Characterization by Belmonte's Gas Esthesiometer of Mechanical, Chemical, and Thermal Corneal Sensitivity Thresholds in a Normal Population

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Purpose. We used a prototype gas esthesiometer to measure corneal threshold sensitivity values for mechanical, chemical, and thermal stimuli. We also evaluated the reproducibility of the esthesiometer measurements, the influence of previous corneal symptoms, and the safety of this technique.

Methods. Forty healthy subjects participated in the study. Mechanical, chemical, and thermal (hot and cold) thresholds were determined at the center of the cornea using a prototype Belmonte's gas esthesiometer. To determine reproducibility of the results, the sensitivity thresholds were measured for each eye on 2 days. Corneal fluorescein staining and bulbar hyperemia after completion of the tests were analyzed.

Results. There were no differences for any sensitivity threshold between eyes or between the first and second esthesiometries. The reproducibilities of mechanical and hot thresholds were higher than for chemical and cold thresholds. Men had significantly higher chemical intensity thresholds than did women (men: 23.50 ± 5.10; women: 10.20 ± 2.16, $P = 0.021$). There were no alterations of the ocular surface after completion of the measurements.

Conclusions. Mechanical, chemical, and thermal corneal sensitivity thresholds in the central cornea have been established in healthy men and women of different age groups. The use of the Belmonte gas esthesiometer is safe and reproducible, with the highest reproducibility in determining mechanical and hot thresholds. (*Invest Ophthalmol Vis Sci.* 2012;53:3154–3160) DOI:10.1167/iovs.11-9304

The cornea is one of the most densely innervated tissues in human anatomy.[1–4] The principal function of the corneal nerve plexi is to preserve vision by ensuring the defense of the ocular surface against external attack. There are numerous eye conditions that can have a direct effect on corneal sensitivity, such as dry eye, conjunctivitis, uveitis, keratitis, contact lens wear, and corneal surgery (especially refractive surgery), among others.[5,6] Traditionally, clinical evaluation of corneal sensitivity has been performed with the Cochet-Bonnet esthesiometer that determines mechanical sensitivity by corneal contact.[7] Belmonte et al.[8] developed a noncontact gas esthesiometer to measure mechanical and chemical corneal sensitivities, and more recently thermal (hot and cold) sensitivity.[9] This new device allows exploration of different types of sensory fibers, such as mechanosensory fibers that respond to mechanical forces; polymodal nociceptive fibers that are the most prevalent and respond to a wide variety of stimuli, including mechanical forces, irritants, extreme temperatures, and endogenous inflammatory mediators; and cold fibers that are less abundant and activated mainly by decreases of temperature.[10–12] As a noncontact instrument, it avoids the risk of producing mechanical damage in hypoesthesic and fragile corneas (Hsu HY, et al. *IOVS* 2008;49:ARVO E-Abstract 1912), as can occur with contact esthesiometers.[13–16]

The Belmonte gas esthesiometer has been previously used to assess corneal sensitivity in different pathologies, such as dry eye[5,17–21] and diabetic and herpetic keratitis,[22,23] in contact lens wearers,[24–26] and to assess the recovery of corneal sensitivity after different types of refractive surgery.[27–30] In the present study, we used Belmonte's gas esthesiometer to establish corneal mechanical, chemical, and thermal sensitivity thresholds in healthy subjects. We also analyzed different aspects of this technique, such as reproducibility and safety, as well as the influence of other factors, such as the previous presence of dry eye symptoms.

Materials and Methods

Subjects

A total of 80 eyes in 40 healthy subjects were explored. To eliminate the biases of age and sex,[31] subjects were distributed in 5 balanced groups of 4 men and 4 women, each according to the following age ranges: 20 to 29, 30 to 39, 40 to 49, 50 to 59, and 60 years and older. The nature of the research and protocols were explained to each subject before written consent was obtained. The study complied with the tenets of the Declaration of Helsinki, and the protocol was approved by the institutional review board of the School of Medicine, University of Valladolid, Valladolid, Spain. Exclusion criteria included previous contact lens wear, any ocular or systemic disease, refractive spherical values over ±6.00 diopters, astigmatism higher than ±2.00 diopters, and use of any topical medications. To exclude subjects with dry eye disease, McMonnies test, Ocular Surface Disease Index (OSDI) questionnaire, tear breakup time (TBUT), Schirmer's tear test without anesthesia, phenol red thread test, and corneal and conjunctival fluorescein stainings were performed. The presence or absence of symptoms evaluated in McMonnies questionnaire[32] (secretions,

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grittiness, dryness, eyestrain, tearing, itching, and redness) was used for correlation analysis with corneal sensitivity thresholds.

