Pressure-Induced Changes in Axial Eye Length of Chick and Tree Shrew: Significance of Myofibroblasts in the Sclera

John R. Phillips and Neville A. McBrien

PURPOSE. To investigate the change in axial eye dimensions resulting from stretching the sclera by acute elevation of intraocular pressure (IOP).

METHODS. IOP was increased to 100 mm Hg for 1 hour through an intravitreal cannula, while ocular component dimensions were monitored every 10 minutes with A-scan ultrasound in anesthetized animals (10 chicks and 10 tree shrews). In addition, immunocytochemical detection of α-smooth muscle actin (α-SMA) using a monoclonal antibody was conducted in the sclera of the tree shrew and the chick.

RESULTS. In both species, axial eye length immediately and significantly (P < 0.0001) increased on elevation of IOP to 100 mm Hg: chick to 103.9%, tree shrew to 101.2% (mean percentage of original measured at 15 mm Hg). After 1 hour of maintained pressure, chick eyes showed a further significant increase in axial length (to 108.6%), but axial length of tree shrew eyes decreased (to 100.3%) to the point that it was not significantly different from the original value at 15 mm Hg. Immunocytochemical studies of age-matched tissue demonstrated the presence of α-SMA-containing fibroblasts (myofibroblasts) within tree shrew but not chick sclera.

CONCLUSIONS. Elevation of IOP caused axial elongation of chick eyes, but a consistent reduction in axial length of tree shrew eyes. The presence of myofibroblasts, demonstrated in tree shrew but not chick sclera, suggests that the reduction in axial length of tree shrew eyes may have been caused by activation of a contractile mechanism involving scleral myofibroblasts. Such a mechanism may play a role in the regulation of eye size and refractive development.

Articles


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Axial elongation of the eye with associated myopia can readily be induced in developing animals by visual form deprivation1-4 or by raising the animal with negative lenses in place.5,6 In the avian (chick) eye, in which the sclera consists of an inner cartilaginous layer and an outer fibrous layer, eye elongation involves increased proteoglycan synthesis and growth of the inner cartilaginous layer.10,11 However, in the mammalian eye, in which the sclera comprises a single fibrous layer, ocular expansion does not involve net growth but rather a remodeling of the sclera with decreased proteoglycan synthesis and a net loss of scleral tissue.12,13 In the tree shrew, experimental myopia is also associated with changes to the biomechanical properties of sclera, in particular an increase in the creep rate,14,15 and it has been proposed that this may account for eye elongation by reducing scleral resistance to the expansion forces of normal intraocular pressure (IOP). However, the mechanical properties of sclera have not been sufficiently characterized to allow quantitative prediction of the actual changes to eye shape or length that would result from a change in scleral creep properties. The changes in creep rate that have been reported are from experiments involving in vitro, uniaxial (one directional) mechanical stretching of scleral strips cut from the posterior pole of myopic and normal tree shrew eyes.14,15 In the living eye, the mechanical behavior of sclera may be quite different. IOP stretches the sclera in many directions, and the presence of nonvascular contractile myofibroblasts reported within human and monkey choroid and sclera16 raises the possibility that in vivo sclera may exhibit some active contractile behavior in addition to its passive elastic and creep properties. Indeed, unexplained absolute reductions in axial length and vitreous chamber depth have been reported for tree shrew eyes recovering from induced axial myopia17,18 and such reductions in axial length would be consistent with activation of a contractile process within the sclera.

The purpose of the present study was to examine the association between axial eye length and in vivo stretching of the sclera induced by acute elevation of IOP. A second purpose was to investigate the presence of myofibroblasts in the sclera. To do this we used immunocytochemical methods to detect the presence of α-smooth muscle actin (α-SMA) in sclera, because the expression of α-SMA is currently considered to be the most reliable marker of differentiated myofibroblast.19,20 Experiments were conducted in the tree shrew and in the chick because both have been widely used as animal models of myopia, and the proposed mechanisms of axial elongation in the two species are different.

