Topical Estrogen Therapy for Hyperopia Correction in Vivo

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Received: April 1, 2020
Accepted: May 17, 2020
Published: June 24, 2020

Citation: Leshno A, Prokai-Tatrai K, Rotenstreich Y, et al. Topical estrogen therapy for hyperopia correction in vivo. *Invest Ophthalmol Vis Sci.* 2020;61(6):55. https://doi.org/10.1167/iovs.61.6.55

Purpose. In vitro studies found that 17β-estradiol (estrogen) modulates corneal biomechanical properties and reduces tissue stiffness. Therefore we hypothesized that topical estrogen might affect the refractive properties of the cornea, inducing a myopic shift.

Methods. Twelve female New Zealand white rabbits 16 weeks old were used. The rabbits were randomly divided to either the treatment group receiving 1.5% (w/v) estrogen eye drops or a control group receiving vehicle only (n = 6 each group). Both groups were given drops (50 µL) to the right eye every 12 hours for 35 days. Ocular examination, pachymetry, intraocular pressure (IOP), keratometry ,and refraction were evaluated at baseline and on a weekly basis.

Results. No significant differences were observed between the two groups at baseline in all outcome measures. Both groups displayed corneal flattening and a hyperopic shift. However, the change rate was slower in the treatment group. Repeated measurements analysis revealed a statistically significant difference in keratometry readings between groups ($P = 0.034$) with steeper keratometry by up to 0.6 diopters in the treatment group. The difference between the two groups diminished and became statistically insignificant after treatment cessation. No significant changes were observed in IOP and pachymetry throughout the study period. No side effects were observed in either group.

Conclusions. Estrogen eye drops induced a myopic shift in keratometry readings. These results suggest that corneal refractive power might be manipulated pharmacologically. Further studies on the physiology behind this change are warranted to facilitate a pathway for development of novel pharmacologic treatments to correct refractive errors.

Keywords: estrogen, keratometry, refraction, myopic shift, cornea, hyperopia

Estrogen is one of the two female sex hormones responsible for the development and regulation of the female reproductive system. Plasma estrogen levels increase during ovulation, in the luteal phase of the menstrual cycle, and during pregnancy, peaking gradually in the third trimester. The literature contains numerous evidence regarding the effect of variations in blood levels of sex hormones on the biomechanical and optical power of the cornea. Several prospective studies have shown an increase in corneal curvature, thickness, and volume during pregnancy.[1,2] Goldich et al.[2] observed a significant increase in corneal steepening in pregnant women versus nonpregnant women (44.81 diopters vs. 44.1 diopters, $P = 0.039$). Pizzarello et al.[3] found that pregnancy can cause a myopic shift of about one diopter. These changes are considered temporary and resolve at the postpartum period.

Corneal changes also occur throughout the menstrual cycle along with the changes in estrogen levels, although they are relatively minor compared with those found during pregnancy. Significant flattening of the corneal curvature associated with estrogen serum levels was observed during menses as compared with after menses ($P < 0.05$).[4,5] Aydin et al.[6] showed that the elevations in corneal topography mainly affected the horizontal curvature. Changes in corneal thickness, refractive parameters, and vision sensitivity were also found to correlate with fluctuation of estrogen levels during the menstrual cycle,[7–9] although the biomechanical properties may not be affected.[10]

Although the direct cause for the above-mentioned corneal changes remains unclear, it is presumed to be driven by direct interaction of estrogen, with its nuclear estrogen receptors (ERα and ERβ) expressed in the corneal epithelial,

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stromal, and endothelial cells.[11–13] Therefore we expected that topical estrogen treatment would result in a myopic shift by increasing corneal steepening.

The use of systemic and topical estrogen has been evaluated previously by several studies as a possible treatment for a variety of eye conditions, including dry eye,[14–16] glaucoma,[17,18] age-related cataracts,[19] diabetic retinopathy,[20] and age-related macular degeneration.[21] However, to the best of our knowledge, the effect of estrogen on corneal topography has not yet been described, especially when applied topically. We therefore aimed in this proof-of-concept study to determine the in vivo effect of estrogen eye drops on corneal refractive power.

