Decreased Proteoglycan Synthesis Associated with Form Deprivation Myopia in Mature Primate Eyes

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Purpose. The rate of proteoglycan synthesis was measured in the scleras of adolescent marmosets that had undergone monocular form deprivation to characterize the scleral extracellular matrix changes associated with the development of myopia in a mature primate.

Methods. Form deprivation myopia was induced in adolescent marmosets by unilateral lid suture for an average of 108 days. After the lids were reopened, the axial lengths and refractions were measured at intervals for up to 39 weeks. At the end of the study period, sclera were isolated and immediately radiolabeled with 35SO4 in organ culture. Proteoglycan synthesis rates were determined by measurement of 35SO4 incorporation into cetylpyridinium chloride-precipitable glycosaminoglycans after digestion of the scleral samples with proteinase K. Collagen content was determined by measurement of total hydroxyproline in scleral digests. Newly synthesized proteoglycans were separated on a Sepharose CL-4B molecular sieve column and identified by their core proteins by Western blot analyses.

Results. Lid suture resulted in myopia due to a significant increase in vitreous chamber depth. After Sepharose CL-4B chromatography, newly synthesized scleral proteoglycans isolated from normal, form-deprived, and contralateral control eyes, resolved into one major peak that eluted in the position of decorin, a small chondroitin-dermatan sulfate proteoglycan. After digestion of the major peak with chondroitinase ABC, an approximately 45-kDa core protein was detected by Western blot analyses, confirming the presence of decorin. Form deprivation resulted in a significant reduction in the rate of proteoglycan synthesis in the posterior sclera (−43.55%, P ≤ 0.001). Proteoglycan synthesis was also significantly reduced in the posterior sclera of form-deprived eyes relative to total collagen content (−36.19%, P ≤ 0.01) and was negatively correlated with the rate of vitreous chamber elongation in the deprived eye (r2 = 0.779, P ≤ 0.05).

Conclusions. Significant extracellular matrix remodeling occurs in the posterior sclera of the adolescent primate eye during vitreous chamber elongation and myopia development. The negative correlation between vitreous chamber elongation rates and the synthesis rates of decorin in form-deprived eyes suggests that proteoglycan synthesis within the posterior sclera plays a role in the regulation of ocular size and refraction in the adolescent marmoset.


The sclera is a dense viscoelastic connective tissue that determines the shape and size of the eye and therefore plays a major role in influencing the refractive state. In mammals, the sclera consists of interwoven lamellae of collagen and elastic fibrils, interspersed with proteoglycans and noncollagenous glycoproteins. Sandwiched between the lamellae are the cell bodies of scleral fibroblasts, which are responsible for the synthesis and degradation of the scleral extracellular matrix.

There is much evidence for the existence of a vision-dependent emmetropization process that regulates the coordinated growth of the separate tissues of the eye to minimize refractive error.1–3 Deprivation of form vision, either experimentally induced in young animals4–14 or occurring in humans as a result of scarring or disease to the anterior ocular segment15–18 has been shown to result in elongation of the vitreous chamber and the development of myopia, presumably by interfering with the normal emmetropization process. Form deprivation has been widely used in studies on birds,4–7 mammals8–12 and primates13,14,19,20 to study the ocular changes associated with vitreous chamber elongation and the development of myopia.

