Estimation of Corneal Endothelial Pump Function in Long-Term Contact Lens Wearers

William M. Bourne,¹ David O. Hodge,² and Jay W. McLaren¹

PURPOSE. To study the effects of long-term contact lens wear on morphologic and physiologic properties of corneal endothelial cells.

METHODS. The endothelial permeability to fluorescein and the rate of corneal deswelling from hypoxia-induced edema were measured in 20 long-term (mean, 17 ± 9 years; range, 5-33 years) contact lens wearers and 20 age-matched control subjects. From these data, the relative endothelial pump rate in each subject was estimated, based on the pump-leak hypothesis of corneal hydration control. Corneal autofluorescence and the aqueous humor flow rate were determined by fluorescein fluorophotometry. Images of corneal endothelial cells were recorded by using specular microscopy, and morphologic indices (cell density, coefficient of variation of cell area, percentage of hexagonal cells, and skewness) were determined.

RESULTS. No statistically significant differences were found between the contact lens and control groups in endothelial permeability, corneal deswelling, relative endothelial pump rate (mean = 1.07 ± 0.33 relative pump units versus 1.01 ± 0.25 relative pump units; contact lens versus control; P = 0.57), and endothelial cell density. Contact lens wearers had a significantly higher aqueous humor flow rate (3.57 ± 1.03 µl/min versus 2.77 ± 0.51 µl/min; P = 0.005), coefficient of variation of cell area (0.35 ± 0.09 versus 0.28 ± 0.04; P = 0.006), and corneal autofluorescence (3.1 ± 0.6 ng/ml versus 2.3 ± 0.3 ng/ml fluorescein equivalents; P < 0.001) than did non-contact lens wearers.

CONCLUSIONS. Despite the known effects of long-term contact lens wear on corneal endothelial morphology, no effect on endothelial function was found. (Invest Ophthalmol Vis Sci. 1999;40:603-611)

Endothelial cells are thought to control corneal thickness by maintaining a barrier to solute movement into the cornea and an active pump of solute out of the cornea, a process that has been referred to as a pump-leak mechanism.¹ As solute crosses this barrier, water moves with it, responding to local osmotic gradients. When solute moves into the corneal stroma (leak), the cornea swells, and as solute is pumped out (pump), the cornea deswells. When the transfer is balanced, cornea thickness remains constant. Solute and fluid movement across the endothelium are thought to take place in the paracellular space between the endothelial cells.¹ It is not known whether changes in the structure of the endothelial cell matrix that may alter the configuration of paracellular space, such as polymegethism (elevated coefficient of variation of cell area), can affect the barrier or the pump functions, or both.

Two approaches have been used to study the function of the corneal endothelium, and several investigators have attempted to associate changes in endothelial function with changes in the appearance of the endothelium. First, endothelial function has been assessed by measuring the time for corneal thickness to return to normal after swelling. In early studies, swelling was induced by cataract surgery, and corneas with polymegethism required more time than normal corneas did to recover.²,³ Subsequently, investigators measured the rate of corneal deswelling after various periods of contact lens wear.⁴-⁷ More recently, Polse et al.⁸ developed a clinical method to measure overall endothelial function, or corneal hydration control, by inducing hypoxic corneal swelling with a contact lens and measuring the rate of deswelling. They found that in an individual patient the thickness of the swollen cornea decreased exponentially toward the normal corneal thickness, which they termed the open-eye steady state (OESS) thickness. By fitting the deswelling data to this first-order model by nonlinear regression, they calculated the deswelling rate and expressed it as the percent recovery per hour (PRPH). The method is purely descriptive and does not depend on assumptions about stromal swelling pressure, pump rate, endothelial or epithelial permeability, or total corneal thickness. If it is assumed that the epithelial barrier is intact, the deswelling rate is a measure of endothelial function, but it reflects both the barrier and the pump. Nieuwendaal et al.⁹ by using a method similar to Polse's method, found abnormally low deswelling rates in long-term contact lens wearers.

In the second approach, the barrier function has been assessed by measuring endothelial permeability to a small molecule, fluorescein. In using this method, it is assumed that permeability to fluorescein is proportional to endothelial permeability to other solutes. Permeability studies have shown no
obvious association between polymegathism and a change in endothelial barrier function. Endothelial permeability to fluorescein has been normal in studies of contact lens wearers, 10, 11 patients with early Fuchs’ dystrophy, 12 and diabetic patients 13–15 despite polymegathism and pleomorphism (a decreased percentage of hexagonal cells) in many of these groups. In other studies of experimental animals, permeability returned to normal within 3 months after the endothelial cells had been damaged, although abnormal cellular morphology persisted. 16 Because the barrier function has been normal in studies of contact lens wearers, 10, 11 the low deswelling rates noted by Nieuwendaal et al. 9 may reflect an abnormal pump function. The pump function can be separated from the barrier function only if endothelial permeability to fluorescein is known in addition to the deswelling rate.