Esthesiometry

Using a prototype Belmonte's gas esthesiometer,[8] the corneal thresholds for mechanical, chemical, and thermal (hot and cold) sensitivities were determined in the central cornea of both eyes of each subject on 2 different days, separated by a minimum of 24 hours and a maximum of 6 days[8]; the period of time between inclusion visit and the first esthesiometry was between 24 and 48 hours. Three-second air pulses of adjustable flow rate, composition (CO_2%), and temperature were applied to the center of the cornea for determining corneal sensitivity thresholds. Mechanical stimulation consisted of a series of variable flows of medicinal air (0–200 mL/min). Air was heated at the tip of the probe at 50°C so that it reached the ocular surface at 34°C to prevent a change in corneal temperature caused by the airflow.[8,9,33–35] For chemical threshold, we used a mixture of medicinal air with different concentrations of CO_2 (0%–90% CO_2, 99.8% purity), at 50°C at the tip of the probe and with a flow rate of 10 mL/min below mechanical threshold.[5,8] Thermal thresholds were determined by heating or cooling the air to produce changes in basal corneal temperature of ±0.1°C,[8,35] with a 10 mL/min flow below mechanical threshold.[5,8]

Threshold Measurements

The probe of the esthesiometer was mounted on a base adapted to a slit lamp. Subjects were instructed to look at a fixation target at 3 m, and the tip was placed perpendicular to the center of the cornea, 5 mm from the surface, measured with a transparent ruler.[5,8] Subjects were allowed to blink at will during the test, but were instructed to close and open their eyes just before launching the stimulus. Each eye remained opened during the 3 seconds of airflow, which was easily identified by the click produced by the opening and closing of a valve inside the probe.[5,8]

The mean temperature and humidity of the examination room were 22.17 ± 1.07°C and 36.27% ± 1.98%. All measurements were performed by the same operator between 3:30 PM and 8:00 PM to reduce diurnal variations.[24,36–38] All threshold levels were determined by using methods previously described.[8,17] On the first day of measurements, each subject was trained in intensity evaluation by exposing him or her to different intensities of each stimulus. During every procedure, pulses of zero flow were interspersed to detect false-positive results.[8] When a stimulus or a false stimulus was delivered, subjects were requested to verbally indicate the presence or absence of sensation and the degree of intensity. To avoid alignment errors between the tip of the esthesiometer and the center of the cornea, an auxiliary operator controlled the alignment constantly during all the procedure. The subject indicated verbally with a number between 0 and 100 the degree of sensation that was felt. The auxiliary operator recorded the current value indicated by the patient on a visual analogue scale from 0 to 100, for which 0 indicated the "absence of sensation" and 100 indicated "maximum sensation."[8,35,39]

The mechanical threshold was always determined first,[5,8] and the order in which chemical, heat, and cold thresholds were determined, as well as the starting eye, was randomized. To avoid biasing the subject with respect to the modality and intensity of the stimulus, the subjects were not informed of this information. With the exception of the chemical stimulus, 15-second pauses were given between pulses. To determine the chemical threshold, 2-minute pauses were given between CO_2 pulses to avoid chemical sensitization of polymodal nociceptors.[17,40] Each of the tests took 10 to 15 minutes per eye and were alternated between eyes. Thus, after a test, while the contralateral eye was being tested, the first eye rested. Afterward, the next test was applied to the first eye and so on.

Bulbar hyperemia and fluorescein corneal staining were evaluated and graded on a 0 to 4 scale[41] before and after the measurements to evaluate the safety of the technique.

Statistical Analysis

Data were expressed as means ± SEMs. Statistical analyses were performed using the Statistical Package for the Social Sciences software (SPSS 18.0 for Windows; SPSS Inc., Chicago, IL) and R software[42] by a licensed statistician (I.F.). *P* values of 0.05 or less were considered statistically significant. The Shapiro-Wilk test was used to check the assumption of normal data distribution. Differences of sensitivity thresholds were evaluated by Mann-Whitney *U* and Kruskal-Wallis tests for qualitative variables. Spearman Rho correlation coefficient was used for quantitative variables. To determine if there were differences between measurement days, a Wilcoxon signed-rank test was used.

Reproducibility was evaluated by analyzing variability between two measurements of the same individual on different days. Esthesiometry variability could be due to three variables: esthesiometry day, eye, and subjects and the interactions among them. Thus, the data were analyzed using these variables in a three-way ANOVA with three independent variables: day, eye, and the subject; and all three pairs of two-way interaction terms. It is expected that most sampling variations are due to the variability between subjects and their interactions with esthesiometry days and eyes. The reproducibility was quantified by the esthesiometry day variable. If the percentage of variability explained by the esthesiometry and its interactions with the other two variables was below 10%, then it was considered acceptable. If above 30%, it was considered unacceptable.

To assess the concordance between measurements, Bland-Altman analysis and the determination of the intraclass correlation coefficient (ICC) was performed. The ICC represents the proportion of the total variability due to the variability between subjects, and it takes values between 0 and 1. For ICC = 1, all of the observed variability would be attributed to differences between subjects and the different measurement days. For ICC = 0, the observed variability would be attributed to random effects. We classified the ICC values as follows: higher than 0.90, very good; 0.71 to 0.90, good; 0.51 to 0.70, moderate; 0.31 to 0.50, mediocre; less than 0.30, poor.[43] Bland-Altman plots represent the differences between two measurements and the mean of those measurements, with limits of agreement obtained from the 95% confidence interval of the difference of two measurements. For good agreement between the measurements, the average difference would be about 0. Such measurements would be within the limits of agreement with the data points not following any trend.

