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Diurnal Pattern of Tear Osmolarity and Its Relationship to Corneal Thickness and Deswelling

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Purpose: To identify the diurnal variations of tear osmolarity (TO) and its relationship with central corneal thickness (CCT) and corneal deswelling over a 14-hour period.

Methods: TO and CCT were measured using the TearLab Osmometer and Bioptigen spectral domain optical coherence tomography, respectively, on 38 healthy neophytes (mean age, 21.5 ± 2.2 years). TO and CCT were measured at bedtime (baseline), upon awakening, 20 minutes, 40 minutes, 1 hour, 2 hours, 4 hours, and 8 hours after awakening. Deswelling rate was estimated and expressed as percent recovery per hour (PRPH). Mixed-effect linear regression models describe the relationships among TO, CCT, and PRPH.

Results: The tear film upon wakening (264 ± 14 mOsm/L) was hypoosmotic compared with baseline (297 ± 15 mOsm/L, $P < 0.001$). TO (in mOsm/L) at 20 minutes, 40 minutes, 1 hour, 2 hours, 4 hours, and 8 hours were 287 ± 10, 292 ± 16, 293 ± 12, 292 ± 10, 289 ± 10, and 286 ± 10, respectively. CCT (mean ± SD) at baseline was 552.2 ± 35.9 μm and increased to 572.0 ± 38.7 μm after sleep. CCT returned to baseline thickness 4 hours after awakening ($P < 0.000$) and remained stable throughout the day. A small but statistically significant association was found between higher TO and lower CCT ($P < 0.0001$) and between lower baseline TO and higher PRPH (faster deswelling; $P < 0.0001$).

Conclusions: The diurnal pattern of TO has been established. The association of TO with corneal thickness and deswelling suggests that the tear film tonicity may be partly responsible for corneal hydration control; however, the effect may not be of clinical significance in a normal study cohort.

Key Words: tear osmolarity, corneal thickness, corneal deswelling rate, optical coherence tomography, TearLab, diurnal, ethnicity

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Numerous theories have been proposed to explain corneal hydration control after overnight sleep. Many agree that the endothelium is primarily responsible for corneal deswelling as a physiological recovery process to maintain corneal transparency. However, in diseased eyes such as Fuchs dystrophy, corneal deswelling can also be facilitated by applying hypertonic solutions to the ocular surface. Although the effects of humidity on both tear osmolarity (TO)[1–5] and corneal deswelling[6–8] have been investigated, the relationship between TO and corneal thickness and deswelling is not well understood.

In the 1970s, freezing point depression methodology was the gold standard for measuring TO.[9] Gilbard et al[10] examined the diurnal variation of TO in normal subjects and found that tears are hypoosmotic upon wakening, but sample size and measurement time points were restricted because of the cumbersome process of freezing point depression. A new device, TearLab osmometer, allows fast and noninvasive measurements of TO using electrical conductivity.[11,12] With this technology, our study is the first to directly associate TO and corneal changes throughout the day. The aims of this study were to identify the diurnal variations of TO after overnight eye closure and to investigate the relationship between TO, central corneal thickness (CCT), and corneal deswelling over a 14-hour period.

MATERIALS AND METHODS

Subject Recruitment Requirements

Neophytes, noncontact lens wearers within the year before study participation, aged 18 to 39 years, were recruited by the Clinical Research Center (CRC) at the University of California, Berkeley. Subjects were free of anterior surface diseases, eye trauma or surgery, lagophthalmos, and were not taking oral or topical ocular medications. All subjects scored less than 13 on the Ocular Surface Disease Index (OSDI)[13] and were nonsmokers. Subjects included Asian (Japanese, Chinese, and Korean) and non-Asian (European white and Hispanic) ethnicities.

