

Changes in the Evaporation Rate of Tear Film After Digital Expression of Meibomian Glands in Patients With and Without Dry Eye

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Purpose: To evaluate the effect of excess meibum on tear evaporation rate in patients with and without dry eye.

Methods: Eleven healthy subjects and 16 patients with dry eye were tested. The dry eye group was divided into 2 subgroups: classic keratoconjunctivitis sicca (KCS) with clear and easily expressed meibum and KCS with meibomian gland dysfunction (MGD) with turbid secretions and difficult-to-express meibum. Evaporative measurements were performed at baseline and after digital expression of meibomian glands at 12, 24, 36, and 48 minutes. Two ranges of relative humidity were used, 25% to 35% and 35% to 45%. The data were expressed as microliters per square centimeter per minute.

Results: An increase in the evaporation rate of the tear film was noted for all measurements at both relative humidities in the classic KCS and KCS with MGD groups compared with healthy subjects ($P < 0.05$). The average evaporation rates at relative humidities of 25% to 35% and 35% to 45% were 0.056 ± 0.016 and 0.040 ± 0.008 for the classic KCS group; 0.055 ± 0.026 and 0.037 ± 0.019 for the KCS with MGD group and 0.033 ± 0.012 and 0.023 ± 0.008 for the healthy group. Also, a decrease in the evaporation rate was observed in the healthy and KCS with MGD groups between baseline and the first measurement after digital expression for both relative humidities ($P < 0.05$). The classic KCS group did not show any changes after expression.

Conclusions: Classic KCS and KCS with MGD groups showed an increase in tear evaporation rates compared with the healthy group. Aqueous tear evaporation diminished in the healthy and KCS with MGD groups after expression of meibomian glands. However, this effect was transient and negligible after the second measurement.

Key Words: dry eye, meibum, tear evaporation, meibomian gland dysfunction

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Dry eye (DE) is a common, chronic, and debilitating disease affecting millions of people worldwide. The disease occurs when there is an inadequate balance between tear production and evaporation from the ocular surface. In both cases, increase in osmolarity of the tear film and exposure of the corneal and conjunctival epithelium to the environment are major factors for pathogenesis of the disease.^{1–3}

An adequate volume of tears is fundamental for a healthy ocular surface. However, in some patients with DE, tear production has been found to be normal, or even higher, compared with that of healthy individuals.⁴ Understanding of the process of tear production, its distribution over the ocular surface by blinking, drainage into the nasolacrimal duct, and ocular surface evaporation is essential for the development of new diagnostic tools and therapies. Tear production and dynamics have been studied for clinical and experimental purposes using the Schirmer test,⁵ tear film break-up time, tear turnover rates,⁶ meniscometry,⁷ tear interferometry,⁸ and tear evaporimetry.^{9,10} This last diagnostic tool has been especially useful in studying the evaporative type of DE (also known as lipid tear deficiency DE).¹¹

Meibomian glands are a specialized type of sebaceous glands located in groups within the eyelids; their duct orifices are at the eyelid margin. Meibomian glands are responsible for secretion of the lipids that contribute to the precocular tear film. Between blinks, these lipids form the outermost part of the tear film and function as a protective barrier against evaporation of the aqueous component of the tear film from the ocular surface. The contribution of evaporation to aqueous tear (AT) volume loss ranges from 20% to 60% and is dependent on environmental conditions such as relative humidity (RH).¹²

In our recent publication, we characterized the lipids present in human meibomian gland secretions in healthy individuals, finding a large quantity of nonpolar lipids of the wax esters and cholesteryl esters families.¹³ The physiological structure of the tear film lipid layer is thought to be composed of a hydrophobic nonpolar lipid phase that creates a barrier against evaporation and a polar lipid phase that provides a supporting structure.

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A new clinical approach was designed for this study to quantify the evaporation rates of the AT using a tear evaporimetry method over time in patients with and without DE. The evaporative measurements were performed before and after the increase in tear film lipid volume by warming and digitally expressing the meibomian glands. The results of this study provide a clear view about the changes in the evaporative rates produced by the meibomian lipid in normal and pathological circumstances such as DE disease.

