Mammalian Model of High Myopia

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PURPOSE. The development of high myopia is associated with scleral thinning and changes in the diameter of scleral collagen fibrils in humans. In the present study, the association between these scleral changes and the losses in scleral tissue that have previously been reported in animal models were investigated to determine the relationship between changes in collagen fibril architecture and thinning of the sclera in high myopia.

METHODS. Myopia was induced in young tree shrews by monocular deprivation of pattern vision for short-term (12 days) or long-term (3–20 months) periods. Scleral tissue from normal animals over a wide age range (birth to 21 months) was also collected to provide data on the normal development of the sclera. Light and electron microscopy were used to measure scleral thickness and to determine the frequency distribution of collagen fibril diameters in the sclera. Tissue loss was monitored through measures of scleral dry weight.

RESULTS. Significant scleral thinning and tissue loss, particularly at the posterior pole of the eye, were associated with ocular enlargement and myopia development after both short- and long-term treatments. However, collagen fibril diameter distribution was not significantly altered after short-term myopia treatment, whereas, from 3 months of monocular deprivation onward, significant reductions in the median collagen fibril diameter were noted, particularly at the posterior pole.

CONCLUSIONS. The results of this study demonstrated that loss of scleral tissue and subsequent scleral thinning occurred rapidly during development of axial myopia. However, this initial tissue loss progressed in a way that did not result in significant alterations to the collagen fibril diameter distribution. In the longer term, there was an increased number of small diameter collagen fibrils in the sclera of highly myopic eyes, which is consistent with findings in humans and is likely to contribute to the weakened biomechanical properties of the sclera that have previously been reported. (Invest Ophthalmol Vis Sci. 2001;42:2179–2187)

The prevalence of high myopia (myopia in excess of 6 diopters [D]) in world populations has been estimated to be between 0.3% and 9.6%. However, this figure has recently been shown to be as high as 16% in certain young Asian populations, and evidence suggests that the prevalence is increasing. These figures are a cause for concern, given that 30% to 70% of high myopes display at least some lesions of the retina and choroid. Furthermore, pathologic changes in high myopia, such as macular degeneration, subretinal hemorrhage and retinal detachment, are assumed to be a result of mechanical stresses related to the excessively enlarging eye. This conclusion is supported by evidence that the incidence of retinal disease in high myopia is greater with increasing eye size.

Excessive ocular enlargement must be facilitated by the outer coat of the eye, namely the sclera, and high myopia in humans has been found to be associated with a thinner sclera, particularly at the posterior pole of the eye. Previous studies have shown that this scleral thinning is associated with a narrowing and dissociation of the collagen fiber bundles and a reduction in collagen fibril diameter. In addition, it has been reported that there is an increase in the occurrence of collagen fibrils that are stellate in cross-sectional profile. Changes in the biochemical structure of the sclera, such as altered glycosaminoglycan (GAG) and collagen content, have also been reported in the sclera of highly myopic post-mortem human eyes and are further evidence of scleral pathology in high myopia. The thinning of the sclera in highly myopic humans was previously believed to occur as a result of passive stretching of the tissue to cover the enlarged globe, however, data from animal models of refractive development have forced a reinterpretation of this hypothesis.

A study in monkeys with experimentally induced high myopia has established that marked scleral thinning is associated with smaller collagen fibril diameters, as found in humans. Furthermore, the study defined inner, middle, and outer layers of the sclera and determined that there is a gradient in fibril diameter across these layers and that this gradient is absent in myopic eyes. More recent studies in animals, such as the tree shrew and chick, have demonstrated that scleral dry tissue weight alters in conjunction with changes in scleral thickness and that this is associated with both biochemical and biomechanical changes in the scleral extracellular matrix.

Collagen accounts for at least 90% of the dry weight of the mammalian sclera, and the tree shrew model of myopia exhibits a fibroblast-maintained scleral extracellular matrix that is similar to that of humans, consists of predominantly type I collagen associated with smaller amounts of other fibrillar and fibril-associated collagens. Studies in the tree shrew have shown that during development of myopia the vitreous chamber enlarges, the sclera thins at the posterior pole, and there is a reduction in scleral collagen and GAG content changes that are also found in the sclera of human high myopes. In addition, studies have shown that this scleral thinning is associated with tissue loss, even during the earliest stages of myopia development. This loss of scleral tissue occurs in conjunction with rapid reductions in scleral GAG synthesis and cell proliferation. In addition, investigators have shown that the activity of matrix metalloproteinase (MMP)-2, an enzyme involved in the breakdown and turnover of collagen, is rapidly upregulated in the sclera of eyes with developing myopia.

