Choroidal Thickness Changes during Altered Eye Growth and Refractive State in a Primate

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PURPOSE. In the chick, compensation for experimentally induced defocus involves changes in the thickness of the choroid. The choroid thickens in response to imposed myopic defocus and thins in response to imposed hyperopic defocus. This study was undertaken to determine whether similar choroidal changes occur in the primate eye with induced refractive errors.

METHODS. Thirty-three common marmosets were used. Eyes in 26 monkeys served as untreated control eyes, and eyes in 7 received 3 weeks of monocular lid suture to induce changes in eye growth and refractive state. Refractive errors were measured using refractometry and retinoscopy, and axial ocular dimensions, including choroidal thickness, were measured using high-frequency A-scan ultrasonography. Eyes were measured before the lids were sutured and at frequent intervals after lid opening.

RESULTS. In the marmoset, choroidal thickness ranges from 88 to 150 μm and increases significantly during the first year of life. Monocular lid suture initially results in short, hyperopic eyes that then become elongated and myopic. In these animals the choroids of both the experimental and the fellow control eyes also increase in thickness with age but additionally show interocular differences that vary significantly with the relative changes in vitreous chamber depth and refraction. In eyes that are shorter and more hyperopic than control eyes the choroids are thicker, and in eyes that are longer and more myopic than control eyes the choroids are thinner.

CONCLUSIONS. In marmosets, the thickness of the choroid increases during postnatal eye growth. Superimposed on this developmental increase in choroidal thickness there are changes in thickness that are correlated with the induced changes in eye size. These changes are small (<50 μm) in comparison with those observed in the chick, contributing to less than a diopter change in refractive error. (Invest Ophthalmol Vis Sci. 2000;41:1249–1258)

Evidence from several species, including primates, indicates that the growth of the eye is an active, visually guided process. The strongest evidence for this visual regulation comes from studies showing that eyes wearing spectacle lenses compensate for the imposed refractive errors (chicks,1,2 macaques,3,4 marmosets,5 and tree shrews6).

In chicks, compensation for imposed defocus occurs through two mechanisms that affect vitreous chamber depth. One involves modulation of the growth of the sclera,7–11 and the other involves changes in the thickness of the choroid.12,13 Specifically, eyes wearing positive lenses, in which the image plane is in front of the retina, show a thickening of the choroid that pushes the retina forward toward the image plane, which is followed by changes in scleral extracellular matrix synthesis and decreased eye growth. Conversely, eyes wearing negative lenses, in which the image plane is behind the retina, show a thinning of the choroid that pulls the retina back toward the image plane, followed by changes in scleral extracellular matrix synthesis and increased eye growth.

There is circumstantial evidence from the chick model suggesting that the choroidal thickness and scleral growth mechanisms are causally related. First, the compensatory changes in choroidal thickness occur within a few hours and precede the compensatory axial growth changes.14 Second, the phase relationships between diurnal rhythms in choroidal thickness and axial length differ, depending on the rate of eye growth; in normal and fast-growing eyes, the two are in antiphase with one another, whereas in slow-growing eyes the two are in phase with one another.15 Third, in tissue culture experiments,16 the condition of the choroid (thin from fast-growing form-deprived myopic eyes or thick from slow-growing eyes recovering from form deprivation myopia) was found to alter the rate of scleral proteoglycan synthesis. Although these findings suggest a causal relationship between choroid thickness, eye growth, and, ultimately, refractive state, it is also possible that choroidal thickness change in chick eyes is a form of long-term accommodation12,17 and is unrelated to the mechanism underlying the visually dependent changes in eye growth.

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It is unknown whether choroidal thickness changes occur in mammalian and primate eyes during development and how they relate to the control of ocular growth. Similar to chicks, mammalian scleras undergo changes in proteoglycan synthesis that are associated with induced changes in refractive state. Proteoglycan synthesis in tree shrews and marmoset monkeys is reduced during form deprivation myopia\textsuperscript{18,19} and is increased in tree shrews during recovery from form deprivation myopia.\textsuperscript{20} If changes in the choroid are causally related to changes in scleral proteoglycan synthesis and, consequently, eye growth and refractive state, we would expect to see changes in the thickness of primate choroids after experimentally induced refractive errors.

We report on the choroidal thickness changes of the marmoset monkey measured in vivo during normal ocular development and experimentally manipulated eye growth. We show that choroidal thickness in the marmoset can be reliably measured and that small but significant changes are correlated with induced changes in eye growth and refractive state.

