Evidence for the Major Contribution of Evaporation to Tear Film Thinning between Blinks

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PURPOSE. To determine the contribution of evaporation to the thinning of the precorneal tear film between blinks.

METHODS. The rate of tear film thinning after a blink was measured using spectral interferometry from the right eyes of 37 subjects. Data were obtained under two different conditions: free air and air-tight goggles.

RESULTS. The mean ($\pm$ SD) tear film thinning rates for subjects was $3.22 \pm 4.27 \mu\text{m}/\text{min}$ in free air and $-0.16 \pm 1.78 \mu\text{m}/\text{min}$ (i.e., a slight but not significant thickening) for the same subjects wearing air-tight goggles; this reduction in thinning rates was significant ($P < 0.0001$).

CONCLUSIONS. The large reduction in thinning rate caused by wearing goggles indicates that evaporation is the major cause of thinning between blinks. The mean thinning rate in free air is greater than reported evaporation rates; it is argued that the preocular chambers used in evaporimeters restrict movement of air over the tear film and reduce evaporation compared to our free air condition. (*Invest Ophthalmol Vis Sci.* 2010;51:6294–6297) DOI:10.1167/iovs.09-4772

According to the Report of the Dry Eye Workshop,¹ tear film breakup is one of the two “core mechanisms” of dry eye, the other being hyperosmolarity. Whereas it is evident that hyperosmolarity is caused by increased evaporation and/or reduced tear production,¹ the causes of tear film breakup are less evident. To help elucidate the mechanisms of breakup, we have used spectral interferometry to study the thinning of the tear film over an extended period after blinking.² After some transient thinning or thickening for approximately the first 2 seconds after a blink (probably associated with an upward drift of the aqueous layer of the tear film³), we found that the tear film thins as a rather linear function of time with rates of up to approximately $20 \mu\text{m}/\text{min}$. This high thinning rate is fast enough to explain tear film breakup; for example, if the tear film were $3 \mu\text{m}$ thick initially (2 seconds after a blink), then, at a thinning rate of $20 \mu\text{m}/\text{min}$, it would thin to zero thickness in a further 9 seconds for a total time of 11 seconds, which is comparable to observed noninvasive breakup times.⁴ Thus it is arguable that tear film breakup is the result of this linear thinning between blinks. Note that our thinning rate measurements correspond to just one location at the center or the cornea; thus, even if little or no thinning is observed at this

location, thinning could be rapid enough in other locations (e.g., due to lipid layer deficiency) to explain observed noninvasive breakup times.

From considerations of the three-dimensional nature of space, it may be concluded that thinning of the tear film could be due to flow of tears (or water) in three directions: outward (i.e., evaporation), inward, into the epithelium, and tangentially, over the ocular surface.² Tear film thinning did not cause a detectable thickening of the corneal epithelium,² which would indicate a flow of tear fluid into the epithelium; the analysis of Levin and Verkman⁵ indicates that any flow across the corneal surface would tend to be in the opposite direction, driven by osmotic flow from the epithelium into hyperosmolar tears.

Thus evaporation and tangential flow will be the possible mechanisms of tear thinning considered here. It was noted that reported rates of evaporation were typically less than half of the observed average thinning rate, suggesting that tangential flow made a greater contribution than evaporation.² However, a problem with this conclusion is that tangential flow would be expected to have variable effects; if tears tend to flow out of some areas (divergent flow), this will indeed cause local thinning, but in other areas, convergent flow of tears will cause thickening. Thus tangential flow could cause either thinning or thickening and would tend to contribute to the variability of thinning rates rather than to the mean rate.⁶ Additionally, movement of the lipid layer at times >2 seconds after a blink was found to be slow, and the corresponding movement of the underlying aqueous layer was insufficient to explain the observed rate of tear film thinning.⁷ Movement of the underlying aqueous layer, due to gravity and pressure gradients, is possible without any movement of the lipid layer, but, again, these effects were found to be considerably smaller than the observed thinning rate.⁷ To help explain the discrepancy between observed evaporation and thinning rates, it has been suggested that evaporation rate measures using open chambers or unventilated closed chambers restrict air currents over the cornea, and hence evaporation is slower than in the more open environment used for tear thinning rate measurements.⁶ The proposal that tear film thinning is largely due to evaporation is supported by the inverse correlation between tear thinning rate and lipid thickness (increasing lipid thickness would be expected to reduce evaporation).⁸

In this study, the contribution of evaporation to tear film thinning was studied by measuring the thinning of the tear film behind air-tight goggles. These were expected to eliminate or greatly reduce tear film evaporation; thus, it was hypothesized that little or no thinning would be observed with wearing goggles if thinning is mainly due to evaporation.