Results

Forty subjects (80 eyes) with a mean age of 44.1 ± 2.4 years were examined (Table 1). All of the ocular surface metrics,

Table 1. Clinical Characteristics of the Subjects

Metric	Value
Age,* y	44.1 ± 2.4 (range: 20–72)
OSDI	1.03 ± 0.31
McMonnies	5.53 ± 0.59
T-BUT, s	
OD	10.53 ± 0.62
OS	11.11 ± 0.72
Phenol red thread test, mm	
OD	26.26 ± 0.79
OS	26.50 ± 0.63
Schirmer's test, mm/5 min	
OD	18.00 ± 2.12
OS	16.97 ± 2.03

OSDI, Ocular Surface Dry-eye Index; T-BUT, tear breakup time; OD, right eye; OS, left eye.

* $n = 40$.

Table 2. Sensitivity Thresholds of the Central Cornea by Age Groups for the Right Eye in the First Esthesiometry

		Sensitivity Thresholds			
Age Groups	***n***	**Mechanical Threshold**	**Chemical Threshold**	**Hot Threshold**	**Cold Threshold**
20–29	8	58.26 ± 17.84	25.59 ± 11.70	1.56 ± 0.99	−1.32 ± 1.52
30–39	8	69.73 ± 19.83	24.80 ± 13.46	1.47 ± 1.17	−2.14 ± 1.27
40–49	8	56.44 ± 17.70	18.06 ± 5.31	1.25 ± 1.02	−1.65 ± 1.41
50–59	8	56.11 ± 26.93	14.93 ± 6.56	1.44 ± 0.96	−1.62 ± 1.31
≥ 60	8	56.20 ± 20.51	18.25 ± 9.52	1.39 ± 1.43	−1.65 ± 1.27
Mean ± SEM		59.35 ± 6.30	20.27 ± 3.01	1.42 ± 1.07	−1.68 ± 1.32

including dry eye questionnaires, TBUT, phenol red thread test, and Schirmer's test, were within the range of normal eyes. There were no significant differences in the esthesiometry values between right and left eyes of the same subject, or between esthesiometry values on the first and second visits. For this reason and to simplify the statistical analysis, only right eye data for the first esthesiometry are shown (Table 2).

For the mechanical threshold, the percentage of variability explained by the interactions of esthesiometry with the eye and the subject was 9.17% (Table 3). Thus, the reproducibility of the technique for the detection of this threshold was acceptable. Also, the effect of the eye and the subject was significant ($P = 0.024$ and $P = 0.001$, respectively). The concordance for this threshold, ICC = 0.77 (Table 3), was good. Bland-Altman analysis (Fig. 1A) showed that the values measured on the first and follow-up visits were centered on the "0" value, indicating that there was no clear trend for changes in the threshold between the first and second esthesiometry.

For the chemical sensitivity, the percentage of variability explained by esthesiometry and its interactions with the other variables was 18.06% (Table 3), a value intermediate between acceptable (<10%) and unacceptable (>30%). In this case, only the subject effect was significant ($P = 0.005$). The strength of concordance, ICC = 0.49, was mediocre. Bland-Altman analysis (Fig. 1B) showed that the measurements on the first and follow-up visits were centered on the "0" value in ranges between 0% and ±40%. Above this value, the differences were mostly negative, suggesting that values for the second esthesiometry were lower than the first.

For heat sensitivity, the percentage of variability explained by esthesiometry and its interactions with the other variables was 2.88% (Table 3), within the acceptable threshold. The only significant effect was associated with the subject ($P = 0.001$). The strength of concordance for this threshold, ICC = 0.64, was moderate. Bland-Altman analysis (Fig. 1C) showed that the values measured on the first and follow-up visits were centered on the "0" value, suggesting that there were no significant differences between measurements.

Table 3. Analysis of the Reproducibility and Concordance between Measurements

	Esthesiometry Variability, %	ICC	95% CI
Mechanical threshold	9.17	0.77	0.63–0.87
Chemical threshold	18.06	0.49	0.18–0.71
Thermal threshold–hot	2.88	0.64	0.42–0.79
Thermal threshold–cold	16.01	0.64	0.44–0.77

Esthesiometry variability was determined by ANOVA and represents the variability explained by the interactions of esthesiometry with other variables. CI, confidence interval; ICC, intraclass correlation coefficient.

The reproducibility for the cold threshold was questionable. The percentage of variability explained by esthesiometry and its interactions with the other variables was 16.01% (Table 3). The interaction effects of esthesiometry with the eye ($P = 0.012$), subject ($P = 0.001$), subject × eye interaction ($P = 0.027$), and esthesiometry × subject interaction ($P = 0.001$) were all significant. For this threshold, the strength of concordance, ICC = 0.64, was moderate. In the Bland-Altman analysis of changes between the initial and follow-up visits (Fig. 1D), the data were centered on the "0" value, again indicating that there was no clear trend for changes between the first and second esthesiometries. Greater differences between measurements were present in the intermediate thresholds taken between 20°C and 40°C, however.