**METHODS**

**Subjects**

Use of the animals in this study was in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and was approved by the New England College of Optometry Institutional Animal Care and Use Committee in accordance with Public Health Service Policy on Humane Care and Use of Laboratory Animals and US Department of Agriculture Animal Welfare Regulations.

Thirty-three common marmosets (*Callitrichus jacchus*) were used in this study. Untreated eyes in 26 animals provided normal control data for various ages (control eyes). Refractive errors were induced monocularly in the remaining seven animals by lid suture (experimental eyes). The contralateral untreated eyes of these animals served as interocular controls (fellow control eyes).

All marmosets were housed in family groups in our marmoset breeding colony. Artificial lighting was provided by daylight-balanced fluorescent lamps (Vita-Light, Fairfield, NJ) on a 12-hour light–12-hour dark cycle. Temperature was maintained at 75 ± 2°F and humidity at 45% ± 5%. Food and water were provided ad libitum. Food consisted of formulated dry pellets (Marmoset Lite; Animal Spectrum, North Platte, NE) with fresh fruit and protein supplements. Vitamin D₃ supplementation was provided twice weekly.

**Treatments and Measurements**

Monocular lid suture was performed at 10 to 17 days of age (mean, 13 days). The lids were reopened 14 to 28 days later (mean, 23 days). After this manipulation, axial hyperopia (experimental eye shorter than normal) is typically observed at lid opening and changes over time to axial myopia (experimental eye longer than normal) without recovery.\textsuperscript{21}

Lid suture surgery was performed on anesthetized animals (0.2 ml/100 g: alphaxalone (0.9%), alphadoline acetate (0.3%) [Saffan]; Pitman-Moore, UK) as previously described.\textsuperscript{21} The lid margins were trimmed, and the tarsal plate was separated from the lid. The upper and lower tarsi were sutured together with 7-0 polyvicryl, and the upper and lower lids were then suftered together with 5-0 silk, leaving a small (1–2 mm) drainage opening at the nasal canthus. The outer sutures were removed 5 to 7 days after lid fusion. To conclude the deprivation period, the lids were reopened along the fusion line, with animals under Saffan anesthesia, and healed without notable lid dysfunction.

Refractive errors were measured by refractometry and streak retinoscopy in animals under Saffan anesthesia. A lid retractor was used to hold the lids open. Cycloplegia was induced with 2 drops of 1% cyclopentolate applied topically 5 to 10 minutes apart; refractions were measured 60 minutes later. This procedure reliably produces maximal cycloplegia in young marmosets.\textsuperscript{22} Retinoscopy was performed first, followed by refractometry with a Hartinger Coincidence Refractometer (Carl Zeiss, Oberkochen, Germany). The two measurements were always performed independently by the same two investigators. All refraction data presented are the means of the retinoscopic and refractometry data expressed as equivalent spherical refractive errors derived by averaging the two principal meridians in each case.

Axial ocular dimensions, including choroidal thickness, were measured using high-frequency A-scan ultrasonography. Our system is based on that developed by Wildsoet and Wallman\textsuperscript{13} (Department of Biology, City College of New York, NY). We used a 33-MHz piezoelectric immersion transducer (model PZ500.25SU-R1.00; Panametrics, Waltham, MA) driven by an ultrasound pulser receiver (model 5072 PR-15U; Panametrics). The transducer was coupled to the eye with a 16-mm water-filled stand-off that positions the focal zone of the sound wave inside the vitreous chamber of marmoset eyes at all ages. The ultrasound signal was digitized for analysis using a 100-MHz analog-to-digital conversion board (model STR-8100; Sonix, Springfield, VA). The high-frequency transducer coupled with the sampling rate of the digitizing board provided good resolution of the echoes returning from the posterior ocular tunics. Figure 1 shows an example of a typical ultrasound trace from a marmoset eye. The echoes from the posterior ocular tunics are expanded to show the thicknesses of the retina, choroid, and sclera. Each echo gives rise to a complex of peaks. The specific peaks used as measurement reference points were selected based mainly on their consistent appearance within repeated measurements and between different individuals (see Fig. 2). In chicks, peaks similarly identified have also been cross-referenced to histology and other in vivo measurements of the posterior ocular tunics.\textsuperscript{23} Echo latencies arising from the different ocular surfaces were converted to distances between surfaces using estimates of the velocity of sound in the different media of chick eyes.\textsuperscript{24} The thicknesses of the retina, choroid, and sclera were calculated using the velocity of sound in vitreous humor (1.534 m/sec).