Scleral thinning and changes in scleral collagen fibril morphology have been reported, in abstract form, in the eyes of tree shrews developing myopia over the short term.
However, most data on the human sclera have been obtained from older post-mortem eyes. In the present study, we investigated changes in scleral thickness and collagen fibril morphology, during both short and extended periods of myopia development, to examine how the short-term changes in scleral biochemistry observed in young tree shrews develop into long-term scleral pathology in adult animals. These findings bring us closer to an understanding of the mechanisms of scleral pathology in highly myopic humans.

**Materials and Methods**

**Experimental Subjects and Treatments**

The animals used in this study were maternally reared tree shrews (Tupaia belangeri), raised in our own breeding colony. Animals were maintained on a 15-hour light–9-hour dark cycle, and food and water were available ad libitum. Tree shrew pups were randomly assigned to one of seven experimental groups, three of which were used to investigate scleral collagen fibril morphology and scleral thickness and four of which were used to monitor changes in scleral dry weight. Differences in the tissue processing requirements dictated that collagen fibril and dry weight data could not be collected from the same animals.

Myopia was induced by monocular deprivation (MD) of pattern vision, either through the use of a head-mounted goggle and translucent diffuser, for short periods of monocular visual deprivation (up to 3 months), or by monocular eyelid closure using lid suture, for longer periods of monocular visual deprivation. The untreated contralateral eye served as a genetic control. Treatment commenced 15 days after natural eye opening, the time from which tree shrews are most susceptible to the induction of axial myopia. Although the use of a diffuser is preferable in this type of study, because it does not induce the changes in corneal curvature found after eyelid closure, it is impractical to maintain the head-mounted goggle and diffuser over longer periods. It is important from the point of view of the present study to note, however, that both of these methods of myopia induction result in a refractive error that is caused predominantly by enlargement of the vitreous chamber. All the procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Collagen Fibril Morphology and Scleral Thickness Groups**

Myopia was induced in two of the three groups of animals allocated to this part of the study. Animals in the first group wore a diffuser over a 12-day period (short-term–deprived; n = 5) whereas animals in the second group either wore a diffuser for 3 months or underwent eyelid closure for periods of 6 to 20 months (long-term–deprived, n = 5). The final group consisted of animals that had normal visual experience and varied in age from birth to 21 months (normal, n = 10). Some of these animals served as age-matched control subjects for the monocularly deprived animals, and the remaining animals were used to establish the normal course of scleral development. The ages examined covered a normal course of scleral development. The ages examined covered a period when animals are most susceptible to myopia induction); 45 months, 13.5 months, and 21 months (control subjects for long-term–deprived group).

**Scleral Dry Weight Groups**

Myopia was induced in two of the four groups that were used to monitor scleral dry weight changes. These animals were treated identically with those just described, in that monocular deprivation was induced, either over a 12-day period, through the use of a head-mounted goggle and translucent diffuser (n = 11), or for periods of 6 to 8 months, through the use of monocular eyelid closure techniques (n = 10). Two groups of age-matched normal animals were used as control subjects (n = 4 each group).

**Refractive and Biometric Ocular Measures**

After the designated period of treatment, ocular refraction and biometry data were gathered and the ocular tissue collected for processing. As previously described, ocular refractive error was measured by retinoscopy and corneal curvature by keratometry. Internal ocular dimensions were measured through the use of A-scan ultrasound. Measures were performed with animals under ketamine-xylazine (90 and 10 mg/kg, respectively), anesthesia and 1% topical atropine sulfate was used as a cycloplegic-mydriatic to aid the measurement process. The only variation from this procedure was necessary in animals killed at birth, in which ocular biometry measurements were not possible with a sufficient degree of accuracy.

**Processing of Ocular Tissue for Microscopy**

After ocular refractive and biometric data had been collected, the animals were administered a lethal dose of pentobarbital sodium (120 mg/kg) and perfused by intracardial administration of 4 mg/ml heparin, 4 mg/ml isoxsuprime in rabbit Ringer solution at 37°C, and 3.5% glutaraldehyde buffered with 0.15 M sodium cacodylate. A small mark was made at the 12-o’clock position on the cornea and limbus, with an indelible ink marker pen, to allow orientation of the eye cup after enucleation. Eyes were enucleated and residual orbital tissue carefully removed. The eyes were immersed in the sodium cacodylate-buffered glutaraldehyde for 1 hour. At the end of this period, the cornea and lens were dissected away, with care taken to leave the mark in the limbal region. Guided by the limbal mark and the position of the optic nerve, a 1.5-mm diameter trephine was used to excise a punch of retina, choroid, and sclera from the posterior pole of the eye, to enable the measurement of scleral thickness. A crescent-shaped sector was then cut, extending anteroposteriorly along the medial aspect of the eye cup from the limbus to the cut edge of the posterior punch. Tissue samples were postfixed in 1% osmium tetroxide for 2 hours at 4°C, and then rinsed and dehydrated in graded ethanols before embedding in Araldite.