Subjects with previous symptoms of gritty sensation had significantly lower mechanical thresholds ($n = 5$, 26.00 ± 10.17 mL/min, Fig. 2A) than those without ($n = 35$; 64.11 ± 6.70 mL/min; $P < 0.05$). Subjects with previous symptoms of red eyes had significantly lower chemical thresholds ($n = 15$, 13.12% ± 2.58% CO_2; Fig. 2B) than those without ($n = 25$, 32.89% ± 5.37% CO_2; $P = 0.011$). Subjects experiencing itching had lower chemical thresholds; however, the difference was not significant. There were no differences for thermal thresholds for any of the symptoms studied.

When a chemical stimulus was delivered, the subjective intensity perceived, indicated by the patient on the visual analogue scale, was significantly higher in men than in women (men: 23.50 ± 5.10; women: 10.20 ± 2.16; $P = 0.021$). There were no significant differences for the other thresholds.

The total duration of the tests for both eyes in the first esthesiometry session was 60.8 ± 2.6 minutes and 58.1 ± 2.1 minutes in the second. The range of the number of stimuli needed to determine all sensitivity thresholds varied between 6.6 ± 0.4 pulses for chemical threshold and 8.8 ± 0.6 pulses for mechanical threshold. There were no significant differences in the duration of the tests or the number of stimuli needed to reach the threshold between the two measurements. Nevertheless, the data showed a trend toward decreased test durations.

To evaluate the safety of the technique, corneal staining and hyperemia after esthesiometry measurements were compared with those values obtained during the initial screening visit. There were no significant differences between these values for either first or second esthesiometry sessions.

Discussion

The Cochet-Bonnet esthesiometer is the most common instrument used to evaluate corneal sensitivity[7,44]; however, the procedure is invasive and explores only the corneal mechano-nociceptors. The development of noncontact esthesiometers,[8,45,46] such as Belmonte's gas esthesiometer,[8] allows the exploration of different corneal sensory fibers in a