The distance from the posterior lens surface to the inner surface of the sclera was used as our measure of axial length. This was chosen as our axial length measure, because this dimension includes the choroid and is therefore a direct measure of the growth of the back of the eye. In addition, excluding the anterior chamber eliminates the additional variable of corneal curvature (short-duration lid suture has been shown to affect transiently both corneal curvature and therefore anterior chamber depth in marmosets).\textsuperscript{21}

Ultrasound data for each eye are the average of a minimum of eight individual traces. The eye was typically realigned with the ultrasound probe several times over the course of measurement. We used as a criterion for proper alignment echoes of
approximately equal amplitude from the anterior and posterior lens surface. On average, the standard errors of the mean of repeated measurements were less than 10 μm for vitreous chamber depth, and less than 5 μm for choroidal thickness. The precision of the ultrasound measures was determined by measuring one eye of an individual marmoset eight times during a 15-minute period. The SD of the mean difference between repeated measurements was 3.6 μm for vitreous chamber depth and 11 μm for choroidal thickness.

RESULTS
Development of Choroidal Thickness in Control Eyes
Complete refractive error and ocular dimension data were obtained from 26 untreated marmosets between the ages of 26 and 308 days. The data for right and left eyes did not differ significantly for any of the parameters measured. Therefore, the data shown from untreated animals (Figs. 3 and 4) are from the right eyes only.

The eyes of normal untreated marmosets undergo emmetropization during the first postnatal year.21 There is a rapid increase in axial length (Fig. 3, top) that accounts for the concurrent reduction in hyperopia (Fig. 3, bottom). The mean choroidal thickness pooled over all ages was 120 ± 15 μm and ranged from 88 to 150 μm. Figure 4 (top) shows that during the period of emmetropization, the thickness of the choroid increased significantly (ANOVA, P < 0.01) by 28% from infancy (100 ± 7 μm) to 300 days of age (128 ± 16 μm). Most of the increase in choroidal thickness (69.1%) occurred during the first 150 days when most of the axial growth (70.5%) also occurred (Fig. 4, bottom) and corresponded to the period of rapid emmetropization. During this period the rate of choroidal thickening was approximately 0.3 μm/mm of axial growth.
It has been reported in young marmosets that 3 weeks of visual form deprivation by lid suture results in axial hyperopia that eventually becomes axial myopia over time after the opening of the lids. The present results confirm this observation. At the time of lid opening, experimental eyes were, on average, more hyperopic and shorter than their fellow control eyes. By the end of the monitoring period, the experimental eyes had become more myopic and longer than the control eyes. Figure 5 shows, plotted against time, the refractive error (top) and vitreous chamber depth (bottom) expressed as the interocular difference between experimental eyes and their contralateral control eyes. The changes over time in both refractive error and vitreous chamber depth were statistically significant (ANOVA, \(P < 0.01\)).

Significant interocular differences in choroid thickness were correlated with experimentally induced changes in refraction (Fig. 6, top: \(r = 0.361; \text{ANOVA, } P < 0.01\)) and axial length (Fig. 6, bottom: \(r = 0.434; \text{ANOVA, } P < 0.01\)). The choroids of both the experimental and fellow control eyes increased significantly in thickness with age as shown earlier for untreated marmoset eyes (for experimental eyes: \(y = 0.070 + 0.025\log(x); \ r = 0.551; \text{ANOVA, } P < 0.01\)).

Individual responses to form deprivation by short-duration lid suture were variable. Figures 7 and 8 show the interocular differences (experimental eye – fellow control eye) for refractive error (left column) and axial length and choroidal thickness (right column) from individual experimental animals plotted over time. In four of the seven (Fig. 7) experimental animals, a clear shift from axial hyperopia to axial myopia was seen, although the timing and amplitude of the shift varied significantly between animals. In two of the three remaining subjects, axial length was initially longer in the experimental eye when the lids were reopened (Fig. 8, top and middle). The hyperopia in the subject plotted in the middle panel was apparently due to a persistently flatter cornea (relative to the fellow control eye) not observed in the other subjects. The mean radius of curvature throughout the measurement period was 3.41 mm in the experimental eye compared with 3.30 mm in the control eye (paired \(t\)-test, \(P < 0.05\)). In the third subject shown in Figure 8 (bottom), axial hyperopia was maintained for the main part of the postdeprivation period before isometropia was attained approximately 1 year after the end of the lid-suture period.
DISCUSSION

In the chick, changes in choroidal thickness contribute to emmetropization in response to retinal defocus imposed by lenses or form deprivation.\textsuperscript{12,13} In this study we asked whether similar choroidal changes occur in primates with experimentally induced changes in refractive state. In marmosets, short-duration lid suture early in life initially results in axial hyperopia that eventually progresses to axial myopia.\textsuperscript{21} We measured the changes in refractive state, axial dimensions, and choroidal thickness in the experimental eyes after lid opening to ascertain whether changes in choroidal thickness are associated with the induced changes in axial length and refractive error in the same manner as seen in chicks. Normal animals were also measured from infancy to maturity to determine the course of normal choroidal development.

