

# Changes in Corneal Detection Thresholds After Repeated Tear Film Instability

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**PURPOSE.** To use a human-based model to study the effects of repeated tear film instability on corneal detection thresholds to cold, mechanical, and chemical stimuli.

**METHODS.** Twenty-five subjects participated in three study visits. A computer-controlled Belmonte esthesiometer was used to estimate corneal detection thresholds to cold, mechanical, and chemical stimuli before, after, and 30 minutes following 10 consecutive sustained tear exposure (STARE) trials. Subjects turned a pain knob (0-10) to indicate discomfort during STARE trials. The area of tear breakup and thinning in each trial was analyzed. Symptoms were evaluated by the Current Symptom Questionnaire (CSQ).

**RESULTS.** There was a significant time effect on CSQ symptoms during both visits (Friedman test,  $P < 0.001$ ), with immediately after repeated STARE and 30 minutes later significantly differing from before STARE (Wilcoxon,  $P < 0.017$ ). Tear breakup occurred in every trial, ranging from 25% to 88% of the exposed corneal area and all subjects indicated discomfort during trials. There was a significant time effect on mechanical thresholds between before STARE mechanical thresholds and 30 minutes later (repeated measures analysis of variance [ANOVA]  $P < 0.001$ ), but not cold ( $P = 0.057$ ) or chemical ( $P = 0.565$ ) thresholds.

**CONCLUSIONS.** In this study, tear breakup during STARE trials was associated with discomfort, which when repeated, resulted in increased symptoms of ocular discomfort and alterations of mechanical sensory thresholds after 30 minutes. These results suggest that tear film instability, which is thought to occur repeatedly during normal blinking among dry eye patients over the day, can produce neurosensory alterations.

**Keywords:** Tear film instability, corneal detection thresholds, sensory response, symptoms of ocular irritation, dry eye

Dry eye disease (DED) is a condition that affects millions in the United States<sup>1-4</sup> and worldwide.<sup>5-9</sup> It adversely affects quality of life<sup>2,10</sup> and poses a considerable economic burden, estimated at \$55.4 billion yearly, to the US economy.<sup>11</sup> According to the Dry Eye Workshop (DEWS) II report,<sup>12</sup> DED involves a loss of homeostasis of the tear film and is accompanied by ocular symptoms, with tear film instability, hyperosmolarity, inflammation, and neurosensory abnormalities playing an etiological role. However, as the definition suggests, the connection between the etiological factors and their impact on tear film homeostasis and symptoms remains speculative,<sup>12-14</sup> perhaps due to the potential multifactorial causes of DED.<sup>12</sup>

Theoretically, tear film homeostasis is disrupted in a proposed cycle of events leading to DED, sometimes referred to as the "vicious circle of DED."<sup>12-14</sup> The circle or cycle includes tear film hyperosmolarity, inflammation, neural stimulation, and surface alterations and damage. It can be entered at multiple locations, but an important entry point is tear film instability, which includes both tear breakup and excessive tear thinning.<sup>12-14</sup> It is thought that the sequelae of tear film instability and associated ocular stress could repeatedly stimulate ocular surface sensory neurons and may lead to sensitization, increasing neural damage and functional disturbances that can ultimately lead to neuropathic pain.<sup>15</sup> Bron et

al.<sup>16</sup> suggested that the predicted natural progression of dry eye follows this course, beginning with tear film instability and increased tear film osmolarity, which provides noxious stimulation to ocular surface sensory nerve endings and increase sensory drive, followed eventually by increasing neural damage and decreased sensation.

Thus, studying neurosensory abnormalities in DED and their relationship to other potential etiological factors can be challenging due to the potential for ocular surface damage and neurosensory alterations in the condition.<sup>12</sup> An alternative approach is to create a method to study DED etiological factors in healthy subjects with an unaltered ocular surface and normal sensory response to understand potential initiating factors in the condition. In previous studies,<sup>17-19</sup> we used the technique of sustained tear exposure (STARE) to induce tear film instability, a known etiological factor in DED, and study its effects. Using STARE, we have shown that tear film instability, or more specifically tear breakup, is associated with immediate pain, discomfort, burning, and stinging in both healthy and DED subjects, suggesting that tear breakup can directly stimulate underlying corneal neurons. Furthermore, we have recently shown that ratings of intensity to cooling stimuli decreased, whereas irritation ratings to mechanical stimuli increased following repeated STARE trials, which suggested that tear film instability affects neurosensory function.<sup>20</sup>



However, the link between symptoms and neurosensory function has yet to be demonstrated, and is likely not to be a direct one. In this study, we use the STARE technique to induce and measure tear breakup<sup>17,18,21,22</sup> in healthy subjects to test the hypothesis that repeated episodes of tear film instability and breakup are associated with discomfort and lead to symptoms of ocular irritation and alterations in corneal thresholds.