MATERIALS AND METHODS

Study Population

Before the commencement of the studies, study protocols, consent forms, and data accumulation methods were approved by the University of Texas Southwestern Medical Center's Institutional Review Board. Informed consent was obtained from each patient at screening and Health Insurance Portability and Accountability Act (HIPAA) regulations were followed. The study was conducted according to the tenets of the Declaration of Helsinki.

The eligibility criteria for patients with DE included previous clinical diagnosis of DE with: (1) symptoms of foreign body sensation or dryness; (2) interpalpebral fissure conjunctival positive vital dye staining with 1% lissamine green, according to the National Eye Institute (NEI)/Industry Workshop Scale (1995),¹⁴ detected by slit-lamp examination; and (3) absence of concurrent disease including eyelid or ocular surface inflammation beyond 1+ bulbar conjunctival injection. Exclusion criteria were patients with previous ocular surgery, alterations of the lachrymal drainage system, or eyelid abnormalities. Patients with any systemic disease that might affect the ocular surface, as well as pregnant or lactating women, were excluded. Likewise, research volunteers using ophthalmic medications or systemic medications affecting tear secretion were excluded.

Sixteen patients with DE disease were recruited (5 men and 11 women), with a mean age of 56 ± 17 years (74–28 years). Patients with DE were assigned to 2 groups depending on their clinical examination. There was a classic keratoconjunctivitis sicca (KCS) group for normal-appearing meibomian secretions (clear and easily expressed) and a KCS with meibomian gland dysfunction (MGD) group for meibomian glands that were difficult to express with turbid secretions. The classic KCS group included 4 patients (1 man and 3 women) with an average age of 50 ± 3 years (54–46 years). The KCS with MGD group included 12 patients (4 men and 8 women) with an average age of 57 ± 17 years (74–28 years). After enrollment, and in between the screening and study visits, patients were instructed to continue with their usual DE therapy, except on the day of evaluation.

The control group consisted of a total of 11 healthy subjects (5 men and 6 women) with a mean age of 33 ± 8 years (54–23 years). The inclusion criteria were no symptoms or signs of DE or other ocular disease detected by history, slit-lamp examination, vital dye staining, and Schirmer test. Volunteers with systemic diseases that might affect the ocular surface or individuals who used any ocular or systemic medication were excluded.

Measurements of the Evaporation Rate

In a controlled laboratory setting, tear evaporation studies were performed using an evaporimeter (Oxdata, Portland, OR) that used a pump to direct room air through a drying tube into a form-fitting eye goggle that created a closed environment. The goggle included a humidity/temperature sensor.¹⁰ Dry air was pumped into the goggle to reduce the RH to 15%, at which time the pump was turned off. The RH in the goggle was allowed to increase. The increase in humidity, because of evaporation from the skin and evaporating tears, was measured. The test was performed first with the eye closed and then with it open. During this latter part, the blinking rate was controlled every 3 seconds with a metronome; both measurements were calculated and the difference represented the ocular AT evaporation rate.¹⁵ Measurements were taken at a controlled room temperature of $25^{\circ}\text{C}(\pm 1)$. Using the formula from Rolando and Refojo,⁹ the evaporation rates were calculated under 2 different ranges of RH, 25% to 35% and 35% to 45%. The area of the interpalpebral ocular surface was used to calculate the evaporation per unit area (calculated with the aid of computer software, Photoshop 6.0; Adobe Systems, San Jose, CA¹⁵) and expressed as microliters per square centimeter per minute.

