring and either a translucent or a +15 D lens goggle to induce myopia and hyperopia, respectively. Translucent goggles were used because they have been previously shown to induce the greatest amounts of myopia. Additional experiments with -15 D lenses were not done in the interests of reducing the number of chicks used and because how the myopias are ultimately manifested (increased vitreous chamber depth and thinner choroid) is the same regardless of method of induction (Wallman et al., 1995; Wildsoet & Wallman, 1995). Ungoggled, contralateral eyes served as controls for the goggling procedure. Chickens were cared for according to the Guidelines of the Canadian Council on Animal Care; therefore, their management conforms to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. After 7 days, both eyes were measured for refractive errors using streak retinoscopy; then chickens were killed by decapitation. Eyes were enucleated in oxygenated (95% oxygen, 5% carbon dioxide) Tyrode's solution (TS: 134 mM NaCl, 3 mM KCl, 20.5 mM NaHCO<sub>3</sub>, 1 mM MgCl, 3 mM CaCl<sub>2</sub>) and the back of the eye was removed except for the portion containing the ciliary nerves and ganglion (Figure 1). Equatorial and axial lengths were measured using calipers for a subset of eyes (23 of 31 pairs for form-deprived birds, 33 of 38 pairs for +15 D-treated birds) prior to removal of the posterior portion.

Eyes were pinned cornea facing down to a Sylgard® (Dow Corning) washer (Figure 1) and were then placed into a silicone mould that formed the bottom of a chamber. The base mould was fitted with a rectangular glass tube that had a second smaller, open-ended tube attached. A hand-made

silver chloride suction electrode with Tygon® tubing tip was passed through the smaller tube, and the ciliary nerve was suctioned into the electrode tip. The rest of the open-ended tube was plugged with petroleum jelly. The chamber was filled with 8% (v/v) fetal bovine serum in Tyrode's solution to visualize the refracted beams.

Lenses were scanned as previously described (Choh, Sivak, & Meriney, 2002). A low-power helium-neon laser entered through the front of each eye at 0.13-mm steps from the optical axis, with the number of beams recorded dependent on irideal aperture size (Figure 1). Images of the refracted beams leaving the back of the eye were recorded using software included with the scanning laser device developed at the University of Waterloo. Back vertex focal length was calculated as the distance from the back vertex of the lens, which was previously determined from a camera image of the posterior lens surface, to the point where a refracted beam crossed the optical axis. For each eye, a baseline, prestimulus scan was made, followed by a scan during accommodation. Accommodation of the intact lens and ciliary apparatus was induced by electrical stimulation

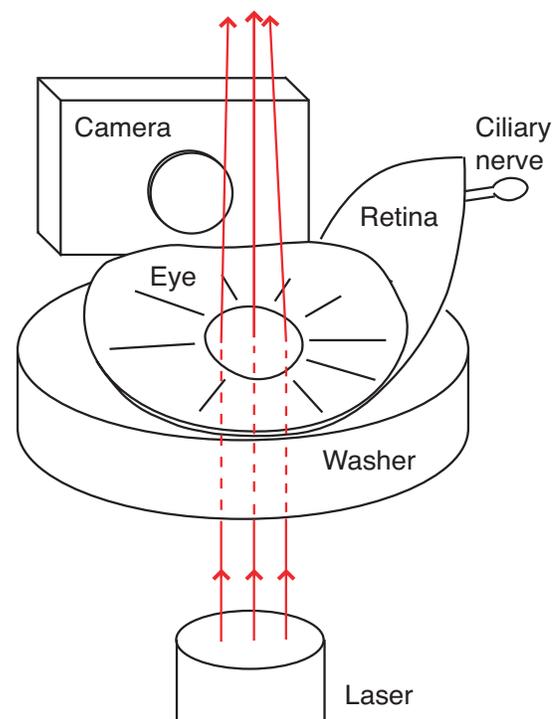


Figure 1. Schematic drawings of the scanning laser monitor preparation. The anterior segment of the eye is placed cornea facing down onto a washer and into a scanning chamber (not shown) containing Tyrode's solution. The ciliary nerve is suctioned into the tip of a suction electrode to allow investigator-controlled accommodation. Laser beams enter the eye through the bottom of the chamber, passing through the pupil and lens at various eccentricities from the optical center. Refracted beams are captured by a camera. Note that because the eye is surrounded by Tyrode's solution, optical effects of the cornea are neutralized (please see text).

of the ciliary nerve (30 Hz, 0.1-1.5 mA; Figure 2). These parameters, which induced maximal irideal contractions, were chosen on the basis of previous work (Choh et al., 2002; Pilar, Nunez, McLennan, & Meriney, 1987). A third, final scan was made to measure back vertex focal lengths during a poststimulus unaccommodated state. The scans across all eccentricities were between 1 and 2 min in duration. During collection of the data, the three most central rays were omitted to avoid spurious variability associated with sutural regions of the lens (Bantseev, Herbert, Trevithick, & Sivak, 1999; Kuszak, Peterson, Sivak, & Herbert, 1994; Sivak, Herbert, Peterson, & Kuszak, 1994). As the lens is a radially symmetrical organ, inferences of the magnitudes for lenticular focal lengths, spherical aberrations, and nonmonotonicity from spherical aberration were based on the two-dimensional scans.

Note that because eyes were submerged in physiological saline, the corneal power from differences in corneal surface curvatures must be small and differences during corneal accommodation even smaller. Calculations using a schematic chicken eye (Schaeffel & Howland, 1988) show that the maximum corneal accommodative change in air (9 D) (Glasser, Troilo, & Howland, 1994) is equivalent to 0.10 D in water, a magnitude that is beyond the resolving power of most keratometers (Appendix). Thus, the back vertex focal lengths measured were taken to represent lenticular back vertex focal lengths.

Although all eyes were optically scanned at 0.13-mm intervals, the number of eccentric points across the lens varied as a result of differences in pupil aperture sizes (Table 1). Pupil size differences arose due to natural or inherent pupil size variation, or due to accommodation-associated pupillary constriction (Figure 2). As different pupil sizes can artificially increase or decrease the mean lenticular focal length if any spherical aberration exists (Jenkins & White, 1957; Smith, 2000), means were calculated for a constant irideal aperture size prior to comparisons; focal lengths at eccentricities for which corresponding data did not exist in the fellow eye nor during accommodation were eliminated from calculations of the mean.

