Age Differences in Corneal Hydration Control

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Dynamic changes in corneal thickness were measured in eight young and eight older normal subjects (mean ages 24.4 ± 4.3 years and 71.9 ± 7.3 years, respectively) to provide data for quantitative assessment of corneal hydration control and thereby provide information for studying age differences in this important aspect of corneal function. For each subject, pachometry data were obtained by (A) monitoring corneal recovery following hypoxic stress, and by either (B1) measuring recovery after sleep or (B2) by measuring corneal thickness in the late afternoon. The combined data from A and B1 or A and B2 were analyzed through an exponential model to provide information on the: (1) percent recovery per hour (PRPH) following induced corneal hydration; (2) open-eye steady-state (OESS) corneal thickness; (3) residual corneal swelling just before the hypoxic stress test; (4) amount of corneal edema induced by hypoxic stress; and (5) time to reach 95% recovery back to the OESS thickness level (T95%). The results show that between the two age groups, there are substantial differences in some characteristics of corneal hydration while other aspects are similar. For example, the mean PRPH values (58.9 ± 7.8% and 34.2 ± 6.4%/hr) were significantly higher in the younger subjects (P = 0.0002) and the mean time for 95% recovery to OESS thickness (207 ± 42 min and 452 ± 117 min) was significantly lower in the younger vs. the older group (P = 0.0002). Also, differences between the two age groups were observed at midmorning before starting the hypoxic stress test; the mean corneal thickness of the older subjects was 32.1 ± 4.8 μm above the OESS thickness, and this was significantly greater than the results (1.84 ± 7.1 μm) found with younger subjects (P = 0.0002). However, the absolute amount of induced corneal swelling following hypoxic stress, 64.2 ± 10.6 μm in younger vs. 66.0 ± 9.3 μm in older subjects, did not differ significantly (P = 0.96), nor did the mean OESS thickness levels, 516 ± 34 μm in younger vs. 537 ± 28.9 μm in older subjects (P = 0.234).

These results provide strong evidence that corneal hydration control decreases with age. The test methods used in this study show good promise for applicability in the clinical setting so that certain "at risk" patients can be tested and monitored in the ophthalmological office. However, to prove clinically applicable, more work is needed to refine the test procedures to make them as convenient and efficient as possible. Invest Ophthalmol Vis Sci 30:392–399, 1989
In an earlier set of experiments we reported preliminary results using a corneal function test that was administered to ten young subjects (mean age 26.7 years) and ten older subjects (mean age 65.7 years). The basic principle of the test was to increase the level of corneal hydration using a hypoxic stimulus and then monitor the rate of corneal recovery (ie, corneal dehydration) after removal of the stimulus. From these experiments, we obtained recovery data that were sufficiently reliable for overall group comparisons. These data were based on the mean rate of change in corneal thickness during the first 2 hr of recovery after approximately 60 \( \mu \text{m} \) of corneal swelling was induced by the hypoxic stimulus. These results showed that the recovery rate was significantly slower in the older vs. younger group (10.5 \( \mu \text{m/hr} \) vs. 15.0 \( \mu \text{m/hr} \), \( P < .001 \)) and suggested that hydration control was age-dependent.

For clinical and more refined research applications, procedures that make it possible to reliably assess an individual cornea's capacity for hydration control are needed. In this paper, we report results based on recently developed test procedures that provide estimates of an individual's overall corneal hydration control capacity. The measurements resulting from these newer test procedures are used to compare younger and older subjects with respect to: (1) the amount of swelling induced by a standardized hypoxic stress stimulus; (2) the percent recovery per hour that characterizes the exponential recovery after the hypoxic stress stimulus ends; (3) the time to 95% recovery (T95%); and (4) the open-eye steady-state corneal thickness.

**Materials and Methods**

In this section, the study subjects used in this investigation and the hydration control test procedures, along with associated modeling and analysis techniques, are briefly described.

**Subjects**

Sixteen subjects were recruited from the general community, eight in a younger group whose mean age was 24.4 ± 4.3 years, and eight in an older group whose mean age was 71.9 ± 7.3 years. The procedures of the study were explained fully to all subjects and then an informed consent was obtained. (The U.C. Berkeley Committee for the Protection of Human Subjects had granted approval for the research project and for the Informed Consent Form and Medical Subjects' Bill of Rights given to all participants.) All subjects passed a complete eye examination that indicated normal ocular health. On a separate day, each subject was given a detailed slit-lamp examination to again ensure that the cornea was free of disease. Additional examination of the cornea was done by specular photomicroscopy and each photomicrograph was digitized and analyzed for cell count, cell size and cell size variation (ie, polymegathism). The morphometric analysis indicated that all three endothelial characteristics were within the normal ranges.