METHODS

Ten normal tree shrew pups (Tupaia belangeri) maintained under a 15:9-hour light–dark cycle were studied between 25 and 35 days after eye opening. Ten normal chicks (White Leghorn: Black Australorp cross, 12–18 days old) maintained under a 12-hour light–dark cycle were also used in this study. Animals were anesthetized intramuscularly (tree shrews, 90 mg/kg ketamine HCl with 10 mg/kg xylazine; chicks, 50 mg/kg ketamine HCl with 3.5 mg/kg xylazine) and maintenance doses of anesthetic were given as required to maintain areflexia. Animal procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.
The methods used to study changes in axial eye length during elevated IOP were the same for both species. In each case, the head was supported in the normal upright position with a bite bar of dental acrylic. The upper lid of the eye was cut and folded back to expose the vitreous chamber. A 27-gauge hypodermic needle, connected to a rubber sphygmomanometer bulb and punctured the superior equator of the globe at an angle of 45° to the optic axis (Fig. 1) to avoid contact with the crystalline lens. The position of the needle in the vitreous chamber was checked with an ophthalmoscope to ensure that the tip remained clear of the retina and that it was sufficiently far from the optic axis that it would not interfere with the A-scan measurements. Tubing and needle were filled with Ringer’s solution before insertion into the eye. On insertion of the needle, IOP was adjusted to 15 mm Hg. It was then increased gradually to 100 mm Hg over a period of 2 minutes with the sphygmomanometer bulb and then held steady at 100 mm Hg for 1 hour. A high pressure of 100 mm Hg was chosen to increase signal to noise ratio because in tree shrew eyes in particular, pressures much lower than this resulted in very small changes in ocular dimensions which would have necessitated much longer periods of in vivo monitoring, which was considered undesirable. Axial dimensions of the eye were determined with A-scan ultrasonography using a 15 MHz transducer, as has been described previously.21 Axial length (AXL) of the eye was determined as the sum of the anterior chamber depth (ACD), the lens thickness (Lens), and the vitreous chamber depth (VCD). Thus, AXL was measured to the inner limiting membrane of the retina and did not include retinal or choroidal thickness. A-scan measures were made immediately before insertion of the needle into the vitreous chamber and immediately after insertion once the pressure had been adjusted to 15 mm Hg. Further measures were made immediately after raising the pressure to 100 mm Hg and thereafter every 10 minutes while the pressure was elevated (60 minutes). Final readings were taken after reducing the pressure to 15 mm Hg, before removing the needle from the eye. Particular care was taken to ensure that IOP did not decline during the 1 hour of sustained elevated pressure. Leakage at the point where the needle entered the globe was easily detected because the local fascia covering the eyeball swelled and, in addition, the mercury level in the manometer decreased, as fluid volume was lost. In the few experiments in which such leaks developed, data collection from that eye was abandoned. In the tree shrew, ophthalmoscopic observation of the blood vessels at the disc indicated that at IOP higher than approximately 65 mm Hg, the retinal arteries and veins remained empty of blood. The state of the blood vessels was checked every 10 minutes, immediately after ultrasound determination of ocular dimensions, to ensure that the vessels remained empty of blood, indicating that the pressure remained elevated and that the intravitreal needle was patent. When IOP was reduced to 15 mm Hg at the end of the 1-hour period of elevated pressure the axial length of the eyes decreased rapidly, consistent with a sudden reduction in IOP. In addition, blood immediately filled retinal arteries and veins. Animals were killed by intraperitoneal injection of pentobarbital sodium (100 mg/kg), while under anesthesia.