## METHODS

### Subjects

This study was reviewed and approved by the Indiana University Institutional Review Board and was conducted at the Indiana University School of Optometry in Bloomington, Indiana. Informed consent was obtained from all subjects before beginning the study, which was conducted in compliance with the tenets of the Declaration of Helsinki. Twenty-five subjects with no self-reported history of dry eye, other ocular diseases, ocular surgeries, systemic diseases, or use of topical or systemic medications that could affect ocular sensation were included in the study. Before admission into the study, subjects were screened to exclude those with evidence of ocular surface disease.

### Procedures

This study involved three visits on three different days. Visit 1 involved examination of the ocular surface to screen for damage or disease, measurement of tear breakup time, and the Schirmer's tear test. Subjects were trained in the procedures to be used in visits 2 and 3, including sustained eye exposure (STARE) and threshold measurements by the Indiana University Belmonte esthesiometer.<sup>23</sup> If subjects were unable to perform the latter two procedures adequately, they were determined ineligible for the study.

Visits 2 and 3 were experimental visits, which occurred within 2 weeks of each other. Subjects filled out three questionnaires. The Demographic Questionnaire asks subjects about their age, sex, race, and ocular and general health. The Dry Eye Questionnaire (DEQ-5) queries habitual symptoms of dry eye over the past month, with a cutoff score of 6 dry eye and 12 for suspected Sjögren syndrome.<sup>24</sup> The Current Symptom Questionnaire (CSQ) asks about the intensity of symptoms of ocular irritation associated with dry eye at the time of testing (total summed score = 50).<sup>25</sup>

Sensory thresholds to pneumatic cool stimuli were measured by the ascending method of limits<sup>26</sup> using our custom, computer-controlled, modified Belmonte esthesiometer.<sup>23</sup> Subjects fixated on a target with the untested eye during the experiment. The tip of the esthesiometer was 5 mm from the central corneal surface of the tested eye and was continuously monitored by a calibrated video camera. Before stimulus presentation, a sound signaled the subject to blink and fixate on the target. The stimulus duration was 2 seconds with an interval of 10 seconds between stimuli. Subjects pressed a knob to indicate stimulus detection. Initial starting flow rates were 30 mL/min, with steps of at least 10 mL/min above the initial flow rates. This procedure was repeated three times and the final threshold was determined as the average flow rate. The order of study visits was always cool stimulus in visit 2 and mechanical/chemical in visit 3, because of the length of time required to cool the esthesiometer.

The pneumatic cool stimulus in visit 2 consisted of room temperature air (approximately 20°C) with a flow rate varying from 0 to 200 mL/min. During visit 3, the mechanical stimulus

was measured first (flow rates from 0 to 200 mL/min), with the esthesiometer air heated to approximate the corneal temperature at the eye and minimize the possibility of a thermal (cooling) effect.<sup>27</sup> After measurement of the mechanical stimulus threshold, the chemical stimulus was delivered at 50% flow rates beneath the subject's threshold for the mechanical stimulus to minimize the likelihood of mechanical effects contaminating the chemical threshold.<sup>28</sup> The chemical stimulus was produced by adding CO<sub>2</sub> to the stimulus air column<sup>29</sup> (0% to 80% CO<sub>2</sub>) to induce a local pH change in the tear film. The initial level of 10% CO<sub>2</sub> was increased in increments of 5% CO<sub>2</sub>.

After threshold measurements, subjects were seated behind a slit lamp biomicroscope custom fitted with a Tamron (Saitoma, Japan) (M111FM16) C1.1 16 mm F/1.8 12 MegaPixel Fixed Focal lens (Tamron, USA). Images of the tear film of the tested eye (untested eye held shut) were taken after instilling 5 µL 2% fluorescein dye with a micro-pipette onto the superior conjunctiva. Subjects then performed 10 consecutive STARE trials, during which subjects kept the tested eye open for as long as possible.<sup>18</sup> During each STARE trial, subjects turned a pain knob to indicate the level of ocular discomfort, from 0 (no discomfort) to 10 (pain level that requires blinking), with a 5-second break between each trial. Additional fluorescein dye was instilled when necessary to ensure adequate fluorescence of the tear film.