**Assessment of Scleral Tissue**

Scleral thickness measurements were obtained from thin sections stained with toluidine blue under light microscopy. Oblique sectioning was avoided by monitoring photoreceptor orientation in the sections, and variability was minimized by taking thickness readings from 25 serial sections, beginning approximately 0.4 to 0.5 mm into the tissue punch. Ultrathin sections were cut from both the posterior tissue punches and the equatorial (2.0 mm from the limbus in long-term–deprived animals only) region of the tissue crescents, collected on coated copper grids, and stained for transmission electron microscopy with uranyl acetate, lead citrate, and phosphotungstic acid. Electron micrographs were taken of collagen fibrils in transverse section from the outer (fourth collagen fibril bundle inward from the sclera–episcleral boundary), middle (center bundle), and inner layers (fourth layer out from the lamina fusca). Four electron micrographs (×40,000 magnification) of approximately equal area were taken of each defined scleral layer. Two of these micrographs were obtained from separate areas in one section and two from a later section. Care was taken to ensure that each of these sample micrographs constituted a different collagen fiber bundle. As a result, approximately 400 fibrils were sampled per defined scleral layer, which amounted to approximately 1200 scleral fibrils being sampled from each eye. Fibers were measured with a digitizing tablet, and where fibers were elliptical, the smallest diameter was measured.

Electron micrographs (×100,000 magnification) were also taken of scleral collagen fibrils in longitudinal section, cut from the posterior region of the scleral tissue collected from two long-term–deprived animals. Collagen D-periodicity was measured in 90 to 160 fibrils from...
the treated and control eye sclera of these animals, using the digitizing tablet. Low-magnification scleral electron micrograph montages were assembled from a selection of short- and long-term-deprived and normal animals. Estimates of both the number of collagen fibril bundles along a perpendicular through the scleral thickness and the mean maximum thickness of these bundles were obtained from multiple measurements across the montages.

**Scleral Dry Weight**

In the separate groups of animals that were used to assess scleral dry weight, ocular refraction and biometry measurements were collected, as detailed earlier, before eyes were enucleated in animals under deep anesthesia. Extraneous orbital tissue was dissected from the globe, and the cornea was removed by careful dissection around the pigment ring that defines the limbus in the tree shrew, by an investigator viewing through an operating microscope. The iris, lens, and vitreous were carefully removed, and a 5-mm surgical trephine was used to remove a region of the posterior pole of the eye. Retina and choroid were carefully removed, and a 5-mm surgical trephine was used to remove the cornea was removed by careful dissection around the pigment ring that defines the limbus in the tree shrew, by an investigator viewing through an operating microscope. The iris, lens, and vitreous were carefully removed, and a 5-mm surgical trephine was used to remove a region of the posterior pole of the eye. Retina and choroid were carefully removed, and a 5-mm surgical trephine was used to remove the cornea.

**Data Analysis**

Ocular refraction and biometric measures, as well as scleral thickness and dry weight data, are represented as the mean of the right or left treated or contralateral control eyes ± SEM or as the mean or percentage mean (treated − control/control) difference between paired right and left or treated and control eyes ± SEM. In the absence of evidence for a skewed distribution, these values were analyzed using paired t-tests, and comparison between groups was performed using a one-way ANOVA with a Tukey post hoc test. Collagen fibril diameter distributions in the sclera were analyzed using a Kolmogorov-Smirnov normality test and found to be positively skewed. As a result, nonparametric statistics were applied to these data. Median fibril diameter and the first and third quartiles were used to represent the fibril data in individual eyes and grouped data. The Mann-Whitney test was used to compare individual or grouped right and left or treated and control eye data. The Kruskal-Wallis test with a Dunn post hoc test were used to make comparisons between multiple data sets. Fibril frequency distribution plots were used to represent the data spread graphically after data had been normalized to the total number of fibrils sampled in each eye.

**RESULTS**

**Ocular Biometry and Refractive Error**

Representative ocular biometric data from the animals in this study are presented in Table 1. These refractive error data were not corrected for the small-eye artifact of retinoscopy and, consequently, it should be noted that the true values of ocular refraction in the treated and control eyes of both young and adult tree shrews are likely to be approximately 5 D more myopic than those reported in Table 1.