Instrumentation

The TearLab Osmometer (TearLab Corp, San Diego, CA) and Bioptigen spectral domain optical coherence tomography (SD-OCT; Bioptigen, Inc, Research Triangle Park, NC) were used to measure TO and CCT, respectively. The TearLab Osmometer was modified, and an algorithm was

engineered in collaboration with TearLab to obtain hypo-osmolarity values below 275 mOsm/L. Strict precautions for ambient temperature control were implemented to ensure accurate readings. The Bioptigen SD-OCT has a 3.2-mm deep imaging window (in air) with a 32 kHz A-line rate with reference arm position optimized for anterior chamber imaging. The preset custom scan parameters used in the study included an automated 3 mm lateral scan length with 500 A-scans per frame and 3 B-scan frames. Images were captured with the subject aligned in our custom optical coherence tomography (OCT) mount while the subject fixated on the center of the scanning beam inside the OCT probe. The operator aligned the OCT probe with the perpendicular light reflex from the cornea at the horizontal and vertical center of the SD-OCT aiming windows. Mean CCT was manually calculated using the InVivoVueLab prototype software program by tracing the entire anterior and posterior corneal surface and correcting for the refractive index of the cornea. Noninvasive tear break-up time (NITBUT) was measured using the Medmont Topographer (Medmont International Pty Ltd, Vermont, Victoria, Australia).

Part I: Pilot Study and Sample Size Calculation

A pilot study was conducted to assess the repeatability and reproducibility of the Bioptigen SD-OCT. OCT images were repeatedly captured by 2 operators and analyzed by 2 readers on 2 separate days. Data were obtained to assess the interoperator, interreader, and interday variability. Compared with limits of agreement of interoperators and interday variance, the mean difference and the SD of difference from interreaders were larger, implying that image readers were crucial in obtaining precise and consistent CCT. Therefore, only 1 investigator analyzed all CCT images. A sample size of 16 in each study group was selected to detect a minimum difference of 8 mOsm/L in TO and 15 μm in CCT, with an 80% power and 95% confidence interval. The calculation was based on the SD of 10.5 mOsm/L and 19 μm for the TearLab Osmometer[13a] and Bioptigen SD-OCT,[14] respectively.

Part 2: Diurnal TO and CCT

Forty-five medically healthy neophytes with unremarkable ocular health and no symptomatic ocular dryness, determined by OSDI, were enrolled. Subjects arrived in the evening, and informed consent was obtained. This project adhered to the tenets of the Declaration of Helsinki, and the research protocol was approved by the institutional review board. Subjects reported to the CRC an average of 14 ± 2.0 hours (7–17 hours) after awaking for baseline measurements. Subjects slept at the CRC, thereby allowing for uniform environmental exposure (eg, humidity and temperature) and timely collection of measurements upon awakening.

NITBUT, Schirmer I test, and slit-lamp biomicroscopy were performed to further exclude asymptomatic subjects who had ocular surface disease or lagophthalmos, which might confound our study results. Before sleep, a randomly selected eye was gently patched[15] to ensure complete eye closure during sleep and to control measurement intervals independent of wake time. The subjects slept a minimum of 6 hours at the CRC.

The next morning, TO of the unpatched eye was measured upon awakening, followed by patch removal and immediate measurement of TO in the fellow eye. Subsequently, CCT of both eyes were imaged. Serial TO and CCT were measured at approximately 20 minutes,[16] 40 minutes, 1 hour,[16a] 2 hours, 4 hours, and 8 hours after awakening. Measurements were taken with caution to minimize reflex tearing. For a small subset of subjects (n = 10), tear osmolarities were repeated in 5-minute intervals for 20 minutes after awakening.

After measuring the osmolarity and CCT upon awakening, 3 consecutive NITBUT measurements of each eye and a slit-lamp evaluation were performed to detect corneal desiccation, irregularities, or haze after overnight sleep. Examination with fluorescein and visual acuity assessment were performed at the last visit before study completion.

Statistical Analysis

Mixed-effect models with random effects of eyes were used to test for the diurnal variation of TO and CCT. Post hoc *t* tests with Bonferroni correction were performed to explore the difference between each pair of the measurement visits. Deswelling rate, expressed as percent recovery per hour (PRPH),[17] was estimated from a previously defined nonlinear regression (exponential model). Mixed-effect multivariate regressions were used to assess the relationships among diurnal variations of TO, CCT, and PRPH while taking into consideration the potential influence of subject demographics and baseline ocular characteristics. The analysis was generated using SAS software (V 9.2 of the SAS System for Windows, Copyright 2012 SAS institute Inc).

