Hypoxic Effects on Corneal Morphology and Function

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Normal corneal metabolism depends on a critical level of oxygen, below which a series of acute corneal responses occur, including an increase in stromal lactate, a reduction in intercellular pH, and an increase in corneal hydration. These acute responses are reversible when normal oxygen is restored; however, it has been shown that chronic exposure to low oxygen levels can result in permanent morphologic changes in the corneal endothelium. Clinicians have expressed concern that these observed structural changes may also be accompanied by alterations in corneal physiology. Whether such effects occur is not known, since it has been difficult to assess human corneal function accurately. Recently, we have developed an in vivo test, able to measure overall corneal hydration control, that can be used to study the effects of hypoxia on corneal function. This test provides information on several characteristics of hydration control, one of which is the percent corneal thickness recovery per hour (PRPH) after inducing corneal swelling. In this study, we assumed that corneal hypoxia accompanies both extended and polymethylmethacrylate (PMMA) contact lens wear and that the dose received is related to the years of past lens wear. Using this paradigm, we explored the relationship of hypoxic dose to an endothelial polymegathism index (EPI), endothelial cell density (ECD), and PRPH in 36 subjects with varying contact lens wearing histories. Based on multiple regression analysis, the relative change (expressed as percent per year) associated with hypoxic dose (adjusted for age and gender) was found to be dose-dependent and corresponded to estimated changes of 1.70%/yr, −0.25%/yr, and −1.26%/yr, with 95% confidence limits of (−0.3, 3.7), (−1.4, 0.9), and (−2.6, 0.06) for EPI, ECD, and PRPH, respectively. These preliminary data suggest that hypoxic exposure alters endothelial morphology and reduces corneal function; however, it is important to indicate that this was an exploratory investigation with several limitations, and that therefore these results should be viewed as preliminary until more definitive studies are completed. Invest Ophthalmol Vis Sci 31:1542−1554, 1990

Normal corneal metabolism depends on a critical level of oxygen, below which a series of acute metabolic events occur, including an increase in stromal lactate, a reduction in intercellular pH, and an increase in corneal hydration.1–3 These acute responses appear to be reversible when normal oxygen levels are restored.3 However, chronic exposure to low oxygen levels apparently can cause structural changes that may take several months to reverse (epithelial microcysts) or that may be permanent (endothelial polymegathism).4–9

Although the currently available information is limited, many clinicians have expressed concerns that chronic hypoxic exposure results in endothelial polymegathism, which might be connected with altered corneal physiology. Evidence that polymegathism may be accompanied by a functional impairment in corneal function comes primarily from the work of Rao and co-workers, who found that cataract patients with preoperative polymegathism had a longer postoperative degree of corneal edema compared to cataract patients who did not demonstrate such morphologic changes.10 More recently, Lass and colleagues11 and Dutt and colleagues12 used fluorophotometry to demonstrate that extended- and long-term polymethylmethacrylate (PMMA) contact lens wearers with polymegathism had a decrease in endothelial barrier function and an increase in endothelial pump rate. Also, other studies, in which animal corneas were exposed to hypoxia, found a reduction in endothelial pump site density.13 If this loss also occurs in humans after hypoxic exposure, impairment to corneal hydration control may be expected.

Although these studies suggest that contact lens wear may result in a functional deficit to the cornea, there is also evidence that indicates that the low oxygen levels are not accompanied by adverse effects on corneal physiology. For example, in experiments sim-
ilar to those conducted by Lass et al.\textsuperscript{11} Carlson and co-workers did not find differences in endothelial permeability between contact and non-contact lens wearers.\textsuperscript{14} Other studies have shown that in individuals who had worn extended-wear (EW) lenses for several years, corneal thickness returned to normal levels about 2 days after lens wear was discontinued—a result that would not be expected if there were a functional deficit in the endothelium.\textsuperscript{7}

Conclusions about the effects of long-term hypoxic exposure to corneal structure and function remain unclear, and important questions remain unanswered. For example, is there a measurable structural and physiologic response from chronic exposure to low oxygen levels, and if so, how marked are these changes compared to those that occur in subjects who have not had chronic hypoxic exposure? If chronic exposure has an effect, does it depend on the degree, duration, and timing of the low oxygen dose?

These and related issues need to be explored in part because there seems to be an evolving interest in EW contact lenses among patients, and among clinicians and manufacturers in prescribing and marketing them. However, since most currently available EW materials do not meet required oxygen levels during eye closure, the cornea will typically be exposed to hypoxic levels for a number of hours every day the lenses are worn.\textsuperscript{15,16}

It has been possible to study structural changes that result from contact lens wear through morphometric analysis of endothelial photomicrographs; however, in vivo measurements of corneal function have not been readily available for assessing the physiologic effects of hypoxic exposure.\textsuperscript{6,8,11,14} Recently, we described a method for in vivo assessment of overall corneal hydration control that provides one approach for studying the effects of contact lens wear on corneal physiology.\textsuperscript{17} In the current report we present results from an exploratory study, the purpose of which was to provide preliminary estimates of the relationships among various quantitative measures of lens wear, several measures of corneal morphology, and this new measure of corneal hydration control. This exploratory study was conducted to obtain information to determine whether or not further investigation seems warranted and to guide the design of studies that can yield more definitive assessments of the possible impact of contact lens wear on corneal structure and function.