After 12 days of monocular deprivation, treated eyes of short-term-deprived animals had developed significant amounts of myopia relative to contralateral control eyes (−11.8 ± 0.7 D, P < 0.01). The treated eyes of monocularly deprived animals were also significantly more myopic than the right and left eyes of age-matched normal animals (−2.1 ± 1.0 D vs. +7.7 ± 0.8 D, P < 0.01). Development of myopia in the treated eye was found to be a consequence of increased axial length relative to contralateral control eyes (7.56 ± 0.03 mm vs. 7.15 ± 0.04 mm, P < 0.01). This ocular enlargement was

| Table 1. Ocular Biometry in Tree Shrews during the Development of Induced Axial Myopia |
|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| **Ocular refractive error** | **Center of Power** | **Anterior Chamber** | **Lenticular Chamber** | **Axial Orbit (mm)** |
| Right | Left | Right | Left | Right | Left | Right | Left | Right | Left |
| 4.65 | 4.75 | 0.5 | 0.6 | 0.6 | 0.7 | 0.0 | 0.0 | 0.0 | 0.0 |
| 7.7 | 7.1 | 0.6 | 0.6 | 0.6 | 0.6 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3.28 | 3.28 | 0.3 | 0.3 | 0.3 | 0.3 | 0.0 | 0.0 | 0.0 | 0.0 |
| 7.39 | 7.36 | 0.04 | 0.04 | 0.04 | 0.04 | 0.0 | 0.0 | 0.0 | 0.0 |

Absolute values represent the group means of treated and control or right and left eyes ± SEM. Interocular differences are expressed as the group mean of the differences between treated and control or right and left eyes ± SEM.
primarily due to a significant increase in the vitreous chamber depth of the treated eye (2.99 ± 0.02 mm vs. 2.75 ± 0.03 mm, \( P < 0.01 \)), as previously reported.\(^{18}\) No significant differences were found between treated and control eyes for any other measured intraocular parameter.

After longer periods of form deprivation, the relative myopia between treated and contralateral control eyes was significantly greater than that found in short-term–deprived animals (−14.7 ± 0.9 D vs. −11.8 ± 0.7 D, \( P < 0.05 \)). As was the case in short-term–deprived animals, development of myopia was primarily a result of increased axial length in the treated, relative to the contralateral control, eye and this was mainly due to increased vitreous chamber depth (3.21 ± 0.05 mm vs. 2.71 ± 0.05 mm, \( P < 0.01 \)). However, there was also a small, but significant, thinning of the lens in these animals (Table 1), as previously reported,\(^{18}\) which when modeled was found to account for just 1 D of the induced myopia. The induction of myopia through the use of eyelid closure has previously been shown to result in a slight flattening and subsequent reduction in power of the cornea in the treated eye.\(^{18}\) The measured degree of myopia in the treated eye was reduced by 4.9 ± 0.7 D in the present study, due to this corneal flattening (Table 1).

Scleral Thickness and Collagen Fibril Morphology in the Normal Developing Eye

Dehydrated scleral thickness at the posterior pole was found to increase from 33 \( \mu \)m at birth to 79 \( \mu \)m at eye opening. Thereafter, scleral thickness remained relatively steady at approximately 80 \( \mu \)m, indicating that the sclera had reached its adult thickness by the time of eye opening (Fig. 1A). The age at which scleral thickness at the posterior pole reached adult levels was in good agreement with the age at which both the number of collagen fiber bundles across the scleral thickness (~23 bundles, Fig. 1B) and the median collagen fibril diameter at the posterior pole (78 nm [62–98] median value with interquartile range of first and third quartiles in brackets; Fig. 1C) also reached adult levels. The distribution of collagen fibril diameters present at birth, although approximating a normal distribution, was not found to be gaussian when tested for normality (\( P = 0.01 \)). The distribution changed rapidly up to the age of 45 days, with a positively skewed profile developing (Fig. 2A). During this period, there was a significant increase in the median collagen fibril diameter from 62 nm (interquartile range, 53–70) at birth to 80 nm (interquartile range, 62–115) by 45 days (\( P < 0.001 \)), although the distribution profile remained skewed toward the smaller diameter fibers. The appearance of larger diameter fibers and the skewing of fibril distribution was found to occur in each of the defined scleral layers (inner, middle, and outer) during development. Between the ages of 6 and 21 months, no significant alterations were found in the median fibril diameter (Fig. 2B; at 6 months, 74 nm [51–130], and at 21 months, 85 nm [52–129]; \( P = 0.36 \)). Median collagen fibril diameter was assessed between the defined inner, middle, and outer scleral layers to determine whether differences were present across layers. It was generally found that the median fibril diameter was significantly smaller in inner scleral layers than in middle layers and that the fibril diameter in outer layers was significantly larger than in middle layers (\( P < 0.001 \)). In the present study, the specific variation in fibril diameter across layers is referred to as a gradient, and it was found that the gradient at birth was minimal (inner 59 nm vs. middle 62 nm vs. outer 64 nm), whereas at 21 months the gradient was pronounced (68 vs. 93 vs. 117 nm, respectively).