Material and Methods

Materials

Estrogen eye drops 1.5% (w/v) were formulated as described previously by using 20% (w/v) hydroxypropyl-β-cyclodextrin (Steraloids, Inc. New Port, RI, USA) in saline solution as a vehicle.[18] Stable isotope-labeled estrogen (17β-estradiol-13,14,15,16,17,18-$^{13}C_6$) with an isotopic purity of 99% was supplied by the Cambridge Isotope Laboratories, Inc. (Andover, MA, USA) and was used as an internal standard for drug quantitation by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Other chemicals were obtained from Sigma Chemical Company (St. Louis, MO, USA).

Animals

Twelve female New Zealand White rabbits (Envigo Laboratories, Rehovot, Israel), 16 weeks old and weighing 2.4 ± 0.2 kg were used. All animal procedures and experiments were conducted with the approval and under the supervision of the Institutional Animal Care Committee at Tel Aviv University, Tel Aviv, Israel, and conformed to the recommendations of the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. All animals underwent a complete ocular examination before study initiation to exclude any signs of ocular pathology.

Intervention

Based on previous studies we estimated that the effect of estrogen treatment would be apparent after four weeks of treatment. An additional week of treatment was added to ensure a margin of safety. An equal time frame was used for follow-up after treatment cessation to check whether the effects were transient or permanent. The animals were examined at baseline (day 0) and once a week until the seventieth day. After baseline examination, the rabbits were randomly divided in an equal manner to either the treatment group receiving 1.5% (w/v) estrogen eye drops or the control group receiving a vehicle only. Both groups were given drops (50 µL) to the right eye every 12 hours during the first 35 days of the study only. Blood was collected from the marginal ear vein one week before study initiation and at day zero to establish baseline blood estrogen levels. Blood was also collected at each time point during follow-up, approximately one to two hours after the morning dose. The blood serum was used for estrogen quantitation by LC-MS/MS based on liquid-liquid extraction and principles of isotope dilution as published before.[22] All researchers involved in the study were masked as to animal group assignment and content of treatment vials until the end of the study.

Ocular Assessment

Corneal curvature and refraction were evaluated using Righton Retinomax K-plus 3 (Righton, Tokyo, Japan). Multiple measurements were recorded by the machine with a final display of a single best result that was determined automatically. To avoid measurement errors, keratometry readings (i.e., K1-flat, K2-steep, and K-avg) and refraction (i.e., sphere, cylinder power, and cylinder axis) were measured five times by the same observer, and the readings were averaged for each time point for statistical analysis.

A complete ocular examination, pachymetry (PachPen; Accutome, Malvern, PA, USA), and intraocular pressure (IOP; Tono-Pen AVIA; Reichert, Buffalo, NY, USA) were evaluated at each time point for both eyes. Anterior segment ocular coherence tomography (AS-OCT; Spectralis, Heidelberg Engineering, Franklin, MA, USA) imaging was performed with the animals under anesthesia with an intramuscular injection of ketamine (35 mg/kg) and xylazine (5 mg/kg) at baseline and at two, five, and ten weeks' follow-up. Corneas were kept moist with 2.5% (w/v) hydroxypropyl methylcellulose (Fisher Pharmaceuticals Labs, Bnei Brak, Israel) during OCT measurements. Central corneal thickness (CCT) was obtained using the Spectralis software in both the longitudinal and horizontal AS-OCT imaging lines for each eye. The average of the two lines measurements was calculated and used for statistical analysis.

Statistical Analysis

Data for drug quantitation are expressed as mean ± SEM, n = 6 per treatment groups. Statistical software (SPSS version 25.0; SPSS, Inc., Chicago, IL, USA) was used for data analysis. Spearman's test was used to determine correlation between variables in the entire data. Repeated measures ANOVA (RMANOVA) analysis was performed to compare the overall differences in measured parameters between groups during the entire study period and during the intervention period. Variables found to have a significant correlation with K-avg in the Spearman's test used as covariates. Post-hoc analysis with parameter estimates was applied for comparison of continuous variables at each time-point between the two groups to establish duration of treatment required for statistically significant difference. Differences were considered significant if $P < 0.05$.