Materials and Methods

Subjects

Subjects were recruited from the campus community of the University of California at Berkeley. All candidates received a complete eye examination, and only those subjects who were free of ocular disease were accepted into the study. The emphasis in recruiting was to obtain a subgroup of patients with no history of contact lens wear and a subgroup who had some experience with a lens-wearing mode that presumably would have caused some level of corneal hypoxia. No volunteers whose past lens wear consisted entirely of soft contact lens daily wear (SCLDW) or rigid gas-permeable daily wear (RGPDW) were accepted. Recruitment efforts produced 36 subjects including 7 with no history of lens wear. Before beginning any of the testing procedures, each subject was given a description of the study, and informed consent was obtained.

Measurement Procedures

Endothelial morphology: Endothelial photomicrographs were taken with a Nikon AS-1 Enhanced Graphics Adapter (EGA) noncontact specular microscope. The 35-mm slides were rear-projected onto a 24 × 24-inch screen, and a Bio-Optics (Bio-Optics Inc., Arlington, MA) digitizer coupled to an IBM-XT computer with an enhanced graphics adapter (EGA) board was used to trace each cell within a defined area of the endothelium (approximately 100 × 500 μm). The analyzed cell parameters were mean cell density (cells/mm\(^2\)) and mean and standard deviation of cell areas (mm\(^2\)). These last two measures were used to compute the coefficient of variation of cell area (standard deviation of cell size divided by mean cell area), which provides an endothelial polymegathism index (EPI).

Corneal thickness: Optical pachometry was done on each subject with a modified slit lamp to provide improved corneal thickness measurements. A more detailed description of this instrument is provided elsewhere.\textsuperscript{18}

Corneal Hydration Control

Assessment of corneal hydration control was based on two different data sets obtained on separate days. One test consisted of two repeat measurements of corneal thickness every 15 min over a period of 1–2 hr to assess the open-eye steady-state (OESS) thickness. These OESS measurements were made in the late afternoon after the subject had been awake for 6 or more hr.

The second procedure required that the subject report to the laboratory in the morning for a "stress" test that involves wearing a 400-μm-thick 40% water hydrogel contact lens for 2 hr with the eyes closed. The estimated oxygen tension under this lens during eye closure was at or near 0 mmHg.\textsuperscript{15} Before inserting
the contact lens, baseline pachometry measurements were taken. After the 2-hr stress period, the contact lenses were removed, and two repeat corneal thickness measurements were made at approximately 30-min intervals for 4–5 hr. For 23 of 36 subjects, the OESS assessment was made before the stress test. For those 13 subjects who had the stress test done first, two had discontinued their lens wear for 3 days while the other 11 had their lenses out 6 or more days.

Endothelial photographs were taken usually on the day the stress test was given, approximately 3–5 hr after the removal of the stress lens. Specular micrography was timed in this manner to minimize possible short-term effects of the subjects’ own contact lenses on corneal clarity or endothelial morphology. Although it is possible that the cell density or EPI may have changed over the 3-day period after contact lens discontinuance, most current information suggests that such changes would be unlikely. No attempt was made to quantify these effects.5,6,8,9,11,12,14 It also has been shown that wearing soft lenses can have a small transient effect (eg, blebs) on some individuals; however, since this transient change lasts less than 30 min, it is not likely to have had any affect on the determination of endothelial cell morphology done in this study.19

These two data sets were then combined by means of a composite exponential model that provides estimates of several aspects of an individual’s overall corneal hydration control. The key characteristic that was analyzed for this study measures the rate at which the cornea returned to OESS thickness (deswelled) from an induced level of edema. This quantity, called the percent recovery per hour (PRPH), describes the amount of recovery (by percentage) that the cornea undergoes in any 1-hr time period during exponential recovery. A more complete description of the composite experimental model and its use, along with sample deswelling curves, are provided elsewhere.17,20

Contact Lens Wear

Obtaining complete and accurate information about lens wear history is an important element in a study of this type, which depends on establishing one or more operational definitions of lens wear to provide variables for statistical analysis. Choosing one or more lens wear measures involves a working assumption that other aspects of lens wear can safely be ignored when the chosen measures are studied relative to corneal structure or function. For example, an analysis that ignores daily wear (DW) would be valid only if DW is unrelated to the corneal properties under study or is not associated with the measure of lens wear that is being studied.