All optical scans showed negative spherical aberration (SA), regardless of refractive error or accommodation state (see Results). For each scan, back vertex focal lengths at each eccentricity were converted to dioptric values (vergences) prior to being fitted to second-order polynomial

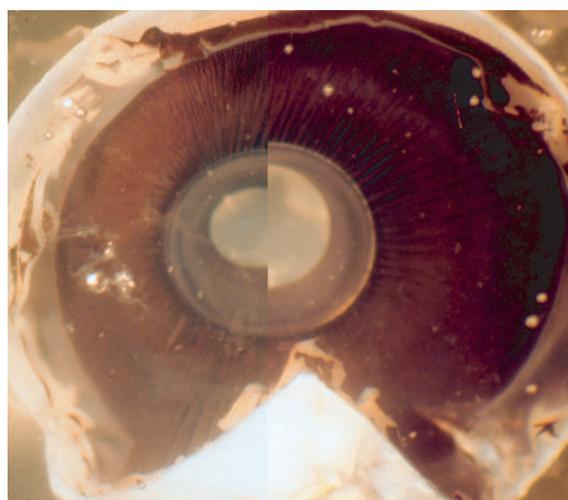


Figure 2. Slightly angled top view of a dissected accommodating chicken eye. Note ciliary muscle movement, contraction of the crystalline lens, and constriction of the pupil during nerve-stimulated accommodation.

regression functions ( $y = Ax^2 = Bx = C$ ). The A-coefficient, which defines the shape (i.e., steepness) of the parabola, was used to quantify lenticular spherical aberration, where steeper parabolas represent scans with greater spherical aberration (Jenkins & White, 1957). Most of the scans (91.8% or 190 of 207) were significantly correlated to the quadratic polynomial equation ( $p < .05$ ). Not unexpectedly, the 17 scans with poorer regression correlations were those for stimulated eyes, with 10 from form-deprived birds and 7 from +15 D-treated birds; poorer correlations were expected for these eyes because accommodation was associated with pupillary constriction and therefore a lower number of beams passing through the pupil. These results indicate that although not perfect, lenticular spherical aberrations fit well with the parabolic function. Although this method of determining the spherical aberration is not traditional, it has worth in allowing comparisons over a range of eccentricities from the optical axis rather than exclusively at a specific point or marginal ray. This is an advantage in the work presented here, given the differences in aperture size (Smith, 2000) and absence of paraxial back vertex focal lengths. Moreover, spherical aberrations calculated for

Treatment		Number of beams (mean:range) entering the pupil for each accommodative state			
		pre	stim	post	
Translucent goggle	control	16: 14 to 19	13: 10 to 17	15: 12 to 19	15: 12 to 19
	treated	15: 14 to 17	12: 10 to 15	15: 12 to 17	15: 12 to 17
+15 D goggle	control	16: 13 to 17	12: 10 to 15	15: 13 to 17	15: 13 to 17
	treated	16: 13 to 18	12: 10 to 15	15: 13 to 17	15: 13 to 17

Table 1. The number of beams (mean:range) entering the pupil for form-deprived myopic and +15 D-treated hyperopic chicks for each state of accommodation.

specific pupil sizes (in the traditional manner) revealed exactly the same relative associations within the groups and yielded exactly identical statistical results (see Results).

Nonmonotonicity from spherical aberration was assessed using the residual root mean square (RMS) of each scan against the best-fitting quadratic function. As the RMS is a measure of the amount of deviation from the ideal, or best-fitting function, higher RMS values indicate greater amounts of deviation and therefore more aberrant rays.

The effects of induced ametropias and accommodation were analyzed using two-way repeated measures ANOVA (analysis of variance) tests at two-tailed  $\alpha$  levels of  $p \leq 0.05$  with both refractive error and accommodative state as repeated, dependent within-subjects factors. Comparisons between ametropia-induced eyes and their respective controls were analyzed using paired  $t$  tests. Changes associated with accommodation were assessed using one-way repeated measures ANOVA, followed by paired  $t$  tests with Bonferroni corrections (Bonferroni multiple comparison test) to account for multiple testing.

## Results

### Effects of refractive error and accommodation

Form-deprivation resulted in induction of myopia, an observation that is consistent with other reports. After 7 days of goggle-wear and prior to enucleation, refractive errors in form-deprived eyes ( $n = 31$ ) ranged from  $-4.50$  to  $-24.50$  D and averaged ( $\pm SD$ )  $-13.63 \pm 5.53$  D (mean change from day 0:  $-19.69 \pm 5.76$  D), whereas the contralateral (control) unoggled eyes ( $n = 31$ ) were hyperopic, with refractive errors ranging from  $+1.50$  to  $+6.75$  D and averaging  $+3.81 \pm 1.16$  D (mean change from day 0:  $-1.79 \pm 3.15$  D). As expected, axial lengths for form-deprived eyes ( $n = 23$ ) were longer, at a mean length of  $9.63 \pm 0.36$  mm, compared to those for control eyes, which averaged  $8.88 \pm 0.22$  mm ( $n = 23$ ). The mean equatorial diameters of myopic eyes ( $12.23 \pm 0.32$  mm;  $n = 23$ ) were greater ( $p < .0001$ ) than those for their controls ( $12.01 \pm 0.24$  mm;  $n = 23$ ). Eyes imposed with  $+15$  D lenses ( $n = 38$ ) became hyperopic, ranging from  $+6.25$  to  $+19.00$  D and averaging to  $+14.36 \pm 2.50$  D (mean change from day 0:  $+9.01 \pm 3.66$  D), whereas refractive errors for their contralateral, unoggled eyes ( $n = 38$ ) ranged from  $+1.75$  to  $+6.00$  D and averaged to  $+3.45 \pm 0.90$  D (mean change from day 0:  $-1.53 \pm 2.90$  D). Axial lengths of defocus-imposed eyes ( $n = 34$ ) were shorter, at  $8.58 \pm 0.18$  mm, compared to their controls ( $n = 34$ ), at  $8.85 \pm 0.20$  mm. Mean equatorial diameters in lens-treated eyes ( $11.90 \pm 0.24$  mm;  $n = 33$ ) were statistically similar ( $p = 0.4604$ ) to those of their controls ( $11.88 \pm 0.22$  mm;  $n = 33$ ).

Differences in the mean back vertex focal length were detected as a function of accommodative state ( $p < .0001$ ). For both form-deprived and control eyes, mean lenticular

focal lengths for stimulated eyes were shorter than for those at rest (Figure 3), an indication that, as expected, stimulation of the ciliary nerve was able to induce a lenticular accommodative response. Poststimulus focal lengths were also shorter than their prestimulus counterparts, an indication that there were significant hysteresis effects in both treated and control groups ( $p < .0001$  for both).

Overall, mean lenticular focal lengths for form-deprived eyes were shorter than for their controls ( $p = 0.0003$ ; two-way repeated measures ANOVA) but significant interaction was also detected ( $p = .0028$ ), indicating that differences between eyes were dependent on the accommodative state of the eye. Specifically, for both the pre- and poststimulus accommodative states, mean focal lengths for form-deprived eyes were significantly shorter than for their controls ( $p < .0001$  for both accommodative states), with differences in mean length at about 1 mm (for both accommodative states; Figure 3) or in power at about 3 D (assuming thin lens in water,  $n_w = 1.33$ ; calculation not shown). However, mean lenticular focal lengths for stimulated form-deprived eyes were similar to those for stimulated control eyes ( $p = .4721$ ). Together with the finding that focal lengths for lenses from myopic eyes are inherently shorter (Figure 3), the results indicate that lenticular accommodation was also affected by induction of myopia. Indeed, changes in accommodative amplitudes were affected by both refractive error ( $p = .0138$ ) and by the hysteresis effects ( $p < .0001$ ), with treated eyes showing smaller accommodative amplitudes than their controls, and with amplitudes during recovery from accommodation smaller than those for accommodation (Figure 4).