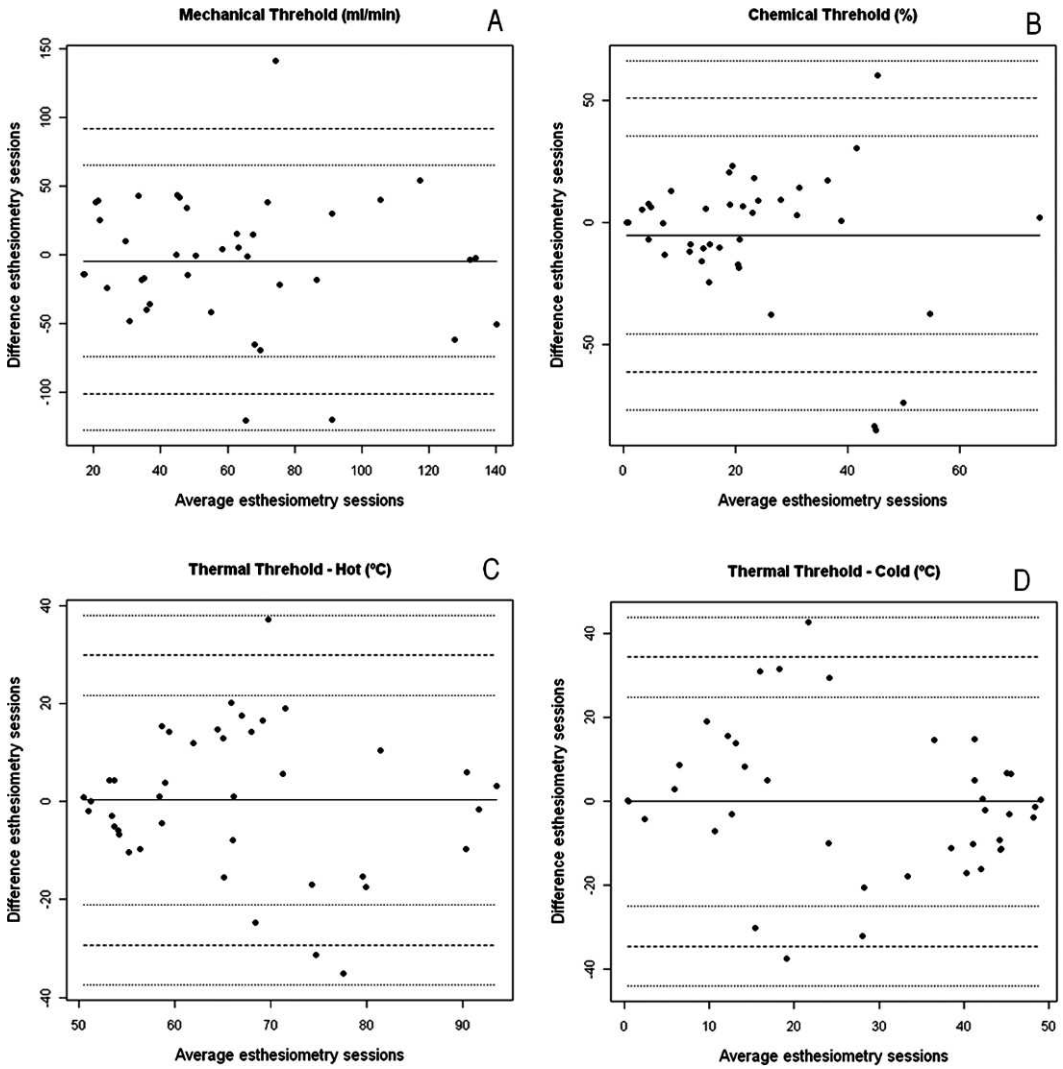

Figure 1. Bland-Altman analysis for mechanical (**A**), chemical (**B**), thermal-hot (**C**), and thermal-cold (**D**) sensitivity thresholds. These types of graphs represent the differences between two esthesiometries against their mean. The x-axis represents the mean of the measurements for each subject at the initial and follow-up visit. The y-axis represents the difference between the two esthesiometries for each subject. *Dashed lines*, limits of agreement; *dotted lines*, 95% confidence interval for the limits of agreement.

noninvasive way, and thus provides a greater amount of information.

In this study, we used a prototype Belmonte gas esthesiometer to establish threshold values of corneal sensitivity for mechanical, chemical, and thermal stimuli in the central cornea for a healthy population that covered a wide range of ages and both sexes. The resulting data were repeatable and the methodology was safe.

The sensitivity thresholds obtained in our study of healthy subjects were slightly lower than data from previous reports with Belmonte's gas esthesiometer.[8,9,17,47] Studies using different esthesiometers report different results. These discrepancies could be because of differences in the procedures or in the characteristics of the instrument used, such as units of measurement,[25,29,45,46] the stimuli,[48] diameters of the probes,[24,49] distances to the ocular surface,[17,24,49] pulse stimulus durations,[24,44,46,49] and time of the day when measurements were taken.[5,9,25]

Most studies report that corneal sensitivity decreases with age[5,22,31,50–53]; however, this is not a universal finding.[17,47,53] Also, sex is a variable that can influence in the sensitivity thresholds.[5,17,31,50,52] We have designed this study with age and sex balance to avoid these effects on the variables studied.

Over intervals of 1 to 6 days, we measured the reproducibility of the esthesiometry for all sensitivity thresholds. All experiments were performed during the same hour range to minimize the effects of diurnal variations described by previous authors.[24,36–38] The reproducibility of mechanical threshold measurements using noncontact esthesiometers has been previously reported[46,49]; however, to the best of our knowledge, this is the first study of the reproducibility of Belmonte's gas esthesiometer for all of the corneal sensitivity thresholds at the same time (i.e., mechanical, chemical, thermal-hot, and thermal-cold). We found no differences between first and second esthesiometry sessions, with the mechanical threshold having the highest ICC and the chemical threshold having the lowest. Thus, there is good concordance between measurements taken on different days and for both eyes of the same individual, as others have shown.[24,35,46,47,49] For some diagnostic techniques, like perimetry, there is a well-known learning factor effect.[54–56] We did not find any difference between days, however, so this technique may be relatively insensitive to any previous experience or training.

