Published in final edited form as:

Eye Contact Lens. 2009 May ; 35(3): 117–119. doi:10.1097/ICL.0b013e31819c2963.

Assessment and Impact of the Time of day on Aqueous Tear Evaporation in normal subjects

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Abstract

Objectives—To determine the impact of the time of day on aqueous tear (AT) evaporation in normal subjects on two consecutive days

Methods—In a controlled laboratory setting, morning and afternoon AT evaporation was tested in 19 normal subjects, at the same time of day on two consecutive days. Evaporimetry was used at two ranges of relative humidity (RH) 25 to 35% and 35 to 45%.

Results—Mean AT evaporation rates were 0.069 ± 0.024 for 25–35% RH and 0.049 ± 0.018 for 35–45% ($p=0.001$). There were significant differences for both RH between time of day ($p<0.05$) on day 1, but not observed on day 2. Variation between days, showed no difference for either RH during the afternoon, but there was a difference during the morning ($p=0.042$).

Conclusions—The data are remarkably consistent between study days but there is more fluctuation during the morning than in the afternoon. Therefore, to further standardize AT evaporation study protocol, we recommend to perform evaporimetry measurements during the afternoon rather than the morning, due to the fact that our results showed less variability during the afternoon test between days.

Introduction

The tear film is the primary interface of the ocular system between the ocular surface (corneal and conjunctival epithelium) and the outside environment. It plays a critical role in optical function. Maintenance of the quality and quantity of an adequate tear film requires a dynamic system, consisting of tear production, tear drainage through the nasolacrimal duct, fluid absorption or exchange through the conjunctival and corneal epithelium and evaporation to the air.¹ An adequate volume of tears is a prerequisite for a healthy ocular surface, and a reduction in the volume of tears gives rise to a greater chance of developing signs and symptoms of ocular dryness causing a disturbance of the optical surface for the cornea.

The key mechanisms of dry eye syndrome that lead to ocular surface damage are initiated by tear hyperosmolarity and tear film instability. The major cause of tear hyperosmolarity is reduced aqueous tear (AT) flow due to lacrimal gland failure or increased evaporation from the tear film or a combination of both.^{2,3}

Evaporation of AT has been studied as a major factor in tear film dynamics and its impact in dry eye syndrome. Evaporation of molecules from a liquid's surface depends on the

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temperature, humidity and air velocity.⁴ Environmental conditions of low humidity⁵ and high air flow contribute to hyperosmolarity. The contribution of evaporation to AT volume loss ranges from 20% to 60% and is dependent on environmental conditions such as relative humidity (RH).⁶

Many physiological systems oscillate during the day. Symptoms of ocular dryness vary widely during the day and are more severe at the end of the day.⁷ Some diurnal variations in other tear film parameters including pH, osmolarity, and tear volume^{8–16} has been studied. However, little is known regarding the impact of diurnal changes on physiology of AT evaporation rate. Human AT evaporation rates have been reported, and differences between the results in normal subjects and patients with dry eye vary with the method used.^{5,6,17–20}

If the diurnal pattern of AT evaporation rate is known, the time of measurements during the day can be assessed. This will allow us to gain a better understanding of AT evaporation and to elucidate the effect of time of day on evaporation rate measurements to further standardize study protocol.

Material and Methods

Study Population

The study protocol, consent form, and data accumulation methods used in these studies were approved by the University of Texas Southwestern Medical Center Institutional Review Board prior to the initiation of these studies. The eligibility criteria for patients entering the study were no symptoms or signs of dry eye or other ocular disease, detected by history slit lamp examination, vital dye staining and Schirmer test. Subjects with other systemic disease that might affect the ocular surface, the use of any eye-drops or oral medication were excluded from the study. A total of 20 healthy subjects non-contact lens wearers were enrolled in the study. By protocol design, the left eyes only of all subjects were tested.

Measurement of Evaporation Rate

An evaporimeter (Oxdata, Portland, Oregon) utilized a pump to direct room air through a drying tube into a form-fitting goggle that created a closed environment and contained a humidity/temperature sensor.¹⁸ Dry air was pumped into the firm-fitting eye goggle to reduce RH to 15%, at which time the pump was turned off. The RH within the goggle was allowed to rise. The increase in humidity due to evaporation from skin or evaporation tears was measured. The process was carried out first with the eyelids closed and then with them open; the difference represented the ocular surface AT evaporation rate.¹⁹

At the final step (open eyes), blink interval was controlled every 3 seconds with a metronome. Patient visual fixation and the fixation target were parallel to the floor and ceiling. The tests were performed by the same technician in a controlled room temperature (25 C°). Patients were allowed an interval of thirty minutes before running the test to adjust to room temperature. Using the original formula published by Rolando and Refojo²¹, we calculated the evaporative rates under two different ranges of increasing RH, 25% to 35% and 35% to 45%. The area of the interpalpebral ocular surface was used to calculate AT evaporation per unit area; the image of the area was captured with the use of a digital camera, and the area was calculated directly with the aid of computer software (Adobe Photoshop, version 6.0.1.2001; Adobe Systems, San Jose, California)¹⁹, expressed as $\mu\text{L}/\text{cm}^2/\text{min}$.

To investigate the impact of the time of day on AT evaporation rates were measured on 2 consecutive days during the morning, between 8:00 and 9:00 AM and repeated in the afternoon between 4:00 and 5:00 PM on each day.

