In Vivo Human Corneal Hydration Control Dynamics: A New Model

Monica T. P. Odenthal,¹ Carla P. Nieuwendaal,¹ Hans W. Venema,² Johannes Oosting,³ Jan H. C. Kok,⁴ and Aire Kijlstra¹

PURPOSE. To introduce a new model describing human in vivo corneal deswelling after hypoxic contact lens wear, based on a damped harmonic oscillator, which can describe an overshoot in corneal deswelling, to compare this new model with the currently used exponential model, and also to test whether a diurnal variation in baseline corneal thickness exists that would have to be taken into consideration when calculating corneal deswelling curves.

METHODS. In nine healthy young adults, corneal thickness was measured every 30 minutes for 11.5 hours using modified optical pachometry (natural test). On another day, corneal deswelling was monitored for 11.1 hours on average after 2 hours of hypoxic contact lens wear (stress test). The damped harmonic oscillator model and the exponential model were used to calculate best-fitting deswelling curves. Natural test data were analyzed for the presence of a trend. Goodness of fit of the curves to the experimental data was analyzed using the F test.

RESULTS. In 82% of the deswelling curves the new damped harmonic oscillator model provided a better fit to the data than the exponential model (P < 0.05). An average overshoot in corneal thickness recovery of 5 μm (range, 0–11 μm) was found. In 50% of the natural tests significant trends were found, without any consistent similarities. The overshoot could not be explained by these trends.

CONCLUSIONS. The new damped harmonic oscillator model describes corneal deswelling after hypoxic contact lens wear more accurately than the exponential model. No consistent diurnal variation could be demonstrated. (Invest Ophthalmol Vis Sci. 1999;40:312-319)

Corneal hydration control has been the subject of extensive research in recent years. Monitoring the deswelling rate after induced corneal swelling by wearing a low gas-permeable contact lens with the eye closed, the Corneal Hydration Control (CHC) test, has been used extensively to study corneal physiology in human subjects. With this method, corneal function has been assessed in healthy individuals and in persons with a possibly compromised corneal function, for example, due to long-term contact lens wear, persons who have had cataract surgery, those with Fuchs' dystrophy, diabetes, or perforating keratoplasty. More recently, the CHC test was used to study the effect of dorzolamide on corneal endothelial function.

The current model to describe corneal hydration control in the CHC test is a nonlinear model, in which the deswelling rate is considered to be constant. This results in an exponential deswelling curve. This model is based on the "pump-leak" corneal hydration control model by Maurice and was first presented by O'Neal and Poise in 1985. During our investigations in this area we found a phenomenon that could not be described by the exponential model: an overshoot in corneal thickness recovery in which corneal thickness seems to decrease to a value below "open eye steady state thickness" or "baseline corneal thickness" before returning to this baseline thickness. Although this overshoot has been noted by others, it was apparently not considered to be a real phenomenon. In any case, the exponential model remained in use to fit the data, and this model does not take the overshoot-phenomenon into account.

In general it is thought that corneal thickness is solely dependent on eye closure, so that corneal thickness decreases after opening of the eyes in the morning and remains steady after reaching "baseline thickness" or "open eye steady state" corneal thickness. The presence of diurnal variation is important in the context of the CHC test because it might influence calculated deswelling rates. It might also explain the phenomenon of overshoot, when the time at which the overshoot occurs would coincide with the time of the lowest value that occurs naturally during the day.

On the basis of these arguments, we decided to address the following questions:

1. Is a diurnal variation in corneal thickness present, apart from swelling due to eye closure during sleep and deswelling after eye opening during waking hours? If present, can this diurnal variation be attributed to an...
exponential decrease of corneal thickness during the day or to a steady increase or decrease in corneal thickness during the day?
2. Can corneal deswelling data be better described using a new model, which allows for an overshoot in corneal thickness recovery (when present), than by the currently used exponential model?
3. Is this overshoot a real phenomenon, due to the deswelling mechanism, or can it be attributed to a natural variation in corneal thickness?