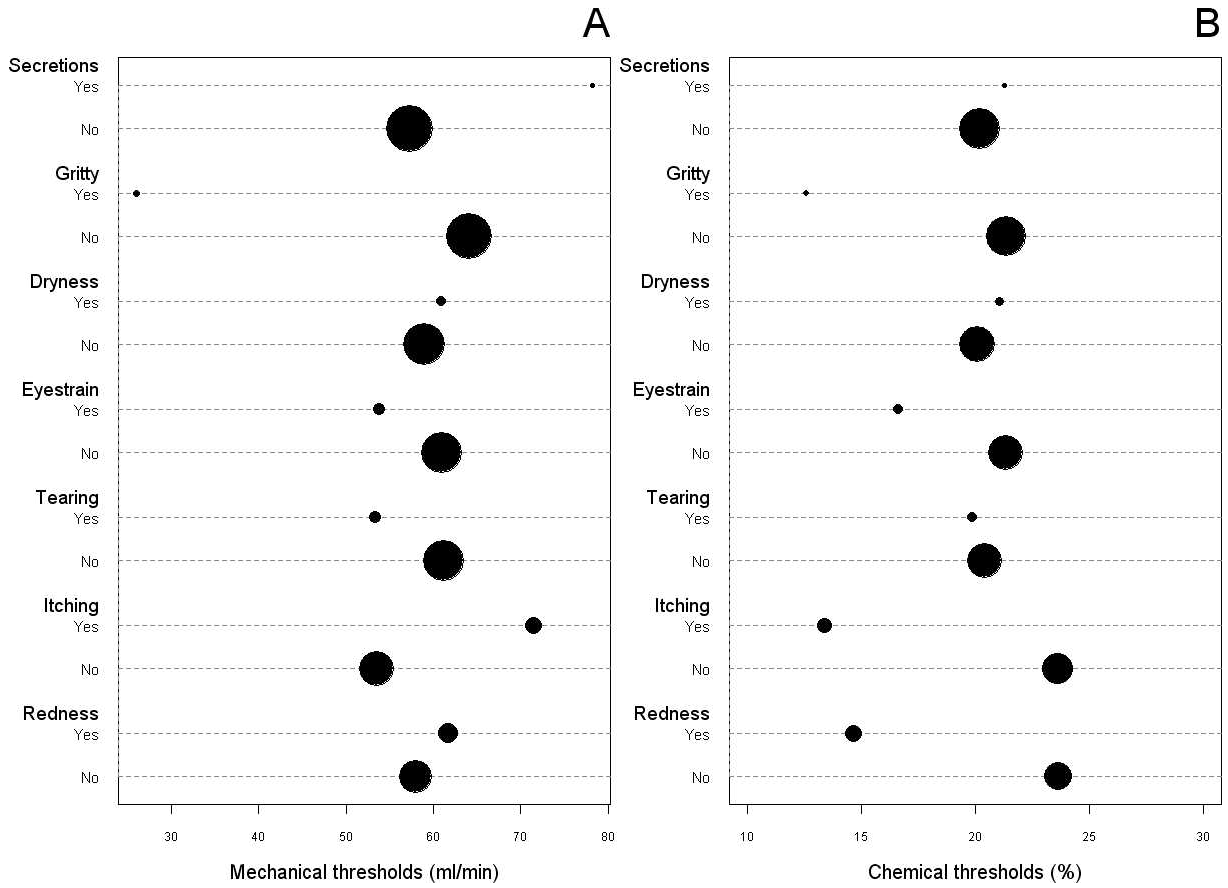

Figure 2. Sensitivity for mechanical and chemical thresholds as functions of previous symptoms. The x-axis represents the mean of (**A**) mechanical and (**B**) chemical thresholds and the size of each bubble corresponds to the number of subjects referring the symptom during the threshold measurements.

Further studies are needed to assess interday variations over at least 3 consecutive days to assess whether or not these tests have a learning factor.

Previous studies have shown that subjects with dry eyes have thresholds for mechanical, chemical, and thermal stimulation significantly higher than healthy subjects[5,20]; however, another study reported the opposite.[17] Recently, Chen and Simpson[57] evaluated contact lens wearers with and without symptoms of dry eye and did not find differences between the two groups in the mechanical threshold. Our study is the first that evaluated the impact of dry eye symptoms on corneal sensitivity thresholds in healthy subjects. We found that the presence of grittiness and redness symptoms were associated with a decrease of mechanical and chemical sensitivity thresholds respectively. Several reports suggest that corneal hypersensitivity may be associated with ocular surface inflammation. Inflammatory mediators, such as IL-1 and prostaglandin E2, have been reported to produce hyperesthesia.[58,59] Also, prostaglandins produced by arachidonic acid metabolism contribute to local inflammatory reaction, sensitization, and excitation of nerve endings.[39] It is possible that in a preclinical stage of dry eye development, the inflammation is responsible for generating the hyperesthesia and the presence of symptoms without any other ocular surface alterations. The sustained inflammation over time could then generate clinical manifestations, such as alteration of the epithelium, resulting in the onset of symptoms. The changes could finally cause overt dry eye disease and corneal hypoesthesia, a hypothesis that is consistent with one suggested recently by Situ et al.[18] This raises the possibility that the symptoms may be the result of an increase of sensitivity of the sensory receptors caused by the presence of inflammatory mediators. The response mechanisms then generate tear film instability and disruption of the ocular surface.

We also studied the safety of the technique. No alterations of the ocular surface with respect to conjunctival hyperemia and corneal fluorescein staining were detected in any of the subjects. These results support previous studies and corroborate the safety of this technique.[8]

The development of a comprehensive database of corneal sensitivity thresholds in the healthy population is essential to know the impact of corneal sensitivity on ocular surface pathologies. Further investigations are needed to determine which factors influence corneal sensitivity in healthy subjects.

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References

1. de Castro F, Silos-Santiago I, López de Armentia M, Barbacid M, Belmonte C. Corneal innervation and sensitivity to noxious stimuli in trkA knockout mice. *Eur J Neurosci*. 1998;10:146–152.