Statistical Analysis

Statistical analyses were carried out using SigmaStat 2.03 Software (Systat Software Inc, Richmond, California, USA) Paired *t*-test were applied with statistical significance determined at the $p < 0.05$ level. Data presented in figures are expressed as means $\pm$ standard deviation. Grubbs test was used for numerical consistency testing.²²

Results

The study group consisted of 20 normal subjects. The mean age was 40 ± 12 years (24 to 59 years). One female subject was eliminated from the mean statistical analyses based upon Grubbs outlier test variance. Thus, the final analyses included data on 19 subjects (11 females and 9 males).

Each subject had four AT evaporation rate determinations at each RH range over two consecutive days. The mean AT evaporation rate at each RH range for all determinations was 0.069 ± 0.024 $\mu\text{l}/\text{cm}^2/\text{min}$ at 25–35% and 0.049 ± 0.018 $\mu\text{l}/\text{cm}^2/\text{min}$ at 35–45% RH ($p = 0.001$). The testing at different RH ranges validates that the lower RH range is associated with higher mean rates of evaporation of AT. Analyses of the effect of the time of day of testing are displayed in Table 1 and Figure 1. The AM test time results, for both RH ranges on day 1 were statistically significantly reduced (RH range 25–35% was $p = 0.029$ and for RH range 35–45% was $p = 0.030$) as compared to their corresponding PM tests. Results of the mean AT evaporation rates at the two study times on day 2 were essentially equal. In addition, they were almost the same as those obtained at the PM study time on day 1.

Discussion

With the exception of the first test (AM day 1), AT evaporation rates were almost identical within each RH range that was tested. These findings are in line with results reported from another laboratory that studied three normal subjects on multiple days to evaluate the stability of the evaporation measurements. They found that normal patients appear to fall within a fairly narrow range of values and remained stable with repeated measurements.¹⁸ Tomlinson et al²³ measured evaporation rates during the day at two hour intervals. The first measurement corresponded to the awaking time where low tear film evaporation rates were detected. These rates rapidly increased to levels that were maintained for the rest of the waking day. They concluded that if the awakening measurement was excluded from the analysis, no significant trend was seen in the remaining 12 hour period of measurements.²³ Aqueous tear film evaporation rates are difficult to compare between laboratories because the recorded absolute values are technique-dependent. For example, our laboratory measures the exposed ocular surface area instead of using a constant or average. Despite this study unique differences, the report by Tomlinson et al²³ is comparable to our findings. The causation of the discrepancies in our study between day 1 AM measurements and those at other time points at both RH ranges are probably more related to fluctuation between AM readings rather than PM readings, but yet to be explained by underlying mechanisms. Therefore, there may be more variability in the early morning hours evaporative rates as reported by others.²³ Diurnal variations are seen in tear film dynamics using other test procedures. Tear film quality and stability measured with a keratometer has been shown to be lowest in the morning and to reach equilibrium between 10:00 am and noon.⁸ Morning tear turnover rates are higher than in the afternoon.⁹ Inferior tear meniscus height assessed by OCT decreased towards the end of the day.¹⁰

However, lacrimal secretions measured with Schirmer test outline the existence of a circadian rhythm peaking at 6pm.¹¹ Measurements performed on collected tear samples have shown that certain properties vary diurnally including levels of pH¹², osmotic pressure,^{13, 14} and

nocturnal inflammatory mediators^{15, 16, 24}. All of these possibly contribute to the common increase symptoms of ocular dryness in the late afternoon or evening.⁷

The results from this study showed a mean value of AT evaporation rates of 0.069 ± 0.024 for 25–35% RH with a range of 0.02 to 0.12 $\mu\text{l}/\text{cm}^2/\text{min}$ and a mean value of 0.049 ± 0.018 for 35–45% RH, with a range of 0.01 to 0.09 $\mu\text{l}/\text{cm}^2/\text{min}$., which are comparable to those previously obtained in our laboratory for normal subjects.^{19,20} The current results as in our previous study confirm that a reduction in RH is correlated with an increase in evaporation rate.²⁰

The results of this study have provided additional data to understand the tear film dynamics in regards to its evaporation rate in normal subjects. It is recommended to standardize evaporation study protocol for comparative or therapeutic clinical trials in the afternoon.

Acknowledgements

This study was supported in part by grants NIH EY12430, EY016664 and an unrestricted grant from the Research to Prevent Blindness, New York, New York.

The authors thank Dr. Igor Butovich for valuable discussions and Mike Molai for technical assistance.

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Figure 1.

Comparison of AM and PM AT evaporation rates on two consecutive days.

The AM test at both RH ranges on day 1 were significantly lower than AM measurements on day 2 ($p < 0.029$ for RH 25–35% and $p < 0.030$ for RH 35–45%). This difference was not seen on day 2 testing where the corresponding morning and afternoon mean AT tear evaporation rates were similar within each range of RH and similar to day 1 pm measurements.

Table 1

Mean AT Evaporation rates ($\mu\text{l}/\text{cm}^2/\text{min}$) at two different times of day in normal subjects, on two consecutive days. n=19 patients

Day	Evaporative Rate at 25–35% RH*			Evaporative Rate at 35–45% RH*		
	AM	PM	P Value†	AM	PM	P Value†
Day 1	0.062 ± 0.019	0.069 ± 0.024	<0.029	0.044 ± 0.016	0.051 ± 0.019	<0.030
Day 2	0.073 ± 0.030	0.072 ± 0.024	≥0.873	0.051 ± 0.020	0.051 ± 0.018	≥0.965

* Values expressed as mean ± SD

† Comparison of the evaporative rates within day for each RH