A patient’s contact lens history is often a fairly complex sequence that may involve periods of lens wear with different types of lenses or modes of wear, and possibly interspersed periods of no contact lens wear. Also, the impact of lens wear may differ depending on the number of hours of lens wear each day, the nature of the lens fit, the companion solutions used, and other behavior connected with wearing and maintaining the lenses. Consequently, a contact lens wear history can be complex and multidimensional.

For this preliminary analysis we have made the working assumptions that the historic time when particular types of lenses were worn is immutable, and that the sequencing of types of lens wear also is immutable in cases with histories of more than one type of lens wear. We have chosen to examine only the total amount of previous lens wear in each of several broad categories. We considered this to be the kind of information that could be obtained most reliably in an exploratory study based on self-reported lens wear. Since we were attempting to investigate long-term effects, it seemed appropriate to focus on the amount of wear without regard to the timing of wear.

Because we were interested in studying hypoxic effects, it was important to establish an operational definition of the types of contact lens wear that involve hypoxia. For the purposes of this study, we considered either PMMA, soft contact lens extended wear (SCLEW), or rigid gas-permeable extended wear (RGPEW) to expose the subject to some level of hypoxia, since there is considerable evidence to show that none of these wearing modalities supplies sufficient oxygen for normal corneal metabolism in at least some part of the wearing cycle.7,16,21 No attempt was made to determine the oxygen level under the contact lens; rather, we presumed that corneal hypoxia occurred to some extent during some period of the day when these lenses were worn. The hypoxic dosage was defined simply by the number of years that the subject wore any of these lens materials/wear-mode combinations.

It is also possible that SCLDW or RGPDW may result in a modest level of hypoxia, since many DW hydrogel materials have only modest oxygen transmissibilities (Dk/L).12 Several of the rigid gas-permeable (RGP) materials that currently are worn or that have been worn in the past also have low enough Dk/L values to possibly cause some oxygen deprivation.15,22 The period of use (years) of SCLDW or RGPDW also was studied for its influence on corneal properties.

Lens Wearing Before Test Measurements

The principal characteristics evaluated in this study were determined from information gathered from the
endothelial photomicroscopy and optical pachometry measurements. Conceivably, acute effects of current contact lens use by wearers who had not removed their contact lenses for sufficiently long periods before testing could have influenced either of these measurements. Unfortunately, there is little information to indicate how long contact lens wear must be discontinued to eliminate any possible acute effects that lens wear may have on test outcomes. We attempted to eliminate the acute effects of contact lens wear by having subjects who were current contact lens wearers discontinue any hypoxic wear for 3 days before testing. Three days was selected based on previous studies indicating that corneal thickness changes stabilize after discontinuing lens wear for 2 days or more and that transient morphologic changes typically clear within 2 hr after lens removal. At the time the protocol was designed, we were less concerned about SCLDW or RGPDW, so lens removal for 1 or more days before testing was planned for this type of wear.

The actual experience with lens removal before testing differed for the OESS and stress tests because they occurred on different days. The five subjects whose OESS tests were performed the soonest after discontinuation of hypoxic lens wear had been without lenses for 2, 3, 6, 30, and 64 days. Most subjects had not worn hypoxic lenses for a considerable length of time, and the average time without hypoxic lenses was 793 days among the 29 subjects with past hypoxic wear. The five shortest durations between removal of hypoxic lenses and the stress test were 0, 3, 3.1, and 78 days, and the average was 794 days. After checking that the two subjects with protocol violations did not dominate the findings from this study, they were used for data analysis.

SCLDW or RGPDW tended to occur in much closer proximity to the OESS and stress test times. The number of days without lens wear prior to the OESS test for the 21 subjects with past SCLDW or RGPDW were 0 or 1 (2 subjects), 2 (6 subjects), 3 (5 subjects), 4 (2 subjects), 7 or 9 (2 subjects), and 370 or more days (4 subjects). The corresponding number of days in connection with the stress test were 1 (12 subjects), 2 or 3 (2 subjects), 4 (2 subjects), 14 (1 subject), and 364 or more days (4 subjects).

For the 29 subjects with contact-lens-wearing history, 24 had endothelial photomicroscopy done on the day of the stress test (after the lenses had been removed for 4 or more hr). Of the remaining 5 subjects, 1 had photos taken on the day of the OESS, 3 had photos taken within 8 weeks of completing the stress test, and 1 was photographed 2 months before other testing was done. Since data from previous studies have shown that endothelial cell coefficient of variation and cell density change relatively slowly, the photos of these 5 subjects most likely are valid indicators of the subjects’ endothelial morphology at the time of the stress test. In general, the timing of measurements related to last lens wear is an aspect of study design that needs to be carefully reconsidered before confirmatory studies are conducted.