METHODS

Subjects
Nine volunteers, young adults with no history of eye or systemic disease and no previous or current contact lens wear, participated in this experiment, eight men and one woman, age 19 to 26 years (mean, 22 years). Informed consent was obtained from each subject after the nature and possible consequences of the study were explained. This study followed the tenets of the Declaration of Helsinki, and permission to perform the study was obtained from the Medical Ethics Committee of the Academic Medical Center of Amsterdam.

Corneal Thickness
Corneal thickness was measured using modified optical pachometry with a Haag Streit (Bern, Switzerland) optical pachometer attached to a slit lamp, equipped with fixation and alignment lights to increase accuracy. The pachometer was attached to an electronic recording system and linked to a personal computer, which allowed immediate calculation of corneal thickness. Measurements took place in a darkened room, after 2 minutes of dark adaptation, to allow the use of a narrow slit of light, and thus to increase accuracy. The slit width was fixed during all measurements. On each measuring day, the pachometer was calibrated with eight polymethylmethacrylate contact lenses of known thickness (300–650 μm). Each corneal thickness measurement, at a certain time point, was obtained as two sets of 10 readings, carried out within 4 minutes. Between the two sets of readings the investigator and test subject both had a short pause of a few seconds. Each set of 10 readings was averaged; hence, at every time point, two mean values of 10 readings were obtained. The SD of one set of readings had to be within 10 μm, to be accepted. If this was not the case, which happened only rarely, the set of readings was repeated immediately. Typically, the SD of one set of readings ranged between 4 μm and 6 μm.

Procedures
Subjects were instructed to wake up at 7:00 am on test days with measurements starting at approximately 9:30 am at the earliest to allow for a waking period of more than 2 hours before the start of measurements.

Measurements took place on two separate days. The first day a “natural test” was performed as follows: corneal thickness was measured every 30 minutes, from 10:00 AM to between 8:30 PM and 11:00 PM, without any application of contact lenses or other intervention. This natural test was performed to monitor corneal thickness under natural or “baseline” conditions, to evaluate possible deswelling due to residual swelling from eye closure during sleep and to detect a possible diurnal variation in corneal thickness. The natural tests had an average duration of 11.5 hours (range, 10.5–13.0 hours).

On a second day the “stress-test” was performed. This “stress test” was never performed on a day directly after the first test day, to eliminate any possible effect due to an abnormally short period of sleep the night before. Experimental soft low gas-permeable (38% water content) contact lenses with a thickness of 0.45 mm were used as “stress lenses.” These stress lenses were applied between 9:30 AM and 10:00 AM, worn for 2 hours with the eyes closed and lightly patched, and then removed. Corneal thickness measurements were subsequently performed every 30 minutes after removal of the “stress lenses” up to between 10 PM and 12 PM. The mean duration of deswelling measurements was 11.1 hours (range, 10.1–13.0 hours).

Natural test data were available from 18 eyes of 9 subjects. Stress-test data were available from 17 eyes of 9 subjects. In one subject the stress lens in one eye had been decentered from the cornea for a substantial period before scheduled removal of the stress lens, resulting in a significantly lower percentage of induced swelling. Stress-test data from this eye were excluded from analysis.

Description of Models
In all models, thickness is expressed in micrometers, and time is expressed in hours.

Models Used for Analysis of Natural Test Data. The data from the natural test were used to detect possible diurnal corneal thickness changes. An analysis was made for data for each eye from each subject separately.

Two possible deviations from a constant corneal thickness during the day were considered. First, the data were tested for the presence of an exponential decrease of corneal thickness due to residual deswelling after eye opening in the morning. This exponential decrease is described by the same model as the exponential model (see below). In case this deswelling could not be demonstrated, the presence of a slow drifting of corneal thickness during the day was tested, described by a linear trend: a constant decrease or increase in corneal thickness during the day when the eyes are open, described in the following model:

\[ \text{th}(t) = \text{th}(t_0) + c(t - t_0) + \epsilon, \]

where \( \text{th}(t) \) is thickness at time \( t \), \( t_0 \) is the time of the first measurement, \( c \) is the rate of corneal thickness change per hour (expressed in micrometers per hour), and \( \epsilon \) is the error term.