In this study we measured endothelial permeability to fluorescein and the corneal deswelling rate and devised a method to estimate the relative endothelial pump rate. With this method we examined long-term contact lens wearers, who had corneas never operated on and were known to have severe polymegathism, 17–20 and compared them with corneas of normal subjects with normal endothelium who had never worn contact lenses.

MATERIALS AND METHODS

Subjects

We recruited 20 subjects from our contact lens clinic who had used daily-wear contact lenses in both eyes for at least 5 years. Twenty age-matched (within 5 years) subjects who had never worn contact lenses were also recruited from Mayo Clinic patients and staff and their families, to serve as control subjects. All 40 subjects were examined, including slit lamp examination, tonometry, and undilated fundus examination, and had none of the following conditions: any ocular disease, previous ocular surgery, topical ocular medications in the previous month, systemic medications that can affect corneal thickness (e.g., birth control pills, diuretic drugs), ptosis, keratoconjunctivitis sicca, superficial corneal vascularization extending more than 2 mm inside the limbus, or diabetes mellitus. All subjects were at least 18 years old. All contact lens wearers had always worn similar lens types in both eyes. Types of lenses worn and duration of wear are given in Tables 1 and 2. This study followed the tenets of the Declaration of Helsinki and was approved by our Institutional Review Board; all subjects provided written informed consent to participate.

Measurements

Subjects were studied on 2 days, and identical procedures were used in all subjects. Contact lenses were not worn during either of the study days. On the afternoon of day 1, autofluorescence of the central cornea was measured at an excitation wavelength of 488 nm (emission between 515 nm and 600 nm) by using a two-dimensional scanning ocular fluorophotometer. 21, 22 Corneal fluorescein was measured three times, and the mean was accepted as the autofluorescence. Anterior chamber volume of each eye was then measured by using a photogammetric technique, 23 and 5 to 10 photographs of the central corneal endothelium of each eye were recorded by a wide-field contact specular microscope.

On day 2, subjects instilled 2% fluorescein in both eyes beginning at 2 AM. The drops were instilled at 5-minute intervals, and the number of drops was determined by the subject’s age: 5 drops were instilled by subjects less than 26 years of age, 4 drops by those 26 to 35 years of age, and 3 drops by those more than 35 years of age. 13 Subjects were instructed to return to sleep. All subjects reported to the clinical research area by 8 AM for baseline pachometry and fluorophotometry of each eye.

Corneal thickness was measured by using a modified Haag-Streit optical pachometer equipped with fixation lights and a potentiometer that registered measurements directly into a computer memory. 24 The operator was not aware of the individual thickness measurements as they were recorded. The mean of 10 consecutive measurements was accepted as the corneal thickness. The process was repeated if the SD of the 10 measurements was 10 μm or more. The instrument was calibrated daily by measuring a set of contact lenses of known thickness. We noted that some examiners used slightly differ-

<table>
<thead>
<tr>
<th>Table 1. Contact Lens Wearers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of Contact Lens</td>
</tr>
<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>Soft (n = 10)</td>
</tr>
<tr>
<td>Polymethylmethacrylate (n = 3)</td>
</tr>
<tr>
<td>Rigid gas permeable (n = 1)</td>
</tr>
<tr>
<td>Mixed (n = 6)*</td>
</tr>
<tr>
<td>Total (n = 20)</td>
</tr>
</tbody>
</table>

Values are means ± SD, with range in parentheses. * See Table 2.

<table>
<thead>
<tr>
<th>Table 2. Lenses Worn by Mixed Contact Lens Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wearing Time (y)</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>Subject</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
</tbody>
</table>
sent alignment end points when measuring contact lenses than they did when measuring corneas. To compensate, we derived a correction factor for each cornea based on its thickness, measured with the specular microscope during the afternoon of study day 1.

At approximately 8:20 AM each eye was fitted with a soft contact lens (38% water content; +20.00 D; 8.3-mm base curve) and patched. After 2 hours, at approximately 10:20 AM, the patches and contact lenses were removed, and corneal thickness and corneal and cameral fluorescence were measured in both eyes. Starting at approximately 11:30 AM, corneal thickness was measured in each eye and repeated every 10 minutes for 1 hour, then every 20 minutes for 1 hour, and then every 30 minutes until 5 PM. Corneal and anterior chamber fluorescence were measured at 11:30 AM and noon, and then hourly until 5:00 PM. After the final fluorescence and thickness measurements were completed, intraocular pressure was measured three times by applanation tonometry, and the mean was recorded. The study was then complete for that subject. Autofluorescence was subtracted from each fluorescence measurement.