Statistical Analysis Methods

The relationships between corneal properties and measures of lens wear were analyzed by standard methods of multiple regression analysis that were implemented with a statistical package on an MS-DOS personal computer. In these analyses, the average of right and left eye measurements of corneal properties on each subject provided the dependent variables used in the analysis. In this way errors in the measurement of corneal properties were diminished and statistically independent data were obtained for analysis. In all cases, the lens wear measurements, which provided the predictor variables for analysis, were the same for both eyes of all the subjects. For purposes of this exploratory study and analysis, estimates of the magnitudes of specified relationships of interest along with associated confidence intervals have been emphasized for summarizing results.

Results

The presentation of the results is organized in three parts. First, the lens wear experience in the study group is described. Then differences in lens wear by age and gender are examined. Finally, multiple linear regression methods are used to investigate the relationship between lens wear variables and three corneal properties; EPI, ECD, and corneal hydration control as measured by the PRPH.

Lens Wear Experience

The study of past lens wear was based on five component measures extracted from the self report provided by the subjects. These measures are SCLDW, SCLEW, RGPDW, RGPEW, and PMMA.

Twenty-nine of the 36 subjects had a history of lens wear that included either extended or PMMA wear. Many of these subjects also had a history of DW. A summary of the amount of past lens wear for each of the five lens-wear categories is provided in the upper portion of Table 1. Subjects who had more than one type or mode of lens wear contributed data to more than one category.

EW is one important type of past wear that presumably led to some degree of corneal hypoxia. In this category, only a small amount of past wear involved RGP lenses. Except for two subjects who re-
Table 1. Past lens experience among the 29 study subjects with history of lens wear

<table>
<thead>
<tr>
<th>Type of lens wear</th>
<th>Number of subjects</th>
<th>Years of lens wear</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Min</td>
<td>Max</td>
<td></td>
</tr>
<tr>
<td>SCLDW</td>
<td>9</td>
<td>2.5</td>
<td>1.7</td>
<td>0.3</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>SCLEW</td>
<td>14</td>
<td>3.4</td>
<td>1.6</td>
<td>0.5</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>RGPDW</td>
<td>12</td>
<td>2.4</td>
<td>1.3</td>
<td>0.3</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>RGPEW</td>
<td>2</td>
<td>0.9</td>
<td>0.2</td>
<td>0.7</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>PMMA</td>
<td>20</td>
<td>7.3</td>
<td>2.6</td>
<td>2.0</td>
<td>12.0</td>
<td></td>
</tr>
<tr>
<td>Combined measures</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DW</td>
<td>19</td>
<td>2.7</td>
<td>1.8</td>
<td>0.3</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>EW</td>
<td>16</td>
<td>3.1</td>
<td>1.7</td>
<td>0.5</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>EW + PMMA</td>
<td>29</td>
<td>6.8</td>
<td>3.3</td>
<td>2.0</td>
<td>15.1</td>
<td></td>
</tr>
</tbody>
</table>

* Subjects with multiple types of past lens wear contribute to more than one category.
† DW = SCLDW + RGPDW; EW = SCLEW + RGPEW.
‡ Two subjects had both SCLDW and RGPDW episodes. No subject had both SCLEW and RGPEW episodes.

reported RGPEW of 9 and 12 months as their only episodes of EW, all subjects with a history of EW had only SCLEW episodes. The more commonly encountered SCLEW was reported by 14 (38.9%) of the 36 study subjects. The total duration of SCLEW ranged from 0.5 to 5.2 yr, and the mean and standard deviation of the length of wear were 3.4 and 1.6 yr, respectively. Because there is so little RGPEW experience, for subsequent analyses the 16 subjects with some history of EW all were assigned a value for total years of EW without regard to the type of lens material. In the interpretation of results, it must therefore be remembered that the EW variable is heavily dominated by SCLEW.

As mentioned above, we have assumed that both PMMA and EW would lead to some degree of corneal hypoxia. In order to examine the contribution of both PMMA and EW as components of hypoxic wear, the joint distribution of these measures of past lens wear is presented in Figure 1. Most subjects had either PMMA or EW episodes, but not both. The exceptions are four subjects who had predominantly PMMA wear along with 1 yr or less of SCLEW and three additional subjects who had a substantial amount of both PMMA and SCLEW. The single point in Figure 1 at the origin where SCLEW = PMMA = 0 represents the subgroup of seven subjects who had no history of prior lens wear.

Under the assumption that EW and PMMA wear operate similarly in their impact on corneal properties, it would be appropriate to sum these two measures to give the combined total years of hypoxic wear. Graphically, the sum would be represented by the projection of the data points in Figure 1 onto the 45° line shown there. For each of the corneal properties studied, this summary measure of hypoxic wear has been used in one of the regression models used to study the effect of past lens wear. Use of this summary measure of hypoxic wear is motivated by the potential gain in statistical precision that can be expected from entering the combined information about past hypoxic wear into the analysis in an efficient way. However, if the assumption that EW and PMMA wear operate similarly is false, then analysis with combined hypoxic wear may be misleading, because it will give only a weighted average of the individual impacts of the two hypoxic wear components. Thus, the relationship of one component measure will be understated and the other will be overstated. Consequently, in connection with each corneal property we also have used a second regression model in which each of these component measures is studied as a separate predictor. This provides some indication of the independent relationship between each component and the corneal property under study, but some of these estimated component relationships have reduced precision.