Immediately after the 10th STARE trial, the CSQ was completed and threshold measurements were repeated, using the same procedures. Subjects then waited for 30 minutes in the laboratory, engaging in activities of their choice (reading or electronic device usage). The 30-minute time period was chosen based on previous studies<sup>18,20</sup> using the STARE technique, during which subjects noted that most ocular symptoms were greatly diminished after 30 minutes (data not published). After the 30-minute break, subjects again filled out the CSQ and threshold measurements were made as before.

Ten subjects from the pool of experimental subjects participated in a control experiment, in which the procedures in visits 2 and 3 were repeated, except without the instillation of fluorescein and STARE trials. As in the experimental visits, control subjects filled out questionnaires at the beginning of each visit and then took initial threshold measures. Afterward, subjects sat in the laboratory and participated in the activity of their choice for 30 minutes (reading or electronic device), which was the average time required for 10 STARE trials. Then thresholds for either cool or mechanical/chemical stimuli were measured and again after 30 minutes, as in experimental visits. Control experiments were designed to control for the effect of extended eye opening and delayed blinking (STARE) versus normal blinking during activities chosen by subjects.

### Image Analysis

To estimate the area tear breakup and thinning in each trial, the first image, taken immediately after eye opening and the last image, just before the blink that ended the trial, were extracted from trial videos. A custom MATLAB program (The MathWorks, Inc., Natick, MA, USA) was used to convert images to grayscale, align them, isolate the exposed cornea as the region of interest, and calculate the pixel intensity distribution.<sup>21</sup> As in a previous study,<sup>21</sup> we used a cutoff from the pixel intensity distribution of the first image in the trial to define the percentage of tear breakup in the last image of the trial, given the known linear relationship between decreasing fluorescence and tear film thickness during eye opening.<sup>30</sup> We chose the fifth percentile of the pixel intensity distribution in the first image as the cutoff, so that the percentage of pixels less than that value were defined as tear breakup in the last image. The maximum

TABLE. Demographic and Clinical Data for Subjects in This Study

Subjects	25 (12 males, 13 females)
Age, y	25 ± 2 (22-30)
DEQ-5	4 ± 3 (0-16)
Tear breakup time, s	10 ± 6 (4-21)
Schirmer's test (without anesthesia), mm	25 ± 9 (7-35)

blink interval<sup>31</sup> was defined as the difference in time between the first and last image of each trial.

### Statistical Analysis

Statistical analyses were performed using SPSS 24 (IBM SPSS 24.0.; IBM Corp., Armonk, NY, USA). Corneal thresholds were transformed by conversion to the natural log to achieve normality of the data distribution.<sup>32</sup> A repeated measure ANOVA was used to analyze any differences between times. Huynh-Feldt corrected *P* values ( $P < 0.05$ ) were reported to minimize the effects of violating assumptions about data sphericity. Post hoc two-tailed paired *t*-tests with Bonferroni correction were used to estimate the significance at  $P < 0.017$  for the repeated measures ANOVA. The Friedman test was used to analyze the difference between times for the CSQ and a Wilcoxon signed rank test with Bonferroni correction for pairwise comparisons.

### RESULTS

The Table summarizes subject demographics and some clinical measures. Approximately half of the subjects were female with an average age of 25 years. The average DEQ-5 score was close to the cutoff for dry eye of 6, with six subjects falling below the cutoff, despite the lack of a diagnosis of dry eye in this group of subjects. The tear breakup time and Schirmer's tear test values fell within the range for a healthy (non-dry eye) subject population.<sup>33</sup>

### Symptoms During Testing

The box plots in Figures 1A and 1B show the experimental visit CSQ sum scores for all subjects before, immediately after repeated STARE trials, and 30 minutes later. There was a significant time effect for both visits (Friedman test,  $P < 0.001$ )

with CSQ sum scores significantly higher immediately after STARE trials and 30 minutes later, compared with before (Wilcoxon signed rank test with pairwise comparisons,  $P < 0.002$ ). In contrast, there was little or no change in the CSQ symptom scores over time during control visits. Median CSQ scores for the first and second control visits (before, immediately after, and 30 minutes later) were 0.5; 0 and 0.5; and 0, 0, and 0, respectively. There was no significant time effect for either of the control visits ( $P = 0.465$  and  $P = 0.225$ , respectively).