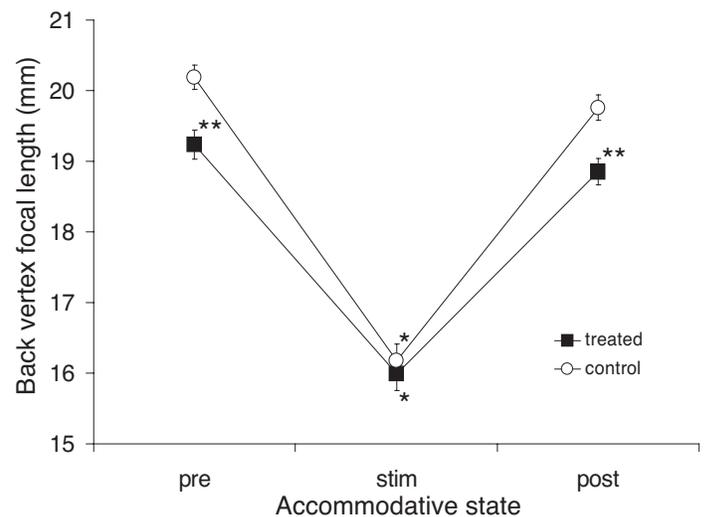


Figure 3. Mean back vertex focal lengths ( $\pm SEM$ ) for lenses from form-deprived eyes (filled squares) and from their controls (open circles) at each accommodative state. For each eye, focal lengths denoted by asterisks were shorter than for those not marked ( $p < .05$ ; Bonferroni multiple comparison test). Means denoted by double asterisks were significantly shorter than those for control eyes at the same accommodative state ( $p < .05$ ; Bonferroni multiple comparison test).

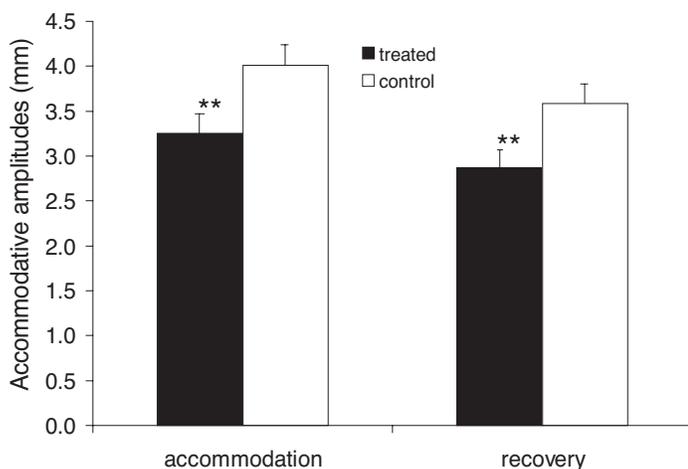


Figure 4. Mean accommodative and recovery amplitudes ( $\pm$ SEM) for lenses from myopic eyes (filled bars) and their controls (open bars). For each accommodative state, means denoted by double asterisks were significantly reduced compared to those not marked ( $p < 0.05$ ; paired  $t$  test).

Accommodation effects on mean focal lengths for +15 D-treated eyes and their controls were significant ( $p < .0001$ ) and similar to those observed for form-deprived eyes. For both +15 D-treated and control eyes, lenticular focal lengths for stimulated eyes were shorter than for their respective eyes at rest, and hysteresis effects were also significant ( $p < .0001$  for treated and control eyes). An overall refractive error effect ( $p = .0178$ ; two-way repeated measures ANOVA) and significant interaction ( $p = .0190$ ) of the refractive error and accommodation effects were also detected, again indicating that differences between +15 D-treated and control eyes were dependent on the accommodative state of the eye (Figure 5). Induction of hyperopia resulted in opposite effects to those observed for form-deprivation. For both the pre- and poststimulus accommodative states, focal lengths for the +15 D-treated eyes were longer than for their respective control eyes ( $p = .0069$  and  $p = .0053$ , respectively), by about 0.5 mm, or 1.75 D in power for both accommodative states. The findings that focal lengths for stimulated +15 D-treated were similar to those for stimulated control eyes ( $p = .3956$ ) but that means for resting eyes +15 D-treated eyes were inherently longer suggests that induction of hyperopic refractive error also affects lenticular accommodation. In contrast to results for form-deprived chickens, accommodative changes were greater, rather than smaller, for treated eyes than for their controls ( $p = .0415$ ; Figure 6). As in form-deprived birds, however, the amplitudes for recovery from accommodation in +15 D-treated birds were smaller than the amplitudes observed during accommodation ( $p < .0001$ ).

The results suggest that resting, baseline lenticular focal lengths are correlated to the refractive error of the eye. Prestimulus lenticular focal lengths were linearly regressed

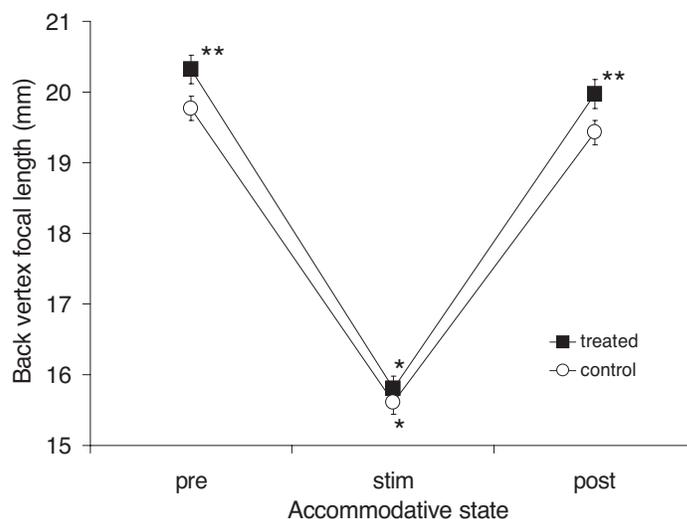


Figure 5. Mean back vertex focal lengths ( $\pm$ SEM) for lenses from +15 D lens-treated eyes (filled squares) and from their controls (open circles) at each accommodative state. For each eye, focal lengths denoted by asterisks were shorter than for those not marked ( $p < .05$ ; Bonferroni multiple comparison test). Means denoted by double asterisks were significantly longer than those for control eyes at the same accommodative state ( $p < .05$ ; Bonferroni multiple comparison test).

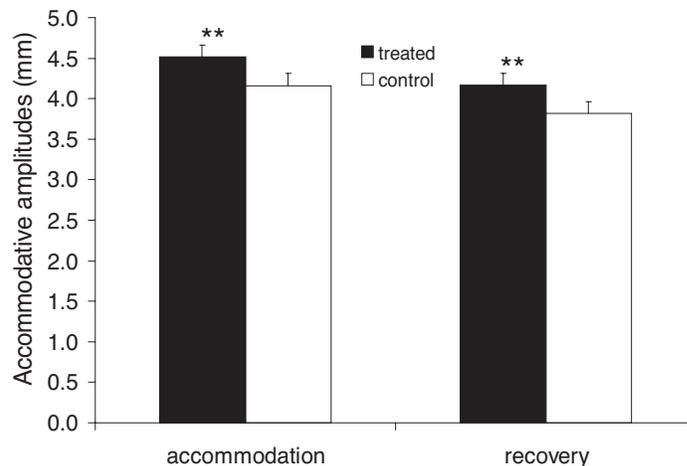


Figure 6. Mean accommodative and recovery amplitudes ( $\pm$ SEM) for lenses from +15 D lens-treated eyes (filled bars) and their controls (open bars). Mean amplitudes denoted by double asterisks were significantly longer than those for control eyes at the same accommodative state ( $p < .05$ ; Bonferroni multiple comparison test).

as a function of refractive error. For both form-deprived and +15 D-treated birds, correlations were extremely poor ( $R_2 = 0.027$  and  $R_2 = 0.038$ , respectively; data not shown) but significant ( $p < .0001$  and  $p = .0510$ , respectively).