Scleral Thickness in Myopia

Significant reductions in scleral thickness were apparent in the posterior pole region of myopic eyes, compared with contralateral control, eyes in short-term–deprived animals (Fig. 3A; myopic eye 95 ± 12 \( \mu \)m vs. control eye 120 ± 13 \( \mu \)m; −21%; \( P = 0.001 \)). Significant differences in scleral thickness between myopic and control eyes were also apparent in long-term–deprived animals (Figs. 3A, 3B; 68 ± 5 \( \mu \)m vs. 89 ± 4 \( \mu \)m; −23%; \( P < 0.05 \)), whereas no differences were found in the thickness of the posterior sclera between control or age-matched normal eyes (\( P = 0.42 \)). Furthermore, no significant difference was found between the thickness of the sclera in the equatorial region of myopic and control eyes of long-term–deprived animals (64 ± 8 \( \mu \)m vs. 66 ± 3 \( \mu \)m, \( P = 0.85 \)), which suggests that scleral thickness changes in myopic eyes were regionally specific.

Scleral Dry Weight

Total scleral dry weight was found to be significantly reduced in the myopic eyes, compared with contralateral control eyes, of both short-term (5.21 ± 0.10 mg vs. 5.43 ± 0.10 mg; −3.7%; \( P < 0.05 \)) and long-term (5.68 ± 0.19 mg vs. 6.09 ± 0.17 mg; −6.8%; \( P < 0.05 \))-deprived animals, demonstrating that scleral tissue had been lost, rather than redistributed (Fig. 3C), a finding that has previously been reported by this laboratory.\(^{20}\)
The interocular differences in scleral dry weight in deprived animals were significantly larger than the normal interocular variation found between the right and left eyes of the appropriate age-matched normal animals. The largest change in scleral dry weight was always found at the posterior pole of myopic eyes in both short-term (0.87 ± 0.04 mg vs. 1.04 ± 0.04 mg; -16.9%; P < 0.001) and long-term (0.77 ± 0.04 mg vs. 1.03 ± 0.06 mg; -24.5%; P < 0.001)–deprived animals. It should be noted that the mean percentage reductions in dry scleral weight found at the posterior pole were consistent with the percentage changes in scleral thickness.

**Scleral Collagen Fibril Distribution in Myopia**

Scleral fibril diameter distributions were found to be positively skewed, with a longer tail of larger diameter fibrils, in both treated and control eyes of short-term– and long-term–deprived animals (Figs. 4A–C), as was the case in the two groups of age-matched control subjects. No significant differences were found in the median collagen fibril diameter between treated and control eyes of short-term–deprived animals (Fig. 4A) in the posterior scleral region (95 nm [64–156] vs. 97 nm [64–154]; P = 0.94). As might be expected from these data, no differences in median fibril diameter were found in the inner (P = 0.96), middle (P = 0.52), or outer (P = 0.52) scleral layers between myopic and control eyes. A significant gradient in fibril diameter was found in both myopic (inner 84 nm versus middle 106 nm versus outer 128 nm, P < 0.001) and control eyes (84 vs. 111 vs. 120 nm, respectively; P < 0.001) of short-term–deprived animals.

After 3 months of monocular deprivation, a significant difference was apparent in the median collagen fibril diameters in the posterior sclera (Fig. 4B) between the myopic and contralateral control eyes (82 nm [61–126] vs. 89 nm [63–135]; P < 0.05). However, the reductions between myopic and control eyes in any of the individual scleral layers did not reach significance, with the greatest difference apparent in the outer posterior scleral layer (94 nm [63–150] vs. 106 nm [67–183]; P = 0.056). In the animals that had been monocularly deprived for 6 months or more, there were significant changes in the median fibril diameter of myopic eyes, compared with control eyes.

**FIGURE 2.** Scleral collagen fibril diameter distribution at the posterior pole of the normal tree shrew eye from birth to adulthood. (A) Birth to 45 days of age; (B) 6 to 21 months of age. Each distribution profile represents data collected from the right and left eyes of a single animal and has been normalized to the number of fibrils sampled.