Results

Baseline ocular characteristics were considered normal in all animal subjects. No statistically significant differences were observed between the two groups in keratometry readings, pachymetry, and IOP measurements (Table).

Overall, significant correlations were observed between K-avg and body weight (0.850, $P < 0.001$), pachymetry (−0.280, $P = 0.002$), and IOP (−0.206, $P = 0.025$). Both groups displayed corneal flattening and hyperopic shift in keratometry readings over time (Fig. 1).

After adjustment for weight and pachymetry, both groups had similar baseline K-avg readings (50.3 ± 0.28 vs. 50.5 ± 0.28, $P = 0.5357$); however, the decline was slower in

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Table. Baseline Characteristics Of Experimental Animals

Group	Study (1.5% w/v Estrogen), n = 6 Mean ± SDEV	Control (Vehicle Alone), n = 6 Mean ± SDEV	*P* Value
Body Weight (kg)	2.4 ± 0.3	2.4 ± 0.3	0.808
K-avg (diopter)	50.4 ± 0.7	50.4 ± 0.6	0.855
Spherical Equivalent (diopter)	+2.4 ± 0.7	+2.2 ± 1.2	0.873
Pachymetry (µm)	357.3 ± 10.6	359.8 ± 23.3	0.748
IOP (mm Hg)	13.5 ± 1.1	13.8 ± 2.4	1.000

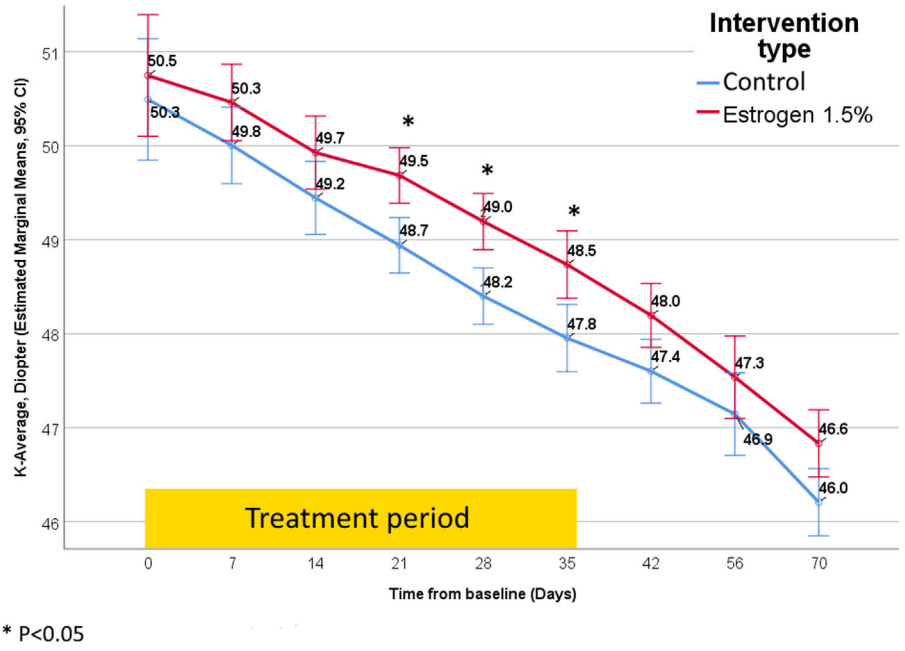

Figure 1. Mean K-average readings for the treatment and control groups.