**Immunocytochemistry**

Ocular tissues were collected for immunocytochemistry from age-matched tree shrews and chicks. Animals were sedated with ketamine-xylazine then killed with pentobarbital sodium. Eyes were dissected, cleaned of extraneous orbital tissue, and cut around the equator. A 7-mm surgical trephine was used to isolate a punch comprising retina, choroid, and scleral tissue from the region of the posterior pole. Tissue was fixed in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS, pH 7.4), for 50 minutes at room temperature. After fixation, the tissue was washed three times in PBS for 15 minutes, followed by immersion for 30 minutes each in 10% and 20%, and immersion overnight in 30% (wt/vol) sucrose in PBS. Tissues to be sectioned were mounted in embedding medium for frozen tissue sections (optimal cutting temperature [OCT] compound, Tissue-Tek; Sakura Finetek, Torrance, CA). The tissue block was cut in frozen sections (6 μm thick) on a cryostat (Leica, Heidelberg, Germany) at −20°C and collected on poly-l-lysine-coated glass slides (MenzelGlaser, Braunschweig, Germany) and stored at −20°C. α-smooth muscle actin (α-SMA) was detected using the α-SMA immunohistology kit (Product No. IMMHH-2, monoclonal, clone no. 1A4; Sigma-Aldrich, St. Louis, MO) according to the manufacturer’s protocol. The primary antibody was mouse monoclonal anti-smooth muscle actin (code A0307; Sigma-Aldrich). The secondary antibody used was biotinylated goat anti-mouse IgG (code B4398; Sigma-Aldrich). Briefly, sections were rehydrated and endogenous peroxidase activity quenched with 3% hydrogen peroxide. The tissue was then blocked with 1% normal goat serum in PBS (catalog no. G9023; Sigma-Aldrich). Either the primary antibody (mouse monoclonal α-SMA) or PBS as a negative control was applied to the sections for 1 hour. The sections were washed with PBS to remove excess unbound primary antibody. The bound primary antibody was then visualized by application of the biotinylated secondary antibody (goat anti-mouse IgG) for 20 minutes. After a short incubation, slides were washed and an avidin-conjugated peroxidase reagent (Extravidin; Sigma-Aldrich) applied. After a further incubation, a substrate reaction was performed with 3-amino-9-ethylcarbazole (AEC) chromogen in 5% hydrogen peroxide (to produce a red-brown precipitate in the cytoplasm of α-SMA-positive cells). The slides were rinsed in deionized water for 5 minutes, when sufficient staining had been achieved. The slides were counterstained with Mayer’s hematoxylin (Sigma-Aldrich) for 2 minutes and rinsed gently in running tap water, to blue the hematoxylin. The sections were allowed to dry, coated with glycerol gelatin (Sigma-Aldrich), and attached to a glass coverslip (Menzel-Glaser). All incubations were at room temperature. Cells containing α-SMA were visualized with a ×40 oil-immersion objective on a microscope (Axioplan2; Carl Zeiss Meditec, Oberkochen, Germany) connected to a digital imaging camera (Eastman Kodak, Rochester, NY).

**Statistical Analysis**

Repeated measures analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons tests were used to assess differences with time. Data at each sampling time passed the Shapiro-Wilk normality test and had similar standard deviations and covariance.

**RESULTS**

**In Vivo Changes in Axial Length with Elevated IOP**

Chick. Figure 2A shows the time course of the change in axial length of 10 normal chick eyes resulting from an increase in IOP from 15 to 100 mm Hg for 60 minutes followed by a return to 15 mm Hg pressure. The period of elevated pressure, from time = 0 minutes (T0) to time = 60 minutes (T60) is
indicated by horizontal bars in Figure 2. For each chick, two axial length measures were made before the period of elevated pressure. The first (Normal in Table 1, but not shown in Fig. 2) was the physiological axial length measured before the needle was inserted into the vitreous chamber (see the Methods section). The second (Pre in Table 1) was the axial length measured after the needle had been inserted into the vitreous chamber and with the pressure set to 15 mm Hg. There was no significant change in axial length resulting from insertion of the needle into the eye and the setting of pressure to 15 mm Hg (Normal = 8753 ± 355 μm vs. Pre = 8829 ± 374 μm; mean ± SD; P > 0.5, see Table 1). However, there was a significant increase in axial length associated with the increase in IOP from 15 to 100 mm Hg at T0 (Pre = 8829 ± 374 μm vs. T0 = 9169 ± 335 μm; P < 0.001), which corresponds to an ocular elastic compliance of approximately 1 μm/mm Hg increase in IOP of approximately 4 μm/mm Hg. There was also a significant increase in axial length over the 60-minute period of elevated pressure (T0 = 9169 ± 335 μm vs. T60 = 9585 ± 370 μm; P < 0.001). Then there was a significant decrease in axial length when the pressure was returned to 15 mm Hg (T60 = 9585 ± 370 μm vs. Post = 9281 ± 394 μm; P < 0.001). However, this decrease did not return the axial length to the Pre-pressure value; the Post-pressure axial length remained significantly greater (Pre = 8829 ± 374 μm vs. Post = 9281 ± 394 μm; P < 0.001). Thus, a residual elongation of the eye occurred in chick (mean elongation Post to Pre, 452 μm; range, 809 to 228 μm) that did not display an elastic recovery on return of pressure to 15 mm Hg.