**Analysis**

The methods for calculating the deswelling rate, endothelial permeability, and aqueous humor flow rate have been described in detail elsewhere\(^8\) and are briefly outlined below:

**Deswelling Rate**

We assumed that after removal of the contact lens the stromal swelling, the thickness in excess of the OESS thickness, deswells as a first-order process, as described by Polse et al.\(^8\):

\[
q(t) = B + Se^{-Dt}
\]

where \(q(t)\) is the corneal thickness at time \(t\), \(B\) is the OESS thickness, \(S\) is the induced swelling (the thickness in excess of \(B\) at \(t = 0\)), \(D\) is the deswelling rate constant, and \(t\) is time since removal of the contact lens. Corneal thicknesses were fitted to Equation 1 by nonlinear regression (S-PLUS; Statistical Systems, Seattle, WA) to obtain estimates of \(B\), \(S\), and \(D\) in each subject. Corneal thicknesses measured during the first 50 minutes after removal of the contact lenses were excluded because of the potential effects of decreased pH on the deswelling rate during this period.\(^27\) The PRPH, a more clinically meaningful parameter used to describe the deswelling rate, was also calculated\(^6\):

\[
PRPH = 100(1 - e^{-600})
\]

**Endothelial Permeability**

Endothelial permeability to fluorescein was determined by using the method described by Jones and Maurice\(^28\):

\[
\text{Permeability} = \frac{q_r[t_a, t_1]}{q_s} = \frac{C_e(t_i) - C_e(t_f)}{C_e(t_i) - C_e(t_f)}
\]

where \(t_0\) and \(t_1\) are time at the beginning and end of the interval, \(q_s\) is the mean corneal thickness on the interval, \(r_{ea}\) is the steady state distribution ratio for fluorescein between the cornea and the anterior chamber (assumed to be 1.6 in corneas at normal thickness\(^29\)), \(C_e(t_i)\) is the concentration of fluorescein in the cornea at time \(t_i\), and \(C_e\) and \(C_c\) are the mean concentrations of fluorescein in the cornea and anterior chamber on the interval. Corneal fluorescence was adjusted for changes in corneal thickness.\(^14,15,25\) Mean concentrations \(C_e\) and \(C_c\) were determined from the initial and final concentrations on the interval by assuming that \(C_e\) and \(C_c\) decreased as a single exponential decay.\(^30\)

We determined the permeability to fluorescein during two intervals. The first permeability (the AM, or hypoxic, permeability) was calculated from fluorescein concentrations at 8:00 AM, just before contact lens insertion, and at 11:30 AM, approximately 60 minutes after contact lens removal. The second permeability (the PM, or normoxic, permeability) was the mean of permeabilities during five 1-hour intervals between noon and 5 PM. The PM permeability is similar to the permeability measured when the cornea is not swollen.\(^26\)

**Aqueous Humor Flow Rate**

The clearance of fluorescein was used to calculate the aqueous humor flow rate:

\[
\text{Flow} = \frac{\Delta M}{C_e \times \Delta t} = 0.25 \mu l/min
\]

where \(\Delta M\) is the loss of mass of fluorescein in the combined cornea and anterior chamber during an interval \(\Delta t\), and \(C_e\) is the average concentration in the anterior chamber during the same interval, estimated from the initial and final fluorescence and assuming a single exponential decay. The constant, 0.25 \(\mu l/min\), was subtracted to account for diffusional loss of fluorescein. We calculated aqueous humor flow between 12 noon and 5 PM, during the same intervals used to calculate PM endothelial permeability.

**Relative Endothelial Pump Rate**

The endothelial solute pump rate cannot be measured directly in humans, but the ratio of pump rate to normal pump rate can be estimated if we assume that the deswelling rate is directly proportional to the pump rate and inversely proportional to the endothelial permeability to fluorescein.\(^1\) This simple model can be expressed in terms of the pump rate:

\[
\text{Pump rate} = K \times \text{Deswelling rate} \times \text{Permeability}
\]

where \(K\) is a constant. The absolute value of the pump rate can only be determined if we know \(K\). However, if \(K\) is constant from subject to subject, we can determine the ratio of pump rate of one person's cornea to normal pump rate:

\[
\text{Relative endothelial pump rate} = \frac{\text{deswelling rate}}{\text{deswelling rate}_n} \times \frac{\text{permeability}_i}{\text{permeability}_n}
\]

where the subscript \(i\) indicates an individual, and the subscript \(n\) indicates the normal values, for which we used the mean for the control group.
TABLE 3. Results