Significant changes in scleral extracellular matrix synthesis, accumulation, and turnover are associated with vitreous chamber elongation during the development of experimentally induced myopia in a variety of animals.21–27 In chicks, the development of myopia is associated with increased growth of the cartilaginous scleral layer at the posterior pole of the eye, as evidenced by increases in thickness,28 total protein accumulation,29 proteoglycan synthesis,22,23 and proteoglycan accumulation.21 In contrast, the outer fibrous layer of the chick...
sclera undergoes increased catabolism during the development of myopia, as evidenced by an increased expression of gelatinase, A\textsuperscript{30,31}; decreased expression of tissue inhibitor of metalloproteinase (TIMP)-2, an endogenous inhibitor of gelatinase A\textsuperscript{31}; decreased rate of proteoglycan synthesis\textsuperscript{23,32}; and overall thinning of the fibrous layer.\textsuperscript{26} Similar to the sclera of the chick eye, the sclera of form vision-deprived tree shrew eyes exhibits thinning\textsuperscript{33} and has a reduced rate of proteoglycan\textsuperscript{24} and collagen\textsuperscript{24,34} accumulation at the posterior pole, as well as an increase in the ratio of active-to-latent gelatinase A, compared with control eyes.\textsuperscript{35} Furthermore, decreases in glycosaminoglycan and collagen concentration have also been identified in the posterior sclera of eyes from highly myopic human donors,\textsuperscript{36} suggesting that the changes observed in the fibrous sclera of chicks and tree shrews model the changes that occur in the human sclera during the development of myopia.

Nearly all experimental studies examining scleral extracellular matrix changes associated with myopia development have been performed in neonatal or young juvenile subjects whose eyes are still undergoing significant growth and elongation. Because the onset of myopia in humans most commonly occurs in late childhood or early adolescence,\textsuperscript{37} an age when the eye has grown to nearly adult dimensions,\textsuperscript{38} it is of much interest to examine the extracellular matrix changes associated with vitreous chamber elongation in adolescent primates. Results obtained from these studies may be used to predict the scleral changes associated with the development of myopia in humans during the teenage years.

**MATERIALS AND METHODS**

**Subjects**

Eight common marmosets (*Callithrix jacchus*) were used as experimental subjects for the present study in conjunction with another study (Troilo et al.\textsuperscript{39}). Eyes of animals were monocularly lid sutured to alter ocular growth and refraction, as described in detail elsewhere.\textsuperscript{14} The contralateral untreated eyes of these animals served as interocular controls for the experimental manipulation. All experiments were performed in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Treatments and Measurements**

In five animals, form deprivation by lid suture was introduced between 299 and 315 days of age (mean, 306 days). This age was selected because it is comparable to puberty in humans,\textsuperscript{40} when axial eye growth has slowed, and axial length has stabilized.\textsuperscript{39} The duration of lid suture in the present experiment was between 79 and 135 days (mean, 108 days). After the lids were reopened, refractive error and axial dimensions were measured at intervals between 14 and 78 days.

Two additional marmosets used in the present study underwent unilateral lid suture at 10 and 17 days of age for a period of 3 weeks. These marmosets were euthanatized by an overdose of pentobarbital sodium at the ages of 529 and 538 days, respectively, and the scleras were used for proteoglycan characterization (described later). One untreated marmoset (248 days old) was used as a normal control for proteoglycan characterization. Vitreous chamber depths of treated and control eyes were measured in vivo using a-scan ultrasonography. Complete optometric data are presented in Troilo et al.\textsuperscript{39} and are summarized in Table 1.

**Organ Culture and Radiolabeling**

Sclera were cleaned of adherent adnexa and immediately placed in organ culture in Dulbecco’s modified Eagle’s medium (DMEM) containing 15% fetal bovine serum and \(35\)SO\textsubscript{4} (500 \(\mu\)Ci/ml) and incubated for 6 hours at 37°C, to label newly synthesized proteoglycans, as previously described.\textsuperscript{21,41}