RESULTS

Descriptive Statistics

Forty-five subjects were initially enrolled, and 38 successfully completed the study. Reasons for disqualification included reduced Schirmer I test, undisclosed mixed ethnicity of half Asian and half white at initial screening, and a dislodged eye patch during overnight sleep. In addition, data from 1 subject were excluded because of extremely variable TO of 365 mOsm/L. The results from 38 subjects between the ages of 18 and 29 years (mean ± SD = 21.5 ± 2.2) comprising 21 women, 17 men, 21 Asians, and 17 non-Asians were analyzed.

The sleep time at the CRC ranged from 6 to 8.55 hours, with mean ± SD of 8.03 ± 0.48 hours. TO of the unpatched and patched eye were measured on average of 4.7 ± 4.03 minutes and 5.8 ± 4.38 minutes from awakening, respectively. There was no systematic difference in TO between eyes, indicating that reflex tearing was not induced by patching. The mean ± SD of NITBUT upon awakening was 11.34 ± 8.02 seconds and 9.63 ± 5.77 seconds for the unpatched and

patched eye, respectively, compared with NITBUT of 9.96 ± 7.33 seconds at baseline. NITBUT was the same across all ethnicities. Baseline OSDI was 3.41 ± 3.97. At the last visit, no conjunctival staining was observed. Other ocular findings are summarized in Table 1.

Diurnal Variation of TO

All subjects exhibited a similar diurnal TO variation, shown in Table 2 and Figure 1. Upon awakening, the tear film was significantly hypoosmotic (264 ± 14 mOsm/L) compared with baseline (297 ± 15 mOsm/L, adjusted $P <$ 0.000). Upon awakening, TO changed quickest within the first 10 minutes (Fig. 2) and elevated to baseline levels within the first 40 minutes (P = 0.085). TO gradually decreased thereafter until the 8-hour visit, followed by a relatively hyperosmotic trend toward the end of the day. There were no statistically significant differences in TO between Asians and non-Asians.

Diurnal Variation of CCT and Deswelling

All subjects exhibited a similar diurnal corneal thickness pattern. The central cornea was thickest upon wakening with a 3.58 ± 1.85% overnight swelling from baseline. The CCT deswelled gradually upon eye opening (571.99 ± 38.72 μm), recovered to baseline level (554.17 ± 34.86 μm) at 4 hours, and remained stable thereafter, shown in Table 3. The change in CCT over time was plotted in Figure 3 and followed an exponential curve. Comparing the CCT between ethnicities, Asian subjects had thicker corneas than non-Asians (5–14 μm); however, this difference was not statistically significant ($P > 0.05$). To better describe corneal deswelling dynamics, we fitted the exponential equation as shown below.

$$CCT_t = B + Se^{-Dt},$$

where CCT_t is the CCT at time t measured in minutes and is 0 upon awakening. B is equal to the CCT at open eye steady state (or baseline). S is the swelling upon awakening from baseline. D is the time constant. For each subject, B, S, and D were estimated from a nonlinear regression (exponential model) on each eye. The deswelling rate was calculated as PRPH as follows:

$$PRPH = \left(1 - e^{-60D}\right) \times 100.$$

Using this definition, we obtained the mean PRPH of 61.27 ± 2.68%, with 95% confidence interval.