<table>
<thead>
<tr>
<th>Measurement</th>
<th>20 Control Subjects*</th>
<th>20 Contact Lens Wearsers*</th>
<th>P†</th>
<th>MDD‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>38 ± 14</td>
<td>37 ± 13</td>
<td>0.72</td>
<td>14</td>
</tr>
<tr>
<td>PRPH (%/h)</td>
<td>65.7 ± 12.3</td>
<td>72.5 ± 9.7</td>
<td>0.06</td>
<td>11.3</td>
</tr>
<tr>
<td>Open-eye steady state thickness (μm)</td>
<td>552 ± 53</td>
<td>557 ± 37</td>
<td>0.76</td>
<td>47</td>
</tr>
<tr>
<td>8:00 AM thickness (μm)</td>
<td>556 ± 55</td>
<td>554 ± 39</td>
<td>0.89</td>
<td>49</td>
</tr>
<tr>
<td>Induced swelling (μm)§</td>
<td>61 ± 10</td>
<td>59 ± 7</td>
<td>0.56</td>
<td>9</td>
</tr>
<tr>
<td>Induced swelling (%)§</td>
<td>11.1 ± 2.1</td>
<td>10.8 ± 1.7</td>
<td>0.68</td>
<td>2.0</td>
</tr>
<tr>
<td>Endothelial permeability, AM (×10⁻⁴ cm/min)</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>3.95 ± 0.74</td>
<td>3.39 ± 1.05§</td>
<td>0.07</td>
<td>0.94</td>
</tr>
<tr>
<td>Endothelial permeability, PM (×10⁻⁴ cm/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.04 ± 0.51</td>
<td>3.87 ± 1.08</td>
<td>0.56</td>
<td>0.88</td>
</tr>
<tr>
<td>Relative endothelial pump rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.01 ± 0.25</td>
<td>1.07 ± 0.33</td>
<td>0.57</td>
<td>0.31</td>
</tr>
<tr>
<td>Aqueous humor flow rate (μl/min)</td>
<td>2.77 ± 0.51</td>
<td>3.57 ± 1.03</td>
<td>0.005§</td>
<td>—</td>
</tr>
<tr>
<td>Intraocular pressure (mm Hg)</td>
<td>15.5 ± 2.8</td>
<td>15.6 ± 2.4</td>
<td>0.95</td>
<td>2.6</td>
</tr>
<tr>
<td>Corneal autofluorescence (ng/ml fluorescein equivalents)</td>
<td>2.3 ± 0.3</td>
<td>3.1 ± 0.6</td>
<td>&lt;0.001</td>
<td>—</td>
</tr>
<tr>
<td>Endothelial cell density (cells/mm²)</td>
<td>2736 ± 373</td>
<td>2688 ± 492</td>
<td>0.92§</td>
<td>447</td>
</tr>
<tr>
<td>(2757)</td>
<td>(2794)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coefficient of variation of cell area</td>
<td>0.28 ± 0.04</td>
<td>0.35 ± 0.09</td>
<td>0.006</td>
<td>—</td>
</tr>
<tr>
<td>Skewness of cell area</td>
<td>0.70 ± 0.53</td>
<td>0.77 ± 0.44</td>
<td>0.51#</td>
<td>0.50</td>
</tr>
<tr>
<td>(0.66)</td>
<td>(0.64)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexagonal endothelial cells (%)</td>
<td>62.4 ± 7.0</td>
<td>55.8 ± 14.1</td>
<td>0.07</td>
<td>11.4</td>
</tr>
</tbody>
</table>

* Values are means ± SD, with median in parentheses when data are not distributed normally.
† Two-tailed Student’s t-test for means (except # below).
‡ MDD, minimum detectable difference with 90% power (a = 0.05, β = 0.10).
§ Increase in thickness from 8:00 AM to 10:30 AM, from just before contact lens insertion to just after removal. n = 19 in control group (10:30 AM thickness was not recorded in one subject).
|| n = 19 in control group (no fluorophotometry on study day 2 for one subject).
¶ Significantly less than the evening permeability in the same subjects (P = 0.02; paired t-test).
* Wilcoxon rank sum test.

The relative endothelial pump rate was determined for each subject. Deswelling rate, determined by using Equation 1, and PM, or normoxic, permeability, determined by using Equation 3 for the period between noon and 5 PM, were used in Equation 6.

Endothelial Morphologic Analysis

Apices of 100 central corneal endothelial cells were digitized from photographic negatives magnified 500 times. The mean, SD, coefficient of variation (SD/mean), skewness of cell area, and the percentage of cells with six sides were determined by use of a commercial algorithm (Bambi system; Bio-Optics, Arlington, MA). The endothelial cell density in cells per square millimeter was expressed as the reciprocal of the mean cell area.

Statistical Analysis

The mean of the values for the left and right eyes was considered to be one observation in each subject. This summary was used for each subject to avoid the problems associated with the analysis of correlated data. For example, the correlation in PRPH between left and right eyes in this study was 0.56 (P = 0.0002). We compared contact lens wearers with control subjects by using a two-tailed Student’s t-test for means if the data were distributed normally and by using a Wilcoxon rank sum test if they were not. We also tested correlations between PRPH, induced swelling, permeability, relative pump rate, and the morphologic indices by using a Pearson’s correlation (r_p) for normal data and Spearman’s rank correlation (r_s) for non-normal data. A two-tailed P ≤ 0.05 was considered statistically significant in all tests.

RESULTS

We found no statistically significant differences between the two groups in any functional measurement except for an increase in aqueous humor flow rate in the contact lens wearers. 