### Effects of refractive error and accommodation on lenticular spherical aberration and optical quality

In general, optical scans showed negative spherical aberration (Figure 7). For both form-deprived and +15 D-treated chickens, refractive error had no effect on the amount of lenticular spherical aberration (Figures 8 and 9, respectively;  $p = .8937$  and  $p = .8068$ , respectively). In myopic birds, mean lenticular spherical aberration (SA) in stimulated eyes were greater than those in the pre- and poststimulus states (Figure 8;  $p < .0001$  and  $p = .0001$ , respectively), whereas no differences were detected between the means for the pre- and poststimulus states ( $p = .3798$ ). Eyes for hyperopic birds showed a similar pattern; spherical aberrations were similar for pre- and poststimulus eyes ( $p = .9611$ ), but significantly increased in stimulated eyes

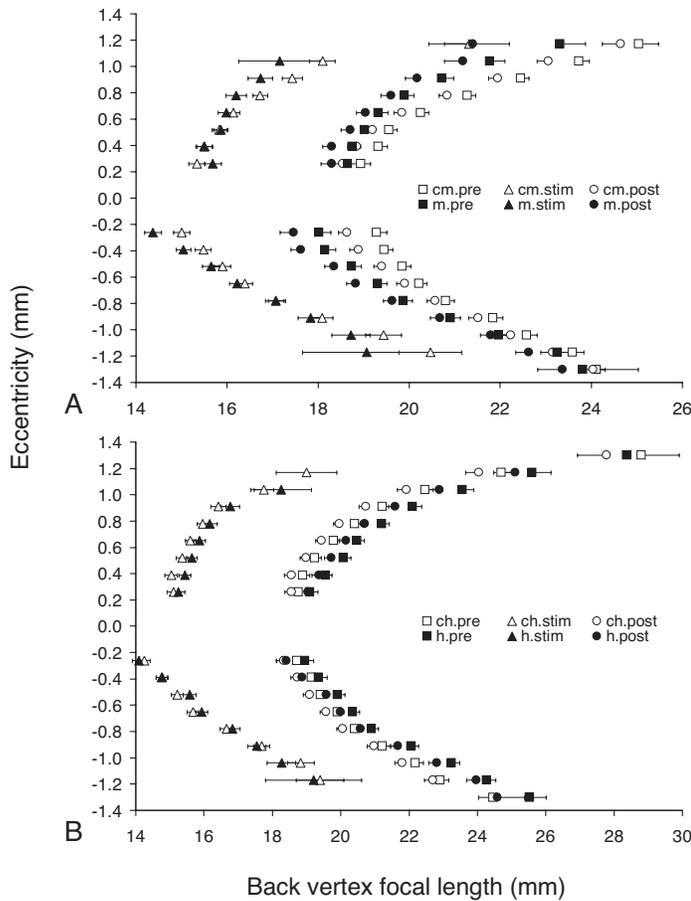


Figure 7. Mean back vertex focal lengths ( $\pm$ SEM) of lenses from form-deprived myopic (A) and +15 D lens-treated hyperopic chicks (B), plotted as a function of eccentricity from the optical center. Each data point represents a mean of a minimum of 3 values measured at that eccentricity. Lenses from treated (filled) and control (empty) eyes were optically scanned prior to stimulation (squares), during stimulation (triangles), and after stimulation (circle). Note that for all accommodative states, spherical aberrations are monotonic and clearly negative.

compared to the pre- and poststimulus states (Figure 9;  $p < .0001$  for both states).

To test the suitability of using the second-order function in the manner described above, SA was calculated in the traditional manner for resting (2.29 mm) and accommodating (1.63 mm) pupil sizes from the vertex of the polynomial function (Table 2). The pupil sizes were calculated by multiplying the eccentricity step size (0.13 mm; see above) by the mean number of beams passing through the pupil in prestimulus eyes and accommodating eyes, respectively (Table 1). The relationships between the all groups and accommodative states were found to be exactly the same statistically as calculations for the A-coefficient (Figures 8 and 9; statistical data not shown).

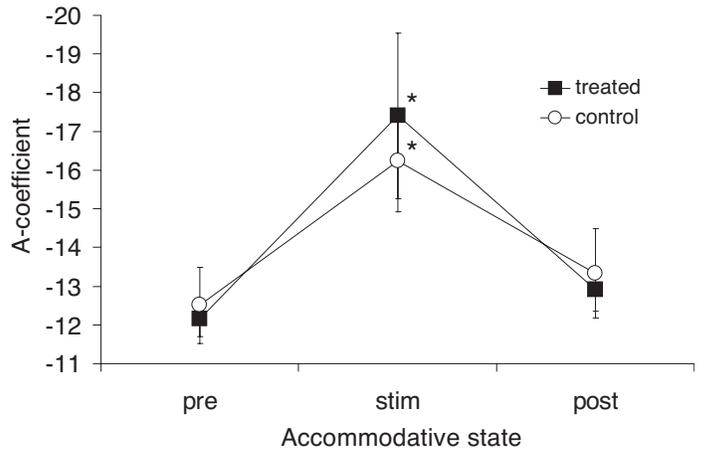


Figure 8. Mean parabolic A-coefficient value ( $\pm$ SEM) representing spherical aberrations for lenses from form-deprived eyes (filled squares) and their controls (empty circles). For each eye, means denoted by asterisks were significantly greater than those not marked ( $p < .05$ ; Bonferroni multiple comparison test).

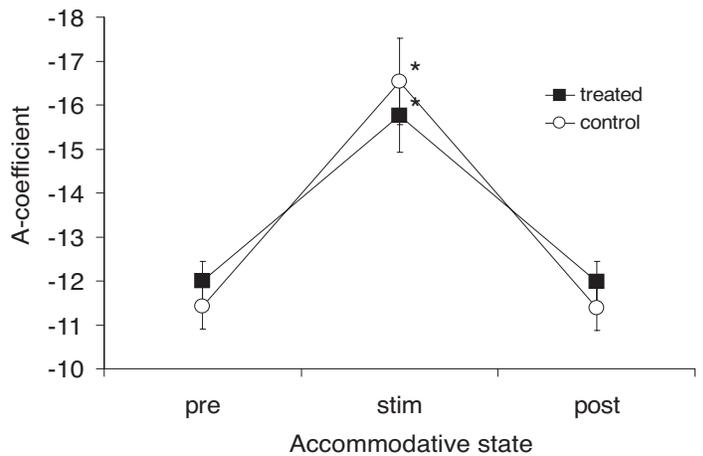


Figure 9. Mean parabolic A-coefficient value ( $\pm$ SEM) representing spherical aberrations for lenses from +15 D lens-treated eyes (filled squares) and their controls (empty circles). For each eye, means denoted by asterisks were significantly greater than those not marked ( $p < .05$ ; Bonferroni multiple comparison test).