Evaporative measurements were performed at baseline and after digital expression of both upper and lower eyelid meibomian glands. Before the digital expression, warm compresses were placed over the eyelid area with the eye closed for 2 minutes at $40^{\circ}\text{C}(\pm 2)$. With the use of a slit lamp, meibomian gland secretions were expressed from the subject's upper and lower eyelids using a cotton swab rolled toward the eyelids' margins until the meibomian secretions were visible. The procedure lasted less than 30 seconds with no negative patient feedback. A total of 4 measurements were taken after digital expression. Each evaporative measurement took $10 (\pm 1)$ minutes to complete, and the interval between the measurements was 2 minutes. Therefore, the final measurement was performed 48 minutes after the baseline determinations.

Statistical Analysis

The data were analyzed using a statistical software (SigmaStat 3.5; Systat Software, Richmond, CA). A *t* test and paired *t* test were applied with statistical significance set to $P \leq 0.05$ for all tests. The data analyzed was from the left eye only.

RESULTS

Table 1 shows the evaporative rates at baseline, and 12, 24, 36, and 48 minutes as mean and SDs for normal and DE groups at RHs of 25% to 35% and 35% to 45%. In all measurements, the DE group had a statistically significant higher evaporation rate compared with the normal group ($P < 0.05$). The mean rate of evaporation was higher at low RH, regardless of which group was tested. The average rates of evaporation at RHs of 25% to 35% and 35% to 45% for the healthy group were 0.033 ± 0.012 and $0.023 \pm 0.008 \mu\text{L}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$, respectively. For the DE group, the average rate of evaporation increased to 0.057 ± 0.026 (42.1%) and $0.038 \pm 0.018 \mu\text{L}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$ (40%) compared with the healthy group ($P = 0.003$ and 0.001 , respectively).

TABLE 1. Evaporation Rates Results

	RH (%)	Normal	DE	P*
Baseline†‡	25–35	0.038 ± 0.012	0.060 ± 0.018	0.002
	35–45	0.024 ± 0.007	0.040 ± 0.014	0.002
12 Minutes†‡	25–35	0.023 ± 0.009	0.039 ± 0.018	0.008
	35–45	0.017 ± 0.007	0.026 ± 0.014	0.04
24 Minutes†‡	25–35	0.034 ± 0.013	0.052 ± 0.026	0.037
	35–45	0.022 ± 0.008	0.036 ± 0.016	0.036
36 Minutes†‡	25–35	0.036 ± 0.011	0.061 ± 0.025	0.005
	35–45	0.023 ± 0.009	0.041 ± 0.019	0.006
48 Minutes†‡	25–35	0.034 ± 0.015	0.067 ± 0.034	0.003
	35–45	0.023 ± 0.011	0.044 ± 0.021	0.003

*Comparison of the evaporative rates between the groups.

†Values expressed as mean ± SD.

‡Units: $\mu\text{L}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$.

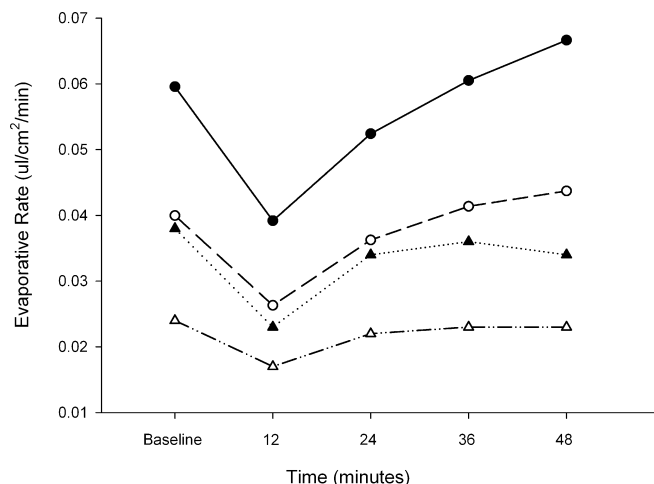
Interesting results were obtained when the DE group was analyzed as classic KCS and KCS with MGD. Compared with the normal group at RHs of 25% to 35% and 35% to 45%, the average rates of evaporation for the classic KCS group increased to 0.056 ± 0.016 (41%) and $0.040 \pm 0.008 \mu\text{L}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$ (37%) ($P = 0.001$ and 0.001), respectively. In addition, the KCS with MGD group showed a significant increase in the average rates of evaporation under the 2 studied RH conditions (25%–35% and 35%–45%) of 0.055 ± 0.026 (40%) and $0.037 \pm 0.019 \mu\text{L}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$ (37%), respectively, compared with normals ($P = 0.032$ and 0.003 , respectively).