2. Müller LJ, Marfurt CF, Kruse F, Tervo TM. Corneal nerves: structure, contents and function [published correction appears in *Exp Eye Res*. 2003;77:253]. *Exp Eye Res*. 2003;76: 521–542.
3. Rózsa AJ, Beuerman RW. Density and organization of free nerve endings in the corneal epithelium of the rabbit. *Pain*. 1982;14:105–120.
4. Felipe CD, Gonzalez GG, Gallar J, Belmonte C. Quantification and immunocytochemical characteristics of trigeminal ganglion neurons projecting to the cornea: effect of corneal wounding. *Eur J Pain*. 1999;3:31–39.
5. Bourcier T, Acosta MC, Borderie V, et al. Decreased corneal sensitivity in patients with dry eye. *Invest Ophthalmol Vis Sci*. 2005;46:2341–2345.
6. Zorab EC. Corneal sensitivity after grafting. *Proc R Soc Med*. 1971;64:117–118.
7. Cochet P, Bonnet R. L'esthésie cornéenne. Sa mesure clinique. Ses variations physiologiquees et pathologiques. *La Clinique Ophtalmol*. 1960;4:3–27.
8. Belmonte C, Acosta MC, Schmelz M, Gallar J. Measurement of corneal sensitivity to mechanical and chemical stimulation with a CO2 esthesiometer. *Invest Ophthalmol Vis Sci*. 1999; 40:513–519.
9. Acosta MC, Tan ME, Belmonte C, Gallar J. Sensations evoked by selective mechanical, chemical, and thermal stimulation of the conjunctiva and cornea. *Invest Ophthalmol Vis Sci*. 2001; 42:2063–2067.
10. Chen X, Belmonte C, Rang HP. Capsaicin and carbon dioxide act by distinct mechanisms on sensory nerve terminals in the cat cornea. *Pain*. 1997;70:23–29.
11. Giraldez F, Geijo E, Belmonte C. Response characteristics of corneal sensory fibers to mechanical and thermal stimulation. *Brain Res*. 1979;177:571–576.
12. Belmonte C, Giraldez F. Responses of cat corneal sensory receptors to mechanical and thermal stimulation. *J Physiol*. 1981;321:355–368.
13. Martin XY, Safran AB. Corneal hypoesthesia. *Surv Ophthalmol*. 1988;33:28–40.
14. Millodot M, Owens H. Sensitivity and fragility in keratoconus. *Acta Ophthalmol (Copenh)*. 1983;61:908–917.
15. Rao GN, John T, Ishida N, Aquavella JV. Recovery of corneal sensitivity in grafts following penetrating keratoplasty. *Ophthalmology*. 1985;92:1408–1411.
16. Mathers WD, Jester JV, Lemp MA. Return of human corneal sensitivity after penetrating keratoplasty. *Arch Ophthalmol*. 1988;106:210–211.
17. De Paiva CS, Pflugfelder SC. Corneal epitheliopathy of dry eye induces hyperesthesia to mechanical air jet stimulation. *Am J Ophthalmol*. 2004;137:109–115.
18. Situ P, Simpson TL, Fonn D, Jones LW. Conjunctival and corneal pneumatic sensitivity is associated with signs and symptoms of ocular dryness. *Invest Ophthalmol Vis Sci*. 2008; 49:2971–2976.
19. Velasco MJ, Bermúdez FJ, Romero J, Hita E. Variations in corneal sensitivity with hydrogel contact lenses. *Acta Ophthalmol (Copenh)*. 1994;72:53–56.
20. Benítez-Del-Castillo JM, Acosta MC, Wassfi MA, et al. Relation between corneal innervation with confocal microscopy and corneal sensitivity with noncontact esthesiometry in patients with dry eye. *Invest Ophthalmol Vis Sci*. 2007;48:173–181.
21. Tuisku IS, Konttinen YT, Konttinen LM, Tervo TM. Alterations in corneal sensitivity and nerve morphology in patients with primary Sjögren's syndrome. *Exp Eye Res*. 2008;86:879–885.
22. Murphy PJ, Patel S, Kong N, Ryder RE, Marshall J. Noninvasive assessment of corneal sensitivity in young and elderly diabetic and nondiabetic subjects. *Invest Ophthalmol Vis Sci*. 2004;45: 1737–1742.
23. Gallar J, Tervo TM, Neira W, et al. Selective changes in human corneal sensation associated with herpes simplex virus keratitis. *Invest Ophthalmol Vis Sci*. 2010;51:4516–4522.
24. Stapleton F, Tan ME, Papas EB, et al. Corneal and conjunctival sensitivity to air stimuli. *Br J Ophthalmol*. 2004;88:1547–1551.
25. Murphy PJ, Patel S, Marshall J. The effect of long-term, daily contact lens wear on corneal sensitivity. *Cornea*. 2001;20: 264–269.
26. Situ P, Simpson TL, Jones LW, Fonn D. Effects of silicone hydrogel contact lens wear on ocular surface sensitivity to tactile, pneumatic mechanical, and chemical stimulation. *Invest Ophthalmol Vis Sci*. 2010;51:6111–6117.
27. Gallar J, Acosta MC, Moilanen JA, Holopainen JM, Belmonte C, Tervo TM. Recovery of corneal sensitivity to mechanical and chemical stimulation after laser in situ keratomileusis. *J Refract Surg*. 2004;20:229–235.
28. Tuisku IS, Lindbohm N, Wilson SE, Tervo TM. Dry eye and corneal sensitivity after high myopic LASIK. *J Refract Surg*. 2007;23:338–342.
29. Murphy PJ, O'Brart DP, Stephenson CG, Oliver KM, Patel S, Marshall J. Effect of hyperopic photorefractive keratectomy on corneal sensitivity: a longitudinal study. *J Refract Surg*. 2003; 19:34–39.
30. Murphy PJ, Corbett MC, O'Brart DP, Verma S, Patel S, Marshall J. Loss and recovery of corneal sensitivity following photorefractive keratectomy for myopia. *J Refract Surg*. 1999;15: 38–45.
31. Acosta MC, Alfaro ML, Borrás F, Belmonte C, Gallar J. Influence of age, gender and iris color on mechanical and chemical sensitivity of the cornea and conjunctiva. *Exp Eye Res*. 2006; 83:932–938.
32. McMonnies CW, Ho A. Responses to a dry eye questionnaire from a normal population. *J Am Optom Assoc*. 1987;58:588–591.
33. Efron N, Young G, Brennan NA. Ocular surface temperature. *Curr Eye Res*. 1989;8:901–906.
34. Chen X, Gallar J, Pozo MA, Baeza M, Belmonte C. CO2 stimulation of the cornea: a comparison between human sensation and nerve activity in polymodal nociceptive afferents of the cat. *Eur J Neurosci*. 1995;7:1154–1163.
35. Acosta MC, Belmonte C, Gallar J. Sensory experiences in humans and single-unit activity in cats evoked by polymodal stimulation of the cornea. *J Physiol*. 2001;534:511–525.
36. Millodot M. Diurnal variation of corneal sensitivity. *Br J Ophthalmol*. 1972;56:844–847.
37. Millodot M, O'Leary DJ. Effect of oxygen deprivation on corneal sensitivity. *Acta Ophthalmol (Copenh)*. 1980;58:434–439.
38. du Toit R, Vega JA, Fonn D, Simpson T. Diurnal variation of corneal sensitivity and thickness. *Cornea*. 2003;22:205–209.
39. Acosta MC, Berenguer-Ruiz L, García-Gálvez A, Perea-Tortosa D, Gallar J, Belmonte C. Changes in mechanical, chemical, and thermal sensitivity of the cornea after topical application of nonsteroidal anti-inflammatory drugs. *Invest Ophthalmol Vis Sci*. 2005;46:282–286.
40. Belmonte C, Gallar J, Pozo MA, Rebollo I. Excitation by irritant chemical substances of sensory afferent units in the cat's cornea. *J Physiol*. 1991;437:709–725.
41. Efron N, Morgan PB, Jagpal R. Validation of computer morphs for grading contact lens complications. *Ophthalmic Physiol Opt*. 2002;22:341–349.
42. R Development Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing; 2010. Available at: http://www.R-project.org/.