Figures 2A and 2B show CSQ scores normalized to zero before testing to allow visualization of individual differences and to highlight the very high variability in CSQ scores among subjects immediately after repeated STARE trials (both experimental visits 2 and 3). As Figure 2 shows, some subjects reported only very minor changes in CSQ symptom scores immediately after repeated STARE trials, as low as CSQ scores of 1 or 2, whereas other subjects' CSQ scores changed by as much as 40 points (50 points possible). These individual changes were consistent between the two visits, with rank correlations before, after, and 30 minutes later (Pearson's correlation,  $\rho = 0.865, 0.877, 0.817$ , respectively) between visits. As, Figure 2 shows, 30 minutes after STARE testing, most subjects' CSQ scores declined, but many did not return to zero.

### Corneal Detection Thresholds

Corneal detection thresholds (mean ± 95% confidence intervals [CI]) for cool, mechanical, and chemical stimuli are depicted in Figure 3. Figure 4 shows individual subject data, normalized to zero before testing, to visualize individual changes in responses after testing.

There were no statistically significant time effects for corneal cold thresholds ( $P = 0.057$ ), although there was a trend toward sensitization after 30 minutes (Fig. 3A). Normalized cold threshold data (Fig. 4A) shows that 11 subjects (44%) decreased in threshold immediately after STARE and 16 subjects (64%) decreased 30 minutes later. Mean (±95% CI) control cold thresholds were 1.77 (1.69-1.84) before, 1.77 (1.70-1.83) immediately after, and 1.77 (1.70-1.84) 30 minutes later (repeated measures ANOVA,  $P = 0.988$ ).

There was a statistically significant main time effect on mechanical thresholds (Fig. 3B), with a significant difference between before STARE and 30 minutes (repeated measures ANOVA,  $P < 0.001$ ; post hoc Bonferroni adjustment,  $P <$

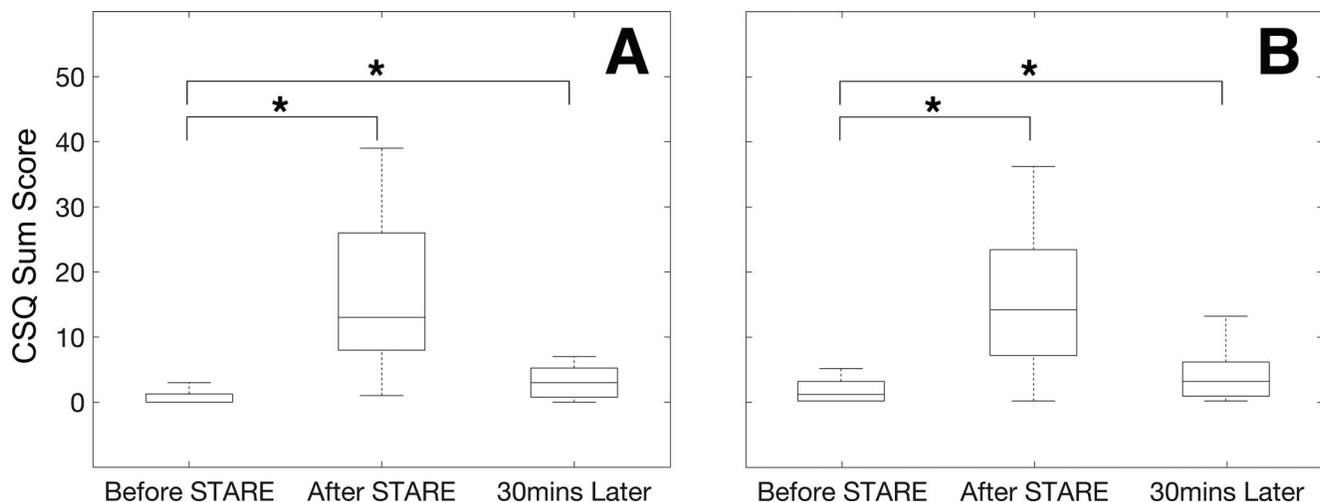


FIGURE 1. Visit 2 (A) and 3 (B) CSQ symptom scores before, immediately after repeated STARE, and 30 minutes later. Box plots represent the median, 25th and 75th percentiles of CSQ data. Bars and asterisks represent statistical significance,  $P < 0.002$ .