The effect of digital expression of meibomian glands on tear evaporation rates over time is shown in Figures 1 and 2. Compared with baseline, there was a statistically significant decrease in the evaporation rate at 12 minutes with RHs of 25% to 35% and 35% to 45% in the normal and DE group, respectively. The normal group diminished by 39.7% and 30.7% ($P = 0.002$ and 0.002), respectively. The DE group showed a decrease by 34.1% and 34.2% ($P = 0.001$ and 0.002), respectively. However, there was a gradual increase in the rate of evaporation in subsequent measurements at 24, 36, and 48 minutes using RHs of 25% to 35% and 35% to 45% in the normal group and the DE group. There were no statistically significant differences between baseline and the 3 subsequent measurements.

Table 2 summarizes the evaporative rate changes for the normal group, classic KCS group, and KCS with MGD group after digital expression of meibomian glands using RHs of 25% to 35% and 35% to 45%. The KCS with MGD group had a significant decrease in the evaporation rate by 41.6% and 40% between baseline and the 12-minute measurements ($P = 0.001$ and 0.001 , respectively). However, the following measurements (24, 36, and 48 minutes) showed a progressive increase in the evaporation rate at both RH levels, without statistical significance when compared to baseline ($P > 0.05$). The classic KCS group showed a similar pattern of evaporation but did not reach the statistical significance in any measurement after digital expression of meibomian glands compared with baseline ($P > 0.05$).

DISCUSSION

At low RH, the evaporation from the ocular surface tends to be higher.^{16,17} This increased rate of tear film



● Dry Eye at RH 25-35%
 ▲ Normals at RH 25-35%
 ○ Dry Eye at RH 35-45%
 △ Normals at RH 35-45%

FIGURE 1. Comparison between AT evaporative rates for DE and normal groups measured at 2 RH ranges. The DE and the normal group showed decreased AT evaporation at 12 minutes after digital expression of meibomian glands by 34.1% ($P = 0.001$) and 39.4% ($P = 0.002$), respectively, under RH of 25% to 35%. Using RH of 35% to 45%, DE group evaporation decreased by 34.2% ($P = 0.002$) and the normal group evaporation decreased by 30.7% ($P = 0.002$). Measurements at 24, 36, and 48 minutes showed no statistically significant differences compared with baseline ($P > 0.05$).

evaporation is particularly important in low-humidity environments such as commercial airplane cabins and arid climates.¹⁸ An earlier publication reported the impact of low RH conditions on evaporation of the AT in patients with and without DE disease and detailed the significant proportion of DE symptoms in such conditions.¹⁸ Our data support the previous study and suggest that lower RH conditions increase evaporation from the AT. A 10% reduction in the RH from 35% to 45% to 25% to 35% resulted in an average evaporation rate increase of 33.3% in patients with DE and 30.3% in healthy subjects.

After blinking, water invariably evaporates from the tear film. The amount of water loss from evaporation is regulated in part by a lipid-rich layer. This layer is composed of meibomian gland lipids that spread over the tear film during blinking.^{19–23} Measurement of evaporation rates from tear film is a useful method for studying the physiology of tear film among healthy subjects and the pathophysiology in patients with DE.