**Chromatography**

The sclera from two marmosets, with lids sutured at 10 and 17 days of age for a period of 3 weeks, and one untreated marmoset (248 days old) were used for proteoglycan characterization. To characterize newly synthesized proteoglycans, radiolabeled sclera from control and form-deprived eyes were minced into small (<2-mm\(^3\)) pieces with a razor blade. The minced tissue was extracted in 4 M guanidine-HCl containing
0.01 M sodium acetate, 0.01 M sodium EDTA, 0.005 M benzamidine-HCl, and 0.1 M ε-aminocaproic acid at 4°C overnight, followed by re-extraction in the same solvent for 2 to 4 hours at 4°C. The two extracts were combined for each tissue and were dialyzed exhaustively in 0.01 M Na2SO4, followed by exhaustive dialysis in distilled water and lyophilization. Lyophilized scleral extracts were reconstituted into column buffer (4 M guanidine-HCl containing 0.02 M Tris [pH 6.8] and 0.1% CHAPS) and applied to a Sepharose CL-4B column (100 × 1.6 cm; Pharmacia Uppsala, Sweden) and eluted with the same buffer at a flow rate of 0.2 ml/min. An aliquot from each fraction was measured for the presence of radioactivity by liquid scintillation counting, and tubes containing the peak fractions were pooled, dialyzed, and lyophilized. Measurements of the areas under each peak of each chromatographic profile were used to calculate the relative amounts of newly synthesized proteoglycans present in scleral extracts.

Quantification of Proteoglycan Synthesis

The sclera of five animals, form deprived between 299 and 315 days of age were used to quantify the rate of proteoglycan synthesis and collagen accumulation in different regions of control and form-deprived eyes. After radiolabeling, 3-mm punches were excised from the anterior, equatorial, and posterior scleral regions of both eyes using a surgical trephine (Storz Ophthalmics, St. Louis, MO). Scleral punches were digested with protease K (protease type XXVIII; Sigma, St. Louis, MO, 0.05% wt/vol in 10 mM EDTA, and 0.1 M sodium phosphate (pH 6.5) overnight at 60°C. This treatment resulted in complete digestion of the tissue. 35SO4-labeled glycosaminoglycans were precipitated by the addition of 0.5% cetylpyridinium chloride (CPC) in 0.002 M Na2SO4 in the presence of unlabeled carrier chondroitin sulfate (1 mg/ml in dH2O), as previously described. Briefly, the samples were incubated for 30 minutes at 37°C and precipitated glycosaminoglycans were collected on Whatman filters (GF/F; Fisher Scientific, Pittsburgh, PA) using a 12 port sampling manifold (Millipore, Bedford, MA). The filters were rinsed with 0.1% CPC containing 0.05 M NaCl and with dH2O. Radioactivity was measured directly on the filters by liquid scintillation counting in 10 ml of scintillation fluid (Ready Safe; Beckman Instruments, Fullerton, CA). The remainder of the protease K digests was used for determining the hydroxyproline concentration in each scleral punch.

Determination of Collagen Concentration

To estimate collagen content, hydroxyproline concentration was measured on an aliquot of each protease K digest. Proteinase K digests were placed in hydrolysis vials (microflex; Kontes, Vineland, NJ) and subjected to acid hydrolysis at 110°C overnight. Hydroxyproline was quantified to the nearest nanogram using chloramidine T.

Electrophoretic Techniques

To survey the marmoset sclera for proteoglycan content, the sclera that remained after 3-mm punches were obtained for proteoglycan synthesis quantification was extracted, and proteoglycans were analyzed by sodium dodecyl sulfate-polycrylamide gel electrophoresis (SDS-PAGE) without purification. Approximately 50 mg of tissue was extracted in a gel sample buffer composed of 3.3% SDS and 16% glycerol in 0.1 M Tris-HCl (pH 6.8; 1 ml/100 mg tissue wet weight in screw-cap microcentrifuge tubes). Extraction was continued for at least 48 hours and included heating to 60°C for 5 hours. This method has previously been shown to extract 90% of tendon proteoglycans. Scleral extracts were electrophoresed on linear 3% to 15% gradient SDS-polyacrylamide gels with a 3% stacking gel with doubled concentrations of Tris and glycine in the electrode buffer (Tris, 0.05 M; glycine, 0.38 M) and run at 6 mA/gel at 15°C. After electrophoresis, the gels were fixed in numerous changes of 35% methanol:5% acetic acid over a 3-day period, stained for 3 hours with 0.5% alcian blue in 7% acetic acid and destained overnight in 7% acetic acid.