![Fig. 1. Scatterplot of the years of PMMA vs EW among 36 subjects. The 0.0 point represents 7 subjects who had no prior lens wear.](image-url)
As shown in Figure 2, many subjects also had a history of SCLDW or RGPDW. Here again, subjects tended to wear one or the other type of lens, except for two subjects who had worn both types of lenses. As with the hypoxic wear components, the SCLDW and RGPDW components could be treated separately or combined to give a single measure. Given the small size of this exploratory study and the expectation that these types of DW probably are not related to corneal properties, it was decided to work with a combined SCLDW and RGPDW measure. Consequently, subsequent analysis with this combined variable (SCLDW + RGPDW) will give only the weighted average of the two component effects.

The description of the amount of lens wear in the combined categories (EW = SCLEW + RGPEW; DW = SCLDW + RGPDW, and hypoxic = EW + PMMA) is provided in the lower part of Table 1. Considering all hypoxic components, 29 subjects had an average of 6.8 yr of wear. Also, there were 19 subjects with an average of 2.7 yr SCLDW and RGPDW. In Figure 3, the scatter diagram of DW vs hypoxic wear shows there is not a strong association between these two aspects of lens wear history. This configuration is fortunate since it facilitates assessment of the separate relationship between each of these measures and the corneal properties under study.

These descriptions of the lens wear history provide information about the data that form the basis for studying the effects of lens wear on corneal properties. Clearly, they apply only to the subjects in this particular study; however, they probably are indicative of the general kind of lens wear patterns that are likely to arise in other studies.

**Fig. 2.** Scatterplot of the years of SCLDW vs RGPDW among 36 subjects. The 0.0 point represents 7 subjects who had no prior lens wear.

**Fig. 3.** Scatterplot of the years of combined SCLDW and RGPDW vs combined PMMA and EW among 36 subjects. The 0.0 point represents 7 subjects who had no prior lens wear.

### Age and Gender Differences in Past Lens Wear

It is also important to consider the possibility that the nature and length of time of past lens wear is likely to be associated with two co-variables: age and gender. Moreover, these co-variables may be related to corneal properties by way of mechanisms that do not involve any of the lens wear history variables. For example, evidence indicates that both endothelial morphology and corneal hydration control show changes with age, in the absence of lens wear. Since one would expect lens wear history to be age-related, it is necessary to make statistical adjustments for age in an effort to obtain a more direct assessment of possible lens wear effects that are not confounded by age differences. Similar possibilities can be imagined in connection with gender.

A full assessment of the relationship between age, gender, and the various measures of past contact lens wear would require numerous tables and graphs. However, to illustrate the potential of these co-variables to confound the relationship between lens wear measures and corneal properties, we can examine the important lens wear variable—years of hypoxic wear—in relationship to age and gender. Figure 4 shows a pair of scatter diagrams that plot separately hypoxic wear (years) vs age for the subgroups consisting of 11 males and 25 females (of the 36 points, 4 are coincident). Not surprisingly, in both the male and female groups, there is a tendency for older subjects to have had a longer duration of hypoxic wear. Consequently, any effects of age will tend to be confounded with length of hypoxic wear. This illustrates the importance of adjusting for age and gender when attempting to isolate the direct association between lens wear and corneal properties.
Corneal Properties

A brief description of the corneal characteristics of the study group is provided in Table 2. Age and corneal properties are described for the two subgroups consisting of 7 subjects with no history of lens wear and 29 subjects with past lens wear. These results give some indication of the overall differences among subjects that depend on the absence or presence of lens wear without specific consideration to type or amount of lens wear or adjustment for age and gender. Also, the mean values for the 7 subjects with no past lens wear will be used in subsequent tables in this paper as a reference value in the construction of measures of association that describe the relationships between several corneal properties and lens wear variables.

Lens Wear Measures in Relation to Corneal Properties

Endothelial polymegathism: The first corneal property studied in relation to past lens wear is the coefficient of variation of the endothelial cell area, which we refer to as the EPI. In Table 3, the results from two multiple linear regression analyses examine measures of past lens wear as predictors of EPI with adjustment for age and gender. In the first regression analysis (model 1), there are only two measures of lens wear—hypoxic wear and DW—in addition to age and gender. The traditional estimated regression coefficients and their estimated standard errors are provided in the third and fourth columns. However, these basic results are not easy to interpret directly, so in column 5 a related but more interpretable measure is given. This is the estimated percentage change in the EPI per year relative to a reference level that is provided by the average EPI observed for the 7 study subjects who had no history of contact lens wear. These estimates are accompanied by 95% confidence intervals (column 6). In the lower half of Table 3 corresponding results are provided for the analysis in which the two components of hypoxic wear, namely EW and PMMA, are both entered into the regression analysis in place of the combined measure.