In our study, we recognize that there is a difference among age groups. The possible effect of age on AT evaporation was studied by Guillon and Maïsa.²⁴ After measuring the evaporation rate of 160 subjects divided by sex and age, Guillon and Maïsa²⁴ reported that tear film evaporation in older patients was significantly higher than that of the younger age group. Moreover, previous publications^{25–27} have demonstrated that the tear film lipid layer is thinner in older subjects and, therefore, less efficient in preventing evaporation. Because age is an important risk factor for DE

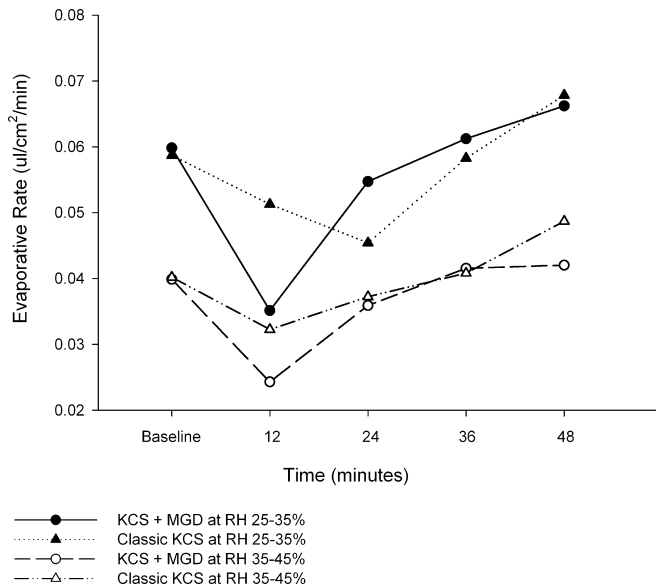


FIGURE 2. Comparison between AT evaporative rates for classic KCS and KCS with MGD groups measured at 2 different RH ranges. The KCS with MGD group showed a decrease in AT evaporation at 12 minutes after digital expression of the meibomian glands by 41.6% ($P = 0.001$) and 40% ($P = 0.001$) at RH levels of 25% to 35% and 35% to 45%, respectively. Measurements at 24, 36, and 48 minutes showed no statistically significant differences compared with baseline. Classic KCS did not reach a statistically significant change at any measurement compared with baseline ($P > 0.05$).

disease,²⁸ our findings suggest that the tear film lipid layer is negatively affected in the older population. This also may be true of other conditions that compromise this oily layer. Bron et al²⁹ have suggested that at late stages of DE disease, a hybrid form evolves in which aqueous-deficient DE takes on features of evaporative DE and vice versa. The authors noted a progressive reduction in the tear film lipid layer spreading in aqueous-deficient DE, which adds an increased evaporative component to the existing aqueous-deficient DE. These observations indicate that the evaporation rate may not always be a good discriminator between the 2 conditions.²⁹

Several investigations using different techniques have measured the evaporation rate from the AT among healthy subjects and patients with DE. A significant increase in tear evaporation rates were reported in patients with DE.¹⁰ Rolando et al³⁰ found an increase in evaporation rates in patients with KCS, which approximately doubled the rate of healthy subjects. In our study, the patients with DE demonstrated a statistically significant increase in the evaporation rate of 42.1% and 40% compared with healthy subjects using RHs of 25% to 35% and 35% to 45% as a stimulant. Our experimental conditions controlled the blink rates, the RH was fixed for the evaporation measurement, and the area between the palpebral fissures was calculated for each subject. Our findings are similar to those reported by other investigators; however, some variation can be explained by the differences in techniques and devices used.^{9,10,31}

Studies in rabbits have shown an increase ranging from a 4- to 20-fold increase in AT evaporation in the absence of meibomian glands.^{21,32} In humans, Mathers³³ described an increase in the evaporation rate in patients with MGD 3-times higher than that of the control group at 30% RH, suggesting diminished quality and structure of the meibomian lipids. In addition, recent publications have found a modification of the meibomian lipids' composition present in patients with MGD, possibly affecting the stability and accelerating the evaporation rate of the tear film.³⁴