Proteoglycans previously separated by Sepharose CL-4B chromatography were characterized by Western blot analyses using antisera generated against a synthetic peptide containing the exon 5 sequence of human decorin (generously supplied by David McQuillan, Center for Extracellular Matrix Biology, Texas A & M University, Houston). Proteoglycan fractions were digested with chondroitinase ABC (Seikagaku America, Ijamsville, MD) in 0.1 M Tris (pH 7.4) containing 500 mM phenylmethylsulfonyl fluoride, 100 mM N-ethylmaleimide, 100 mM EDTA, and 36 mM peptatin A overnight at 37°C, and digested and undigested samples were electrophoresed on 10% SDS-polyacrylamide gels. Proteoglycans were transferred to nitrocellulose, reacted with antibodies, and detected with a chemiluminescent substrate (Western Star; Tropix, Bedford, MA).

Statistical Analyses

Regression lines were fitted to the data using a simple linear model (Statview Student; Abacus Concepts, Berkeley, CA). Comparisons between control and lid-sutured eyes were made using analysis of variance and post hoc t-tests.

RESULTS

Vitreous Chamber and Refractive Error Changes in Experimental Subjects

The eyes of pubescent marmosets were susceptible to form deprivation and became significantly myopic (−3.0 ± 1.49 D interocular difference, P ≤ 0.05) due to significant vitreous chamber elongation (+178 ± 0.05567 μm, P ≤ 0.05) compared with the contralateral untreated control eyes (Table 1; for complete details see Troilo et al). During the period after the deprivation, the vitreous chamber of experimental eyes continued to elongate relative to the control eyes (mean rate, 12.5 mm/d in deprived eyes versus 6 mm/d in control eyes).

Of the two marmosets deprived early in life (used for proteoglycan characterization), one (marmoset C) showed development of myopia that persisted throughout its life. The experimental eye was 0.4 mm longer and 3.6 D more myopic than the fellow eye at the time of death. The other (marmoset D) did not become myopic, although the experimental eye was 0.1 mm longer than the fellow eye (the experimental eye had a flatter cornea than the control eye).

Characterization of Marmoset Scleral Proteoglycans

The major proteoglycan extracted from control and deprived marmoset sclera migrated on SDS-polyacrylamide gels as a single broad band with a molecular weight of approximately 70 to 100 kDa (Fig. 1) consistent with the migration position of
the small chondroitin sulfate proteoglycan decorin. A lighter staining band, migrating at 190 to 220 kDa could be seen in some samples, which corresponds to the migration position for biglycan.

Separation of 35S04-labeled proteoglycans extracted from a normal adolescent marmoset by Sepharose CL-4B chromatography indicated that the majority of newly synthesized scleral proteoglycans eluted in one major peak, consistent with the position of decorin determined from chromatography of human scleral proteoglycans under identical conditions (Fig. 2). Based on previous studies of human scleral proteoglycans, the leading shoulder of the chromatographic profile of newly synthesized proteoglycans in Figure 2 most likely represents a large chondroitin-keratan sulfate proteoglycan related to aggrecan.

To determine whether the population of newly synthesized proteoglycans was altered as a result of visual deprivation, proteoglycans were extracted from the scleras of lid-sutured eyes, as well as from the fellow control eyes and chromatographed on CL-4B (Fig. 3). The elution profiles for newly synthesized proteoglycans from lid-sutured eyes were similar to those of the fellow control, as well as to those of the normal marmoset eyes, indicating that form deprivation did not alter the size or relative composition of newly synthesized proteoglycans. Quantification of newly synthesized proteoglycans by determining the area under the peaks in the chromatograms indicated that the proteoglycans in the sclera of the experimental eye that was elongated and myopic (subject 1) were decreased relative to that of the fellow control sclera. This however, was not the case in subject 2: Although the experimental eye was slightly longer than the fellow control eye, the amount of newly synthesized proteoglycan was slightly higher in the experimental eye. The lower amplitude of the proteoglycan peaks from the sclera of both the control and lid-sutured eyes of subject 2 compared with those of subject 1 most likely reflect differences in the metabolic activity of the tissue at the time of radiolabeling.