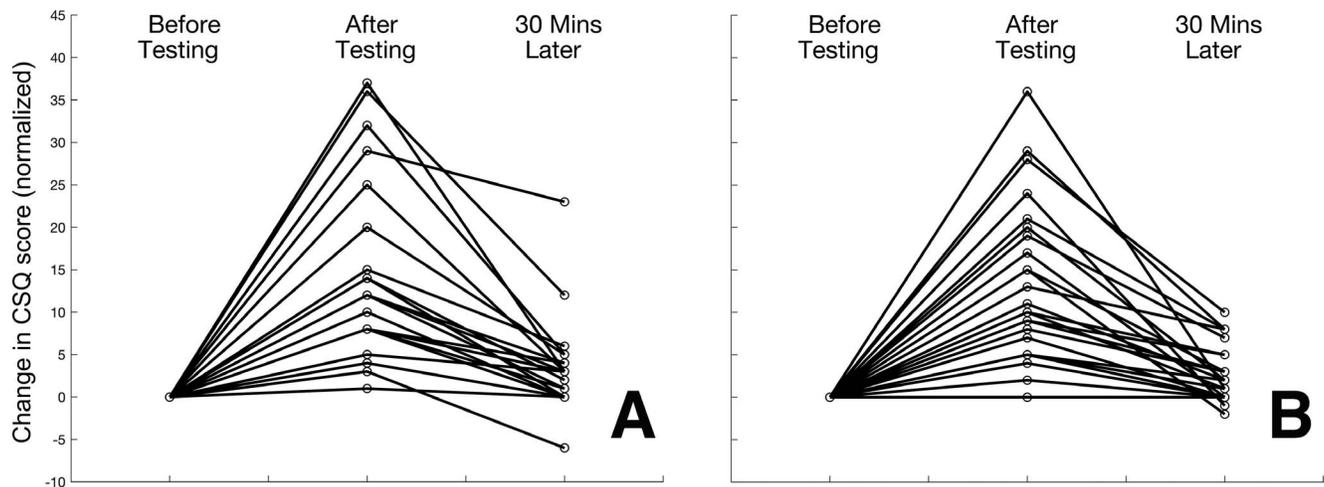


FIGURE 2. CSQ symptom scores normalized to zero before testing to show individual subjects changes in scores for (A) visit 2 and (B) visit 3.

0.001). Figure 4B shows normalized individual responses to mechanical stimuli, illustrating that mechanical thresholds decreased for most subjects (19 subjects or 76% at both time periods) immediately after STARE and 30 minutes later. Mean thresholds for controls were 1.83 (1.76–1.90) before, 1.82 (1.77–1.87) immediately after, and 1.30 (1.70–1.90) 30 minutes later ( $P = 0.666$ ).

Mean chemical thresholds did not change significantly over time (Fig. 3C). There was no significant difference among the three time periods ( $P = 0.565$ ). Figure 4C shows that slightly more subjects decreased in chemical threshold over time, with 15 subjects (60%) decreasing immediately after STARE testing and 30 minutes later. Similarly, there was no significant difference (repeated measures ANOVA  $P = 0.125$ ) among times for control data, with mean (95% CI) of 1.59 (1.49–1.69) % CO<sub>2</sub> before testing, 1.55 (1.46–1.64) % CO<sub>2</sub> after testing, and 1.49 (1.41–1.56) % CO<sub>2</sub> 30 minutes later.

### Tear Breakup and Discomfort During STARE Trials

Overall, all STARE trials for all subjects in this study showed some tear breakup or thinning. Figures 5A–C show some examples of the final frame of videos from three subjects. Figures 5A and B are examples of tear breakup with some tear thinning, whereas Figure 5C shows mainly tear thinning. The area of tear film instability in all subjects ranged from 25% to 88% of the exposed corneal area (Fig. 6A). The mean tear breakup ( $\pm$ SE) for all subjects and trials was 55.24 ( $\pm$ 4.04). The mean ( $\pm$ SE) maximum blink interval<sup>31</sup> was 35.08  $\pm$  6.94 seconds.

Figure 6B shows the mean pain level and the corresponding time for all subjects to reach the level of 10 during visit 2, indicating that all subjects felt pain that required blinking, although the length of trials differed among subjects. A similar observation on the pain response was made on visit 3 (data not shown).

### DISCUSSION

In this study, we hypothesized that repeated tear film instability leads to increased symptoms of ocular irritation and altered sensory thresholds. Although previous studies have associated tear breakup with pain,<sup>19,21</sup> increased symptoms,<sup>18</sup> and altered neurosensory function,<sup>20</sup> this study is the first to show evidence that repeated tear film instability leads to altered sensory thresholds that persist for at least 30 minutes.