**Tree Shrew.** Figure 2B shows the time course of the change in axial length of 10 tree shrew eyes resulting from an increase in IOP from 15 to 100 mm Hg for 60 minutes followed by a return to 15 mm Hg pressure. The procedure for tree shrew experiments was the same as that used for chicks. There was no significant change in tree shrew axial eye length resulting from insertion of the needle into the eye and the setting of pressure to 15 mm Hg (Normal = 7151 ± 92 μm vs. Pre = 7162 ± 107 μm; P > 0.5, see Table 1). As for chicks, there was a significant increase in axial length associated with the increase in IOP from 15 to 100 mm Hg (Pre = 7162 ± 107 μm vs. T0 = 7246 ± 101 μm; P < 0.0001), corresponding to an ocular elastic compliance of approximately 1 μm/mm Hg (compared with 4 μm/mm Hg for chick eyes). Then, for tree shrew eyes there was a progressive and significant decrease in axial length (mean decrease = 64 ± 35 μm) over the 60-minute period of elevated pressure (T0 = 7246 ± 101 μm vs. T60 = 7182 ± 99 μm; P < 0.001). As a result of this decrease, the axial length measurement made at 100 mm Hg after 60 minutes of elevated pressure was not significantly different from the Pre-pressure length measurement made at a pressure of 15 mm Hg (Pre = 7162 ± 107 μm vs. T60 = 7182 ± 99 μm; P > 0.5). There was a further significant decrease in axial length when the pressure was returned from 100 to 15 mm Hg (T60 = 7182 ± 99 μm vs. Post = 7100 ± 107 μm; P < 0.001),

**TABLE 1. Axial Dimensions of Ocular Components**

<table>
<thead>
<tr>
<th></th>
<th>Chick (n = 10)</th>
<th></th>
<th></th>
<th></th>
<th>Post 15 mm Hg</th>
<th>Change (Pre to Post)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal Physiological</td>
<td>Pre 15 mm Hg</td>
<td>T0 100 mm Hg</td>
<td>Change (Pre to T0)</td>
<td>T0 100 mm Hg</td>
<td>T60 100 mm Hg</td>
</tr>
<tr>
<td>ACD</td>
<td>1467 ± 120</td>
<td>1487 ± 112</td>
<td>1536 ± 88</td>
<td>49 ± 49</td>
<td>1554 ± 138</td>
<td>18 ± 72</td>
</tr>
<tr>
<td>Lens</td>
<td>2175 ± 113</td>
<td>2172 ± 112</td>
<td>2140 ± 152</td>
<td>-32 ± 46</td>
<td>2110 ± 103</td>
<td>-30 ± 48</td>
</tr>
<tr>
<td>VCD</td>
<td>5111 ± 145</td>
<td>5170 ± 225</td>
<td>5493 ± 209</td>
<td>323 ± 92</td>
<td>5921 ± 275</td>
<td>428 ± 144</td>
</tr>
<tr>
<td>AXL</td>
<td>8753 ± 355</td>
<td>8829 ± 374</td>
<td>9169 ± 355</td>
<td>340 ± 95</td>
<td>9585 ± 370</td>
<td>417 ± 172</td>
</tr>
<tr>
<td>Tree Shrew (n = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACD</td>
<td>1080 ± 32</td>
<td>1086 ± 38</td>
<td>1056 ± 32</td>
<td>-30 ± 42</td>
<td>1024 ± 44</td>
<td>-32 ± 44</td>
</tr>
<tr>
<td>VCD</td>
<td>2813 ± 67</td>
<td>2839 ± 67</td>
<td>2980 ± 55</td>
<td>141 ± 33</td>
<td>2942 ± 58</td>
<td>-38 ± 32</td>
</tr>
<tr>
<td>AXL</td>
<td>7131 ± 92</td>
<td>7162 ± 107</td>
<td>7246 ± 101</td>
<td>84 ± 21</td>
<td>7182 ± 99</td>
<td>-64 ± 35</td>
</tr>
</tbody>
</table>