![Figure 1] Relative corneal endothelial pump rate (in relative pump units) in long-term contact lens wearers and control subjects. Horizontal lines indicate mean values. There was no significant difference between the groups (P = 0.57).
Aqueous humor flow rate (J.1/min)

Figure 2. Aqueous humor flow rate in long-term soft contact lens wearers, nonsoft lens wearers (polymethylmethacrylate, rigid gas-permeable, and mixed lens wear), and control subjects. Horizontal lines indicate median values. The flow rate was significantly higher in the soft lens wearers than in the control subjects (P < 0.001) and nonsoft lens wearers (P = 0.04). The differences remained statistically significant if the high outlier value in the soft lens group was not included in the analyses.

(1.3.3). The deswelling rate, OESS thickness, induced swelling, endothelial permeability, and relative endothelial pump rate (1.1.1.) were all similar in the two groups. The AM permeability was significantly less than the PM permeability in the contact lens wearers, but not in the control subjects. Among morphologic measurements, the coefficient of variation was increased in the contact lens group. Corneal autofluorescence was also increased in the contact lens wearers.

Corneal Endothelial Pump in Contact Lens Wearers

Figure 3. Plot of PRPH versus age in all subjects. The four contact lens groups are explained in Tables 1 and 2.

Compared with the remainder of the contact lens group, the 10 soft lens wearers had more AM (hypoxic) permeability, more PM (normoxic) permeability, a higher relative endothelial pump rate, higher aqueous flow rate (Figure 2), greater endothelial cell density, fewer years of contact lens wear, and fewer years of age (Table 4). The AM permeability was less than the PM permeability in the nonsoft lens wearers, but not in the soft lens wearers.

In the contact lens group PRPH was correlated with years of contact lens wear (r p = —0.48; P = 0.03), coefficient of

Table 4. Results in Wearers of Soft Contact Lenses versus Wearers of Nonsoft Lenses

<table>
<thead>
<tr>
<th>Measurement</th>
<th>10 Soft Lens Wearers*</th>
<th>10 Nonsoft Lens Wearers*</th>
<th>P‡</th>
<th>MDD§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>29 ± 8</td>
<td>44 ± 13</td>
<td>0.006</td>
<td>—</td>
</tr>
<tr>
<td>Years of contact lens wear</td>
<td>10 ± 5</td>
<td>24 ± 8</td>
<td>0.002</td>
<td>—</td>
</tr>
<tr>
<td>PRPH (%/h)</td>
<td>76.6 ± 8.4</td>
<td>68.4 ± 9.5</td>
<td>0.06</td>
<td>13.0</td>
</tr>
<tr>
<td>Open-eye steady state thickness (µm)</td>
<td>559 ± 41</td>
<td>555 ± 32</td>
<td>0.79</td>
<td>55</td>
</tr>
<tr>
<td>8:00 AM thickness (µm)</td>
<td>558 ± 41</td>
<td>550 ± 38</td>
<td>0.68</td>
<td>58</td>
</tr>
<tr>
<td>Induced swelling (µm)</td>
<td>60 ± 4</td>
<td>59 ± 10</td>
<td>0.60</td>
<td>11</td>
</tr>
<tr>
<td>Induced swelling (%)</td>
<td>10.9 ± 1.4</td>
<td>10.7 ± 2.0</td>
<td>0.80</td>
<td>2.5</td>
</tr>
<tr>
<td>Endothelial permeability, AM (×10^-4 cm/min)</td>
<td>4.10 ± 0.90</td>
<td>2.68 ± 0.65‡</td>
<td>&lt;0.001</td>
<td>—</td>
</tr>
<tr>
<td>Endothelial permeability, PM (×10^-4 cm/min)</td>
<td>4.42 ± 0.98</td>
<td>3.33 ± 0.91</td>
<td>0.02</td>
<td>—</td>
</tr>
<tr>
<td>Relative endothelial pump rate (relative units)</td>
<td>1.26 ± 0.27</td>
<td>0.87 ± 0.27</td>
<td>0.004</td>
<td>—</td>
</tr>
<tr>
<td>Aqueous humor flow rate (µL/min)</td>
<td>4.04 ± 1.09</td>
<td>3.09 ± 0.74</td>
<td>0.04*</td>
<td>—</td>
</tr>
<tr>
<td>(3.88)</td>
<td>(2.96)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraocular pressure (mm Hg)</td>
<td>14.6 ± 2.5</td>
<td>16.5 ± 2.0</td>
<td>0.07</td>
<td>3.3</td>
</tr>
<tr>
<td>Corneal autofluorescence (ng/ml fluorescein equivalents)</td>
<td>3.0 ± 0.4</td>
<td>3.2 ± 0.8</td>
<td>0.32</td>
<td>0.90</td>
</tr>
<tr>
<td>Endothelial cell density (cells/mm²)</td>
<td>2941 ± 271</td>
<td>2435 ± 543</td>
<td>0.04*</td>
<td>—</td>
</tr>
<tr>
<td>(2966)</td>
<td>(2710)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coefficient of variation of cell area</td>
<td>0.34 ± 0.10</td>
<td>0.36 ± 0.09</td>
<td>0.59</td>
<td>0.14</td>
</tr>
<tr>
<td>Skewness of cell area</td>
<td>0.74 ± 0.48</td>
<td>0.81 ± 0.41</td>
<td>0.63*</td>
<td>0.65</td>
</tr>
<tr>
<td>(0.50)</td>
<td>(0.71)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexagonal endothelial cells (%)</td>
<td>60.7 ± 14.7</td>
<td>50.9 ± 12.3</td>
<td>0.12</td>
<td>19.7</td>
</tr>
</tbody>
</table>