Column fractions under the major peak after Sepharose CL-4B chromatography were pooled and subjected to Western blot analyses using anti-human decorin antibodies (Fig. 4). A 45-kDa core protein was detected in chondroitinase ABC-
digested samples (Fig. 4, Case ABC) from both control and lid-sutured eyes that reacted with the antiserum, confirming that decorin is the major proteoglycan in the marmoset sclera.

Comparison of Proteoglycan Synthesis Rates in Control and Myopic Eyes

The rate of proteoglycan synthesis in the sclera of form-deprived and fellow control eyes in the adolescent marmosets was determined after radiolabeling in organ culture and precipitation of $^{35}$SO$_4$-labeled glycosaminoglycans (Fig. 5A). The sclera of control eyes demonstrated significant differences in the rates of proteoglycan synthesis between anterior, equatorial, and posterior regions. Proteoglycan synthesis was significantly higher in the posterior sclera of control eyes than in the anterior (+80.55%, P ≤ 0.01) or equatorial sclera (+229.24%, P ≤ 0.01) of control eyes. In contrast, regional differences were largely absent in form-deprived eyes. The rate of proteoglycan synthesis was significantly higher only in the anterior sclera than in the equatorial sclera (+155.16%, P ≤ 0.05) of form-deprived eyes. Comparison of proteoglycan synthesis rates in different regions of control and form-deprived eyes indicated that the rate of proteoglycan synthesis was significantly lower in the posterior sclera of form-deprived eyes, compared with the posterior sclera of control eyes (–43.55%, P ≤ 0.001). No significant differences were detected in proteoglycan synthesis rates in the anterior or equatorial sclera between form-deprived and control eyes.

To estimate collagen content of the scleral punches, hydroxyproline concentration was determined in aliquots of the scleral digests (Fig. 5B). The sclera of control and form-deprived eyes exhibited similar regional differences in hydroxyproline concentration. Hydroxyproline concentration was significantly lower in the equatorial sclera of control eyes than in the anterior sclera of the same eyes (–60.28%, P ≤ 0.05). Hydroxyproline levels were also lower in the equatorial sclera than in the posterior sclera, although these differences did not reach statistical significance (P = 0.0847). A similar regional variation in hydroxyproline content was observed in form-deprived eyes, with hydroxyproline concentration significantly lower in the equatorial sclera than in the anterior sclera (–70.44%, P ≤ 0.01) and the posterior sclera (–43.61%, P ≤ 0.05). When control and form-deprived eyes were compared, no significant differences were detected in hydroxyproline concentration in anterior, equatorial, or posterior sclera.

When normalized to collagen content, the rate of proteoglycan synthesis per microgram hydroxyproline was significantly higher in the posterior sclera of control eyes than in the anterior sclera (+129.15%, P ≤ 0.01) or equatorial sclera (+92.41%, P ≤ 0.05). In contrast, no significant regional differences were detected in the rates of proteoglycan synthesis per microgram hydroxyproline in the sclera of deprived eyes. When scleras of form-deprived eyes were compared with those of control eyes, the rates of proteoglycan synthesis relative to hydroxyproline concentration were significantly lower in the posterior sclera of deprived eyes than in the same region of control eyes (–36.19%, P ≤ 0.01) (Fig. 5C), suggesting that the decrease in proteoglycan synthesis rates observed in deprived eyes is not simply a reflection of a generalized loss of scleral extracellular matrix.

The decrease in the rate of proteoglycan synthesis observed in experimental eyes relative to the fellow control eye was compared with the average rate of vitreous chamber elongation in the experimental eye during the period after the end of form deprivation. We found that there was a significant negative correlation between the mean daily growth rate of experimental eyes during the period after lid-opening and the interocular difference in proteoglycan synthesis ($r^2 = 0.779$, P ≤ 0.05; Fig. 6). Specifically, the scleras of the fastest growing eyes showed larger decreases in proteoglycan synthesis (measured as counts per minute of $^{35}$SO$_4$) relative to that of the scleras of their slower growing fellow control eyes.