**Hydration Control Test Procedures**

In order to obtain a reliable assessment of the functioning of the corneal hydration control system, it has been found necessary to use one of three kinds of composite test procedures; namely a Stress-Patch test, a Stress-Natural test or a Stress-Direct OESS test. Each of these three types of composite tests consisted of two procedures, conducted on separate days. The component tests involved in these three types of composite tests were conducted as follows.

**Stress test:** Subjects arrived at the laboratory in the morning after typically being awake for 2 or 3 hr. First, initial status pachometric measurements were made to determine the initial corneal thickness, which was one of the properties needed to assess residual swelling remaining after the preceding night’s sleep. Then the cornea was fitted with a “stress lens” designed to induce transient edema by exposing the cornea to an hypoxic environment.

The stress lenses were specially designed hydrogel contact lenses with an oxygen permeability (Dk) of 9 \( \times 10^{-11} \) (cm² x ml O₂)/(sec x ml x mm Hg). Each lens was approximately 400 \( \mu \text{m} \) thick with an oxygen transmissibility (Dk/L) of 4.5 \( \times 10^{-9} \) (cm x ml x O₂)/(sec x ml x mm Hg). When these lenses were worn with the eyes closed, the oxygen tension at the anterior corneal surface was near 0 mm Hg, which produced sufficient hypoxia to cause increased corneal hydration. These lenses were manufactured in three posterior base curve radii to provide a lens selection that would give a well-centered and comfortable lens with minimum movement and least subjective sensation.

After each eye had been fitted with a stress lens, the subject was given approximately 5 min in the open-eye state to adjust to wearing the lens. Allowance for this brief 5 min open-eye adjustment period was found to largely eliminate some problems with subsequent decentering of lenses during the closed-eye phase of the test that arose in pilot development of the Stress test protocol. Following the adjustment period, the eyes were patched to ensure lid closure and kept in the closed-eye state for 2 hr except for brief monitoring at approximately 30, 60 and 90 min into the swelling phase of the test to make sure the lens still remained centered on the cornea.

After the 2 hr closed-eye stress period, the lenses...
were removed and central corneal thickness was monitored for at least 3 hr during the deswelling phase of the test. Typically, two sets of ten readings were made every 20 to 30 mins. The means of the two sets of ten readings, which were separated by resetting the subject and the pachometer, were treated as two statistically independent replicate measurements.

Corneal thickness was measured using a modified slit lamp and Haag-Streit pachometer. Numerous modifications to this instrument improved the reliability and accuracy of the measurements. The pachometer was linked to a Commodore microcomputer (Model #64, Commodore Business Machines, West Chester, PA), which allowed the pachometry readings to be fed directly into the microcomputer for accurate time monitoring and data collection. The details of this instrument and the modifications are described elsewhere.

**Patch test:** Each subject was given a patch and instructed in how to place it correctly over the designated eye before retiring for the night preceding the test visit. Subjects arrived at the laboratory as soon as possible after waking, typically within 2 or 3 hr. Then the patch was checked for proper placement and removed so monitoring of corneal thickness could begin. The central corneal thickness was monitored over the next 5 to 6 hr using the same measurement techniques described above with two replicate sets of ten readings every 20 to 30 min (except for lunch breaks).

**Natural test:** The natural test was essentially the same as the patch test except that no patch was used to cover the eye during sleep. With this test there was an opportunity for the eye to recover partially from overnight swelling before testing started in the morning. In general, subjects had been awake at least 2 hr before pachometric measurements were made. The Natural test was applied to the unpatched eye not before pachometric measurements were made. The central corneal thickness was monitored for at least 3 hr during the deswelling phase of the test begins, and D (the deswelling rate). The first two parameters, B and S2, are directly interpretable, but D is easier to interpret after reducing the error in pachometric measurement is represented by e, which is not directly observable. The model has three parameters; B (the OESS thickness), S1 (the initial swelling present when the deswelling phase of the test begins), and D (the deswelling rate). The first two parameters, B and S1, are directly interpretable, but D is easier to interpret after it has been converted to alternative forms described below.

The deswelling response in the Patch or Natural test can be represented analogously by the model:

\[
TH(t) = B + S_2 \times \exp(-D \times t) + e,
\]

where \( t \geq 0, D \geq 0 \) (1) in this model. TH(t) is the corneal thickness at time t measured in minutes from the start of the deswelling phase of the test which begins with the removal of the stress lens. The error in pachometric measurement is represented by e, which is not directly observable. The model has three parameters; B (the OESS thickness), S1 (the initial swelling present when the deswelling phase of the test begins), and D (the deswelling rate). The first two parameters, B and S1, are directly interpretable, but D is easier to interpret after it has been converted to alternative forms described below.