Normal Development of Choroidal Thickness

In untreated marmosets the thickness of the choroid increases by approximately 28% from birth to puberty, when the eye is nearly adult size.\textsuperscript{27} Similar results have been reported for macaques.\textsuperscript{28} In marmosets, most of the increase in thickness occurs early in development, during the period of rapid ocular growth and emmetropization from infant hyperopia. Because this emmetropization involves the elongation of the back of the eye toward the focal plane, the observed increase in choroidal thickness is in the wrong direction to be a compensatory component of the emmetropization. Furthermore, the increase in thickness cannot be accounted for by a passive mechanical change associated with increasing the size of the eye, which would cause a thinning of the choroid.

We speculate that the normal choroidal thickening during the juvenile period of rapid eye growth may function to slow the growth of the eye, either by providing a diffusion barrier to...
a putative retinally derived growth signal, or as a mechanical buffer for the sclera, as proposed years ago by van Alphen, who showed in experiments on enucleated eyes that the choroid could withstand the effects of significant increases in force, such as that exerted by intraocular pressure (IOP). If a change in force on the sclera could, either by stretching the tissue or by causing a change in scleral biosynthesis, cause a change in eye size, then preventing that force from reaching the sclera could be a means of controlling the effects of that force. Relevant to this hypothesis, Friedman showed that the IOP-related stress on the posterior ocular tunics was inversely proportional to the thicknesses of the tunics. The developmental thickening of the choroid could, in effect, provide a breaking mechanism to slow axial growth of the eye at the appropriate stage in development. Conversely, thinner choroids and weaker (more compliant) scleras may be associated with eyes growing abnormally fast, as discussed in the next section.

Changes in Choroid Thickness Associated with Refractive Errors
If the choroid acts as a modulator of scleral growth, either as a mechanical buffer as just described or, alternatively, as a diffusion barrier to a retinally derived factor, then thinner choroids would be associated with longer eyes, and changing scleras. In fact, there is some circumstantial evidence in support of this view. In human myopia, for example, thinner than normal choroids are associated with weaker scleras. In primates with form deprivation myopia, thinner scleras and changes in extracellular matrix have also been reported. In tree shrews, treatments that alter scleral collagen cross-linking exacerbate form deprivation myopia, and greater scleral compliance has been measured in the sclera of form deprived eyes relative to control eyes. If choroidal thickness changes are responsible for such changes in the sclera, and consequently,
increased eye growth and myopia, we should see choroidal thickness changes with induced refractive errors.

In our study, we used short-duration lid suture to ascertain whether changes in refractive error are associated with changes in choroidal thickness in marmosets. This paradigm results in an initial hyperopia that progresses to myopia. We found that, superimposed on the developmental increase in choroidal thickness, the transient hyperopia seen after lid opening was associated with thicker choroids, and the progression toward myopia was associated with thinner choroids. In

**Figure 7.** Data from four marmosets responding to the lid suture with initial hyperopia. Intercocular differences (experimental eye – control eye) in refractive error (left column), axial length, and choroidal thickness (right column) are plotted as a function of age. Note that all eyes shown shifted from axial hyperopia with thicker-than-normal choroids to axial myopia with thinner-than-normal choroids in the period after the end of monocular visual deprivation by lid suture. *Horizontal bars:* duration of visual deprivation. In all cases lid suture-induced form deprivation was discontinued by 38 days of age.
In this respect, the results in marmosets seem qualitatively similar to those in chicks: Small, hyperopic eyes have thicker choroids, and large, myopic eyes have thinner choroids.\textsuperscript{12,13,40}