TABLE 1. Ocular Characteristic Statistics

	N	Mean	SD	Minimum	Maximum
NITBUT					
Baseline	67	9.96	7.33	1.35	31.1
Awake (unpatched)	32	11.34	8.02	2.4	36.4
Awake (patched)	22	9.63	5.77	2.33	21.07
Bulbar redness					
Baseline	76	0.03	0.18	0	1.25
8 h (unpatched)	38	0.03	0.16	0	1
8 h (patched)	38	0.03	0.16	0	1
Limbal redness					
Baseline	76	0.07	0.28	0	1.50
8 h (unpatched)	38	0.03	0.16	0	1
8 h (patched)	38	0.03	0.16	0	1
Corneal staining					
Type: baseline	76	0.02	0.07	0	0.4
Extent: baseline	76	0.02	0.07	0	0.3
Depth: baseline	76	0.02	0.07	0	0.4
Type: 8 h (unpatched)	38	0.14	0.21	0	1
Extent: 8 h (unpatched)	38	0.10	0.14	0	0.6
Depth: 8 h (unpatched)	38	0.11	0.14	0	0.6
Type: 8 h (patched)	38	0.10	0.17	0	0.6
Extent: 8 h (patched)	38	0.08	0.13	0	0.4
Depth: 8 h (patched)	38	0.07	0.11	0	0.4

TABLE 2. Mean TO Over a 14-Hour Period (n = 76 Eyes)

Period	Mean Osmolarity ± SD (mOsm/L)	Mean Δ% in Osmolarity ± SD*	Adjusted P (vs. Baseline)
Awake	264 ± 14	−10.89 ± 6.32	0.000
20 min	287 ± 10	−3.17 ± 4.55	0.000
40 min	292 ± 16	−1.71 ± 5.24	0.085
1 h	293 ± 12	−1.17 ± 5.46	0.785
2 h	292 ± 10	−1.46 ± 4.79	0.222
4 h	289 ± 10	−2.64 ± 5.12	0.000
8 h	286 ± 10	−3.46 ± 5.26	0.000
14 h	297 ± 15	—	—

*The mean Δ% in osmolarity was calculated as the difference from baseline divided by the baseline osmolarity.

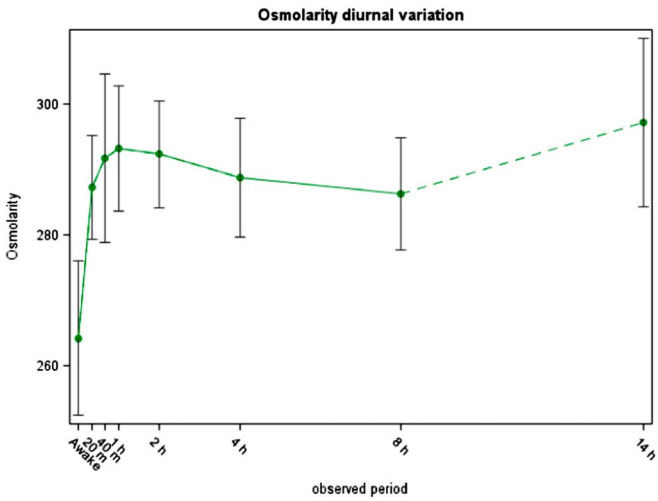

FIGURE 1. Diurnal TO over a 14-hour period. Note: 14-hour measurement was performed at bedtime (baseline) visit the night before, an average time awake of 7 to 17 hours with a mean of 14 ± 2.0 hours the day before.

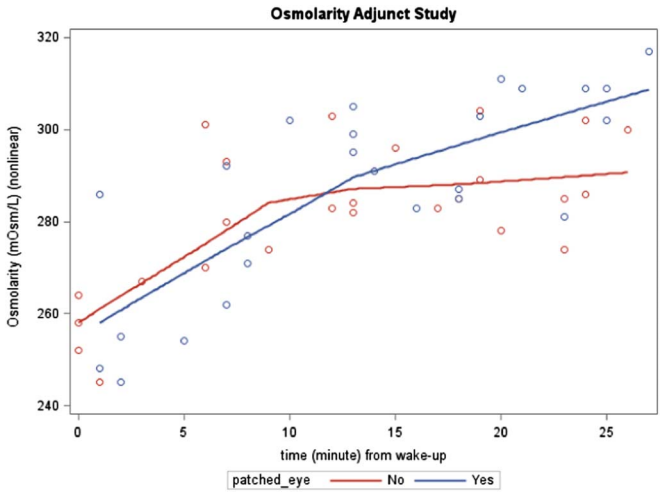

FIGURE 2. Scatter plot of TO in the first 20 minutes (n = 10 subjects).

