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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 after awakening (\( P < 0.0001 \)) was 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 (\( P < 0.0001 \)) 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 toxicity 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

(Cornea 2013;32:1305–1310)

**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)\(^1\) 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 hypotonicity 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 to 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 with the SD of NITBUT upon awakening was 11.34 ± 2.0 hours (7.48 ± 2.2) comprising 21 women, 17 men, 21 Asians, and 17 non-Asians selected eye was gently patched 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 measured. Serial TO and CCT were measured at approximately 20 minutes, 40 minutes, 1 hour, 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), 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 ± 0.03 minutes and 5.8 ± 0.38 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.
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 waking 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 2. Mean TO Over a 14-Hour Period (n = 76 Eyes)

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

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

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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.

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 \(\mu m\) 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.
DIMENSIONS

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who As tear meniscus height is directly related

Consequently, TO is expected to rise

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Until now, it has been unclear

and deswelling within 2 hours16 and 7

hours27 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 PRPH17 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 thick-

ness 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, regard-

less 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 conceiv-

able 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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Consequently, TO is expected to rise
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