Models Used for Analysis of the Stress-Test Data. To describe the deswelling process and to fit a curve through experimental deswelling data from the stress test, two models were used. The first is the currently used exponential model, \(^2^4^3^7\) and the second is a new model, which is based on a damped harmonic oscillator.

The exponential model used to describe corneal deswelling, as first applied by O'Neal and Polse,\(^2^3\) uses the following formula:

\[ \text{th}(t) = a + be^{-\frac{(t-t_0)}{e}} + \epsilon \]

A New Model for Human Corneal Hydration Control 313
where \( th(t) \) is thickness at time \( t \), \( a \) is baseline thickness, \( b \) is induced swelling, \( t_c \) is time constant, \( t_r \) is time of removal of the stress lens, and \( e \) is the error term.

A commonly used parameter in this model to describe corneal hydration control in an individual is the percentage recovery per hour (PRPH), which can be calculated from the time constant, according to the formula below:

\[
PRPH = (1 - e^{-t/\tau}) \times 100\%.
\]

As a new model to describe hydration control, which takes into account an overshoot in deswelling, we used the damped harmonic oscillator model.\(^{21}\) In this model, as in the exponential model, fastest recovery takes place immediately after removal of the stress lenses. Unlike the exponential model, however, an overshoot can take place, followed by a gradual return to baseline corneal thickness:

\[
\text{tb}(t) = a + b \cdot e^{-\left(\frac{t - t_r}{\tau}\right)} \cos(2\pi f (t - t_c)) + e
\]

in which, in addition to the aforementioned parameters, \( f \) is frequency.

Because the deswelling rate in this model is not constant, the percentage recovery per hour cannot be used as a parameter for deswelling dynamics. Instead a characteristic time can be used, for instance the half-recovery time (\( t_{\text{0.5}} \)): the time required after removal of the stress lenses to reach 50% recovery back to baseline corneal thickness.

Simulated Deswelling Data, Derived from the Natural Test. To investigate whether the overshoot, if present, is a real phenomenon caused by the deswelling mechanism or an artifact caused by variations of baseline corneal thickness, we analyzed simulated deswelling curves. The argument for this simulation study is as follows. When corneal deswelling after removal of the stress lenses is in essence purely exponential, and the overshoot is only an artifact as a result of diurnal fluctuation of the baseline thickness, then simulated deswelling data obtained by adding a (calculated) exponential deswelling curve to the measured natural data would show a better fit with the harmonic oscillator model than with the exponential model.

To test this assumption, we added data from a simulated exponential deswelling curve with average characteristics (amount of induced swelling \( b = 66 \mu m \), time constant 1 hour) to each of the 18 experimental natural test data to obtain simulated deswelling data sets. These simulated deswelling data sets were then analyzed by fitting curves using both the exponential and the damped harmonic oscillator model and by comparing the goodness of fit.

Curve Fitting. The different models were fitted to the experimental data with the Levenberg-Marquardt method using our own software. The code of this fitting method was obtained from Numerical Recipes.\(^{22}\) To fit the deswelling curves, only data from the stress test were used, unless specifically indicated otherwise.

Statistical Analysis

For the natural test data and the deswelling data, the goodness of fit of the simple model (constant corneal thickness for the natural data, the exponential model for the deswelling data) was compared with the goodness of fit of the more extended models (an exponential decay and a linear trend for the natural data; the damped harmonic oscillator model for the deswelling data) using the F test.\(^{23}\) The same procedure was followed for the simulated deswelling data.

Parameters derived from the fitted damped harmonic oscillator model were used to detect a possible correlation between induced swelling and magnitude of overshoot, between \( t_{\text{0.5}} \) and magnitude of overshoot, and between magnitude of overshoot and the time needed to reach minimal corneal thickness (this is the time at which the overshoot occurs, when it is present).

RESULTS

Natural Tests

In calculating a possible exponential decrease, in seven of the natural tests estimation procedures did not converge or pro-
produced erratic results. In the other 11 natural tests, no significant exponential decrease could be demonstrated.

In 8 (44%) of 18 natural tests, a significant linear trend was found ($P < 0.05$). In four eyes of two subjects, corneal thickness increased during the measurements, with an average of 0.5 μm to 1.1 μm per hour, and in four eyes of three subjects, corneal thickness decreased, with an average of 0.7 μm to 1.1 μm/h. In the remaining 10 natural tests, no significant linear trend was found.