In our experiment, we analyzed the effects of excess meibum on tear evaporation after warming and expressing the eyelid area in healthy subjects and patients with DE. It is known that a typical melting range of normal meibum as measured in vitro in bulk is 19 to 40°C.^{35,36} Moreover, Butovich³⁷ has reported that the melting range of meibomian lipids is 20 to 45°C, with a transition point of approximately 32 to 33°C. It is thought that raising the temperature of approximately the eyelid area to 40°C before digital expression will increase the temperature of the meibomian lipids and, therefore, modify their consistency from a paste to a liquid-like material. Decreased viscosity would ease the flow through the meibomian gland orifices and create a more uniform, thicker, and stable lipid layer.³⁸

In our study, normal and DE groups experienced similar diminishing evaporation rates during the first measurement (12 minutes) using RHs of 25% to 35% and 35% to 45%. However, the DE group had higher evaporative values.

TABLE 2. Tear Film Evaporative Changes Over Time

	RH (%)	Normal (%)	<i>P</i> *	KCS + MGD (%)	<i>P</i> *	KCS (%)	<i>P</i> *
Baseline vs. 12 min	25-35	↓ 39.7	0.002	↓ 41.6	0.001	↓ 13.5	0.62
	35-45	↓ 30.7	0.002	↓ 40	0.001	↓ 20	0.53
Baseline vs. 24 min	25-35	↓ 11	0.19	↓ 8.3	0.44	↓ 23.7	0.31
	35-45	↓ 7.4	0.27	↓ 10	0.29	↓ 10	0.72
Baseline vs. 36 min	25-35	↓ 5.2	0.58	↑ 1.6	0.78	↓ 1.69	0.96
	35-45	↓ 5.3	0.49	↑ 5	0.771	↑ 2.5	0.88
Baseline vs. 48 min	25-35	↓ 11.5	0.48	↑ 10	0.52	↑ 15.2	0.31
	35-45	↓ 5.8	0.44	↑ 5	0.424	↑ 2.5	0.13

*Comparison between baseline and subsequent measurement.

% Changes = 100% (rate at postexpression - rate at baseline)/rate at baseline.

The effect was temporal and lasted for a short period of time in both groups (Fig. 1). Data obtained when the DE group were divided into classic KCS and KCS with MGD groups further delineated the influence of meibomian gland lipids on tear evaporation (Fig. 2). After the expression of the meibomian glands, the KCS with MGD group showed a significant decrease in the evaporation rate during the first measurement at both RH levels (25%–35% and 35%–45%). Classic KCS, on the contrary, did not show a statistically significant decrease at any measure.

Chemical analysis of meibum from patients with MGD suggests an increase in the proportion of saturated versus unsaturated nonpolar fatty acids.³⁸ This may play an important role in determining the more paste-like consistency of the lipids and an increase in their melting points. The amount of meibomian lipids and their physical properties are fundamental to preventing AT evaporation and consequent DE disease. An increase in the melting point of meibomian lipids above the physiological temperature causes a significant thickening of the meibum within the central duct and produces plugging, stagnation, and pouting of the meibomian gland orifice, thereby reducing secretions to the ocular surface.³⁴

Our data clearly show that enhancing the volume of meibomian lipids over the tear film retards the evaporation rate of the AT, as observed in the normal and KCS with MGD groups. In the case of the classic KCS group, the reason for the absence of a significant decrease in evaporation rates is not obvious but might be because of a deficient amount of polar lipids, including triglycerides. This would produce a clear fluid-like meibum but without the appropriate surfactant properties, which may, in turn, destabilize the tear film by either changing the dynamics of the nonpolar lipid phase involved in blocking water evaporation or altering the interactions between the polar and nonpolar phases that include triglycerides.³⁴

In conclusion, our data indicate that patients with DE have an increase in the evaporation rates from the AT, compared with healthy subjects. Also, we found in both healthy and KCS with MGD groups a statistically significant decrease in the evaporative rate after warming therapy and digital expression of meibomian glands at RHs of 25% to 35% and 35% to 45%. However, this effect was transient. Future work is recommended that includes meibomian lipid analysis and correlation with the rate of evaporation from the AT.

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