In this investigation, we used the STARE technique to induce tear breakup and thinning and study its effects on symptoms and corneal detection thresholds. All subjects had some amount of tear film instability during STARE trials and all subjects reached the highest pain level possible for our scale, which was defined as so painful that they had to blink to end the trial. The association of increasing pain with increasing tear film instability during STARE trials suggests that both tear breakup and thinning produce stress to the ocular surface, thus stimulating underlying corneal nerves. The exact nature of this stress is unknown, but previous experimental studies have suggested that tear film hyperosmolarity may transiently spike as high as 800 to 900 mOsm/kg<sup>17</sup> during tear film instability.

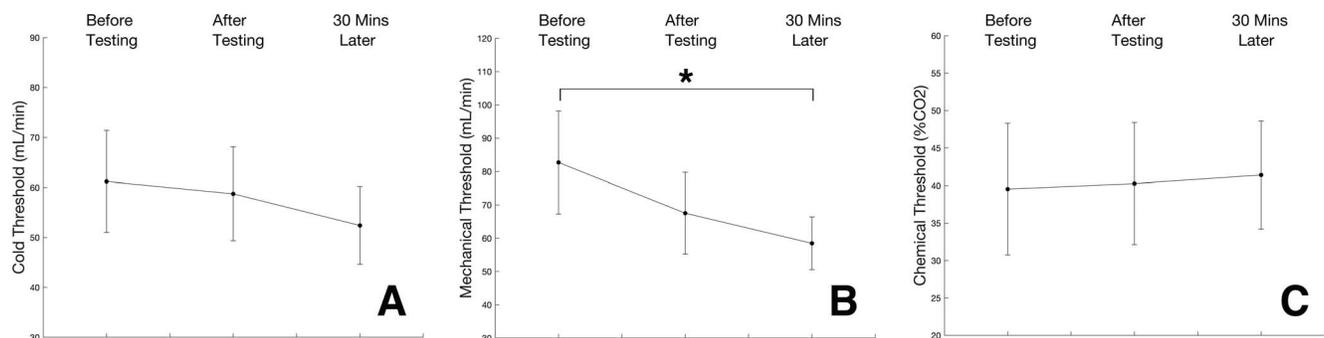


FIGURE 3. Thresholds (mean  $\pm$  95% CIs) before, immediately after STARE, and 30 minutes after STARE for (A) cool, (B) mechanical, and (C) chemical stimuli. The bar and asterisk indicate statistical significance,  $P < 0.001$ .

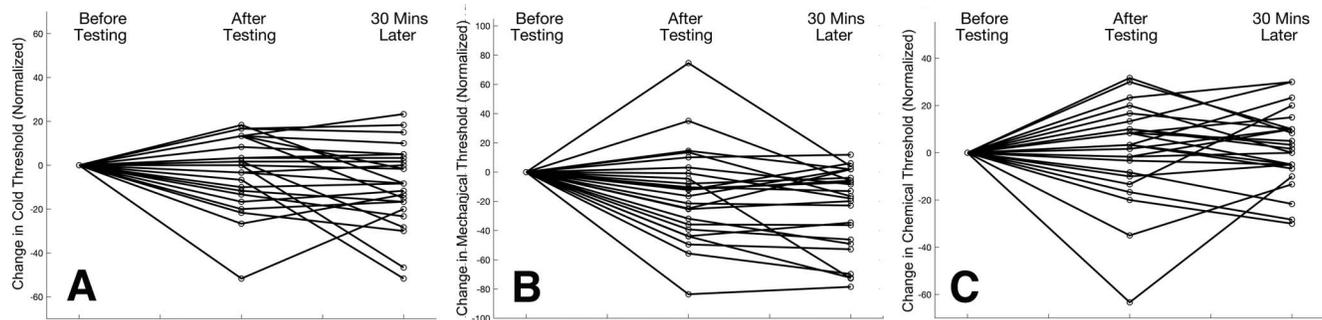


FIGURE 4. Mean threshold changes from before (normalized to zero), immediately after, and 30 minutes after STARE for (A) cool, (B) mechanical, and (C) chemical stimuli.