Treatment		Pupil size (mm)	Lenticular spherical aberration ±SD (dioptres) for each accommodative state		
			pre	stim	post
Translucent goggle	control	1.63	-8.31 ± 3.65	-10.78 ± 4.82*	-8.85 ± 4.29
	treated		-8.07 ± 1.70	-11.56 ± 7.91*	-8.58 ± 2.04
	control	2.29	-16.40 ± 7.20	-21.27 ± 9.52*	-17.48 ± 8.47
	treated		-15.93 ± 3.35	-22.82 ± 15.62*	-16.93 ± 4.02
+15 D lens	control	1.63	-7.76 ± 1.89	-11.02 ± 4.10*	-7.79 ± 1.66
	treated		-7.92 ± 1.86	-10.53 ± 3.50*	-7.93 ± 1.94
	control	2.29	-15.31 ± 3.74	-21.76 ± 8.08*	-15.37 ± 3.28
	treated		-15.63 ± 3.67	-20.78 ± 6.91*	-15.65 ± 3.84

\*Significantly different than prestimulus state ( $p < .05$ ).

Table 2. Lenticular spherical aberrations ±SD (dioptres) for accommodation-sized (1.63 mm) and resting state-sized (2.29 mm) pupils in form-deprived and +15 D-treated birds at each accommodative state.

For both form-deprived and +15 D-treated birds, analysis of the mean residual RMS against the best-fitting quadratic function revealed an overall accommodation effect, with both groups of birds showing increased nonmonotonicity with accommodation (Figures 10 and 11;  $p < .0001$  for both). However, refractive error effects were slightly different; overall, the mean RMS in form-deprived eyes ( $n = 31$ ) was greater than in their controls ( $p = .0513$ ), but while the optical quality in +15 D-treated eyes was also slightly poorer than in their controls, the differences in the RMS were attenuated ( $p = .1325$ ; Figure 11). Together the results indicate that lenticular optical quality is degraded during accommodation, while the effects of refractive error are equivocal.

## Discussion

Given that intraocular structures remained in their natural anatomical configurations and that stimulation of the ciliary nerve resulted in accommodation, as measured by shorter focal lengths (Figures 3 and 5), the results presented here were taken to be representative of the functional optics of intact eyes. The fact that we have found a refractive error-associated difference in lenticular focal length while others have not (Priolo et al., 2000) would seem to indicate that measurements made with the lens in situ differ than when it is in vitro.

The results clearly indicate that much of the loss of lenticular accommodation in myopic eyes (Figure 4) may be

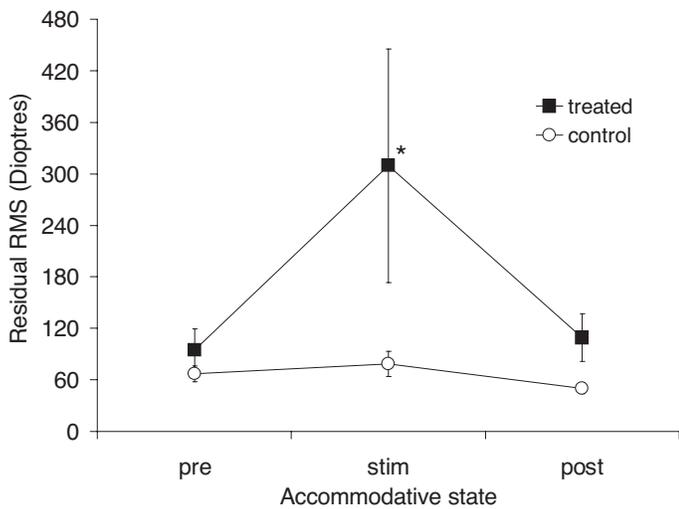


Figure 10. Mean residual root mean squares (RMS) of the best-fitting quadratic function for form-deprived (filled square) and their control (empty circles) eyes. Overall, the RMS for stimulated lenses were greater than those for the pre- and poststimulus states ( $p < .0001$  and  $p = .0002$ ; Bonferroni multiple comparison test), indicating increased nonmonotonicity with accommodation.

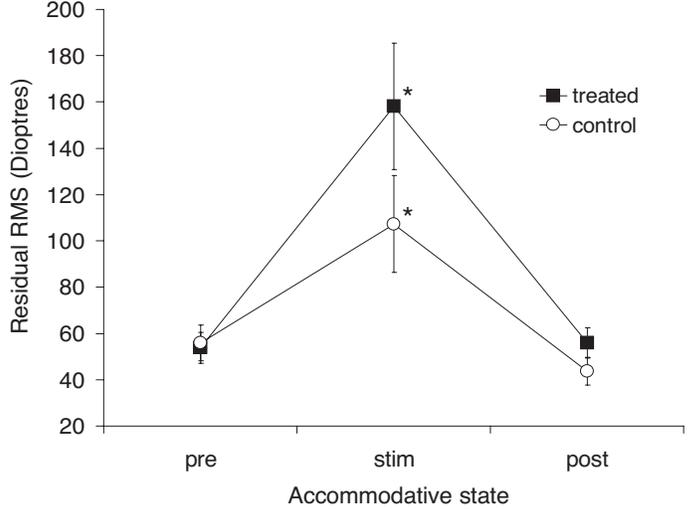


Figure 11. Mean residual root mean squares (RMS) of the best-fitting quadratic function for +15 D-treated eyes (filled square) and their controls (empty circles). Overall, the RMS for accommodating lenses were greater than those for eyes at rest ( $p < .0001$ ; Bonferroni multiple comparison test), indicating increased nonmonotonicity with accommodation.

attributed to the inherently shorter lenticular focal lengths exhibited by myopic lenses at rest (Figure 3). Shorter lenticular focal lengths may be attributed to changes in the lenticular refractive index or in the shape of the lens, or both. To date, no ametropia-associated changes to the total lenticular protein content nor to lenticular  $\alpha$ A- and  $\delta$ -crystallin levels have been found (Pickett-Seltner, Sivak, & Pasternak, 1988; Zaidi, Senchyna, & Sivak, 2002). Instead, the focal length differences in the present study are likely influenced by the shape of the lens. An ultrasound biomicroscopy (UBM) study of lenses from ametropic chickens of the same age that were similarly treated showed that of 12 monocularly form-deprived chicks, all of the resting lenses in situ of the myopic eyes were thicker than in their controls, although differences were too small to be significantly different (Choh, Sivak, Irving, & Wong, 2002). While the results for lenticular thicknesses in hyperopic eyes in the UBM study were equivocal, other studies show that lenses from hyperopic eyes, made so by constant light exposure or optic nerve section, tend to be thinner (Li, Troilo, Glasser, & Howland, 1995; Wildsoet, 2003). Together, the results suggest that if there are lenticular shape changes, they are likely associated with refractive error-associated global changes to the eye.