Table 2. Personal and corneal characteristics of 7 subjects without and 29 subjects with past contact lens wear

<table>
<thead>
<tr>
<th>Variable</th>
<th>Past lens wear</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>Min.</th>
<th>Max.</th>
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<tbody>
<tr>
<td>Age (yr)</td>
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<td>24.4</td>
<td>3.6</td>
<td>18</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>29</td>
<td>27.9</td>
<td>5.9</td>
<td>19</td>
<td>38</td>
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<tr>
<td>EPI*</td>
<td>No</td>
<td>7</td>
<td>0.259</td>
<td>0.034</td>
<td>0.211</td>
<td>0.307</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>29</td>
<td>0.300</td>
<td>0.057</td>
<td>0.192</td>
<td>0.504</td>
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<tr>
<td>Endothelial cell density†</td>
<td>No</td>
<td>7</td>
<td>3192.1</td>
<td>477.7</td>
<td>2564.5</td>
<td>4106.5</td>
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<tr>
<td></td>
<td>Yes</td>
<td>29</td>
<td>3037.8</td>
<td>340.7</td>
<td>2569.5</td>
<td>3754.5</td>
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<tr>
<td>PRPH</td>
<td>No</td>
<td>7</td>
<td>57.3</td>
<td>5.0</td>
<td>51.5</td>
<td>65.2</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>29</td>
<td>53.7</td>
<td>8.2</td>
<td>38.6</td>
<td>66.3</td>
</tr>
</tbody>
</table>

* Coefficient of variation of sizes of endothelial cells in an area sample. † Endothelial cell density in a specified area sample (cell/mm²).
Table 3. Regression analysis of the relationship between the EPI* and measures of past contact lens wear

<table>
<thead>
<tr>
<th>Model</th>
<th>Predictor variable</th>
<th>Est. regression coefficient†</th>
<th>Est. SE</th>
<th>Estimate</th>
<th>95% Confidence interval</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>PMMA + EW (yr)</td>
<td>0.0044</td>
<td>0.0025</td>
<td>1.70</td>
<td>(−0.3, 3.7)</td>
<td>0.088</td>
</tr>
<tr>
<td></td>
<td>DW (yr)</td>
<td>0.0045</td>
<td>0.0048</td>
<td>1.74</td>
<td>(−2.0, 5.5)</td>
<td>0.356</td>
</tr>
<tr>
<td></td>
<td>Age (yr)</td>
<td>0.0008</td>
<td>0.0018</td>
<td>0.31</td>
<td>(−1.1, 1.7)</td>
<td>0.660</td>
</tr>
<tr>
<td></td>
<td>Gender (1 = M, 2 = F)</td>
<td>0.0065</td>
<td>0.0198</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Intercept</td>
<td>0.2282</td>
<td>0.0556</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>PMMA (yr)</td>
<td>0.0047</td>
<td>0.0027</td>
<td>1.81</td>
<td>(−0.3, 3.9)</td>
<td>0.090</td>
</tr>
<tr>
<td></td>
<td>EW (yr)</td>
<td>0.0024</td>
<td>0.0050</td>
<td>0.93</td>
<td>(−3.0, 4.9)</td>
<td>0.635</td>
</tr>
<tr>
<td></td>
<td>DW (yr)</td>
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<td>0.0049</td>
<td>1.81</td>
<td>(−2.0, 5.7)</td>
<td>0.345</td>
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<td>Age (yr)</td>
<td>0.0006</td>
<td>0.0018</td>
<td>0.23</td>
<td>(−1.2, 1.7)</td>
<td>0.741</td>
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<td></td>
<td>Gender (1 = M, 2 = F)</td>
<td>0.0050</td>
<td>0.0203</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td></td>
<td>Intercept</td>
<td>0.2373</td>
<td>0.0590</td>
<td>—</td>
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</table>

Analysis among 36 subjects with adjustment for age and gender. *Coefficient of variation of sizes of corneal endothelial cells in an area sample. †Change in mean EPI per year of lens wear or per year of age, or for females relative to males. ‡Percent change per year relative to 0.259, the mean EPI for the 7 subjects with no history of contact lens wear.

In the interpretation of the results in Table 3, both the estimates of relative percentage change per year and the corresponding confidence intervals are important. The estimates provide the best information that is available from the current data, and the confidence intervals indicate the amount of uncertainty in the estimate due to chance variability resulting from measurement and sampling processes.

Considering both the magnitude and certainty of the estimate, the hypoxic wear variable has the strongest relationship to the EPI. The estimated change of EPI, amounting to 1.70%/yr of hypoxic wear, is substantial, and the 95% confidence interval for this estimate is almost entirely in the positive direction ($P = 0.088$) suggesting that this relationship is likely to persist in a larger study. The corresponding relationship between EPI and DW is surprisingly similar to that found with hypoxic wear, but there is considerably more uncertainty in this estimate. On face value, the estimate suggests there may be some relationship between EPI and DW, but there is clearly less likelihood that this finding will persist in a larger study. The estimated relationship between EPI and age is more modest, at 0.31%/yr, and again there is considerable uncertainty in the estimate.