Mathematical modeling suggests even higher levels,<sup>34–36</sup> with some estimates as high as 1800 mOsm/kg.<sup>36,37</sup>

Although the exact nature of the events occurring during tear film breakup remain speculative, detection thresholds for mechanical stimuli significantly decreased following repeated STARE trials. Although the reason for this alteration in mechanical thresholds is unclear, it is possible that local spikes of tear osmolarity during tear breakup<sup>17</sup> could activate nociceptors, accounting for the pain subjects reported during STARE. In an animal study testing the effect of cooling and hyperosmolarity on corneal sensory nerves, Parra et al.<sup>38</sup> found that corneal polymodal nociceptors were activated by levels of 600 mOsm/kg or greater. It is also possible that transient spikes in tear film hyperosmolarity may lead to shrinkage of underlying ocular surface cells<sup>39</sup> and nerve endings.<sup>15,38</sup> Levin and Verkman<sup>40</sup> found that a nearly twofold drop in relative ocular surface cell volume occurs very rapidly, in less than 5 seconds, when the cellular perfusate was increased in osmolarity from 290 mOsm/kg to 590 mOsm/kg. High concentrations of Na and Cl ions may also modify the ion permeability of the surface membrane and the gating of voltage-dependent ion channels.<sup>38</sup> In addition, a number of inflammatory mediators released in response to ocular surface desiccation are known to sensitize nociceptors.<sup>15</sup> Some are included in an inflammatory “soup” that is used routinely to sensitize nociceptors in animal models.<sup>41,42</sup> Inflammatory mediators released locally are known to either directly or indirectly open ion channels, signaling cascades that open and sensitize TRPV1 and TRPA1 channels.<sup>43,44</sup>

There was a trend for cold thresholds to decrease after 30 minutes, although the difference did not reach statistical significance in this study. One reason may be the complex and variable stimulus likely to be posed by tear film breakup, which

may involve more than one mechanism,<sup>45–48</sup> including evaporation<sup>49</sup> and tangential flow of fluid.<sup>50,51</sup> In addition, we have reported increased irritation to nociceptive supra-threshold stimuli with a reduction in ratings of coolness to innocuous cooling stimuli following STARE. This may suggest alterations in both the nociceptive and innocuous cooling processing pathways,<sup>20</sup> which may point to a dynamic interaction between the complex stimulus likely to be posed by tear film instability and the responses of the corneal sensory nerves.

In contrast to the response of sensory thresholds, symptoms were highest immediately after STARE and then decreased after 30 minutes. Although both time periods were significantly different from pretesting, symptoms were higher immediately after testing, sometimes increasing as much as 40 points on the CSQ. This increase in symptoms immediately after repeated STARE has been shown in previous studies<sup>18,21,22</sup> and presumably reflects the direct stress to the ocular surface by tear breakup,<sup>12–14,16</sup> although it showed little relationship with changes in sensory thresholds in this study. Among the symptoms rated by subjects on the CSQ, the symptoms of discomfort and dryness were the two most highly rated symptoms immediately after STARE (data not shown). Cold thermoreceptors have been suggested as the cause of the symptom of dryness in dry eye patients,<sup>38,52,53</sup> but the cause of symptoms in this study of healthy subjects is unclear.

In this investigation, STARE was used as a human-based model to study the effects of tear film instability on the ocular surface in healthy, nondiseased subjects. Previously, we<sup>17–19,21,22,54</sup> have shown that tear breakup is highly correlated with increasing pain, described as cooling, pricking and stabbing, or burning and stinging,<sup>22</sup> providing indirect evidence for the level of stress to the ocular surface possible