The shorter lenticular focal lengths in myopic eyes in the present study might be related to contraction of the ciliary muscle (the ciliary muscle tone is increased in these eyes). It should be noted that others have shown that accommodation in myopes is reduced compared to emmetropes (Gilmartin & Bullimore, 1991; Gwiazda, Thorn, Bauer, & Held, 1993), a finding that is consistent with our results. However, in their study (Gilmartin & Bullimore, 1991) and in a separate study (Gwiazda, Bauer, Thorn, & Held, 1995), these investigators found that tonic accommodation levels in myopes were lower compared to emmetropes, which is opposite to our results if we consider resting lenticular focal lengths of the present study to be comparable to tonic accommodation. Perhaps the mode of accommodation may be a factor, given that accommodation in chickens is achieved by the ciliary body directly pushing on the lens, whereas in humans, accommodation occurs by relaxation of zonules attached to the ciliary muscle. However, several studies indicate that the ciliary muscle is not involved in experimentally induced ametropias (Schwahn & Schaeffel, 1994; West, Sivak, & Doughty, 1991).

Lenticular shape differences may be independent of the accommodative apparatus. Ronkina, Chabrova, Borisova, Vasin, and Bagrova (1989) suggest that the posterior capsule in myopic eyes is thicker than in emmetropic eyes. It is possible that the lens capsules in the myopic eyes of the present study were also thicker, although capsular changes were never tested. Although less likely, it must be considered that putative changes to the crystalline lens shape are cellular in nature. Given that the lens is enclosed within the eye, it is constantly exposed to and cannot avoid any factors that may be released or up-regulated by the retina in

response to the imposed blurs. Changes to the lens may therefore be a side effect of the growth changes that are occurring within the eye. That the lens responds in a specific manner to distinct visual cues, contributing to the final refractive error of the eye rather than reducing its effect, may imply that the crystalline lens is genetically pre-programmed to respond to specific putative retinal factors, or that the lens itself is capable of distinguishing and up- or down-regulating its own growth changes. It currently remains unknown what these putative signals are, if they exist, and whether regulation involves up- or down-regulation of receptors in the lens or not. It must be noted that there are inherent limitations with altered lenticular fiber growth as a mechanism for lens thinning. Unlike the rest of the eye, the lens grows throughout life, with "shells" or concentric layers of fiber cells continuously added to preexisting layers of the lens, and under normal circumstances, fiber cells do not die or become phagocytosed; the lens contains all of its original cells, with the oldest cells compacted toward the center of the lens. There is no additional mechanism to alleviate growth changes, unlike the eye itself, which can rely on thickening of the choroid to further reduce retinal distance from the anterior of the eye. Moreover, thinning of the lens cannot rely on compaction of fiber cells toward the center of the lens because the refractive power of the lens would increase and the eye would become more myopic.

Given that positive spectacle lenses were able to induce crystalline lenticular responses, and moreover, that these responses were opposite to those for myopic birds, it may be speculated that imposition of a hyperopic defocus, by a negative or concave lens, would result in similar effects as for form-deprivation myopia. Although studies exist that show that the eye is capable of discriminating between, and responds differently to, form-deprivation and lens-induced myopia (Bartmann, Schaeffel, Hagel, & Zrenner, 1994; Kee, Marzani, & Wallman, 2001; Schaeffel, Bartmann, Hagel, & Zrenner, 1995; Schaeffel, Hagel, Bartmann, Kohler, & Zrenner, 1994), the majority of studies indicate that the ultimate overall growth effects (enlarged vitreous chamber, thinner choroids) of the two treatments are similar (Irving et al., 1992; Norton, 1999; Troilo & Wallman, 1991; Wallman et al., 1995; Wildsoet & Wallman, 1995). However, as this paradigm was untested, the effects of hyperopic defocus on lenticular accommodation remain unknown.

The results presented here clearly show that lenticular focal lengths are affected by form-deprivation and by myopic defocus, whereas in a previous study, Priolo and colleagues (2000) did not find any refractive error-related differences in lenticular focal length. It should be noted that in addition to the greater number of chickens tested here, there are a couple of other differences between the study here and the other report (Priolo et al., 2000). In the present study, focal lengths were measured from lenses in situ, whereas previously, lenses were excised. It has been shown that excision of lenses causes them to "round up" (Glasser,

Murphy, Troilo, & Howland, 1995). Given the possibility that the refractive error-associated differences in focal length reported here may be attributable to ciliary muscle-associated changes of the eye, isolation of the lens from the accommodative apparatus may have inadvertently caused neutralizing effects in the prior study. Calculations of mean lenticular focal lengths also differ; unlike in the previous study, focal lengths at the lens sutures were omitted because of the unpredictably variable focal lengths associated with this region (Bantsev et al., 1999; Kuszak et al., 1994; Sivak et al., 1994), which can result in the masking of smaller effects. The combination of these experimental and analytical modifications may together account for the different results observed for this and the previous study (Priolo et al., 2000).

The findings that the already negative lenticular spherical aberrations increase with accommodation in both myopic and hyperopic birds are consistent with several studies showing more negative spherical aberrations with accommodation in human eyes (Collins, Wildsoet, & Atchison, 1995; Ninomiya et al., 2003; Ninomiya et al., 2002) and with a wavefront aberration study by He and colleagues (2003) showing that RMS values for lenticular spherical aberrations increase with accommodation. However, we could not find a refractive error association for mean spherical aberrations, a result that is also consistent with the report by Collins et al. (1995).

Our finding that lenticular optical errors were greater in form-deprived but not so much in +15 D-treated birds differs slightly from a previous report that showed lenticular optical quality was degraded in lenses of both form-deprived and +10 D-treated eyes (Priolo et al., 2000). Again there are some differences in the calculations between the two studies; Priolo and colleagues (2000) used focal length variability as a measure of optical quality, where high variability indicated poor optical quality, whereas we have used the deviation (residual RMS) from the best-fitting parabola to indicate the amount of nonmonotonicity. The advantage of using parabola-based calculations lies in the ability to account for the sign and amount of the spherical aberration, which may be important when considering aberrations of the whole eye.

Because refractive effects of the cornea were neutralized in the work presented here, it remains unknown whether the cornea would otherwise play a role in counteracting or augmenting spherical aberration in chicken eyes in toto. Several studies report that aberrations of the crystalline lens are eliminated by equal and opposite aberrations of the cornea, resulting in zero aberrations for the whole eye (Artal, Guirao, Berrio, & Williams, 2001; Sivak, 1982). Chickens also undergo corneal accommodation (in air), which has additional implications in the amount of spherical aberration of the whole eye during accommodation. Glasser and colleagues found a steepening of the central portion of the cornea and a flattening at the peripheral portion in electrically stimulated, excised chick eyes (Glasser et al., 1994), indicating that (in air) the accommo-

dating cornea would contribute negative spherical aberration to the whole eye. Interestingly, He and colleagues (2003) also report an accommodation-associated change in corneal shape and wavefront aberrations in humans, a species in which corneal accommodation has traditionally been thought to be nonexistent. While the contributions of the corneal aberrations in humans were found to be very small in comparison to the contribution of the lens, the shape changes of the cornea (steeper center and flatter periphery) that accompanied accommodation again indicate a negative spherical aberration contribution to the whole eye (He et al., 2003).