In model 2, when the components of hypoxic wear are studied separately, the PMMA has a stronger association with EPI than the combined hypoxic measure, whereas EW is more weakly associated. The confidence interval again indicates that the true EPI-PMMA relationship is likely to be in the positive direction; however, the estimated relationship between EPI and EW is subject to considerable uncertainty. The findings for age and gender are essentially the same as those found with model 1.

A graphic assessment of the results in Table 3 is provided by the collection of partial regression leverage plots in Figure 5. Each of these plots is designed to reveal the direct relationship between a specific predictor variable and the dependent variable, in this case the EPI. Each plot is constructed by a five-step process as follows: 1) a multiple regression equation is constructed to predict the dependent variable, EPI, from the remaining predictors after excluding the specific predictor of interest; 2) for each subject, the predicted EPIs from this analysis are subtracted from the observed EPIs to give residual EPIs; 3) a multiple regression equation is constructed to predict the specific predictor variable of interest from the remaining predictor variables; 4) for each subject, residual values for the specific predictor are constructed by a calculation analogous to the one used to construct residual values for the EPI; and 5) the residual EPI values are plotted against the residual values for the specific predictor to give a graphic impression of the "direct" relationship between the EPI and the specific predictor after adjustment for the remaining predictors.

The slopes of the best fit lines relating the residual EPI values to the residual values (in years) for PMMA wear (Fig. 5A), EW (Fig. 5B), DW (Fig. 5C), and age (Fig. 5D) are equal to the (partial) regression coefficients obtained from the model 2 analysis presented in the lower portion of Table 3. For each predictor variable included in Figure 3, the horizontal scales in the component plots were chosen to best reveal the partial regression relationship and data scatter. However, since these scales differ, the slopes of the plotted trend lines are not comparable visually even though they are numerically correct. Instead, the numerical...
values given for percentage change per year in Tables 3, 4, and 5 should be used to compare the predictive strength of the lens wear variables and age.

In general, these plots show the amount of trend in the data relative to the scatter. They also show that the scatter is fairly evenly distributed on either side of the trend line, and indicate that the linear multiple regression model is adequate for purposes of this analysis.

Endothelial Cell Density

The estimates for the relationship between ECD and measures of past lens wear are shown in Table 4, based on an analysis approach identical to that used for endothelial polymegathism. In this case, the estimated relationships all are in a plausible direction corresponding to a small decrease in cell density with age or lens wear. However, none of these estimates has confidence intervals that give a very strong indication of whether positive or negative relationships will occur in confirmatory studies ($P \geq 0.269$).

Corneal Hydration Control

With PRPH as the operational measure, corneal hydration control was studied in relationship to past lens wear and two co-variables, age and gender, and the results are given in Table 5. Combined hypoxic wear is substantially related to PRPH, with an estimated 1.26% reduction per year ($P = 0.060$). The corresponding 95% confidence interval is located primarily in the negative direction, giving fairly good certainty that this finding can be replicated. When the components of hypoxic wear are analyzed separately, in model 2, both have estimated relationships of the same order of magnitude as the combined measure. However, the estimate for the PMMA component is more convincingly positive because of the location of its confidence interval. The corresponding partial re-

Fig. 5. Partial regression leverage plots that show the direct relationship between the EPI and (A) PMMA wear, (B) combined EW, (C) combined SCLDW and RGPDW, or (D) age, among 36 subjects after adjustment for the other variables in model 2.
Table 4. Regression analysis of the relationship between endothelial cell density and measures of past contact lens wear