* Values are means ± SD, with median in parentheses when data are not distributed normally.
† Nonsoft: polymethylmethacrylate, rigid gas-permeable, or mixed lens wear (see Table 1).
‡ Two-tailed Student’s t-test for means (except # below).
§ MDD, minimum detectable difference with 90% power (α = 0.05, β = 0.10).
|| Increase in thickness from 8:00 to 10:30 AM, from just before contact lens insertion to just after removal.
¶ Significantly less than the evening permeability in the same subjects (P = 0.04; paired t-test).
* Wilcoxon rank sum test.
Although age and years of contact lens wear were significantly correlated with years of contact lens wear (r_p = -0.52; P = 0.02) and age (r_p = -0.50; P = 0.03; Fig. 3). Relative endothelial pump rate was correlated with years of contact lens wear (r_p = -0.45; P = 0.03; Fig. 4). Years of contact lens wear was also associated with age (r_p = 0.85; P < 0.001) and percentage of hexagonal cells (r_p = -0.64; P = 0.002), but not with coefficient of variation (r_p = 0.36; P = 0.12). Although age and years of contact lens wear were significantly related to PRPH and to relative endothelial pump rate, age and years of wear contained similar information (r_p = 0.85; P < 0.001) and percentage of hexagonal cells (r_p = -0.64; P = 0.002), but not with coefficient of variation (r_p = 0.36; P = 0.12). After adjusting for age, therefore, the years of contact lens wear were not significantly related to PRPH or to relative endothelial pump rate (multiple regression analysis). Statistically significant correlations were not found in the control group.

DISCUSSION

In this study, as in other studies, long-term contact lens wearers had a higher than normal coefficient of variation of endothelial area, but normal cell density, compared with subjects who had never worn contact lenses. In contrast to these changes, there were no significant functional differences in their corneal endothelia; neither recovery from swelling (PRPH), nor permeability to fluorescein, nor relative endothelial pump rate in this group was significantly different from normal subjects. It is not clear why these physiologic properties did not change. Perhaps the increase in paracellular area resulting from the greater variability of cell size and shape was not great enough to change the pump and barrier. Alternatively, the density of active pump sites may have adjusted to compensate for a change in total paracellular area. Also, either the pump or barrier function could be distributed across the cells rather than restricted to the paracellular spaces.

Recovery from swelling induced by contact lenses has also been measured in long-term contact lens wearers by others. Nieuwendaal et al. found that PRPH was significantly less in contact lens wearers than it was in control subjects, whereas we found that it was more, although not significantly so (P = 0.06). It is not clear why results from our experiment and that of Nieuwendaal et al. differ, although in our protocol, thickness during the first hour of deswelling was not used to calculate PRPH, whereas Nieuwendaal used thickness every 15 minutes after the lens was removed. We purposely did not include fluorescein or corneal thickness during this period because of the potential changes in fluorescence and deswelling rate induced by the lower stromal pH.27,32,33 As a test of whether this difference in methods could account for the difference in the results of these two studies, we reanalyzed our data and included the thickness measured immediately after removal of the contact lens. By this method, PRPH values for contact lens wearers and control subjects were 58.0% ± 7.3% and 47.7% ± 11.6%, respectively. These values are lower than those shown in Table 3, presumably because of the effect of decreased pH during the first 50 minutes after contact lens removal.27 However, PRPH in the contact lens wearers was still not significantly lower than PRPH in the control subjects when calculated in this way. The decreased PRPH found by Nieuwendaal et al.9 must have resulted from either an increased endothelial permeability or a decreased pump rate.1 Nieuwendaal et al. did not measure endothelial permeability, but it has not been abnormal in other studies of long-term contact lens wearers.10,11

Another possible explanation for the differences between the results of our study and that of Nieuwendaal et al.9 is that more of their subjects wore polymethylmethacrylate lenses (17/21) than in our study (8/20) and therefore may have been exposed to more severe hypoxia. Also, the average wearing time of polymethylmethacrylate or soft contact lenses was longer in their study (195 years) than in ours (14.2 years in 19 subjects). Therefore, we tested separately the 9 subjects in the polymethylmethacrylate- and mixed-lens-wear groups (Table 1) and the 12 subjects who had worn contact lenses for more than 12 years (mean wearing time, 23.3 years). Neither group differed significantly from their age-matched control subjects in PRPH or relative endothelial pump rate. These findings are explained by the data in Figures 3 and 4, in which the contact lens and control groups are seen to overlap considerably.