Figure 1 shows two examples of natural tests from two eyes of different subjects: one without a trend (Fig. 1A) and one with a significant increase of corneal thickness during the day (Fig. 1B).

**Stress Tests**

For 14 (82%) of 17 deswelling curves, the fit of the new damped harmonic oscillator model was better than the fit of the exponential model at the 5% level and for 13 (76%) of 17 at the 1% level. Thus, in the vast majority of cases the new damped harmonic oscillator model has a significantly better fit to the observed deswelling data from the stress test than the exponential model. Typical examples of curves fitted to the experimental data with the exponential model and the new damped harmonic oscillator model are shown in Figure 2 for two eyes of different subjects.

Average parameter values from these 17 curves calculated with both models are given in Table 1. The $t_{0.5}$ is systematically longer when calculated with the damped harmonic oscillator model than with the exponential model.

The magnitude of the overshoot, determined with the damped harmonic oscillator model, varied from 0 μm to 11 μm (5 ± 3 μm; mean ± SD). In percentage of baseline thickness, this is 0% to 2.3%, with an average of 0.9%. In one eye, zero overshoot was calculated with the damped harmonic oscillator model. In this case, therefore, the damped harmonic oscillator model yielded the same curve as the exponential model.

No significant correlation was found between induced swelling and magnitude of overshoot ($r = -0.44$; Fig. 3). Also no correlation was present between $t_{0.5}$ and magnitude of overshoot ($r = 0.40$; Fig. 4). Time from removal of the stress lenses to the time point at which the overshoot occurred was 4.1 ± 0.7 hours (mean ± SD; range, 3.2–5.6 hours; Fig. 5).
TABLE 1. Average Parameter Values of 17 Corneal Deswelling Curves, Calculated with the Exponential Model and the Damped Harmonic Oscillator Model

<table>
<thead>
<tr>
<th></th>
<th>Exponential Model</th>
<th>Damped Harmonic Oscillator Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline thickness (μm)</td>
<td>535 ± 26 (483–564)</td>
<td>537 ± 26 (486–565)</td>
</tr>
<tr>
<td>Induced swelling (μm)</td>
<td>66 ± 6 (55–82)</td>
<td>60 ± 6 (50–72)</td>
</tr>
<tr>
<td>PRPH (%)</td>
<td>60 ± 6 (46–71)</td>
<td>NA</td>
</tr>
<tr>
<td>t0.5 (h)</td>
<td>0.77 ± 0.15 (0.55–1.13)</td>
<td>0.93 ± 0.16 (0.62–1.23)</td>
</tr>
<tr>
<td>tc (h)</td>
<td>1.11 ± 0.21 (0.80–1.63)</td>
<td>1.78 ± 0.41 (0.91–2.53)</td>
</tr>
<tr>
<td>f (h−1)</td>
<td>NA</td>
<td>0.092 ± 0.024 (0.024–0.127)</td>
</tr>
<tr>
<td>Overshoot (μm)</td>
<td>NA</td>
<td>5 ± 3 (0–11)</td>
</tr>
</tbody>
</table>

Values are mean ± SD with range in parentheses.
PRPH, percent recovery per hour; NA, not applicable.

Simulated Deswelling Data, Derived from the Natural Test

In only 2 (11%) of the 18 simulated deswelling data sets, the damped harmonic oscillator model had a better fit at the 5% level and 1 of these 2 at the 1% level. This is in contrast to actual experimental deswelling data, as was mentioned previously, in which the damped harmonic oscillator model provided a better fit in 82% of the eyes at the 5% level.

DISCUSSION

Our new model to describe corneal hydration control, based on a damped harmonic oscillator and taking into account an overshoot in deswelling, results in a more accurate fit of the experimental data to a calculated deswelling curve than the current exponential model, which is based on the assumption that corneal deswelling takes place at a constant speed.