the estrogen treatment group (Fig. 1). By the third week of treatment, the K-avg reading curves separated. As shown, the differences in K-avg readings between the two groups were statistically significant in the 21-, 28-, and 35-day examination points (i.e., until the end of the treatment period) and diminished to a nonsignificant level after discontinuation of treatment at later time points (Fig. 1). Repeated-measure analysis, adjusted for baseline body weight and pachymetry, revealed a significant difference in keratometry readings between the two groups (df = 1; F = 9.7; $P = 0.014$). During the treatment period, keratometry values were significantly higher in the treatment group by a mean 0.65 ± 0.16 diopter (df = 1; F = 10.8; P = 0.011), as shown in Figure 1. The changes were uniform across meridians maintaining statistical significance in both K1 and K2 values without the development of corneal astigmatism (Figs. 2A–2C). A similar trend was observed in refractive values, although the difference was not statistically significant (Figs. 2D–2F). No significant differences between groups were observed in pachymetry and IOP measurements throughout the study period (Figs. 2H, 2I, respectively). Repeated-measures analysis of the contralateral eye also revealed a slight relative increase in corneal steepening in the treatment group with keratometry values higher by a mean 0.393 ± 0.239 diopter. However the differences did not reach statistical significance (df = 1; F = 2.695; $P = 0.139$).

CCT measurements taken from the OCT scans correlated significantly with keratometry values (Fig. 3, $R^2 = 0.432$, $P < 0.001$). The topical five-week twice-daily estrogen treatments also brought about a 4.5-fold (1292 ± 162 pg/mL) increase in serum estrogen level compared to in the control group (261 ± 61 pg/mL).

None of the rabbits showed any signs of inflammation or other side effect in either group during the study period by biomicroscopy. AS-OCT images of representative rabbits from treatment group are shown in Figure 4. No apparent pathological changes were observed in any of AS-OCT scans.

Discussion

Results from both observational and in vivo studies suggest that estrogen can induce corneal structural[1–3,5–7] and biomechanical[8,23] changes affecting the corneal curvature and therefore its refractive power. In this proof-of-concept study, we found that treatment with topical estrogen induced a relative myopic shift. This shift was transient and reversible after discontinuation of treatment. No other significant effects were observed, including changes in IOP, pachymetry, or

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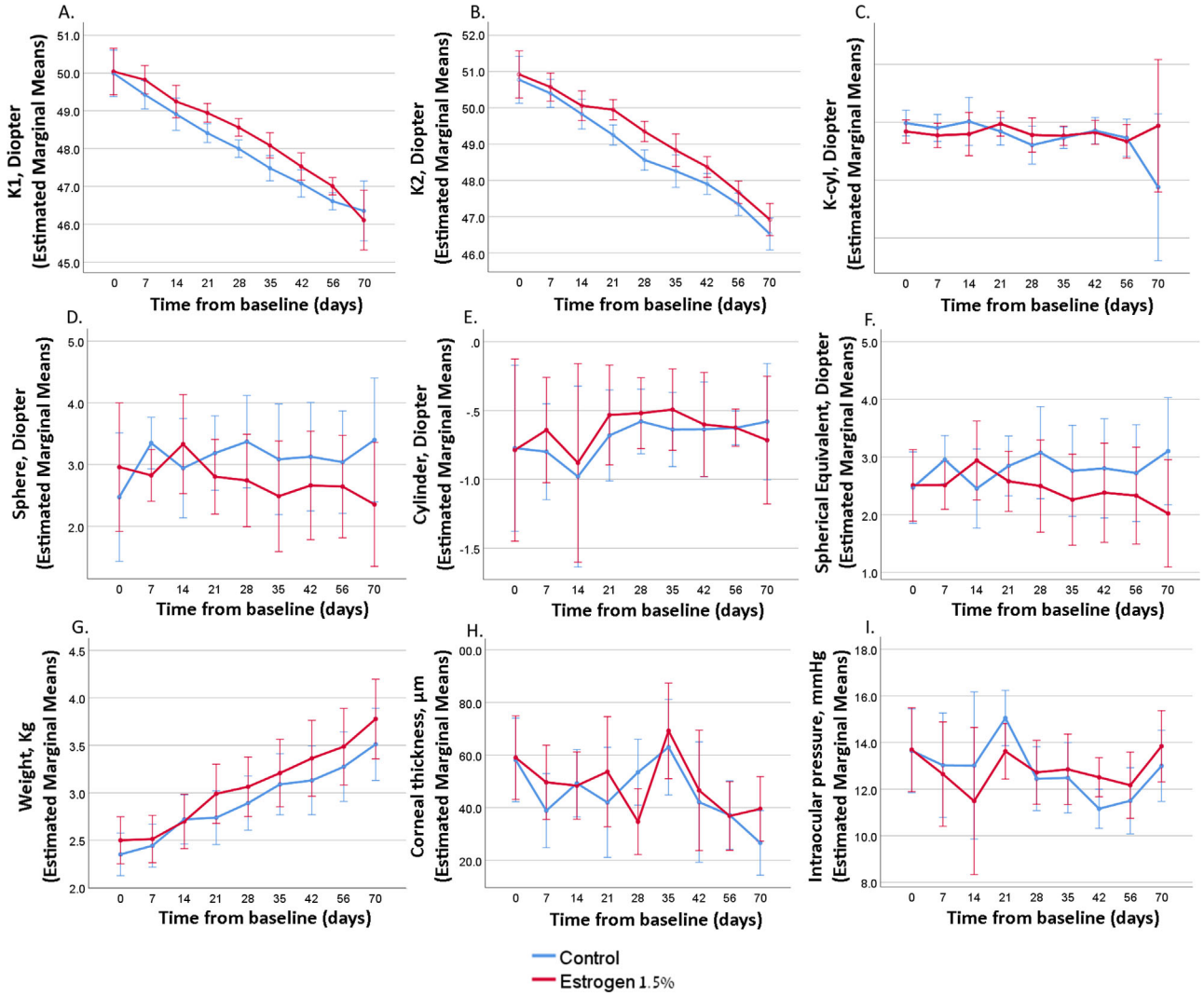