43. Fleiss JL. The design and analysis of clinical experiments. In: *Reliability of Measurement*. New York, NY: John Wiley & Sons; 1986:1–32.
44. Millodot M, Larson W. New measurements of corneal sensitivity: a preliminary report. *Am J Optom Arch Am Acad Optom*. 1969;46:261–265.
45. Murphy PJ, Patel S, Marshall J. A new non-contact corneal aesthesiometer (NCCA). *Ophthalmic Physiol Opt*. 1996;16: 101–107.
46. Vega JA, Simpson TL, Fonn D. A noncontact pneumatic esthesiometer for measurement of ocular sensitivity: a preliminary report. *Cornea*. 1999;18:675–681.
47. Golebiowski B, Papas E, Stapleton F. Corneal mechanical sensitivity measurement using a staircase technique. *Ophthalmic Physiol Opt*. 2005;25:246–253.
48. Situ P, Simpson TL, Jones LW, Fonn D. Conjunctival and corneal hyperesthesia in subjects with dryness symptoms. *Optom Vis Sci*. 2008;85:867–872.
49. Murphy PJ, Lawrenson JG, Patel S, Marshall J. Reliability of the non-contact corneal aesthesiometer and its comparison with the Cochet-Bonnet aesthesiometer. *Ophthalmic Physiol Opt*. 1998;18:532–539.
50. Jalavisto E, Orma E, Tawast M. Ageing and relation between stimulus intensity and duration in corneal sensibility. *Acta Physiol Scand*. 1951;23:224–233.
51. Boberg-Ans J. On the corneal sensitivity. *Acta Ophthalmol (Copenh)*. 1956;34:149–162.
52. Millodot M. The influence of age on the sensitivity of the cornea. *Invest Ophthalmol Vis Sci*. 1977;16:240–242.
53. Millodot M, Owens H. The influence of age on the fragility of the cornea. *Acta Ophthalmol (Copenh)*. 1984;62:819–824.
54. Pierre-Filho Pde T, Gomes PR, Pierre ET, Pierre LM. Learning effect in visual field testing of healthy subjects using Humphrey Matrix frequency doubling technology perimetry. *Eye (Lond)*. 2010;24:851–856.
55. Horani A, Frenkel S, Yahalom C, Farber MD, Ticho U, Blumenthal EZ. The learning effect in visual field testing of healthy subjects using frequency doubling technology. *J Glaucoma*. 2002;11:511–516.
56. Heijl A, Lindgren G, Olsson J. The effect of perimetric experience in normal subjects. *Arch Ophthalmol*. 1989;107: 81–86.
57. Chen J, Simpson TL. A role of corneal mechanical adaptation in contact lens-related dry eye symptoms. *Invest Ophthalmol Vis Sci*. 2011;52:1200–1205.
58. Taiwo YO, Goetzl EJ, Levine JD. Hyperalgesia onset latency suggests a hierarchy of action. *Brain Res*. 1987;423:333–337.
59. Ferreira SH, Lorenzetti BB, Bristow AF, Poole S. Interleukin-1 beta as a potent hyperalgesic agent antagonized by a tripeptide analogue. *Nature*. 1988;334:698–700.