Others have found a delay in the return to normal thickness after cataract extraction in eyes with preoperative polymegathism.2,3 The polymegathism and pleomorphism that were present in our sample of contact lens wearers apparently do not limit the pump function. The slower recovery of corneal thickness after cataract extraction may have been the result of slower recovery of the polymegathous endothelium after surgical trauma or inflammation.

The OESS thickness was similar between the two groups, as was the 8 AM thickness. These findings confirm those of Nieuwendaal et al.9 Holden et al.19 found a decrease in stromal thickness of 11 μm (2.3%) in the lens-wearing eye of unilateral contact lens wearers, but it was manifest only after discontinuing lens wear for 7 days. When the lenses were initially removed, the stromal thickness was increased 2.5% in the lens-wearing eyes.19 Our subjects had discontinued their lenses only overnight, and residual edema may therefore have been present.

The contact lens and control groups had similar swelling induced by two hours of hypoxic contact lens wear. Nieuwendaal et al.9 found less induced swelling in their contact lens wearers. This difference in findings, similar to the difference in PRPH, remains unexplained. Erickson et al.34 found a strong correlation between the percentage of induced swelling
and endothelial cell density in 15 adapted lens wearers. We
found no correlation between these two parameters in the 20
contact lens wearers in the present study ($r_s = 0.05; P = 0.83$).

In this study we assumed that all fluorescein and fluid was
transferred across the endothelium and that losses to the epi-
thelial surface were negligible. The epithelial contribution to
corneal hydration control is probably small in normal eyes. It
is possible that changes in the epithelium affected our mea-
surement of endothelial permeability in contact lens wearers,
because their epithelia are more fragile. Because epithelial
permeability is not elevated in contact lens wearers, however,
our estimates of endothelial permeability should be valid.

The AM permeability was significantly less than the PM
permeability in the contact lens wearers, but not in the control
subjects. The AM permeability in the contact lens wearers was
also less than that in the control subjects, but the difference did
not reach statistical significance ($P = 0.07$). The AM perme-
ability was calculated for the period when the eye was wearing
an aphakic soft contact lens with the lids closed, and therefore
the cornea was hypoxic and acidic. The AM, or hypoxic,
permeability is also decreased in diabetes mellitus. The
mechanism of this abnormal response to acidosis in contact
lens wearers and people with diabetes is unknown; swelling of
endothelial cells with narrowing of the paracellular pathway
has been suggested. Contact lens wear and diabetes in-
duce endothelial cell polymegethism, so that this morphologic
change may be associated with the decrease in hypoxic per-
meability. The AM permeability was not decreased, however,
in seven phakic eyes with corneal transplants that also had
polymegethism, although the endothelial cells were much larg-
er.

There was no significant difference in PM permeability
between the contact lens wearers and the control subjects.
These results exclude, with 90% confidence, a difference of
$0.88 \times 10^{-4}$ cm/min (Table 2). The PM, or normoxic, per-
meability was measured during the period when the cornea was
exposed to normal oxygen concentrations and was similar to
that measured during the unswollen state. These findings
confirm those of earlier studies, in which no effect was found of
long-term contact lens wear on endothelial permeability to
fluorescein.

We calculated a relative endothelial pump rate by using
normalized values and assuming that the deswelling rate was
directly proportional to the pump rate and inversely propor-
tional to the permeability. These assumptions arise from the
pump-leak hypothesis of corneal hydration control: In steady
state, the rate of active solute (and passive fluid) transfer from
the stroma to the aqueous humor by the endothelial pump is
balanced by the passive leak of solute and fluid across the
endothelium into the stroma. This analysis ignores the move-
ment of solutes and fluid across the epithelium and limbus,
which is thought to be of minor importance. The endothelial
permeability to the small molecule, fluorescein, is assumed to
be proportional to the leak. It follows that when the swollen
cornea deswells, the rate of deswelling is slower in the pres-
ence of a decrease in the pump rate or an increase in perme-
ability.

Two fluorophotometric parameters were evaluated in con-
tact lens wearers, corneal autofluorescence and aqueous hu-
mor flow rate. Similar increases in corneal autofluorescence
have been found in patients with diabetes mellitus and in those who have had corneal transplants. Autofluores-
cence is thought to arise from mitochondrial flavoproteins, and its major portion most likely originates in the epithelium. Elevated autofluorescence suggests increased native fluoro-
phore or a change in local conditions that affect its fluores-
cence, such as an alteration in oxidative state, pH, tempera-
ture, light-filtering characteristics of the overlying stroma, or interactions with other molecules.