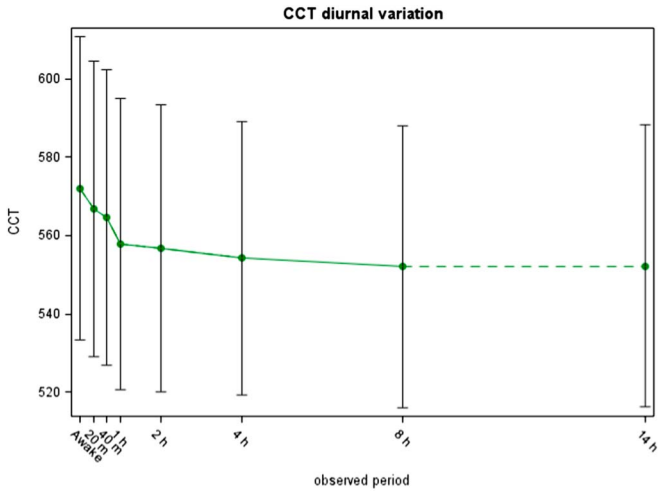

FIGURE 3. Diurnal CCT over a 14-hour period.

Mixed-Effect Regression Models Evaluating Factors Correlated With TO, CCT, and Their Diurnal Variations

These models evaluated TO, CCT, and PRPH as outcome variables and assessed their association with these covariates: demographics (age, gender, and ethnicity), ocular health parameters (corneal staining and redness), humidity, temperature, hours after awakening, and possible interaction between covariates. Only statistically significant covariates are displayed in the models.

Model 1 describes the influence of hours after awakening (awake), baseline TO, and humidity on TO levels:

$$\text{Osmolarity} = 197 + 0.89(\text{awake}) + 0.3(\text{baseline TO}) - 0.09(\text{humidity}).$$

As expected, the TO increased throughout the day ($P < 0.000$). People with higher baseline TO had higher TO ($P < 0.000$), holding other conditions constant, and TO was higher in drier environments ($P = 0.027$). Although these covariates had a statistically significant effect on TO, from a clinical perspective, their effect was not significant.

TABLE 3. Mean CCT Over a 14-Hour Period (n = 76 Eyes)

Period	Mean CCT ± SD (μm)	Mean Δ% in CCT ± SD*	Adjusted *P* (vs. Baseline)
Awake	571.99 ± 38.72	3.58 ± 1.85	0.000
20 min	566.82 ± 37.76	2.65 ± 1.69	0.000
40 min	563.56 ± 37.85	2.12 ± 1.70	0.000
1 h	558.67 ± 37.04	1.42 ± 1.36	0.000
2 h	556.65 ± 36.62	0.81 ± 1.36	0.000
4 h	554.17 ± 34.86	0.38 ± 1.2	>0.05
8 h	551.98 ± 35.98	−0.03 ± 1.19	>0.05
14 h	552.19 ± 35.92	—	—

*The mean Δ% in CCT was calculated as the difference from baseline divided by the baseline CCT.

Model 2 shows that CCT was significantly correlated with the length of time awake, TO, and baseline CCT. Unlike TO, CCT was not associated with humidity or temperature:

$$\text{CCT} = 59 - 4.5(\text{awake}) + 0.23(\text{awake}^2) - 0.13(\text{TO}) + 0.99(\text{baseline CCT}) - 0.08(\text{PRPH}) + 5.43(\text{for Asian if within 2 hours after awakening}).$$

The main effect of *awake* and *awake*^{2} were statistically significant (both, $P < 0.000$), indicating that the rate of corneal deswelling decreases throughout the day. This trend was confirmed by a steeper CCT curve immediately after awakening compared with a flatter curve toward the end of the day. CCT and TO were negatively correlated ($P < 0.000$), indicating that subjects with higher TO had thinner corneas in general. However, the effect size of TO was minimal. This model also demonstrated that people with a higher baseline CCT had thicker corneas at any time point ($P < 0.0001$) and that a thicker cornea was associated with slower deswelling. Additionally, the mean CCT for Asian subjects were 5.43 μm greater than non-Asians within 2 hours after awakening ($P = 0.006$). Thereafter, no CCT difference between Asian and non-Asian was found ($P = 0.54$).