The phenomenon of overshoot in corneal thickness recovery in the deswelling curves in these young healthy subjects cannot be explained by residual overnight swelling or by possible fluctuations in corneal thickness in the natural test. Therefore, it appears to be a real deswelling phenomenon. The magnitude of the overshoot is very small, on average only 0.9% of baseline thickness or 7.8% of induced swelling. Nevertheless, we believe that the use of the new damped harmonic oscillator model has some distinct advantages over the old exponential model, for practical and theoretical reasons, as will be discussed below.

Corneal hydration control testing, as performed in this study, is time-consuming and demanding for both researchers and test persons. To achieve optimal deswelling data, a long deswelling period (11 hours as in this study) is preferable over a shorter one. However, in all protocols describing this kind of testing that appear in the literature, for practical reasons a shorter deswelling period after removal of the stress lenses is used most often in combination with a natural test on a separate day to determine baseline thickness.

To evaluate the surplus value of the extremely long testing time as performed in this study, we did some additional calculations. We compared t0.5 determined with our procedure (the 11.5-hour stress test) with t0.5 determined with another more commonly used procedure: using data from the first 5 hours of deswelling after removal of the stress lenses plus baseline thickness from the natural test from 2:00 PM to 6:00 PM. In this manner, two values for t0.5 were calculated for each data set: one using all deswelling data and one using only the data that would have been available if we had used the shorter, more

![Figure 3](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933430/)  
**Figure 3.** With the damped harmonic oscillator model, no correlation could be established between induced swelling and magnitude of the overshoot.

![Figure 4](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933430/)  
**Figure 4.** In data fitted with the damped harmonic oscillator model, no correlation could be established between t0.5 (half-recovery time, or the time required after removal of the stress lenses to reach 50% recovery back to baseline corneal thickness) and magnitude of the overshoot.

Downloaded From: http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933430/ on 02/18/2018
conventional protocol. These calculations were made for the exponential and the new damped harmonic oscillator models.

The results are depicted in Figure 6. It is apparent that the new damped harmonic oscillator model is much less sensitive to variations in the estimated baseline thickness and the reduction of data points in a shorter test procedure, because \( t_{0.5} \) for both measuring protocols with this model show a much better correlation than with the exponential model (\( r = 0.97 \) versus \( r = 0.79 \)).

In one article, exclusion of measurements of corneal thickness during the first 30 minutes after removal of the contact lenses in calculating deswelling rates is advocated, to avoid the effects of decreased pH during this period. In three recent articles, even more data, the first 50 minutes of deswelling, are discarded in the calculation of deswelling rates. Since the overall corneal deswelling response after removal of a contact lens is the result of several mechanisms, and because the relative importance of each mechanism is not yet known, we feel it is not advisable to exclude a time period to avoid the possible influence of one specific parameter.

However, we did repeat our calculations on deswelling data with exclusion of the first 30 minutes (first pachometry reading). This resulted in 12 (71%) of 17 curves for which the damped harmonic oscillator model still provided a significantly better fitting curve than the exponential model, in contrast to 14 (82%) of 17 curves when all measurements were used.

By eliminating the first measurements, other calculated parameters were influenced. When data from the first 30 minutes of deswelling were discarded, average estimated induced swelling changed from 66 \( \mu \)m to 79 \( \mu \)m (13 \( \mu \)m difference) using the exponential model and from 60 \( \mu \)m to 64 \( \mu \)m (4 \( \mu \)m difference) using the damped harmonic oscillator model. \( t_{0.5} \) changed from 0.77 to 0.66 hour (0.11-hour difference) using the exponential model and from 0.93 to 0.89 hour (0.04-hour difference) using the damped harmonic oscillator model. We therefore conclude that even when we excluded the first 30 minutes, the damped harmonic oscillator model was significantly better in a majority of cases. An additional advantage therefore of this new model is the apparent fact that parameters such as \( t_{0.5} \) and estimated induced swelling are less affected when the first measurement is discarded.

Leaving out the first 50 or 60 minutes by elimination of early pachometry readings decreases the statistical precision in the estimation of deswelling curves. Furthermore, because the average \( t_{0.5} \) is less than 1 hour (see Table 1), we do not think that it makes sense to leave out the first hour of the deswelling from the analysis. However, even when the data were reanalyzed with exclusion of the first hour, the damped harmonic oscillator model produced a significantly better fit in 8 of the 17 cases.