**FIGURE 3.** (A) Mean scleral thickness at the posterior pole of short (12 days) and long (6–20 months)-term myopic, contralateral control and age-matched normal tree shrew eyes. (B) Toluidine blue-stained transverse section of the sclera at the posterior pole of a myopic and contralateral control eye of a long-term–deprived tree shrew. **Arrows:** thickness of scleral tissue in each eye. (C) Mean scleral dry weight at the posterior pole of short (12 days) and long (6–8 months)-term myopic eyes, contralateral control eyes, and age-matched normal eyes. **P < 0.01; *P < 0.05 by paired t-test. Error bars are 1 SEM.**
tralateral control eyes, both in the posterior sclera overall (Fig. 4C) and in each of the defined scleral layers (Fig. 5). Median fibril diameter in the posterior sclera of these long-term–deprived animals was significantly reduced in myopic, compared with contralateral control, eyes (Fig. 4C; 61 nm [41–108] vs. 75 nm [50–127]; \( P < 0.001 \)). This reduction in fibril diameter was most pronounced in the outer layers of the sclera (65 nm [45–114] vs. 98 nm [49–186]; \( P < 0.001 \)), however, significant differences were found in all three of the layers of treated eyes (Fig. 5; inner, 58 nm [40–95] vs. 70 nm [50–101] and middle, 59 nm [39–132] vs. 77 nm [50–145]; \( P < 0.001 \)). The median fibril diameter in long-term–deprived eyes was also significantly less than that in right or left eyes (76 nm [51–125] and 76 nm [50–125]; \( P < 0.001 \)) of age-matched normal animals.

There was no significant difference between contralateral control eyes of long-term–deprived animals (\( P > 0.17 \)). Mean D-periodicity of scleral collagen fibrils, as determined in 454 collagen fibrils

term–deprived animals (\( P < 0.001 \)); however, this gradient was markedly reduced in highly myopic eyes when compared with contralateral control or age-matched normal eyes (Fig. 6). In long-term–deprived animals, a significant decrease in median collagen fibril diameter was also found in the outer layer only of the equatorial scleral region of myopic, relative to control, eyes (67 nm [36–152] vs. 72 nm [41–177]; \( P < 0.005 \)). Of particular note, there was a significant negative correlation between axial eye length and median collagen fibril diameter when the long-term and short-term myopic, control and normal eyes were analyzed together (\( R = -0.69, P < 0.005 \) with a power of 0.81). The correlation between eye length and collagen fibril diameter was found to be particularly strong in myopic eyes (\( R = -0.88 \), compared with control and normal eyes (\( R = -0.57 \)); however, the power of these associations was low.

Scleral collagen fibril D-periodicity was not significantly different between myopic and control eyes in either of two long-term–deprived animals (\( P > 0.17 \)). Mean D-periodicity of scleral collagen fibrils, as determined in 454 collagen fibrils

![FIGURE 4. Scleral collagen fibril diameter distribution at the posterior pole of the myopic and control eyes of tree shrews deprived for (A) 12 days (data from three animals), (B) 3 months (data from one animal), and (C) 9 to 9.5 months (data from three animals). Each distribution profile represents data from all scleral layers and has been normalized to the number of fibrils sampled.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933590/)
consistent with reports in other species.29 No statistical differ-
ment and under conditions that induce myopic eye growth.
the regional collagen fibril diameter in the develop-
mammalian sclera alters, both during normal develop-
in eyes developing myopia, occurred
from the four eyes, was found to be 66.6 \pm 2.6 \text{ nm}, which is
consistent with reports in other species.29 No statistical differ-
ences were found in the number of fibril bundles across the
scleral thickness or in the median fibril bundle thickness in
short- or long-term–deprived animals. However, the number of
collagen fibril bundles across the scleral thickness was slightly
less in myopic eyes of both groups of deprived animals, when
compared with the contralateral control eyes (short-term, 44
vs. 52 bundles, \( n = 1 \); long-term, 41 vs. 45 bundles, \( n = 3 \)).
Furthermore, median fibril bundle thickness was less in myo-
pic eyes of long-term–deprived animals (1.17 \( \mu \text{m} \) [0.74–1.93]
vs. 1.51 \( \mu \text{m} \) [0.68–2.24]) but not in short-term–deprived ani-
mals (1.61 \( \mu \text{m} \) [0.76–2.53] vs. 1.52 \( \mu \text{m} \) [0.78–2.40]).

DISCUSSION

The present study provides a comprehensive picture of how
the regional collagen fibril diameter distribution in the develop-
oping mammalian sclera alters, both during normal develop-
ment and under conditions that induce myopic eye growth.
The main finding of this study was that the changes in scleral
thickness and dry weight, in eyes developing myopia, occurred
before significant changes in the collagen fibril distribution
profile, as indicated by changes in the median collagen fibril
diameter in defined regions of the sclera. The long-term
changes in scleral fibril diameter in highly myopic tree shrew
eyes are consistent with those reported in highly myopic hu-
man and monkey eyes, particularly with respect to the marked
reduction in collagen fibril diameter in the posterior
sclera.7,11,30 The present findings give insight into the time
course of the scleral fibril diameter changes in human myopia
and, as a consequence, probable changes in the biomechanical
properties of the sclera.