METHODS

The study adhered to the Declaration of Helsinki. The Ohio State University Institutional Review Board approved the recruitment of subjects and the research protocol for this study. Thirty-nine subjects,

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18 years of age or older, were recruited for this study. Individuals that were pregnant or breast-feeding, or current contact lens wearers, were excluded. Dry eye patients were not excluded, but the study was not statistically powered to show a difference between dry eyes and normal eyes.

Spectral interferometry was used to record tear thickness values over time for each subject's right eye using the method, equipment, and analysis reported previously.² The wavelength range was 550 to 1060 nm, spectra were obtained at a rate of 10 per second, and the measurement spot on the tear film was $25 \times 33 \mu\text{m}$ (probably larger in practice due to aberrations, eye movements, and any defocus).

To record tear film thinning, subjects were asked to blink 1 second after the beginning of a 20-second recording and to try to keep their eyes open for the remaining 19 seconds. Initially, two such thinning trials were done with a 3-minute rest period in between. After the second free air recording the subjects put on a pair of air-tight swimming goggles and waited 3 minutes, with their eyes open, for the humidity inside the goggles to equilibrate. The goggles were preheated to body temperature, and an anti-fog agent was applied to the right window to reduce the amount of fogging during the subsequent thinning trials. During this equilibration period with the goggles in place, the subjects completed an Ocular Surface Disease Index (OSDI) questionnaire to determine their dry eye symptom status. Subsequently two more measurement trials, separated by a 3-minute rest period, were captured under the air-tight goggles condition.

Thinning rates were derived by fitting a linear regression line to thickness versus time plots, starting 2 seconds after the blink (thus avoiding any initial transient thinning or thickening). Criteria for acceptable thinning rate measurements have been described previously.² Of the 39 subjects originally enrolled, one subject had no acceptable measurements, and one other had no acceptable measurements with goggles in place, leaving 37 subjects with appropriate data for analysis.

For most subjects, the two measurements for the free air condition were averaged as were the two measurements under the wearing goggles condition; in two subjects, only one measurement was acceptable in free air conditions, and in five subjects, only one measurement was acceptable for the wearing goggles condition.

Statistical analysis was performed using SPSS statistical software (SPSS, Chicago, IL). Because tear film thickness and thinning rates did not follow normal distributions, nonparametric statistics—the Wilcoxon signed-ranks test and Spearman correlation—were used to evaluate the data. $P < 0.05$ was considered to be statistically significant.

RESULTS

Data were analyzed from 37 subjects (14 females), with a mean age of 30.0 ± 9.5 years. The mean room temperature was $20.9 \pm 0.5^\circ\text{C}$, and the mean relative humidity of the examination room was $34.2\% \pm 9.9\%$. The mean OSDI score for subjects in this study was 10.8 ± 7.1 . Four of the subjects were classified as dry eye by OSDI standards with a score greater than 22 and, as discussed above, were included in the data analysis.

The circles in Figure 1 give tear thickness as a function of time after a blink for two subjects, for the free air (Figs. 1A, 1C) and wearing goggles (Figs. 1B, 1D) conditions. The thinning rate in free air for the first subject (Fig. 1A), was particularly rapid and so would be expected to show a considerable reduction if thinning were due to evaporation. The thin curve at the bottom of each graph shows reflectance from the eye; dips in reflectance indicate the timing of blinks. Filled circles give the thickness measurements used for linear regression analysis, starting 2 seconds after a blink. It is seen that wearing goggles

FIGURE 1. Tear thickness as a function of time. The *thin curve* at the bottom of each graph shows reflectance from the eye, dips indicating the timing of blinks. *Filled circles* give the thickness measurements used for linear regression analysis, starting 2 seconds after a blink. (A) Free air measurements for a 67-year-old white male with an OSDI score of 22.5, indicating mild dry eye. Supplementary meibography in this subject indicated meibomian gland dysfunction. (B) Corresponding measurements wearing goggles. (C) Measurements in free air for a 25-year-old white male classified as normal by OSDI score. (D) Corresponding measurements wearing goggles.