<table>
<thead>
<tr>
<th>Model</th>
<th>Predictor variable</th>
<th>Estimated regression coefficients*</th>
<th>Estimated SE</th>
<th>Estimate</th>
<th>95% Confidence interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PMMA + EW (yr)</td>
<td>-8.03</td>
<td>18.27</td>
<td>-0.25</td>
<td>(-1.4, 0.9)</td>
<td>0.663</td>
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<tr>
<td></td>
<td>DW (yr)</td>
<td>-6.29</td>
<td>34.77</td>
<td>-0.20</td>
<td>(-2.4, 2.0)</td>
<td>0.858</td>
</tr>
<tr>
<td></td>
<td>Age (yr)</td>
<td>-11.16</td>
<td>12.65</td>
<td>-0.35</td>
<td>(-1.2, 0.5)</td>
<td>0.384</td>
</tr>
<tr>
<td></td>
<td>Gender (1 = M, 2 = F)</td>
<td>132.92</td>
<td>142.14</td>
<td>132.92</td>
<td>3215.39</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Intercept</td>
<td>3215.39</td>
<td>399.48</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>PMMA (yr)</td>
<td>-2.72</td>
<td>18.85</td>
<td>-0.09</td>
<td>(-1.3, 1.1)</td>
<td>0.886</td>
</tr>
<tr>
<td></td>
<td>EW (yr)</td>
<td>-41.06</td>
<td>35.24</td>
<td>-1.29</td>
<td>(-3.5, 1.0)</td>
<td>0.253</td>
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<tr>
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<td>DW (yr)</td>
<td>-3.28</td>
<td>34.77</td>
<td>-0.10</td>
<td>(-2.3, 2.1)</td>
<td>0.925</td>
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<tr>
<td></td>
<td>Age (yr)</td>
<td>-14.65</td>
<td>13.00</td>
<td>-0.46</td>
<td>(-1.3, 0.4)</td>
<td>0.269</td>
</tr>
<tr>
<td></td>
<td>Gender (1 = M, 2 = F)</td>
<td>108.86</td>
<td>143.68</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>Intercept</td>
<td>3373.56</td>
<td>423.62</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Analysis among 36 subjects with adjustment for age and gender.
* Change in mean endothelial cell density per year of lens wear or per year of age. or for females relative to males.
† Percent change per year relative to 3192.1 cells/mm², the mean endothelial cell density for the 7 subjects with no history of contact lens wear.

Regression leverage plots for the prediction of PRPH in model 2 are provided in Figure 6.

In this analysis the DW results go in a direction that is inconsistent with the EP1 findings and also opposed to effects, if any, that might be anticipated from this type of lens wear. However, since the confidence interval for this estimate is quite large, these findings well may reverse in a confirmatory study.

The estimated relationship of PRPH with age is quite low and the confidence intervals are not excessively large. This suggests that for subjects in the age range of 18–35 yr, like those in this study, there is not a major decline of PRPH with age. Since previous work has indicated that, by an average age of 70 yr, people show a substantial decline in PRPH, there may be some nonlinearity in the change of PRPH with age in which there is little decline before age 40 and an accelerated decline sometime thereafter.

Discussion

In this report we have provided results from an exploratory study that was conducted to obtain a preliminary assessment of the impact of contact lens wear on corneal properties, with particular emphasis on corneal hydration control. On the basis of the results that have been obtained so far, there seems to be a real possibility that chronic corneal hypoxia causes permanent changes in corneal structure and function. Although the magnitude of these effects de-
Fig. 6. Partial regression leverage plots that show the direct relationship between the PRPH and (A) PMMA wear, (B) combined EW, (C) combined SCLDW and RGPDW, or (D) age, among 36 subjects after adjustment for the other variables in model 2.

...
then remained stable. In another study, in which corneal thickness change was monitored on EW subjects, the cornea returned to baseline thickness 2 days after discontinuing lens wear. Although more information is needed to provide convincing evidence that the corneal thickness is stable after 2–3 days of discontinuing lens wear, for this current exploratory study, we assumed that the cornea was stable for the function test assessments.

A second limitation of the current study is the substantial uncertainty in some of the estimated relationships, due to the relatively small number of subjects. A clear limitation of the data set is the absence of a solid basis for assessing the impact of any substantial past history of a mixture of PMMA and SCLEW, since there were not enough subjects who had experience with both modes of wear.

A third concern is that the subjects in this study were a convenient sample of volunteers, so there is no guarantee that they provided valid estimates of corresponding relationships in the general population of contact lens wearers, for which we would like to make inferences. Thus, the possibility of selection bias that may be an influence in the findings cannot be discounted. It should be noted, however, that in this type of study it is not necessary to have a random sample of individuals from the target population. Instead, a valid estimate of the relationships between the predictor and corneal variables depends only on having a fair sample from the distributions of outcome variable values conditional on the predictor variables, for the subjects used. In practice, studies of the type involved here always depend on volunteer samples, and it is virtually impossible to rule out the possibility that a particular study is subject to some bias. Consequently, it is essential to do replicate studies under diverse conditions to see if consistent results can be obtained.

Finally, the measures based on self reports of lens wear used in the study were fairly crude. We had no specific data on their validity compared to that of clinical records. Also, we did not consider the average amount of wear per day or any other aspects of lens wear behavior. Intuitively, however, it seems more likely that weakened rather than strengthened associations would result from these measurement problems, which probably would tend to obscure the true relationships.

Throughout the analysis and interpretation of results, we took the position that this is a preliminary study, in which estimated strengths of relationships play the primary role. Use of these estimates was then tempered by considering their uncertainty as indicated by confidence intervals. Possible sources of bias were also considered. Although the results of this exploratory study cannot be considered definitive, they are, in our opinion, sufficient to indicate the need for further investigation of the structural and functional changes that occur as a result of wearing contact lenses that cause corneal hypoxia.

Key words: corneal function, corneal endothelial morphology, hypoxia, contact lens, corneal edema

References