Normal Scleral Development

The normal tree shrew sclera was found, at birth, to consist of
a collagen fibril population that displayed a relatively normal
distribution profile of fibril size. With increasing age, the dis-
tribution of fibril size became positively skewed, due to the
appearance of larger diameter collagen fibrils. Scleral thickness
increased steadily from birth, in conjunction with these fibril
diameter changes, and stable adult thickness was reached at a
time comparable to that at which collagen fibril distribution
stabilized (~45 days). It is interesting to note that 45 days was
also the age at which previous reports showed both ocular
axial growth and scleral type I collagen production in young
tree shrews to have markedly slowed.16,26 After this period of
rapid development, the collagen fibril diameter distribution did
not alter significantly with time. A gradient in median scleral
fibril diameter was encountered across the defined layers of the
sclera at all ages, with larger fibrils found in the outer scleral
layers, which is consistent with reports of the human31 and
monkey11 sclera. However, this gradient was minimal at birth
and became substantially more pronounced in older animals.

Short-Term Development of Myopia

In tree shrews that had relatively high degrees of axial enlarge-
ment and myopia induced over only 12 days, significant thin-
ing of the posterior sclera and comparable reductions in both
the posterior and total scleral dry weight were found. No
significant changes in the median collagen fibril diameter were
detectable after this period of induced myopia, even in the
outer layer of the posterior pole region of the sclera. A gradient
in median collagen fibril diameter was found across the layers
of the sclera in both myopic and control eyes and was similar
to that seen both in normal animals and normal human
sclera.31 The findings demonstrate that early loss of scleral
tissue, and consequent scleral thinning, is not associated with
detectable changes in the scleral collagen fibril diameter distri-
bution. Indeed, because some 90% of scleral dry weight is
accounted for by scleral collagen17 and the activity of collagen-
degrading enzymes is elevated early in myopia development,22
this implies that most of this rapid tissue loss in the posterior
segment of short-term–treated animals must be attributable to
a general scleral collagen fibril degradation, rather than to
degradation of specific fibril populations of certain diameters.
Furthermore, limited evidence from electron micrograph mon-
tages of the myopic and control eyes from a single animal
suggests that short-term scleral thinning and tissue loss may
occur through degradation of whole collagen fibril bundles
across the scleral thickness, rather than through diffuse degra-
dation of fibrils within existing bundles.

Long-Term Development of Myopia

In tree shrews with high myopia for 6 months or more, it was
found that the thinned sclera persisted at the posterior pole of
the eye, as did the reduced total scleral dry weight. The
magnitude and pattern of scleral thinning and scleral dry
weight reduction in short- and long-term–deprived animals
suggests that scleral thinning and loss of tissue is most aggressive during the early stages of myopia development. However, it is also apparent that scleral tissue lost during this more aggressive phase is not replaced if the myopia persists. In previous studies it has been found that animals allowed to recover from induced myopia replace the lost scleral tissue as emmetropia is re-established.\textsuperscript{20} Of particular note in the long-term group of animals was the marked reduction in scleral fibril diameter in myopic eyes, found particularly at the posterior pole of the eye, which is consistent with previous findings of the outer layer of the sclera, which is illustrated by the fact that the normal fibril diameter gradient across the defined scleral layers had markedly declined at the posterior pole of myopic eyes (Fig. 6). This finding is consistent with reports in highly myopic monkey eyes, which also lose the gradient in scleral collagen fibril diameter at the posterior pole.\textsuperscript{11}

It is unlikely that the change in size of myopic eyes in the present study affected scleral collagen fibril diameter per se, given that similar degrees of relative axial enlargement in a short-term (0.29 mm after 12 days), mid-term (0.34 mm after 3 months), and long-term (0.37 mm after 9 months) animal were associated with marked differences in the significance of the interocular change in collagen fibril diameter (P = 0.86 short-term, P = 0.03 mid-term; and P = 0.002 long-term). However, a significant correlation was found between eye size and fibril diameter, particularly in myopic eyes, perhaps suggesting that scleral collagen fibril diameter plays some role in determining eye size. The shift in collagen fibril distribution in myopic eyes may either indicate that the primary target in a continuing degradative process are larger diameter fibrils, or that long-term changes in the synthesis of the scleral extracellular matrix result in a predominance of smaller diameter fibrils. Given that there is no apparent wholesale disappearance of large diameter fibrils in these myopic sclerae, it seems that, in the long term, the process at work is one of aberrantly regulated remodeling of the sclera, resulting in smaller diameter fibrils. Indeed, similar proportions of larger diameter fibrils were found in both the myopic and control eyes of short-term–deprived animals (Fig. 4A) and in longer term normal (Fig. 2B) and deprived (Figs. 4B, 4C) animals, demonstrating that deprivation per se does not prevent the development of larger diameter fibrils. Furthermore, evidence from a limited sample suggests that, after the initial short-term losses of fibril bundles across the sclera, bundle thickness also reduced over time, supporting the hypothesis that initial short-term degradation of whole fibril bundles is followed by subtler long-term changes in scleral remodeling.

