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: (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

A practical and reliable method for assessing corneal function in the clinical setting would be useful for evaluating and predicting the effects of trauma, inflammation, dystrophy, surgery and medical intervention on corneal health. Initially, it was hoped that direct morphological examination of the corneal endothelium using specular microscopy would provide a good indication of the physiological status of the endothelium. The most important evidence for an association between endothelial morphology and function was presented by Rao et al., who showed that after cataract extraction, individuals with considerable preoperative polymegathism tended toward longer periods of postoperative corneal edema, compared to those who did not show polymegathism. Unfortunately, there have been no additional studies to either substantiate this finding or to provide additional information on the relationship between endothelial morphology and corneal function. On the other hand, there is evidence which suggests that morphometric analysis does not always provide sufficient information for this purpose. In fact, a paradox in clinical ophthalmology may sometimes occur when a cornea appearing relatively normal decompensates after surgical intervention, whereas another cornea that appears to have substantial changes in endothelial morphology remains relatively unaffected by surgery. What is needed is a test that gives quantitative information about the cornea's overall capacity to regulate its hydration. This could provide the basis for a more accurate evaluation of the effects of medical or surgical interventions and also give prognostic information about the physiological status of the cornea before medical or surgical intervention.
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 µm 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 µm/hr vs. 15.0 µm/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 × 10^-11 (cm² × ml O₂)/(sec × ml × mm Hg). Each lens was approximately 400 µm thick with an oxygen transmissibility (Dk/L) of 4.5 × 10^-9 (cm × ml × O₂)/(sec × ml × 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 microcomput er (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.10

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 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).

Direct OESS test: The open-eye steady-state (OESS) thickness of the cornea is the thickness level the cornea will approach and will eventually reach if the eye is kept open sufficiently long after removing a stimulus (eg, hypoxia, sleep) that induces an increase in hydration. Getting reliable and valid information about the OESS thickness is one of the keys to obtaining reliable information about the cornea’s capacity to control hydration. The Direct OESS test is a collection of corneal thickness measurements, which, except for random measurement errors, give direct measurements of OESS thickness. Although this test provides a relatively efficient way to obtain useful data to contribute to the assessment of hydration control, it can only provide accurate data when there are convincing reasons to believe that the cornea is really in the OESS when the test measurements are made.

The Stress-Direct OESS test was used to assess hydration control in young normal corneas. The Direct OESS measurements were made on these subjects late in the afternoon after their eyes were open for at least 6 hr. For older subjects the Stress-Patch or Stress-Natural test combinations were used except for two subjects who were given a Direct OESS test late in the day because they could not be scheduled for the longer Patch or Natural test. Upon review, their Direct OESS data was judged valid for analysis purposes. However, based on currently available data, the Direct OESS test is not generally recommended for older subjects because of their relatively slow recovery from overnight swelling.

Analysis of Composite Hydration Control Test Data

Analysis of the combined data from both components of a composite hydration control test was based on coupled exponential models that are briefly described below. In the Stress Test, the deswelling response after the stress lens is removed can be described reasonably well by the exponential model:

\[ TH(t) = B + S_1 \times \exp(-D \times t) + e, \quad t \geq 0, \quad D > 0 \quad (1) \]

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), S_1 (the initial swelling present when the deswelling phase of the test begins), and D (the deswelling rate). The first two parameters, B and S_1, 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:

\[ TH(t) = B + S_2 \times \exp(-D \times t) + e, \quad t \geq 0, \quad D > 0 \quad (2) \]

which is structurally the same as the stress test deswelling response model, but it has a different initial swelling parameter, S_2. The Direct OESS test measurements can be modeled by:

\[ TH(t) = B + e \quad (3) \]

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.13 Sample programs that illustrate the analysis of composite test data with SAS procedure NLIN12 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:

$$\text{PRPH} = \frac{\text{TH}(t) - B - \text{TH}(t + 60) - B}{\text{TH}(t) - B} \times 100 \quad (4)$$

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

The deswelling rate $D$ can also be converted to give the time required to reach 95% recovery back to OESS thickness. The time to 95% recovery, $T_{95\%}$, can be computed from $D$ by the conversion formula, $T_{95\%} = [-\ln(0.05)]/D = 2.996$. For example, when $D = 0.015$ (1/min) then $T_{95\%} = 2.996/0.015 = 200$ min.

Two derived quantities, which are used in the results section, are the residual swelling prior to the Stress test and the swelling stimulated by the Stress test. The residual swelling was determined by subtracting the estimated OESS thickness from the mean of the initial status pachometry measurements made just before fitting the stress lens. The swelling stimulated by the Stress test was estimated by subtracting the estimated residual swelling from the estimated initial swelling provided by the estimate of the $S_1$, a parameter in the exponential deswelling model for the Stress test.

The calculation of the statistical significance of the mean difference between age groups was based on the eight right-left eye averages in each age group in order to account for the statistical dependence of data from two different eyes from the same subject. Significance probabilities were obtained from the distribution-free, Wilcoxon Rank Sum Test.

**Results**

Figure 1A, which provides an example of a typical hydration response profile, shows recovery data obtained from the right eye of an older subject using a Stress-Patch test. The composite test data from the two component test procedures were simultaneously analyzed by a coupled deswelling response model to provide information on various indices of corneal hydration control. For this subject the estimated PRPH was 38.7%/hr (with a 95% confidence interval of 34.2–43.0%/hr). The corresponding estimated time to reach 95% recovery to OESS ($T_{95\%}$) thickness was 436 min. For the contralateral eye the Stress-Natural composite test was done and showed exponential recovery curves similar to those described in Figure 1A except that the Natural test produced less initial swelling than the Patch test, (ie, 15 $\mu$m vs. 25 $\mu$m), presumably because the left eye was open for about 2 hr before monitoring of deswelling began. For the left eye, the estimated PRPH was 35.4%/hr (with a 95% confidence interval from 29.8 to 40.6%/hr) and the corresponding time for 95% recovery to OESS thickness was 410 min.

Data from a younger subject who received the Stress test and Direct OESS thickness measurements are presented in Figure 1B. The estimated OESS thickness is represented by a horizontal line at 552 $\mu$m. 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 49.9%/hr (with a 95% confidence interval from 43.3%/hr to 55.7%/hr) and the corresponding estimated time to 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 $T_{95\%}$ 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 re-
sponses were 66.0 ± 9.25 μm and 64.2 ± 10.6 μm for
the older and younger groups, respectively; this dif-
ference was not significantly different (P = 0.96).

The PRPH estimate and corresponding 95% confi-
dence 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 be-
tween 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 esti-
mates of the exponential hydration control parame-
ters 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 (ie, >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 param-
eters.

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 indi-
vidual. 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 (eg, 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 pro-
vide.

Fig. 4. The open-eye steady-state corneal thickness (OESS) for
the right and left eyes of each older and younger subject calculated
by using the hydration control model. The lines between data
points connect the right and left eye of each subject.

Fig. 5. Residual swelling, the difference between pre-Stress test
and OESS thickness in young and older subjects. The lines between
data points connect the right and left eye of each subject.

Fig. 7.
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.

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. 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
direct assessment of the open-eye steady-state corneal thickness (e.g., 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