Model 3 examined potential factors influencing PRPH (deswelling rate):

$$\text{PRPH} = 133 - 0.19(\text{baseline TO}) - 0.79(\text{baseline NITBUT}) - 10.3(\text{if Asian}).$$

PRPH was associated with baseline TO ($P = 0.004$), baseline NITBUT ($P < 0.000$), and ethnicity ($P < 0.000$). Subjects with lower baseline TO had higher PRPH. In addition, smaller baseline NITBUT, indicating a less stable tear film, was correlated with faster deswelling rate. Asians had slower deswelling rates with all other variables being equal compared with their non-Asian counterparts.

DISCUSSION

Establishing a diurnal pattern of TO in a normal study cohort serves as a basis for comparison in cases of pathology. Deviations in diurnal TO from normal, such as in dry eye or with contact lens wear, can be attributed to factors other than normal variation of TO. This study has shown that in a normal population, tears are hypoosmotic immediately after overnight sleep, increase quickly to baseline levels in 40 minutes, and fluctuate around 297 ± 15 mOsm/L throughout the rest of the day. These results are consistent with freezing point depression[9] and microosmometric[18] studies that found hypoosmotic tears upon awakening. Our TO is also consistent with the normal average TO of 301.8 ± 10.5 mOsm/L reported by TearLab[13a] and Li et al[19] who reported normal daytime TO of 298.0 ± 14.2 mOsm/L also measured by TearLab Osmometer with a similar subject mean age of 27 ± 7 years. Of interest, the results from our study support the hypothesis that normal eyes have a robust homeostatic system, which maintains a stable tear film in response to ordinary environmental stresses over waking hours. We speculate that the diurnal pattern of subjects with dry eye may be different because of a loss of homeostatic control, which manifests as higher between-eye and within-eye variability among dry eye patients compared with those of a normal study cohort.[20]

Eye closure creates a hypoosmotic environment because of reduced tear evaporation, reduced tear production, and reduced tear drainage.[21] Until now, it has been unclear how long this hypoosmotic environment persists after eye opening. The rise in TO can be attributed to increased evaporation as the eye responds to the relatively lower humidity of the surrounding environment. This is supported by the studies of both Tsubota and McCulley. Abusharha and Borchman found just the opposite, that no significant differences were observed in TO between normal and dry environments. Reasons for the discrepancy could be attributed to small sample size and difference in study design. Another potential explanation of this rise in TO is the subsiding of reflex tears induced by eye opening and/or patch removal. Reflex tearing decreases osmolarity.[22] Consequently, TO is expected to rise as reflex tearing wanes. Our data showed no difference in TO between the patched and unpatched eye, suggesting that patching did not induce additional reflex tearing. However, it is conceivable that reflex tearing occurs upon awakening as a protective mechanism to wash out unwanted substances accumulated during subclinical ocular inflammation as a result of overnight eye closure.[23] It has been shown that tear meniscus height increases immediately after eye opening in the morning and recovers to baseline approximately 30 minutes after awakening.[24] As tear meniscus height is directly related to tear aqueous volume on the ocular surface, one can presume that the increase in tear meniscus height is because of reflex tearing upon eye opening in the morning. Based on these observations, we conclude that immediately upon awakening, the tear film is physiologically hypoosmotic, then increases as a result of a combination of subsiding reflex tearing and a change in relative humidity in the open eye environment.

Diurnal CCT has already been well established with a 3.0% swelling during overnight sleep reported by Orbscan and pachymetry,[25,26] and deswelling within 2 hours[16] and 7 hours[27] upon awakening. Our study results echo a similar pattern with corneal swelling overnight during eye closure by 3.58% and recovery to steady-state baseline thickness 4 hours later. Our study calculates a PRPH[17] of 61.27%, which is similar to outcomes of multiple cohort studies (PRPH of 59.6% and 58.9%).[28] Our study has found that corneal thickness and deswelling rate are affected by different covariates in the mixed-effect models.