Data are expressed as mean micrometers ± SD. Normal, before any experimental manipulation; Pre, following vitreal cannulation and setting pressure to 15 mm Hg, but prior to elevating pressure; T0, immediately after elevating IOP to 100 mm Hg; T60, after 60 minutes of elevated IOP; Post, immediately after returning IOP to 15 mm Hg. Change, the increase (if negative, the decrease) in ocular component dimensions after initially increasing pressure (Pre to T0), during the period of elevated pressure (T0 to T60) and the residual change after return to 15 mm Hg (Pre to Post).
and this decrease resulted in the Post-pressure axial length being significantly less than the Pre-pressure value (Pre = 7162 ± 107 μm vs. Post = 7100 ± 107 μm; P < 0.001). Thus, a residual shortening in eye-length occurred in tree shrew (mean shortening: Pre to Post = 62 μm; range, 121 to 25 μm) that did not display an elastic recovery on return of pressure to 15 mm Hg. The mean reduction in axial length that occurred during the period of elevated pressure (64 μm) was essentially the same as the residual shortening (62 μm) that remained after return of pressure to 15 mm Hg.

### Changes in Ocular Component Dimensions with Elevated Pressure

To compare the changes that occurred to the ocular component dimensions in chicks and tree shrews as a result of elevated IOP, the ocular component dimensions were expressed as a percentage of their value measured in the Pre-pressure condition (i.e., with the vitreous chamber cannulated and the pressure set to 15 mm Hg). Figure 3 shows that much greater percentage changes in ocular component dimensions were observed in chick eyes than in tree shrew eyes at the same increase in IOP. In chick eyes, the initial axial elongation after increasing IOP was to a mean (± SD) of 103.9% ± 1.2% of the original length, but the maximum elongation (mean = 118.6% ± 2.1%) occurred after 60 minutes of elevated pressure (T60). In the tree shrew, the maximum elongation (mean = 101.2% ± 0.3%) occurred immediately after elevation of pressure (T0). Figures 3B–D illustrate that in chick, 60 minutes of elevated pressure was associated with a progressive increase in vitreous chamber depth (mean at T60 = 115% ± 3.0%), a relatively constant increase in anterior chamber depth (mean at T30 = 107% ± 3.3%) and a relatively constant reduction in lens thickness (mean at T30 = 97% ± 1.7%). In the tree shrew, 60 minutes of elevated pressure was associated with a progressive decrease in vitreous chamber depth from a mean of 105.2% ± 1.5% at T0 down to 103.7% ± 2.0% at T60. During this period, in the tree shrew there was a relatively constant reduction in anterior chamber depth (mean at T30 = 95.1% ± 2.5%) and little change in lens thickness (mean at T30 = 99.2% ± 0.96%).