Data from the present study were equivocal in resolving the issue of whether the untreated control eye of form-deprived tree shrews responds to deprivation of its fellow eye, as has recently been suggested.\textsuperscript{32} Indeed, data from long-term normal and myopic animals unequivocally demonstrate that contralateral control eye values for median scleral collagen fibril diameter, fibril diameter gradient, scleral thickness, scleral dry weight, ocular refraction, and ocular axial length were similar to those of normal animals. However, the smaller data set for short-term normal and myopic animals suggests that values of control eye scleral thickness, dry weight, and ocular refraction differed from those of normal eyes, although changes in ocular axial length and refractive error were mainly a feature of the treated eye.

### Scleral Biochemistry and Collagen Fibril Diameter

Previous studies have shown that certain proteoglycans, such as decorin,\textsuperscript{35} and fibril-associated collagens\textsuperscript{34} are important factors in the control of collagen fibril synthesis—in particular influencing the diameter of newly synthesized fibrils. Indeed, it is implied from measures of sulfate incorporation in the sclera that the synthesis of proteoglycans, such as decorin, is reduced in mammalian eyes with developing myopia.\textsuperscript{20,35–37} and this is supported by the finding that overall proteoglycan content of the scleral tissue also alters over time, as is the case in the sclera of highly myopic human eyes.\textsuperscript{8,12} Therefore, it may be that, in the short-term, the collagen fibril diameter profile in the sclera of myopic eyes is relatively unaffected by the protease-driven degradative process and that longer term shifts in collagen fibril distribution are mediated by the changing scleral extracellular matrix biochemistry.

### Ocular Enlargement and Scleral Collagen Fibrils

Changes in scleral collagen fibril diameter distribution were found to be associated with the most rapid periods of eye growth in normally developing animals, which may suggest that the rate of growth of the eye is related to the profile of collagen fibril diameters within the scleral matrix. It has previously been reported that smaller diameter collagen fibrils are present in developing tissues, giving way to larger diameter fibrils as the tissue matures.\textsuperscript{38} Recent theories explaining this phenomenon suggest that larger diameter fibrils develop through lateral accumulation of collagen on existing fibrils as the collagen matrix matures and the fibril elongates.\textsuperscript{39} We suggest that the large numbers of smaller diameter fibers in the sclera, seen during early ocular development and myopic eye growth in older eyes, are indicative of collagen fibril maturation during eye growth. Indeed, this hypothesis may explain why the developing eye, in which the collagen matrix is developing, has been shown to be most susceptible to the stimuli that induce myopic eye growth.\textsuperscript{18} Furthermore, this hypothesis suggests that the shift to a preponderance of smaller diameter fibers in older animals with high degrees of induced myopia is consistent with a collagen matrix that is still actively changing. However, the fact that no significant change in fibril profile was encountered in young animals in which myopia was developing suggests that additional factors are involved in facilitating accelerated ocular elongation during the early stages of myopic eye growth.

Changes in the material properties of the sclera have recently been reported in tree shrew eyes with developing myopia.\textsuperscript{14,15,40} These changes were detectable after 4 days of myopia development and were, to a large extent, independent of changes in the thickness of the scleral tissue itself. Furthermore, specifically in mammals, the degree of axial myopia was found to correlate with the degree of change in the mechanical properties of the sclera.\textsuperscript{15} Because the results of the present study suggest that the collagen fibril profile does not alter significantly in short-term–deprived eyes, the mechanical properties of the sclera must be controlled by additional factors, such as proteoglycans,\textsuperscript{41} during the initial stages of myopia development. However, we suggest that during the later stages of myopia development, the increased numbers of small-diameter fibrils, in conjunction with the reduced proteoglycan content of the tissue, contribute to a scleral matrix that is less resistant to imposed mechanical stresses (such as the normal intraocular pressure). Indeed, studies in other fibrous matrices have shown that a preponderance of smaller diameter collagen fibrils is associated with lower tensile strength of the tissue.\textsuperscript{42} It may be that these smaller fibrils in the posterior sclera of eyes with long-term myopia underlie both the changes in refractive
error seen in progressive myopia and the formation of posterior staphyloma.

In summary, the findings of the present study showed that ocular enlargement and scleral tissue loss during myopia development were not accompanied by significant changes in scleral collagen fibril diameter in the short term. However, longer periods of myopia development were accompanied by alterations in the collagen fibril diameter profile, resulting in more small-diameter fibrils. These findings may account for changes that are found in the material properties of the sclera from myopic eyes, indicating a mechanism for the formation of posterior staphyloma in highly myopic humans.

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**References**