Mixed-effect models analyzed the relationship between TO, CCT, and deswelling for the entire 14-hour period. Most changes were observed in the first 40 minutes; therefore, the models were also analyzed in 2 time intervals, awake to 40 minutes and 40 minutes to 14 hours. All models, regardless of the time intervals, reflected the overall 14-hour study duration results. Our study showed that there are statistically significant, but small, correlations between TO and overall corneal thickness and with deswelling rate in the normal subject population.

In model 2, CCT as the outcome variable showed that higher tear osmolarities are associated with thinner corneas. This can be explained by a simple osmotic gradient, whereby when TO increases, water is drawn out of the cornea, causing corneal thickness to decrease. This effect is seen clinically with the use of 5% NaCL (Muro 128, Bausch & Lomb) to treat corneal edema secondary to Fuchs dystrophy. If the endothelium is extensively damaged, despite the effects at the anterior surface with Muro 128, corneal edema ensues and replacement of the endothelium through PKP or DSEK is the only therapeutic alternative. This suggests that the tear film plays a minor role at the anterior surface affecting corneal thickness, but the corneal endothelium remains pivotal in regulating and maintaining corneal hydration.

In model 3, the deswelling rate of the cornea is independent of the actual corneal thickness and humidity, rather more significantly affected by characteristics of the tear film. This supports the findings of Bourassa and Cohen that humidity does not contribute to the deswelling function of the cornea. Interestingly though, deswelling was negatively correlated with TO. In other words, our findings demonstrated that lower baseline TO is associated with faster corneal deswelling, an opposite effect from which one would predict if tonicity is the driving force. The effect size is very small and clinically insignificant, suggesting that TO influences overall corneal thickness but has no effect on deswelling rate; rather, the endothelial pump system is primarily responsible for corneal deswelling after eye opening. It is also conceivable that there may be other homeostatic compensatory mechanisms responsible for this phenomenon of corneal hydration regulation in the normal population.

In conclusion, we have established a diurnal variation of TO for normal subjects, which significantly changes upon awakening and remains relatively constant throughout most of the day. In contrast, CCT is greatest at initial eye opening and deswells throughout the day. The association of TO with corneal thickness exists; however, the effect may not be of clinical significance for a normal study cohort.

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REFERENCES

1. McCulley JP, Uchiyama E, Aronowicz JD, et al. Impact of evaporation on aqueous tear loss. *Trans Am Ophthalmol Soc.* 2006;104:121–128.
2. Tsubota K, Yamada M. Tear evaporation from the ocular surface. *Invest Ophthalmol Vis Sci.* 1992;33:2942–2950.
3. Khanal S, Millar TJ. Barriers to clinical uptake of tear osmolarity measurements. *Br J Ophthalmol.* 2012;96:341–344.
4. Abusharha AA, Pearce EI. The effect of low humidity on the human tear film. *Cornea*. 2012;32:429–434.
5. Borchman D, Foulks GN, Yappert MC, et al. Factors affecting evaporation rates of tear film components measured in vitro. *Eye Contact Lens*. 2009;35:32–37.
6. O'Neal MR, Polse KA. In vivo assessment of mechanisms controlling corneal hydration. *Invest Ophthalmol Vis Sci.* 1985;26:849–856.
7. Cohen SR, Polse KA, Brand RJ, et al. Humidity effects on corneal hydration. *Invest Ophthalmol Vis Sci.* 1990;31:1282–1287.
8. Bourassa S, Benjamin WJ, Boltz RL. Effect of humidity on the deswelling function of the human cornea. *Curr Eye Res.* 1991;10:493–500.
9. Benjamin WJ, Hill RM. Human tears: osmotic characteristics. *Invest Ophthalmol Vis Sci.* 1983;24:1624–1626.
10. Gilbard JP, Cohen GR, Baum J. Decreased tear osmolarity and absence of the inferior marginal tear strip after sleep. *Cornea*. 1992;11:231–233.
11. Eperjesi F, Aujla M, Bartlett H. Reproducibility and repeatability of the OcuSense TearLab™ osmometer. *Graefes Arch Clin Exp Ophthalmol.* 2012;250:1201–1205.
12. Tomlinson A, McCann LC, Pearce EI. Comparison of human tear film osmolarity measured by electrical impedance and freezing point depression techniques. *Cornea*. 2010;29:1036–1041.
13. Schiffman RM, Christianson MD, Jacobsen G, et al. Reliability and validity of the Ocular Surface Disease Index. *Arch Ophthalmol.* 2000;118:615–621.