The increased aqueous humor flow confirms our earlier
results, although a smaller study of 11 extended-wear contact
lens wearers did not show an elevated flow rate in contact lens
wearers. At least three factors that may be altered in contact
lens wearers could elevate aqueous humor flow. First, larger
eyes may require a higher flow rate for adequate function. This
possibility is consistent with the absence of difference in in-
traocular pressure in the two groups, despite the increased
aqueous humor flow (Table 2). Our control subjects were not
matched for refractive error, and the contact lens wearers were
more myopic (mean spherical equivalent correction, $-4.6 \pm 2.3$ D; $n = 19$) than the control subjects (mean spherical
equivalent, $+0.1 \pm 1.0$ D; $n = 12$; $P < 0.0001$). The contact
lens group also had larger anterior chamber volumes ($215 \pm 37 \mu l$) than the control group ($178 \pm 37 \mu l$; $P = 0.004$). After
adjusting for anterior chamber volume, however, there was
still a significant difference between the groups in aqueous
humor flow ($P = 0.02$, analysis of covariance). Therefore, the
difference in anterior chamber volume (larger eyes) does not
seem to explain the difference in aqueous humor flow. In
addition, there was no significant correlation between anterior
chamber volume and flow in either group.

Second, an elevated epithelial permeability in contact lens
wearers could raise the clearance of fluorescein across the
anterior surface of the cornea and increase the apparent flow
rate. As mentioned earlier, however, epithelial permeability is
not elevated in soft or rigid gas-permeable contact lens wear-
ers. Recently, McNamara et al.42 recently measured a 40% elevation in
epithelial permeability after 1 hour of contact lens wear in-
tended to produce hypoxia. When we modeled mathemati-
cally the transfer of fluorescein across the epithelium for a past
presentation, at least a 10-fold increase in epithelial perme-
ability was necessary to increase the estimate of flow by 1%. Moreover, if the epithelial permeability were increased, more
fluorescein should have entered the corneal stroma when flu-
orescein was instilled. The stromal concentration at 8 AM,
however, was not higher in contact lens wearers than it was in
control subjects ($1072 \pm 836$ ng/ml versus $1035 \pm 706$ ng/ml;
$P = 0.88$). For these reasons, even if epithelial permeability
was increased in the contact lens wearers, it should not have
produced the differences in flow that we measured.

Finally, the ciliary body may have been stimulated to
produce aqueous humor at a higher rate by the decrease in
$pH$ or $oxygen$ in the aqueous humor when contact lenses are
worn. Unfortunately, the responses of the ciliary body to
to $pH$ and oxygen levels in the aqueous humor are not known.

Although there was prominent polymegethism (increased
coefficient of variation of cell area) in the contact lens group,
there was no significant difference in the skewness of cell area.
This result suggests that the endothelia of contact lens wearers
have higher proportions of both large and small cells.

The 10 subjects who wore only soft contact lenses were
younger than the remaining 10 subjects, and this difference
may explain their greater endothelial cell densities and higher
pump rates (Table 3). The soft lens wearers also had signifi-
cantly higher aqueous humor flow (Fig. 2) and permeability to fluorescein than did the remaining subjects. Thus, the 10 soft lens wearers accounted for the increased aqueous humor flow rate in the contact lens group compared with that in the control subjects (Table 2). Moreover, the 10 subjects who had worn lenses other than soft lenses accounted for the decreased AM permeability in the contact lens group compared with that in the control subjects (Table 2). We have no reasonable explanation for these findings. In our previous study, in which aqueous humor flow rate was higher in contact lens wearers than in control subjects, the increased flow was also present only in the soft lens wearers. Soft lenses have larger diameters, but we have no plausible hypothesis for why this difference would affect aqueous humor flow rate.

In the contact lens group, we found significant negative correlations between years of contact lens wear and both corneal deswelling rate (PRPH) and relative endothelial pump rate. We could not attribute this negative correlation to an increasingly depressive effect of prolonged contact lens wear on the deswelling and pump rates, however, because of a similar correlation between these rates and age and a stronger correlation between age and years of wear. Therefore, age could not be ruled out as the cause of the decrease in deswelling and pump rates that occurred with increasing years of contact lens wear. The same can be said of the significant negative correlation between PRPH and coefficient of variation of cell area, which also was more strongly correlated with age in the contact lens group. Although we found no significant correlations in the control group between age and PRPH, endothelial permeability, endothelial cell density, coefficient of variation, or percentage of hexagonal cells, such correlations have been found in other studies with more subjects and larger ranges of age.23,24,47

In summary, despite the known effects of long-term contact lens wear on corneal endothelial cell morphology, we could show no effects on endothelial cell function.

References


ANNOUNCEMENT

GRF Names Scientific Advisory Committee Chair
Paul Kaufman, M.D., to Lead Research Funding into 21st Century

The Glaucoma Research Foundation (GRF) has named Paul Kaufman, M.D., Director of Glaucoma Services at University of Wisconsin, Madison, as chairman of its Scientific Advisory Committee.

Kaufman will lead the committee in determining funding for promising research projects to protect sight from glaucoma. Last year, GRF’s Scientific Advisory Committee approved funding of $1.5 million.

“I believe fervently in GRF,” Kaufman said. “GRF programs develop the science and the scientists of the future, at a stage usually too preliminary to light up radar screens of larger organizations.”

“At the same time, GRF resources are now substantial enough to make a real impact.”

GRF is a national not-for-profit organization dedicated to protecting the sight and independence of people with glaucoma. The ultimate goal is a cure.