Figure 2. Changes in mean study parameters in the control and treatment groups: **A–C,** Keratometry readings (K1, K2, and K-cylinder, respectively); **D–F,** Refractive readings (sphere, cylinder, and spherical equivalent respectively); **G,** Weight; **H,** Pachymetry; **I,** Intraocular pressure. Data are presented as mean ± SD.

inflammation. Our results indicate that corneal refractive power can be manipulated pharmacologically.

We used rabbits as an animal model because rabbits are widely used in ophthalmic research. As reported previously by Riau et al.,[24] there is a slow and gradual corneal flattening with an increase in spherical equivalent in the rabbit eye during the first year of life. They showed that keratometry readings and CCT measures changed rapidly from the first to the seventh month and appeared to stabilize after the eighth month. A reduction in corneal curvature of approximately 1.36 diopter (D)/month and an increase of 10 μm/month in CCT measures were also observed during the first seven months of life.

The results of our study are consistent with these observations. Our control group showed a gradual decrease in K reading and an increase in spherical equivalent. Topical estrogen administration altered this flattening effect as long as the treatment was continued. Despite observing a similar trend in refractive parameters between both groups, the differences between groups were not statistically significant. This can be explained by the high variability and the wide range of measures in each sample. This variability is similar to what has been already described by Riau et al.[24] and might be attributed to possible technical difficulties and insufficient cycloplegia before measurements. As a result, we are unable to rule out the possibility that estrogen induces other changes to the eye that may counterbalance in corneal steepening (i.e., axil elongation or reduction in lens refractive index) resulting in milder overall refractive changes.

Our results consolidate findings from previous observational studies in the literature on the effect of estrogen on the cornea. There is ample evidence that, pregnancy is associated with increased corneal thickness and keratometry values in healthy eyes along the progression of pregnancy, corresponding to increase in estrogen blood levels.[2,25–27] Park et al.[26] found that the cornea steepened during the late stages of pregnancy and returned to normal during the postpartum period. Bilgihan et al.[28] described increased keratometry readings during pregnancy among women with keratoconus that were attributed to hormonal changes. Pizzarello et al.[3] detected a transient myopic shift during pregnancy, with return of keratometry readings to