13a. Eldridge DC, Sullivan BD, Berg MD, et al. Longitudinal variability of tear film osmolarity in normal and dry eye patients. *Invest Ophthalmol Vis Sci.* 2010;51:E-Abstract 3379.

14. Harper CL, Boulton ME, Bennett D, et al. Diurnal variations in human corneal thickness. *Br J Ophthalmol.* 1996;80:1068–1072.
15. Staarmann MT, Schoessler JP. Contact lenses and eye closure: comparative analysis using three different methods to induce corneal swelling. *Optom Vis Sci.* 1991;68:374–379.
16. Read SA, Collins MJ. Diurnal variation of corneal shape and thickness. *Optom Vis Sci.* 2009;86:170–180.

16a. Keech A, Senchyna M, Sullivan BD, et al. Impact of time between collection on human tear film fluid osmolarity. *Invest Ophthalmol Vis Sci.* 2010;51:E-Abstract 4174.

17. Egan CA, Hodge DO, McLaren JW, et al. Effect of dorzolamide on corneal endothelial function in normal human eyes. *Invest Ophthalmol Vis Sci.* 1998;39:23–29.
18. Terry JE, Hill RM. Human tear osmotic pressure: diurnal variations and the closed eye. *Arch Ophthalmol.* 1978;96:120–122.
19. Li M, Du C, Zhu D, et al. Daytime variations of tear osmolarity and tear meniscus volume. *Eye Contact Lens*. 2012;38:282–287.
20. Lemp MA, Bron AJ, Baudouin C, et al. Tear osmolarity in the diagnosis and management of dry eye disease. *Am J Ophthalmol.* 2011;151:792–798.e1.
21. Lowther GE. Review of some ocular anatomy and physiology relevant to contact lens practice. Available at: http://www.opt.indiana.edu/lowther/download/anat_phys_review.pdf. Accessed September 28, 2012.
22. Saleh GM, Hussain B, Woodruff SA, et al. Tear film osmolarity in epiphora. *Ophthal Plast Reconstr Surg.* 2012;28:338–340.
23. Sack RA, Conradi L, Krumholz D, et al. Membrane array characterization of 80 chemokines, cytokines, and growth factors in open- and closed-eye tears: angiogenin and other defense system constituents. *Invest Ophthalmol Vis Sci.* 2005;46:1228–1238.
24. Shen M, Wang J, Tao A, et al. Diurnal variation of upper and lower tear menisci. *Am J Ophthal.* 2008;145:801–806.
25. Giráldez-Fernández MJ, Díaz-Rey A, García-Resua C. Diurnal variations of central and paracentral corneal thickness and curvature [in Spanish]. *Arch Soc Esp Oftalmol.* 2008;83:183–191.
26. Holden BA, Mertz GW, McNally JJ. Corneal swelling response to contact lenses worn under extended wear conditions. *Invest Ophthalmol Vis Sci.* 1983;24:218–226.
27. Feng Y, Varikooty J, Simpson TL. Diurnal variation of corneal and corneal epithelial thickness measured using optical coherence tomography. *Cornea*. 2001;20:480–483.
28. Polse KA, Brand R, Mandell R, et al. Age differences in corneal hydration control. *Invest Ophthalmol Vis Sci.* 1989;30:392–399.