The deswelling response in the Patch or Natural test can be represented analogously by the model:

\[
TH(t) = B + S_2 \times \exp(-D \times t) + e,
\]

where \( t \geq 0, D \geq 0 \) (2) which is structurally the same as the stress test deswelling response model, but it has a different initial swelling parameter, S2. The Direct OESS test measurements can be modeled by:

\[
TH(t) = B + e
\]

The pair of models for a composite test are coupled because the B and D parameters are the same in both
models since they represent corneal properties that are presumed to be stable from one component test to another.

An analysis of composite test results, which use all of the test data available for a given eye to estimate B, D, S₁, and S₂ (if needed), can be implemented by using standard nonlinear regression techniques as described more fully elsewhere. Sample programs that illustrate the analysis of composite test data with SAS procedure NLIN are available on request.

The deswelling rate, D, can be more readily interpreted if it is converted to give the percent recovery per hour. The PRPH, which ranges from 0 to 100%, is the deswelling that occurs in any one-hour interval expressed as a percentage of the swelling that exists at the start of the interval. It is defined by:

$$\text{PRPH} = \left[ \frac{\text{TH}(t) - B}{\text{TH}(t + 60) - B} \right] \times 100$$

It can be shown that, for the exponential deswelling model, PRPH = \[1 - \exp(-60 \times D)\] \times 100% when D is expressed in units of (1/min). For example when \(D = 0.015 \text{ (1/min)}\), which is a typical value for a young normal subject, then PRPH = \(1 - \exp(-60 \times 0.015)\) \times 100 = 59.34%.

The deswelling rate D can also be converted to give the time required to reach 95% recovery to OESS thickness. Figure 2 shows the OESS corneal thickness values presented in Figure 1A except that the Natural test procedure NLIN are available on request.

Given the PRPH value for an individual cornea, it is possible to construct mathematically the exponential deswelling response that would be expected from any specified initial percentage swelling. Based on an initial swelling of 10%, these curves are presented in Figure 1A except that the Natural test procedure NLIN are available on request.

The deswelling rate D can also be converted to give the time required to reach 95% recovery to OESS thickness. The time to 95% recovery, T₉⁵%, is represented by a horizontal line at 552 μm. As in the older subjects, recovery follows an exponential pattern, but with considerably faster recovery to OESS thickness. The estimated PRPH for this subject was 39.2%/hr (with a 95% confidence interval from 29.8 to 47.6%/hr) and the corresponding estimated time for 95% recovery was 260 min.

Given the PRPH value for an individual cornea, it is possible to construct mathematically the exponential deswelling response that would be expected from any specified initial percentage swelling. Based on an initial swelling of 10%, these curves are presented in Figure 2 for the PRPH values corresponding to the minimum, mean and maximum values observed in each of the two age groups. From the figure it is apparent that there is very little overlap in hydration recovery between the older and younger groups.

Figure 3A shows the PRPH results for the younger and older subjects; solid lines connect data points for the right and left eyes of each subject. Significant differences were found in the PRPH values for the younger and older groups with values for means ± standard deviations equal to 58.9 ± 7.8%/hr and 34.2 ± 6.4%/hr respectively (\(P = 0.0002\)). These differences in recovery dynamics are also noted in the corresponding T₉⁵% values (Fig. 3B), which were significantly lower for the younger subjects as compared to the older subjects with means and standard deviations equal to 207 ± 42 min and 452 ± 117 min, respectively (\(P = 0.0002\)).

Figure 4 shows the OESS corneal thickness values for both eyes for each of the older and younger subjects. There is a considerable variation in the OESS
levels within each group. Although the mean OESS thickness values of 538 ± 28.9 μm and 516 ± 34 μm for the older and younger subjects, respectively, are not statistically significant ($P = 0.224$), there is some suggestion that older persons may have slightly thicker OESS levels compared to the younger subjects.

Figure 5 shows the residual thickness measurements for each group of subjects, with straight lines connecting right-eye and left-eye data. At midmorning, before starting the Stress test, the residual corneal

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**Fig. 1.** (A) Corneal thickness recovery for the right eye of an older subject: Stress test (circles) and Patch test (triangles). (B) Corneal thickness recovery and Direct OESS data for a younger subject: Stress test (circles) and Direct OESS tests (triangles).