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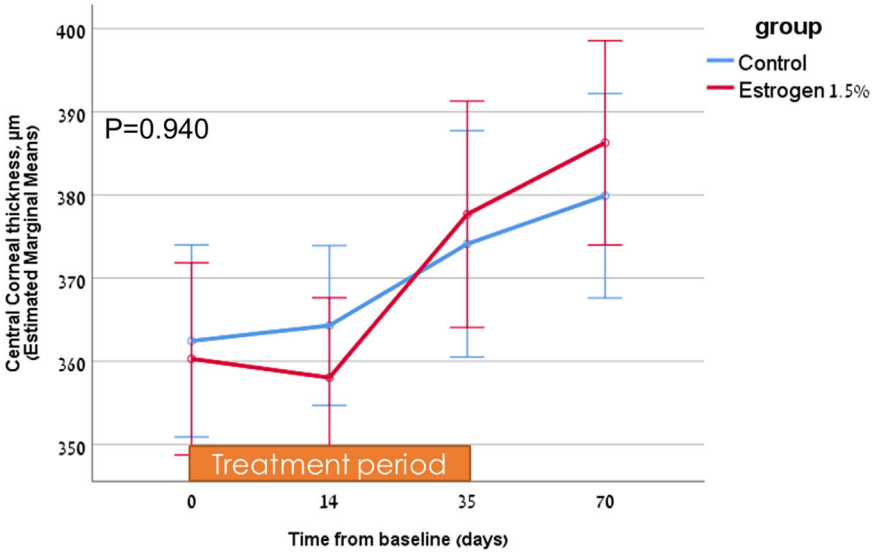

Figure 3. Mean corneal thickness measurements by anterior segment ocular coherence tomography (AS-OCT) for the treatment and control group.

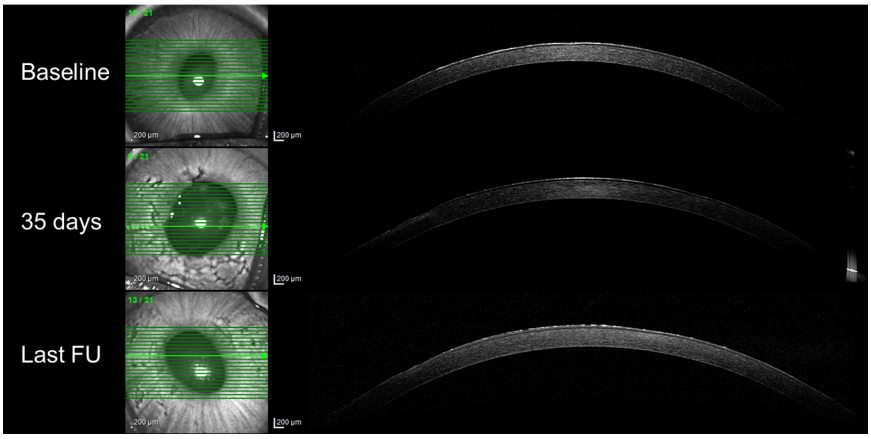

Figure 4. Anterior segment ocular coherence tomography scans at three time points of animal no. 9688 from the treatment group (a representative example).

near-prepregnancy levels after delivery. In a similar fashion, hormonal changes during menstrual cycles have been associated with statistically significant changes in corneal biomechanical parameters and corneal thickness.[8]

A better understanding of the underlying biologic processes causing the observed changes can shed more light on the pathophysiology of corneal ectatic diseases such as keratoconus. Naderan et al.[29] evaluated women with stable keratoconus and compared the corneas between those who became pregnant to nonpregnant age-matched women. They found a significant progression of keratoconus among the pregnant women group, indicating that pregnancy is a risk factor for keratoconus progression. Therefore they concluded that cross-linking should be considered in keratoconus women who intend to become pregnant.

Identification of specific factors that induce such changes can also open a window of opportunities for new therapeutic modalities for pharmaceutical refractive correction. By activating or blocking the correct signal pathways, it would be possible to induce corneal steepening in hyperopic patients and conversely cause corneal flattening to correct myopia. Corneal changes are presumed to result, among other things, from physiological hormonal changes that occur during pregnancy and menstrual cycles.[2,8] Estrogen receptors (ERα and ERβ) have been demonstrated in the nuclei of human corneal epithelial, stromal, and endothelial cells,[11–13] suggesting a possible direct effect of estrogen on corneal cells that may underlie the observed changes in corneal curvature. In addition, estrogen was shown to stimulate proinflammatory cytokines and Matrix metallopeptidase (MMP) 2, 7, and 9 gene expression,[30,31] although the same group found that increased expression did not translate into changes in MMP activity.[32] Hence, it is possible that increased estrogen levels in the corneal tissue affects the biomechanical properties of the cornea through MMP activation or similar pathways that might play a role in the observed changes in corneal curvature. In our study, a statistically significant (approximately 4.5-fold) increase