Several authors reported an overshoot in the deswelling curve after contact lens-induced corneal edema. O'Neal and Polse postulated that this effect might be caused by a disparity between the rate of water removal from the anterior stroma due to evaporation and the fluid movement inward through the endothelium. Generally, it was thought to be due to, for example, the disappearance of a residual amount of
swelling after overnight eye closure. In view of our results, this explanation has to be rejected.

One of the assumptions of the original exponential model is that the endothelial ion-pump functions at one speed. In our new model, the pump rate of the endothelium does not have to be constant. It might be argued that a larger amount of induced swelling could result in a larger overshoot. However, no correlation could be found between induced swelling and magnitude of overshoot. Also no correlation could be established between $t_{0.5}$ and magnitude of overshoot.

Theoretically, the overshoot could be an artifact caused by a naturally occurring low corneal thickness at that time of day, regardless of previous swelling due to hypoxia under a contact lens. The deswelling itself could be a purely exponential process, superimposed on natural data with a naturally occurring low corneal thickness, instead of an overshoot. By adding a simulated exponential deswelling curve to actual natural data, we created mock deswelling data to test this assumption. In these mock deswelling data sets, the exponential model provided a better fit in the majority of cases, in contrast to the real data sets, in which the damped harmonic oscillator provided a closer fit to the data. We therefore conclude that this explanation for the occurrence of an overshoot has to be rejected as well.

An active or passive short-term regulation of the ion pump is not improbable. Long-term adaptations in pump-site density have been described by Geroski et al. (in 1985) in patients with cornea guttata. They stipulate that the increase in pump capacity is partly due to an increase in pump-site density and partly the result of an increased pump-site activity/turnover. In young normal individuals, there must be a large functional reserve in pump capacity that is maybe activated in some way by hypoxic stress and that returns to normal levels only after a certain time lag, causing the overshoot in corneal thickness recovery.

Crawford et al. suggest a regulatory mechanism of the ion pump, dependent on factors that induce corneal endothelial cell swelling. Also, corneal swelling in response to contact lens wear is found to be related to the formation of arachidonate metabolites, especially 12(R)-hydroxy-5,8,10,14-eicosatetraenoic acid (12(R)-HETE), in corneal epithelial hypoxia. 12(R)-HETE inhibits Na-K-ATPase, increases corneal thickness, and reduces ocular pressure in the rabbit. Possibly 12(R)-HETE is also formed in the human corneal epithelium in response to contact lens wear in the closed eye. Riley and coworkers have shown that adenosine can increase net endothelial fluid transport in bovine and rabbit endothelia through an increase in cyclic adenosine monophosphate. They suggest that this regulation of corneal hydration is more probable through stimulation of active transport than through a change in permeability. Fischbarg argues that fluid transport across the endothelial layer might not be a simple process but rather the result of a combination of cell features, including the cytoskeleton, signaling cascades, and the activation of a host of volume regulatory membrane proteins. Many mechanisms to explain the overshoot are therefore imaginable.

We found a significant linear trend in 8 of 17 natural tests. Therefore, we conclude that corneal thickness is not always constant during the day. Also, corneal thickness changes cannot solely be due to the disappearance of swelling from eye closure during the sleeping hours, because corneal thickness increased during the day in some subjects in the present study.

Other factors can possibly explain the unsteadiness in corneal thickness, such as blinking frequency of individuals (which could be reduced during reading between measurements), cortisol levels, tear film quality, and variations in tear production and intraocular pressure during the day. It seems that corneal thickness is more sensitive to subtle internal or external environmental changes in some individuals than in others.

We summarize that, in contrast to the exponential model currently used, corneal deswelling after removal of contact lenses does not necessarily take place at a constant rate. Corneal hydration control is regulated by a complex mechanism that still has to be elucidated in detail.

Acknowledgments

The authors thank Elisabet Pels, PhD, and W. Houdijn Beckhuis, MD, for critically reviewing the manuscript. The authors wish to honor the memory of their friend Jan Kok, who died during the completion of this article.

References