FIGURE 2. Thinning rate with goggles as a function of thinning rate in free air for the 37 subjects. The *thin dashed curve* corresponds to equal thinning rate in free air and with goggles. The *triangle* gives the mean thinning rate with goggles as a function of the mean thinning rate in free air. The *thick dashed line* is the linear regression line of thinning rate with goggles as a function of rate in free air.

reduced the thinning rate by a factor of over 10 times (Fig. 1B), from 19.74 to 1.78 $\mu\text{m}/\text{min}$. Wearing goggles slightly increased the initial thickness, 2 seconds after the blink, from 4.10 to 4.31 μm . For the no goggles condition (Fig. 1A), the regression line has been extrapolated to zero thickness, indicating that breakup might be expected to occur at approximately 15 seconds after the blink. The second subject was more typical, with a thinning rate in free air (Fig. 1C) of 2.87 $\mu\text{m}/\text{min}$, whereas wearing goggles reduced this to $-0.10 \mu\text{m}/\text{min}$ (Fig. 1D).

Figure 2 is a plot of thinning rate with goggles as a function of thinning rate in free air for the 37 subjects; negative values indicate thickening. The thin dashed curve corresponds to equal thinning rate in free air and wearing goggles. It is seen that, when wearing goggles, only three subjects had more rapid thinning (or slower thickening) than in free air, which demonstrates that goggles cause a significant reduction in thinning rates ($P < 0.0001$, Wilcoxon signed-rank test). The triangle gives the mean thinning rate with goggles, $-0.16 \mu\text{m}/\text{min}$ (i.e., a slight thickening) as a function of the mean thinning rate in free air, 3.22 $\mu\text{m}/\text{min}$. The mean thinning rate with goggles was not significantly different from zero ($P > 0.05$, Wilcoxon signed-rank test). The thick dashed line is the linear regression line of thinning rate with goggles as a function of rate in free air; there is a small positive correlation (Spearman $R^2 = 0.166$, $P = 0.012$).

Initial thickness (2 seconds after a blink) and thinning rates are summarized in Table 1. Values given are means $\pm$ SD. Whereas, as noted above, there was a significant difference in thinning rates, no significant difference in initial thickness was observed.

Whereas the subject of Figure 1, who would be classified as mild dry eye by his OSDI score, had a particularly rapid thinning rate, the mean thinning rate for the four subjects with an OSDI score over 22 was 5.75 $\mu\text{m}/\text{min}$, which is slightly but not significantly above the overall mean. It should be noted that although dry eye subjects were not excluded, the study was not powered to show a difference between dry eyes and normal eyes.

CONCLUSIONS

The main conclusion of this study is summarized in Figure 2 and Table 1, namely, that wearing air-tight goggles eliminates or greatly reduces tear film thinning rates. Of the three possible mechanisms of tear film thinning—evaporation, flow into the epithelium, and tangential flow—only evaporation would be expected to be eliminated or reduced by wearing goggles. Transient thinning or thickening of the tear film immediately

after a blink is probably related to the upward drift of the tear film,^{2,7,9} but the current results support the proposal that later thinning, which is a rather linear function of time, is largely due to evaporation. We propose that this steady linear thinning is present throughout the interblink interval and that any transient change after a blink is superimposed on this linear thinning.

If tear thinning is mainly due to evaporation, this implies that evaporation in our conditions was considerably faster than most values reported in the literature. For example, Tomlinson et al.,¹⁰ in a meta-analysis of evaporation measurements, found average rates of 13.57 and $21.05 \times 10^{-7} \text{ g}/\text{cm}^2/\text{s}$ in normal and dry eyes, respectively. Assuming that 1 gram of water is equivalent to 1 cm^3 , these evaporation rates would correspond to thinning rates of 0.81 and 1.26 $\mu\text{m}/\text{min}$, respectively, both of which are considerably less than the mean thinning rate (in free air) of 3.22 $\mu\text{m}/\text{min}$ reported here. As discussed previously,⁶ we think that the origin of this discrepancy is that evaporation rate measurements are typically performed using preocular chambers, which restrict air flow over the tear film surface, permitting a thick layer of humid air to build up, which retards evaporation. In our conditions, air flow over the cornea from convection, and ventilation reduces the thickness of this humid layer and hence increases evaporation.