### Histology

Figure 4 shows localization of α-SMA within the choroid of the chick and within the choroid and sclera of the tree shrew. As expected, both chick (Fig. 4A) and tree shrew (Fig. 4E) tissue showed marked staining for α-SMA in the choroid, particularly in the walls of the blood vessels. However, no staining for α-SMA was detected in the chondrocytes of the cartilaginous layer or the fibroblasts in the fibrous layer of the chick sclera. In contrast, in the tree shrew the red-brown staining indicating the binding of α-SMA antibody was present in the elongated fibroblasts (myofibroblasts) of the sclera (Figs. 4E, 4G). Negative control eyes (Figs. 4B, 4D, 4F, 4H), missing the primary antibody, showed no staining in the chick choroid or the tree shrew choroid or sclera.

### DISCUSSION

In the present study, both chick and tree shrew eyes elongated immediately after IOP was increased. This is the expected result and is consistent with an initial elastic stretching of the sclera by the increased pressure. However, the ocular compliance of chick eyes (previously reported as 8–9 μm/mm Hg) and found to be 4 μm/mm Hg in this study) was much greater than the ocular compliance obtained for tree shrew eyes (approximately 1 μm/mm Hg). This species difference in ocular compliance is the opposite of that which might be predicted from in vitro studies of sclera, which have shown the...
elastic stiffness of chick sclera to be much greater than that of tree shrew sclera when scleral samples from both species were tested (uniaxially) under identical conditions (elastic modulus from 0 to 0.015 strain): chick = 15.4 × 10^6 Pa; tree shrew = 2.7 × 10^6 Pa. After the initial elastic elongation, chick eyes gradually increased in axial length during the period of elevated pressure, which is consistent with results from in vitro studies of the time-dependent creep properties of chick sclera. In contrast, in tree shrew eyes, the initial elastic increase in axial length was followed by a progressive decrease in axial length during the period of elevated pressure. Such an unexpected result is the opposite of that which would be predicted from in vitro creep studies of tree shrew sclera. This observation in the tree shrew is unlikely to be the result of an experimental artifact. First, considerable care was taken (see the Methods section) to ensure that the IOP was maintained at 100 mm Hg for the experimental period. Second, although it is likely that the direct action of 100 mm Hg IOP would have caused compression and thinning of the retina and choroid, as has been shown to occur in chick eyes, the effect of such thinning would be to increase the measured vitreous chamber depth rather than to decrease it. Third, simple displacement of the lens forward or backward would result in equal and opposite changes to the dimensions of the anterior and vitreous chambers, leaving the overall axial length of the eye unchanged. In the tree shrew, both anterior and vitreous chamber dimensions decreased, as did the axial length of the eye. In principle, axial compression of the lens may have contributed to a reduction in overall eye length. In the tree shrew, lens thickness did decrease by a mean value of 19 μm, but this was insufficient to account for the average reduction in eye length of 64 μm. Perhaps the most convincing evidence that an experimental artifact was not involved in these measures is the consistency of the result among animals of the same species and the clear difference in result between the two species when both were subjected to the same experimental manipulation.

In the absence of experimental artifacts, the progressive decrease in axial length may be accounted for either by a change in the shape of the eye (e.g., equatorial expansion accompanied by axial contraction) or by the action of a choroidal or scleral mechanism, which has the effect of reducing axial eye length. In this study, we were unable to make satisfactory measurements of the equatorial diameter of the eye with ultrasound in vivo. However, previous finite element modeling of the change in shape of the tree shrew eye under increased IOP predicts that although axial length would increase, the equatorial diameter of the eye would decrease as the sclera expands elastically. Elastically increased IOP reduces in axial length that was observed in tree shrew eyes is most likely explained by the activation of some process, either within the choroid or sclera, which has the effect of moving the retina forward. It seems unlikely that this forward movement resulted from an increase in choroidal thickness, because increased IOP has been shown to decrease choroidal thickness in the chick (at least for pressures up to 30 mm Hg above normal).