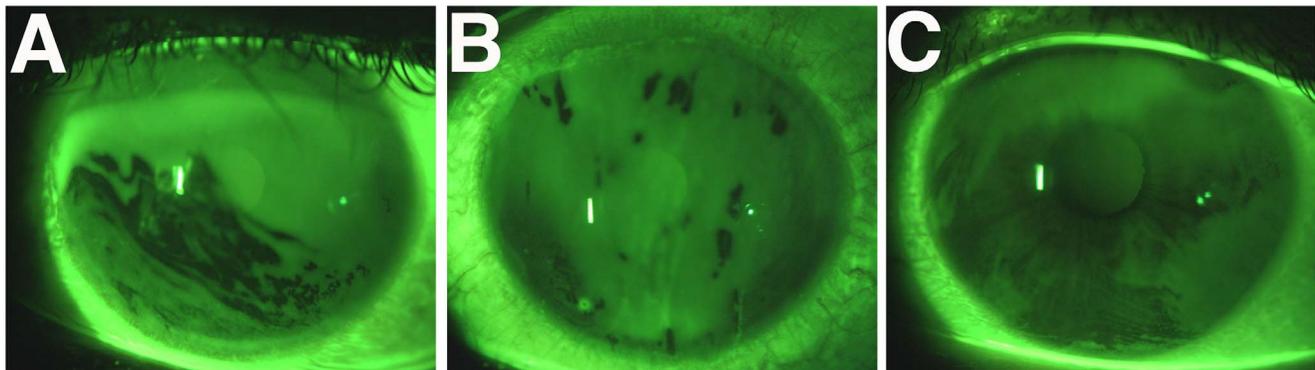


FIGURE 5. Examples of images from the last frame of videos of STARE trials. The analyzed area of tear film instability over the exposed cornea and the time of each trial was (A) 35% in 23 seconds, (B) 16% in 16 seconds, and (C) 65% in 19 seconds.

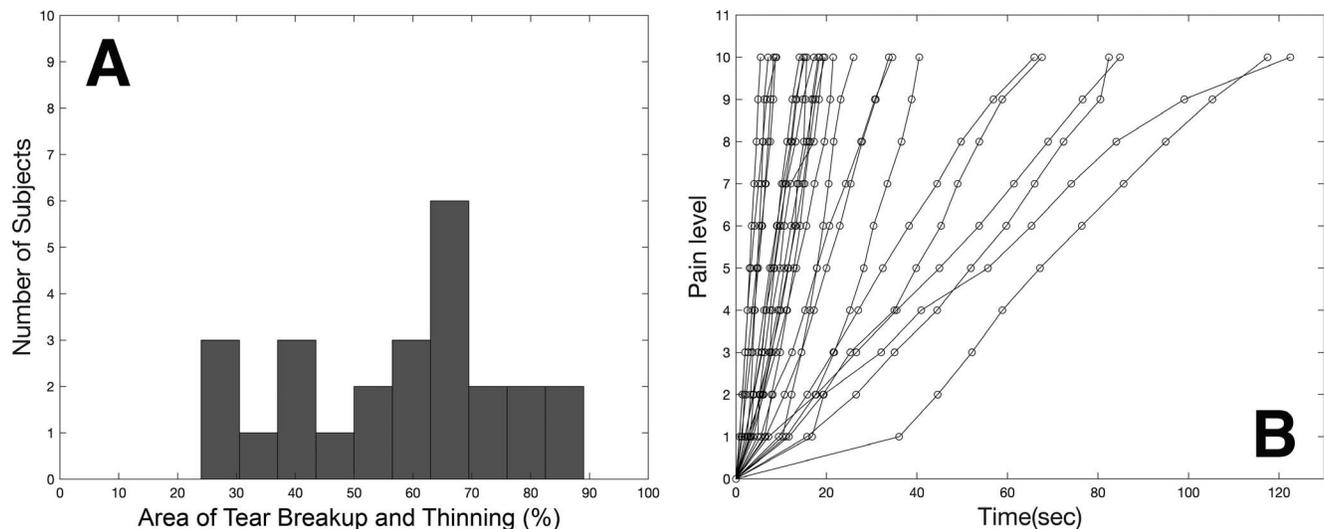


FIGURE 6. (A) Histogram of the percentage area of tear film breakup and thinning over the exposed corneal surface and (B) pain level over time for all subjects in visit 2.

with tear breakup. Wearing a soft contact lens appears to dampen the pain,<sup>18</sup> further implicating tear breakup as a likely cause. However, the STARE technique involves keeping the eye open as long as possible, so that it is conceivable that some other effect, such as the effort of keeping the tested eye open or the instillation of fluorescein dye, could have affected sensory thresholds.

Although STARE does not represent realistic blinking, it can be thought to provide a short-term model to study the effects of the repeated tear film breakup that may be occurring in dry eye patients over the day,<sup>48</sup> and perhaps even among healthy patients during reduced blinking while performing a visual task.<sup>55</sup> If these effects, even with much less marked tear breakup and normal blinking, are extended over the day, it may explain the symptoms of ocular irritation that are known to increase at the end of the day, even among healthy subjects.<sup>56</sup> Among dry eye subjects, this daily stress to the ocular surface by tear film instability could eventually lead to ocular surface epithelial and sensory damage alterations and eventually the downward spiral into the vicious circle of DED.<sup>12,14,16</sup>

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