The question of the relationship between ametropia and accommodation has a very long history, going back to at least the time of Donders (1864), and the matter is still unresolved. The chick eye model described in this work shows that the lenticular accommodative apparatus is affected by ametropia. Thus, this accommodation model may be a useful approach in the effort to resolve this issue.

## Appendix

### Unaccommodating eye

Estimate of corneal radius of curvature for 7-day chicks (Schaeffel & Howland, 1988):

$$y = 2.2904 + 0.0759x - 9.447e^{-4x^2} + 4.583e^{-6x^3} \quad (1)$$

$$= 2.8217\text{mm}$$

Total corneal power in air (effective index = 1.332):

$$F_T = \frac{n' - n}{r_1} = \frac{1.332 - 1}{0.0028217} = 117.65957\text{D} \quad (2)$$

True front corneal surface power ( $n' = 1.373$ ) in air:

$$F_1 = \frac{n' - n}{r_1} = \frac{1.373 - 1}{0.0028217} = 132.18981\text{D} \quad (3)$$

Back corneal surface power, from lensmaker's equation ( $F_T = F_1 + F_2 - F_1 F_2 \delta$ ), Equation 2, Equation 3, and corneal thickness,  $\delta = 0.24$  mm, independent of age (Schaeffel & Howland, 1988):

$$F_2 = \frac{F_T - F_1}{1 - F_1 \delta} = -15.006329\text{D} \quad (4)$$

Back corneal radius of curvature:

$$r_2 = \frac{n' - n}{F_2} = \frac{1.335 - 1.373}{-15.006329} = 0.0025322\text{m} \quad (5)$$

Power of front and back corneal surfaces for eye in water, respectively:

$$F_1 = \frac{1.373 - 1.333}{0.0028217} = 14.175851\text{D}, \quad (6)$$

$$F_2 = \frac{1.335 - 1.373}{0.0025322} = -15.006714\text{D} \quad (7)$$

Total corneal power ( $n' = 1.373$ ) for eye in water using Equations 6 and 7:

$$\begin{aligned} F_T &= F_1 + F_2 - F_1 F_2 \delta \\ &= -0.7798071\text{D} \end{aligned} \quad (8)$$

## Accommodating eye

Maximal corneal accommodative change (9 D) (Glasser et al., 1994) plus normal corneal power in air:

$$F_T = 9 + 117.65957 = 126.65957\text{D} \quad (9)$$

Front corneal radius of curvature from effective index ( $n' = 1.332$ ):

$$r_1 = \frac{n' - n}{F_1} = \frac{1.332 - 1}{126.65957} = 0.0026211\text{m} \quad (10)$$

True front corneal surface power ( $n' = 1.373$ ) in air:

$$F_1 = \frac{n' - n}{r_1} = \frac{1.373 - 1}{0.0026211} = 142.30667\text{D} \quad (11)$$

Back corneal surface power in air, using Equations 9, 10, and 11:

$$F_2 = \frac{F_T - F_1}{1 - F_1 \delta} = -16.200402\text{D} \quad (12)$$

Back corneal radius of curvature:

$$r^2 = \frac{n' - n}{F_2} = \frac{1.335 - 1.373}{-16.200402} = 0.0023456\text{m} \quad (13)$$

Power of front and back corneal surfaces for eye in water, respectively:

$$F_1 = \frac{1.373 - 1.333}{0.0026211} = 15.260768\text{D}, \quad (14)$$

$$F_2 = \frac{1.335 - 1.373}{0.0023456} = -16.200546\text{D} \quad (15)$$

Total corneal power for eye in water using Equation 14 and 15:

$$\begin{aligned} F_T &= F_1 + F_2 - F_1 F_2 \delta \\ &= -0.8804422\text{D} \end{aligned} \quad (16)$$

Maximal corneal power of an accommodating eye in water from Equations 8 and 16:

$$-0.8804422 - (-0.7798071) = -0.1006351\text{D} \quad (17)$$

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## References

- Artal, P., Guirao, A., Berrio, E., & Williams, D. R. (2001). Compensation of corneal aberrations by the internal optics in the human eye. *Journal of Vision*, 1(1), 1-8, <http://journalofvision.org/1/1/1/>, doi:10.1167/1.1.1. [PubMed][Article]
- Bantsev, V. L., Herbert, K. L., Trevithick, J. R., & Sivak, J. G. (1999). Mitochondria of rat lenses: Distribution near and at the sutures. *Current Eye Research*, 19(6), 506-516. [PubMed]
- Bartmann, M., Schaeffel, F., Hagel, G., & Zrenner, E. (1994). Constant light affects retinal dopamine levels and blocks deprivation myopia but not lens-induced refractive errors in chickens. *Visual Neuroscience*, 11(2), 199-208. [PubMed]
- Choh, V., Sivak, J. G., Irving, E. L., & Wong, W. (2002). Ultrasound biomicroscopy of the anterior segment of the enucleated chicken eye during accommodation. *Ophthalmic and Physiological Optics*, 22(5), 401-408. [PubMed]
- Choh, V., Sivak, J. G., & Meriney, S. D. (2002). A physiological model to measure effects of age on lenticular accommodation and spherical aberration in chickens. *Investigative Ophthalmology and Visual Science*, 43(1), 92-98. [PubMed]
- Collins, M. J., Wildsoet, C. F., & Atchison, D. A. (1995). Monochromatic aberrations and myopia. *Vision Research*, 35(9), 1157-1163. [PubMed]
- Donders, F. C. (1864). *On the anomalies of accommodation and refraction of the eye: With a preliminary essay on physiological dioptrics*. London: The New Sydenham Society.
- Gilmartin, B., & Bullimore, M. A. (1991). Adaptation of tonic accommodation to sustained visual tasks in emmetropia and late-onset myopia. *Optometry and Vision Science*, 68(1), 22-26. [PubMed]