**DISCUSSION**

The results of the present study show that form deprivation in adolescent monkeys was associated with a significant reduc-
tion in the rate of proteoglycan synthesis in the posterior sclera. Total collagen content, although somewhat reduced, was not significantly affected by form deprivation. When normalized to hydroxyproline content, the rate of proteoglycan synthesis was significantly lower in the posterior sclera of deprived eyes, suggesting that the reduction in proteoglycan synthesis was not simply a reflection of possible scleral thinning or generalized loss of scleral matrix in form-deprived eyes.

The human sclera is thickest at the posterior pole and thinnest at the equator; it thickens again at the corneal limbus. Although no comprehensive histologic studies have been performed on the marmoset sclera, if the regional differences in scleral thickness are similar in the marmoset, it may account for the regional differences in proteoglycan synthesis and collagen accumulation observed in control eyes. When normalized to collagen content, the posterior sclera exhibited the highest rate of proteoglycan synthesis compared with the anterior and equatorial regions. The higher rate of proteoglycan synthesis in the posterior sclera may be related to the anteroposterior developmental pattern in the primate sclera resulting in the most immature and biosynthetically active cells being located at the posterior pole. Of note, proteoglycan synthesis was significantly reduced in form-deprived eyes only in the posterior sclera. This localized response in the posterior sclera may be related to regional differences in the growth states of the scleral fibroblasts or may be a reflection of a concentration of deprivation-induced changes in the retina, choroid, and sclera along the visual axis.

A similar reduction in proteoglycan synthesis and accumulation has been reported to occur in the sclera of the juvenile tree shrew, suggesting that cellular responses to form deprivation are similar between the two species. A small but statistically significant reduction in collagen accumulation (−11.9%) was also reported in the posterior sclera of the deprived tree shrew eyes. In the present study, no significant differences were detected in collagen accumulation in the marmoset sclera, although a trend toward reduced collagen at the posterior pole was observed in deprived eyes. It is possible that a marmoset sample size comparable to that used in the tree shrew study (n = 10) would also show similar decreases in collagen accumulation at the posterior pole. We cannot, however, exclude the possibility that species and age differences may be related to the differences in collagen accumulation between the two studies.

Results of SDS-PAGE, Sepharose CL-4B chromatography, and Western blot analysis indicated that the major proteoglycan of the marmoset sclera is decorin, a small chondroitin-dermatan sulfate proteoglycan with a core protein of approximately 45 kDa. Decorin has been shown to be present in close proximity along the visual axis.

**Figure 5.** Comparison of the rates of proteoglycan synthesis and collagen accumulation in different regions of control and form-deprived marmoset eyes. (A) Rates of proteoglycan synthesis were significantly lower in the posterior sclera of form-deprived eyes than in the same region of contralateral controls. (B) Total hydroxyproline was measured in experimental and control eyes, as an estimate of total collagen content in each scleral region. No significant differences were detected between experimental and control eyes of any scleral region. (C) Rates of proteoglycan synthesis were expressed relative to total collagen content in the three scleral regions of control and experimental eyes. Proteoglycan synthesis and collagen were significantly reduced in the posterior sclera of form-deprived eyes, compared with control eyes. ***P ≤ 0.001, **P ≤ 0.01 post hoc t-test; n = 5 eyes in each group. n.s., not significant.
association with collagen fibrils of many, if not all, connective tissues, where it regulates collagen fibril formation and organization in the extracellular matrix. Decorin has also been identified in the human sclera where it makes up approximately 74% of the total sulfated proteoglycans. Additionally, the human sclera has been shown to contain biglycan (~20%), another chondroitin-dermatan proteoglycan, and a small amount (~6%) of aggrecan, the chondroitin-keratan sulfate proteoglycan typically found in cartilage. With increasing age in humans, the amounts of aggrecan increase and the amounts of decorin and biglycan decrease, relative to total sulfated proteoglycans. It is speculated that decorin and biglycan function to maintain collagen fibril organization within the scleral extracellular matrix and stabilize intermolecular collagen interactions, whereas aggrecan may function to maintain scleral hydration and pliancy at the posterior pole. In addition, decorin has been shown to suppress cell growth by upregulating the cell cycle inhibitory protein p21, as well as by binding to transforming growth factor-β, thereby neutralizing its growth-promoting activity in Chinese hamster ovary cells. Based on the interactions of decorin with cells and the extracellular matrix characterized in the current study, a decrease in decorin synthesis and reduced accumulation in the scleral extracellular matrix, which may result in decreased mechanical strength and subsequent ocular elongation.