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in circulating blood estrogen was measured during BID treatment with 1.5% (w/v) estrogen eye drops. Nevertheless, the correlation between the actual serum estrogen level in rabbit and the observed myopic shift needs to be further investigated. In vitro, estrogen has also been shown to modulate the biomechanical properties of the cornea, increasing the tissues' laxity.[33] Most recently, Walter et al.[23] compared the relaxing effect of estrogen and progesterone in a porcine eye model using Young's modulus for evaluation of tissue stiffness. Porcine corneas incubated in supraphysiological concentration of estrogen for seven days exhibited a 37% reduction in stiffness, whereas progesterone had a negligible effect. Neither estrogen nor progesterone significantly affected CCT. However, because of the multiple different effects of estrogen on the eye surface and the cornea, they were unable to conclude the exact cause for their observation. Therefore it may be concluded that the increase in keratometry observed in our study most likely resulted from biomechanical corneal changes related to reduced corneal stiffness from exposure to the topical estrogen drops. Because a slight and statistically insignificant corneal steepening was observed in the contralateral eye as well, it is possible that systemic increase in estrogen levels might play a role in causing these changes.

Our study has several limitations. Although we were able to measure relatively reliable keratometry readings reflecting the anterior surface of the cornea, we lack data regarding the posterior corneal surface that might have also been affected by the treatment. Moreover, we were unable to assess topography and tomography scans that could have given supplemental data on corneal curvature and structure. Evaluation of other factors of corneal biomechanics such as hysteresis and corneal resistance factor, which were beyond the scope of this study, might have given us more clues on the exact effect of estrogen on the cornea. In addition, although the overall refraction showed a similar trend to the keratometry changes, we were unable to show a significant difference between the groups and cannot rule out additional refractive changes induced by estrogen in other ocular tissues that counteract the corneal steepening. The normal development of the rabbits eye should also be taken into consideration. Although we were able to show that estrogen reduces the normal flattening of the developing rabbit corneas in the first year of life, we are unable to determine the extent of the effect should it be applied on an already developed eye. Last, the estrogen concentration used in our study was 1.5% (w/v) and was given twice daily. Although a higher concentration was used in the past,[16] most studies used much lower concentrations of estrogen, and the result might be due to a supraphysiological level of estrogen. Because the changes were statistically significant in the treated eyes alone, we can assume that the minor changes in the contralateral eye might have been due to the increase in systemic estrogen levels that was not as strong as the topical treatment; however, further studies are needed to determine the absorption of estrogen in corneal and ocular tissues. Because we explored only one dosage and treatment regimen, we cannot conclude about the effect of different dosages, concentrations or regimen treatments. Furthermore, systemic effects of the estrogen should be taken into consideration. Although investigation of such effects was beyond the scope of this study, the observed increase in blood estrogen levels mandates evaluation of lower dosages and other forms of delivery in future studies.

In conclusion, we have shown that a myopic shift in rabbit eyes can be induced by topical treatment using estrogen-containing eye drops. Our study results may lead to a new approach for a pharmacologic refractive correction. Further studies are needed to better understand the pathophysiology of these processes and to determine the proper treatment regiments.

Acknowledgments

Supported in part by the National Eye Institute and the Office of Research on Women's Health (grant number EY027005, to K.P.-T.).

Disclosure: **A. Leshno,** None; **K. Prokai-Tatrai,** None; **Y. Rotenstreich,** None; **A. Magid,** None; **E. Bubis,** None; **S. Schwartz,** None; **A. Skaat,** None; **O. Zloto,** None; **N. Avni-Zauberman,** None; **A. Barak,** None

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