At first glance, our results appear to support a causal role between choroid thickness and axial growth rate, whereby thick choroids inhibit growth and thin ones accelerate it. However, further examination indicates that the relationship between choroid thickness and eye size in the marmoset is not consistent with a true compensatory mechanism as seen in chicks. First, the choroidal thickness changes in the marmoset are approximately an order of magnitude smaller than those described in the chick. The largest changes would account for less than a diopter of refractive change. Second, and more important, in chicks the smaller hyperopic eyes and thicker choroids occur as the result of compensation for myopic defocus. In marmosets, the initial hyperopia and thick choroids

\begin{figure}[h]
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\caption{Individual data from three marmosets responding to lid suture form deprivation differently from those shown in Figure 7. Interocular difference (experimental eye - control eye) in refractive error (left column), and axial length and choroidal thickness (right column) from two animals in which the experimental eye exhibited axial elongation with thinner-than-normal choroids throughout the period after the end of monocular visual deprivation (top and middle). Bottom: interocular differences in refractions, axial length, and choroidal thickness from a single individual in which the experimental eye showed axial hyperopia with thicker-than-normal choroids throughout most of the postdeprivation period. Horizontal bars: duration of visual deprivation. In all cases lid suture-induced form deprivation was discontinued by 38 days of age.}
\end{figure}
are the result of form deprivation (an open-loop visual condition), which, by definition, is not a compensation but probably represents a default growth pattern. It is interesting, however, that form deprivation produces opposite responses in axial length and choroidal thickness in both species. In the chick, form deprivation produces axial length increases with choroidal thinning, in the marmoset it produces reduced axial growth and choroidal thickening. Restoring form vision results in changes in both choroidal thickness and axial length that are in the appropriate direction for compensation in both species. It could be argued that the thinning of the thick choroids in hyperopic marmosets after the end of deprivation is indeed a compensatory adjustment for the hyperopic defocus. However, that these eyes eventually overshoot emmetropia to become myopic, with thinner than normal choroids, argues against the choroid’s ability to respond actively to a defocus, at least bidirectionally, as seen in chicks. It is possible that the choroidal response to defocus in primates is unidirectional thinning, in the same way that the stronger of the compensatory eye growth responses to spectacle lenses in macaques seem to favor elongation in response to negative lenses rather than slowing growth in response to positive ones. That the marmoset choroid is able to become thicker during normal development and lid suture argues that the unidirectionality of the response is at the level of the signal detector, as opposed to the response generator.

It could be argued that the overshoot of emmetropia to myopia is a consequence of an unchecked “grow” signal that is initiated by the hyperopic defocus, but it is equally plausible that it is a delayed manifestation of earlier form deprivation. In either case, it is possible that the thinning of the choroid over the period after lid opening may be merely a mechanical consequence of an eye elongating abnormally rapidly. If we model the choroid as a simple isovolumetric shell of a sphere, we find that an increase in radius comparable with that seen in induced axial myopia in marmosets predicts approximately 24 μm of thinning, which compares closely with the observed thinning of approximately 20 μm. Conversely, decreasing the size of the eye by an amount seen, on average, in our hyperopic eyes resulted in a predicted increase in thickness of approximately 33 μm compared with the 12 μm observed. Thus, a mechanical effect could account for the observed choroidal thinning with axial myopia but is insufficient to account for the thicker choroids associated with axial hyperopia. In addition, this model does not take into account the increasing choroidal thickness observed with age. If it did, the disparity between the observed and predicted choroidal thickness change in shorter hyperopic eyes would be even greater.

In this study we showed that small changes in choroidal thickness occur in a primate model for eye growth. Exactly how the changes in choroidal thickness are achieved is a matter for speculation. Several hypotheses with varying amounts of supporting evidence have been suggested. These include changes in one or several of the following: choroidal blood flow, choroidal extracellular matrix synthesis, uveoscleral flow, lymphatic or capillary fenestrations, or vascular choroidal smooth muscle tone. From research using chickens to investigate eye growth, it has been postulated that changes in choroidal thickness directly effect changes in scleral growth. The existence in chicks, tree shrews, and monkeys of a similar relationship between choroidal thickness and changes in eye growth (thicker choroids being associated with slower growing eyes and thinner choroids with faster growing eyes) appears to give additional support to a causal relationship. However, that the magnitude of the choroidal thickness changes in marmosets, tree shrews, and macaques are so small compared with those seen in chick implies that large changes in choroidal thickness are not required for altered eye growth in the primate. This does not discount, however, the possibility that the primate choroid is involved in growth regulation, but to a much lesser degree. Alternatively, the choroidal changes in chicks may be part of a local retinal focusing mechanism that is not necessary in a species with foveal retinas in which eye movements and accommodation obviate the need to adjust refraction more peripherally.

In conclusion, we found that the marmoset choroid undergoes small, but significant, changes in thickness that are correlated with the refractive state and size of the eye. Small hyperopic eyes were associated with thicker choroids, and large myopic eyes were associated with thinner choroids. Although the thinning response may be visually guided by the hyperopic defocus initially experienced, we cannot rule out alternative explanations.

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