Changes in scleral creep rates (time-dependent changes in length under constant force) have been reported to correspond to changes in axial elongation rates during the induction and recovery from deprivation-induced and negative lens-induced myopia. It has been speculated that alterations in the levels of the noncollagenous components, such as proteoglycans may affect the structural integrity of the sclera and thereby influence the creep rate of the sclera. The significant changes observed in proteoglycan synthesis rates in form-deprived primate eyes, and the negative correlation of these changes with the rate of vitreous chamber elongation observed in the present study, suggest that the synthesis and accumulation of decorin in the posterior sclera influences scleral distensibility and the rate of ocular elongation in the eyes of adolescent primates.

In the present study, we found that the rate of proteoglycan synthesis in the sclera of the experimental eye relative to the fellow control eye was inversely correlated with the rate of growth: Faster growing eyes had relatively larger decreases in proteoglycan synthesis than slower growing eyes. These changes are presumably related to a restructuring of the extracellular matrix of the sclera. The tissue used in this part of the study (in vitro analysis of synthesis rates) was from animals with previously lid-sutured eyes that had been open for several weeks (2–11 weeks), and the rate of growth used for this analysis was taken as a mean over this period, which differed in different animals. It is known that in marmosets, lid suture produces axial elongation that continues well after the lids are opened and vision is restored. If, as we speculate, the decrease in proteoglycan synthesis is a component of the restructuring of the matrix necessary for eye enlargement, then this negative correlation implies that this component of the restructuring mechanism is directly related to the rate of ocular enlargement.

Because the changes in proteoglycan synthesis occurred in mature marmosets, weeks after late-onset deprivation was discontinued, it is unclear whether the decreased rate of proteoglycan synthesis was responsible for vitreous chamber elongation, or were secondary to another, unidentified scleral response related to the rate of vitreous chamber elongation. The normal postnatal growth of the human eye has been described as having an “early” rapid elongation phase followed by a later

![Figure 6. Relationship between relative proteoglycan synthesis rates in the posterior sclera of experimental eyes compared with control eyes and the rate of vitreous chamber elongation in form-deprived eyes. Linear regression analysis indicated that the magnitude of the reduction in proteoglycan synthesis rates in the posterior sclera of form-deprived eyes was correlated with the rate of vitreous elongation rate in form-deprived eyes during the post-lid-suture period. $R^2 = 0.779; P < 0.05$.](image)
“juvenile” slow-elongation phase. Although the human eye reaches adult size by 10 to 15 years, the concentration of scleral proteoglycans continues to increase significantly in the sclera through the fourth decade of life. Therefore, defects in the regulation of scleral proteoglycan synthesis and/or accumulation in childhood, adolescence, and young adulthood may be expected to cause significant structural abnormalities in the scleral matrix. The results of the present study show that scleral proteoglycan synthesis can be altered in adolescent primates by changes in the visual environment. It is therefore likely that changes in visual experience during adolescence may affect eye growth in humans through alterations in proteoglycan synthesis and extracellular matrix organization in the sclera.

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References


Proteoglycan Synthesis Changes in Primate Sclera