**Fig. 2.** Exponential recovery from 10% initial swelling corresponding to mean, minimum, and maximum PRPH values in the 16 eyes of younger (dashed-line curves) or older (solid-line curves) subjects.

**Fig. 3.** (A) The percent recovery per hour (PRPH) for younger and older subjects following induced corneal edema. The lines between data points connect the right and left eye of each subject. (B) The time required to reach 95% (T95%) of the open-eye steady-state corneal thickness (OESS) for the older and younger subjects. The lines between data points connect the right and left eye of each subject.
swelling in the older subjects was 32.1 ± 4.8 μm above OESS. In contrast, the younger subjects had a mean residual swelling of only 1.8 ± 7.1 μm; these differences between the age groups are statistically significant (P = 0.0002).

The amount of corneal swelling induced by the stress lens was similar for the older and younger groups (Fig. 6) and both developed substantial amounts of corneal edema. The mean swelling responses were 66.0 ± 9.25 μm and 64.2 ± 10.6 μm for the older and younger groups, respectively; this difference was not significantly different (P = 0.96).

The PRPH estimate and corresponding 95% confidence interval for each individual eye tested in the younger and older groups are presented in Figure 7. The connected PRPH estimates for the subject's two eyes illustrate the high degree of concordance between right-eye and left-eye values.

Discussion

By monitoring recovery from increased hydration levels, it is possible to obtain individual estimates of several characteristics of corneal hydration control. When Direct OESS thickness measurements cannot be obtained with certainty, the precision of the estimates of the exponential hydration control parameters can still be made acceptable by doing a composite analysis of data from two different corneal recovery experiments. Based on these studies we recommend that a Stress test plus a Natural or Patched test be done on older individuals (i.e., >40 years) and a Stress test plus a Direct OESS measurement be done on younger individuals to estimate corneal recovery. Regardless of age, if corneal function is believed to be compromised, a Direct OESS test should not be used since residual swelling may be present that would produce biased estimates of hydration control parameters.

Inspection of the paired right-left eye data for the various indices of corneal hydration control indicates noticeable similarity between the two eyes of an individual. These eye-to-eye comparisons are consistent with other measurements and clinical observations that the two eyes of normal subjects tend to be quite similar. The corresponding results were still similar, although the composite test procedures were different for each of the eyes (e.g., one eye received Stress-Patch tests while the other eye received Stress-Natural tests). In principle, the alternative composite tests should differ only in the precision of the estimates they provide.
Fig. 6. Absolute increase in corneal thickness (corrected for residual swelling) for both the older and younger subjects just after the stress lens is removed. The lines between data points connect the right and left eye of each subject.

Visual examination of data obtained for each eye tested in the study indicates that recovery from induced corneal swelling is approximately exponential for both older and younger individuals. However, older individuals have substantially slower recovery rates and require on the average about three times longer to make a 95% recovery to the OESS thickness level. The greater morning residual swelling observed in the older subjects (Fig. 5) most likely results from the substantially lower recovery rate of the older compared to the younger subjects. These age-dependent data are consistent with a recent report on corneal function where permeability to fluorescein was shown to increase with age.13 Barring some remarkable bias in our selection of the particular subjects used to compare PRPH in different age groups, there is a clear indication that hydration control is substantially related to age. However, we do not have detailed information about the year-to-year decline in PRPH with age, which would be of interest.

When the OESS level is reached, the corneal thickness is somewhat greater in older compared to younger subjects; however, these differences are not statistically significant. These findings are slightly different from studies which report that corneal thickness values are fairly constant throughout life.14-16 However, it should be emphasized that most other studies used direct corneal thickness measurements and did not control for time of measurements, so their measurements may not have been equivalent to the OESS thickness assessments that were used in this study. Since the time required to reach OESS levels is age-dependent and appears to be substantially longer than has been previously reported, we recommend that procedures or studies requiring a

Fig. 7. The percent recovery per hour (PRPH) for the right and left eyes for each of the eight younger and eight older subjects. The vertical lines show the 95% confidence intervals for each of the PRPH values.
direct assessment of the open-eye steady-state corneal thickness (eg, refractive keratoplasty, contact lens studies, overnight thickness studies) must be done with care to avoid bias due to residual swelling.\(^{17-19}\)

The test methods used in this study show promise for use in obtaining a clinical assessment of an individual cornea's capacity for hydration control. Efforts are underway to improve the clinical applicability of hydration control testing by refining the hydration control test design and simplifying the analysis procedures.

Key words: corneal hydration, corneal function, corneal edema, endothelial function

References