Relevant to these observations of reduced eye length in the tree shrew is our demonstration of the presence of myofibroblasts in the choroid and sclera of the tree shrew and in the choroid of the chick. Myofibroblasts, which are specialized contractile fibroblasts, are identified by the presence of α-SMA, which is absent in normal connective tissue fibroblasts. Myofibroblasts have been reported in both the choroid and sclera of humans and monkeys and are found in granulation tissue, fibrocontractive diseases and some normal tissues. Morphologically, myofibroblasts are characterized by the presence of a contractile apparatus containing bundles of actin microfilaments and associated contractile proteins such as nonmuscle myosin. The bundles of microfilaments terminate at the cell surface in a specialized adhesion complex (the fibronexus) where transmembrane integrins link intracellular actin with extracellular fibronectin filaments. Functionally, it has been proposed that this transmembrane linkage provides a two-way mechanotransduction pathway through which the myofibroblast can both sense the extracellular mechanical environment and also modify it. Thus, extracellular mechanical signals (e.g., tissue strain) could be transduced into an intracellular signal and in addition, force generated by the intracellular contractile process could be transmitted to the extracellular matrix through this pathway.

In the present study we observed that elevated IOP induced a reduction in axial eye length in the tree shrew, but increased... 

[Figure 4. Localization of α-SMA within the choroid of the chick and within the choroid and sclera of the tree shrew. (A) Chick, +ve. Red-brown stain indicates the binding of α-SMA antibody around the blood vessels of the chick choroid. (C) Chick, +ve. No α-SMA antibody was present in the cytoplasm of the chondrocytes or fibroblasts of the cartilaginous or the fibrous layers of the chick sclera. The negative controls (B, D) were missing the primary antibody; no staining was seen in the chick choroid. (E) Tree shrew, +ve. Red-brown stain indicates the binding of α-SMA antibody around the blood vessels of the tree shrew choroid and also within the fibroblasts of the sclera. (G) Tree shrew, +ve. α-SMA antibody staining was present in the thin processes of the fibroblasts in the tree shrew sclera. Also note that the counterstain stains only cell nuclei, and so the increased visibility of fibroblast processes in the positives is the result of antibody binding only. The negative controls (F, H) were missing the primary antibody and no staining was seen in the tree shrew choroid or sclera. Magnifications: (A, B, E, F) ×500; (C, D, G, H) × 2500. ]
eye length in chick. We have demonstrated the presence of myofibroblasts, through α-SMA immunolabeling, in the sclera of the tree shrew but not in the sclera of the chick eye. Based on this correlation, we propose that it is likely that during our in vivo experiments, tissue stretch resulting from sustained increase in IOP activated, through the mechanotransduction pathway, the contractile elements in the myofibroblasts of tree shrew sclera, causing them to contract. We suggest that this contraction is then transmitted to the surrounding extracellular matrix of the sclera through this pathway and that this in turn results in a reduction in vitreous chamber depth. If such a proposal were correct, it still remains unclear whether the tissue shortening that we observed should be ascribed to myofibroblast contraction alone, or whether some degree of tissue contracture (i.e., semipermanent contraction with extracellular tissue remodeling) might also have occurred during the 1 hour of sustained elevated pressure. Myofibroblasts can contract relatively rapidly (e.g., rapid contraction occurs within 2 to 10 minutes20) and they can also sustain a contractile force for long periods.20 It seems unlikely that significant remodeling (subject to a contractile force for long periods.) would have occurred within a 1-hour period, but the observation that tree shrew eyes were shorter after return to 15 mm Hg IOP than they were before the period of elevated pressure, implies that some of the shortening might have involved extracellular matrix remodeling.

In previous studies of tree shrew eyes recovering from induced myopia, the vitreous chamber depth was found to be shallower after three days of recovery than at the commencement of the recovery period27 and it was shown that the shortening could not be accounted for by an increase in choroidal thickness.28 No explanation was put forward for the observation that the vitreous chamber became shorter in animals recovering from myopia, but the results of the present study provide evidence for the existence of a contractile mechanism in the sclera that, if activated, could reduce vitreous chamber depth in the tree shrew. Further studies of α-SMA expression in the sclera are needed, as scleral myofibroblasts could be important in controlling normal eye growth and the refractive development of the mammalian eye.

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References