- Glasser, A., Murphy, C. J., Troilo, D., & Howland, H. C. (1995). The mechanism of lenticular accommodation in chicks. *Vision Research*, 35(11), 1525-1540. [PubMed]
- Glasser, A., Troilo, D., & Howland, H. C. (1994). The mechanism of corneal accommodation in chicks. *Vision Research*, 34(12), 1549-1566. [PubMed]
- Gwiazda, J., Bauer, J., Thorn, F., & Held, R. (1995). Shifts in tonic accommodation after near work are related to refractive errors in children. *Ophthalmic and Physiological Optics*, 15(2), 93-97. [PubMed]
- Gwiazda, J., Thorn, F., Bauer, J., & Held, R. (1993). Myopic children show insufficient accommodative response to blur. *Investigative Ophthalmology and Visual Science*, 34(3), 690-694. [PubMed]
- He, J. C., Gwiazda, J., Thorn, F., Held, R., & Huang, W. (2003). Change in corneal shape and corneal wavefront aberrations with accommodation. *Journal of Vision*, 3(7), 456-463, <http://journalofvision.org/3/7/1/>, doi:10.1167/3.7.1. [PubMed][Article]
- Hung, L. F., Crawford, M. L., & Smith, E. L. (1995). Spectacle lenses alter eye growth and the refractive status of young monkeys. *Nature Medicine*, 1(8), 761-765. [PubMed]
- Irving, E. L., Sivak, J. G., & Callender, M. G. (1992). Refractive plasticity of the developing chick eye. *Ophthalmic and Physiological Optics*, 12(4), 448-456. [PubMed]
- Jenkins, F. A., & White, H. E. (1957). *Fundamentals of optics* [by] Francis A. Jenkins [and] Harvey E. White (3d ed.). New York: McGraw-Hill.
- Kee, C. S., Marzani, D., & Wallman, J. (2001). Differences in time course and visual requirements of ocular responses to lenses and diffusers. *Investigative Ophthalmology and Visual Science*, 42(3), 575-583. [PubMed]
- Kuszak, J. R., Peterson, K. L., Sivak, J. G., & Herbert, K. L. (1994). The interrelationship of lens anatomy and optical quality. II. Primate lenses. *Experimental Eye Research*, 59(5), 521-535. [PubMed]
- Li, T., Troilo, D., Glasser, A., & Howland, H. C. (1995). Constant light produces severe corneal flattening and hyperopia in chickens. *Vision Research*, 35(9), 1203-1209. [PubMed]
- Lin, L. L., Shih, Y. F., Tsai, C. B., Chen, C. J., Lee, L. A., Hung, P. T., et al. (1999). Epidemiologic study of ocular refraction among schoolchildren in Taiwan in 1995. *Optometry and Vision Science*, 76(5), 275-281. [PubMed]
- Ninomiya, S., Fujikado, T., Kuroda, T., Maeda, N., Tano, Y., Hirohara, Y., et al. (2003). Wavefront analysis in eyes with accommodative spasm. *American Journal of Ophthalmology*, 136(6), 1161-1163. [PubMed]
- Ninomiya, S., Fujikado, T., Kuroda, T., Maeda, N., Tano, Y., Oshika, T., et al. (2002). Changes of ocular aberration with accommodation. *American Journal of Ophthalmology*, 134(6), 924-926. [PubMed]
- Norton, T. T. (1999). Animal models of myopia: Learning how vision controls the size of the eye. *ILAR Journal*, 40(2), 59-77. [PubMed]
- Pickett-Seltner, R. L., Sivak, J. G., & Pasternak, J. J. (1988). Experimentally induced myopia in chicks: Morphometric and biochemical analysis during the first 14 days after hatching. *Vision Research*, 28(2), 323-328. [PubMed]
- Pilar, G., Nunez, R., McLennan, I. S., & Meriney, S. D. (1987). Muscarinic and nicotinic synaptic activation of the developing chicken iris. *Journal of Neuroscience*, 7(12), 3813-3826. [PubMed]
- Priolo, S., Sivak, J. G., Kuszak, J. R., & Irving, E. L. (2000). Effects of experimentally induced ametropia on the morphology and optical quality of the avian crystalline lens. *Investigative Ophthalmology and Visual Science*, 41(11), 3516-3522. [PubMed]
- Ronkina, T. I., Chabrova, L. S., Borisova, L. M., Vasin, V. I., & Bagrova, S. N. (1989). The biomechanical properties of the crystalline lens capsule in emmetropia and myopia. *Oftalmologicheskii Zhurnal*, 7, 420-425. [PubMed]
- Saw, S. M., Chua, W. H., Wu, H. M., Yap, E., Chia, K. S., & Stone, R. A. (2000). Myopia: Gene-environment interaction. *Annals of the Academy of Medicine, Singapore*, 29(3), 290-297. [PubMed]
- Schaeffel, F., Bartmann, M., Hagel, G., & Zrenner, E. (1995). Studies on the role of the retinal dopamine/melatonin system in experimental refractive errors in chickens. *Vision Research*, 35(9), 1247-1264. [PubMed]
- Schaeffel, F., Glasser, A., & Howland, H. C. (1988). Accommodation, refractive error and eye growth in chickens. *Vision Research*, 28(5), 639-657. [PubMed]
- Schaeffel, F., Hagel, G., Bartmann, M., Kohler, K., & Zrenner, E. (1994). 6-Hydroxy dopamine does not affect lens-induced refractive errors but suppresses deprivation myopia. *Vision Research*, 34(2), 143-149. [PubMed]
- Schaeffel, F., & Howland, H. C. (1988). Visual optics in normal and ametropic chickens. *Clinical Vision Science*, 3(2), 83-98.
- Schwahn, H. N., & Schaeffel, F. (1994). Chick eyes under cycloplegia compensate for spectacle lenses despite six-hydroxy dopamine treatment. *Investigative Ophthalmology and Visual Science*, 35(9), 3516-3524. [PubMed]

- Siegwart, J. T., & Norton, T. T. (1993). Refractive and ocular changes in tree shrews raised with plus or minus lenses. *Investigative Ophthalmology and Visual Science*, 34, S1208.
- Sivak, J. G. (1982). The contribution of the crystalline lens to chromatic and spherical aberrations of the eye. *Canadian Journal of Optometry*, 44(2), 89-91.
- Sivak, J. G., Herbert, K. L., Peterson, K. L., & Kuszak, J. R. (1994). The interrelationship of lens anatomy and optical quality. I. Non-primate lenses. *Experimental Eye Research*, 59(5), 505-520. [PubMed]
- Smith, W. J. (2000). *Modern optical engineering: The design of optical systems* (3rd ed.). New York: McGraw-Hill.
- Troilo, D., Gottlieb, M. D., & Wallman, J. (1987). Visual deprivation causes myopia in chicks with optic nerve section. *Current Eye Research*, 6(8), 993-999. [PubMed]
- Troilo, D., & Wallman, J. (1991). The regulation of eye growth and refractive state: An experimental study of emmetropization. *Vision Research*, 31(7-8), 1237-1250. [PubMed]
- Wallman, J., Wildsoet, C., Xu, A., Gottlieb, M. D., Nickla, D. L., Marran, L., et al. (1995). Moving the retina: Choroidal modulation of refractive state. *Vision Research*, 35(1), 37-50. [PubMed]
- West, J. A., Sivak, J. G., & Doughty, M. J. (1991). Role of accommodation in experimental myopia in chicks. *Optometry and Vision Science*, 68(11), 847-852. [PubMed]
- Wildsoet, C. (2003). Neural pathways subserving negative lens-induced emmetropization in chicks—insights from selective lesions of the optic nerve and ciliary nerve. *Current Eye Research*, 27(6), 371-385. [PubMed]
- Wildsoet, C., & Wallman, J. (1995). Choroidal and scleral mechanisms of compensation for spectacle lenses in chicks. *Vision Research*, 35(9), 1175-1194. [PubMed]
- Wu, H. M., Seet, B., Yap, E. P., Saw, S. M., Lim, T. H., & Chia, K. S. (2001). Does education explain ethnic differences in myopia prevalence? A population-based study of young adult males in Singapore. *Optometry and Vision Science*, 78(4), 234-239. [PubMed]
- Young, F. A., Leary, G. A., Baldwin, W. R., West, D. C., Box, R. A., Harris, E., et al. (1969). The transmission of refractive errors within Eskimo families. *American Journal of Optometry and Archives of American Academy of Optometry*, 46(9), 676-685. [PubMed]
- Zaidi, S., Senchyna, M., & Sivak, J. G. (2002). Quantification of chick lens alphaA- and delta-crystallins in experimentally induced ametropia. *Molecular Vision*, 8, 472-476. [PubMed][Article]