In a review of methods for measuring trans-epidermal water loss, Imhof et al.¹¹ define a “saturation flux density” as the rate of evaporation that would be measured for a pure water surface; as an example, for a typical open-chamber evaporimeter, this rate would be approximately 83 $\text{g}/\text{m}^2/\text{hr}$ for skin temperature of 31°C, ambient temperature of 21°C, and relative humidity of 50% (their Fig. 7). Corrected for an eye surface temperature of 35°C,¹² this saturation rate would increase to 110 $\text{g}/\text{m}^2/\text{h}$, equivalent to 1.83 $\mu\text{m}/\text{min}$. Thus an evaporation rate, in free air, equal to the thinning rate of this study, 3.22 $\mu\text{m}/\text{min}$ or even equal to the very rapid thinning in Figure 1 (19.74 $\mu\text{m}/\text{min}$) would be recorded as $<1.83 \mu\text{m}/\text{min}$ on this evaporimeter, in closer agreement with the values reported above for normal eyes and dry eyes. As noted previously,⁶ we propose that the “ventilated chamber evaporimeter,”¹³ which

TABLE 1. Initial Thickness (2 Seconds after a Blink) and Thinning Rates in Free Air and with Wearing Goggles

	Free Air*	Goggles*	P
Initial thickness (μm)	3.46 ± 0.83	3.54 ± 0.83	0.53
Thinning rate ($\mu\text{m}/\text{min}$)	3.22 ± 4.27	-0.16 ± 1.78	<0.0001

* Values are means $\pm$ SD.

maintains a constant flow of air across the corneal surface, provides a value of evaporation rate closer to the free air condition than values from open-chamber or unventilated closed-chamber evaporimeters. In agreement with this, it may be noted that, for normal controls, the evaporation rate of Liu et al.¹⁴ using the ventilated-chamber method, equivalent to 2.36 $\mu\text{m}/\text{min}$, is greater than that of other methods reported by Tomlinson et al.¹⁰ and closer to our mean thinning rate value.

The thickness resolution of spectral interferometry is approximately 1 μm , so it is not possible to follow the thinning of the tear film until breakup occurs, but it seems probable that the linear thinning observed after a blink ultimately leads to a very thin or absent tear film and hence to breakup, as indicated by the extrapolated regression line in Figure 1A. If tear film breakup is a consequence of the linear thinning of the tear film between blinks and if the linear thinning is mainly due to evaporation, this implies that tear film breakup is mainly due to evaporation, rather than tangential flow. (An exception to this conclusion would be the thinning and corresponding breakup of the tear film at the black line near the meniscus, which is caused by tangential flow into the meniscus.^{15,16}) The proposal that breakup is largely due to evaporation is supported by a number of studies. For example, McCulley and Sciallis¹⁷ found that expression of the meibomian glands in meibomian keratoconjunctivitis increased fluorescein breakup time from 7.0 to 29.4 seconds, which was comparable to values for the normal controls. Isreb et al.¹⁸ have demonstrated a correlation between breakup time and lipid thickness, while Craig and Tomlinson¹⁹ showed that breakup time depends on both evaporation rate and lipid layer pattern.

Hyperosmolarity is a consequence of increased evaporation and is considered to be one of the two core mechanisms of dry eye.¹ Although samples of tears from the meniscus show a moderate increase in osmolarity in dry eye, e.g., from 302 to 343 mOsm/L,²⁰ it seems probable that osmolarity could increase to considerably higher levels in the exposed precorneal tear film.²¹ Liu et al.²² compared the discomfort associated with tear film breakup with the discomfort after instillation of hyperosmolar drops; sensations of burning and stinging during breakup were comparable to those from drops of 800 to 900 mOsm/kg, indicating the importance of evaporation in tear film breakup.

If tear thinning is largely due to evaporation, then the high rates of evaporation implied by this study would be expected to cause considerable overall increase in the osmolarity of the tears. It may therefore be asked whether our interpretation is consistent with measured osmolarity, so further studies are warranted involving observed thinning rates and tear osmolarity. In this respect, Levin and Verkman⁵ have proposed that osmotic flow of water from the conjunctiva and cornea into the hyperosmolar tears would help to compensate for the water loss from evaporation, thus reducing the expected increase in osmolarity. In cases of rapid evaporation, such as in evaporative dry eye, the water lost by evaporation may be derived more from osmotic flow through the conjunctiva and cornea than from lacrimal gland secretion.

Are the thinning rate measurements in this study, which were performed in the center of the cornea, representative of thinning at other positions of the exposed precorneal tear film? Tear film breakup has been reported to occur rather randomly and uniformly over all regions of the cornea,²³ so that, if tear thinning is related to tear breakup, this would indicate that tear thinning is similar over different corneal locations. In this regard, more information is needed both about the distribution of precorneal tear film thickness over the cornea and about the distribution of tear film thinning rate.

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