Noninvasive Assessment of Corneal Sensitivity in Young and Elderly Diabetic and Nondiabetic Subjects

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Purpose. To investigate the effect of age and diabetes on corneal sensitivity using the noncontact corneal aesthesiometer (NCCA).

Methods. One hundred sixteen nondiabetic subjects and 111 diabetic subjects (33 type I and 78 type II) were recruited and divided into three age groups: Young (≤29 years), Middle (30–59 years) and Older (≥60 years). The exclusion criteria included patients with severe retinopathy requiring treatment, a history of invasive ocular surgery, or a history of conditions known to affect corneal sensitivity. The corneal cooling sensation threshold, for the right eye of each subject, was assessed with a double-staircase method-of-limits technique with the NCCA. This instrument uses a controlled pulse of air to produce a small, localized reduction in the surface temperature of the eye, which is detected by the nerves in the corneal epithelium.

Results. Analysis of the scatterplot of each subject's central cooling sensation threshold revealed a gradual loss of sensitivity with increasing age (nondiabetic, $r^2 = 0.349$; diabetic, $r^2 = 0.131$). Within the nondiabetic group, inter–age-group comparisons found significant differences between the central corneal cooling sensation thresholds for the three age groups (*t*-test, $P < 0.01$). Within the diabetic group, a significant difference was found between the Middle and Older categories only (*t*-test, $P < 0.05$). In summary, the Young group was more sensitive than the Middle group, which was more sensitive than the Older group. Within both type I and type II diabetic subjects, there was neither a significant relationship between duration of the disease and corneal sensitivity (*t*-test, $P > 0.05$) nor a gender-based difference (*t*-test, $P > 0.05$).

Conclusions. There is a gradual reduction in corneal sensitivity with increasing age in both nondiabetic subjects and diabetic subjects, along with an increasing variation in the measured threshold. There is no relationship between corneal sensitivity and the time since diagnosis of diabetes for a thermally cooling stimulus, suggesting that the A∂ and C fibers of the corneal innervation are affected differently by abnormal glucose metabolism in the diabetic cornea. (*Invest Ophthalmol Vis Sci.* 2004;45:1737–1742) DOI:10.1167/iovs.03-0689

The cornea is innervated by a densely arranged network of fine nerve endings that are located primarily in the epithelial layer and are supplied by the long ciliary nerves, derived from the trigeminal nerve (cranial nerve V). The long ciliary nerves produce a circular limbal plexus in the eye, from which 70 to 80 nerve trunks enter the corneal stroma, to a depth of 150 μm.[1] The trunks contain 900 to 1200 myelinated and unmyelinated axons (diameter 0.5–5 μm), and these ramify to produce a poorly characterized plexus beneath Bowman's layer. Anteriorly directed nerve fibers then enter the epithelium, at an estimated 400 sites,[2] where they combine with peripheral nerves that have entered directly from the limbus. A further plexus is then formed at the epithelial basal cell layer.[3]

From this basal cell layer plexus, nerve fibers ramify anteriorly toward the epithelial surface. The fibers are of two main types, and they are arranged within the epithelium according to their type: myelinated A∂ fibers that run parallel to the corneal surface within the basal cell layer and unmyelinated C fibers that turn upward from the epithelial plexus toward the surface.[4] A∂ fibers are large-diameter, straight nerves that respond primarily to mechanical stimuli, whereas C fibers are small-diameter, beaded nerves that respond to thermal and chemical stimuli.[5–9] The nerves can also be classified according to the mode of stimulation to which they respond. There are A∂ fibers that are mechanosensory receptors that respond to mechanical stimulation; A∂ fibers that mechanoheat receptors that have a high mechanical threshold and respond to heat; polymodal receptors that can be either A∂ or C fibers and respond to mechanical, thermal, and chemical stimuli; and cold receptors that are C fibers and respond to cooling stimuli.[10–12]

Extensive branching of the nerve fibers produces large receptive fields for each sensory axon. Because these fields overlap, a single stimulus will stimulate many receptive fields. This results in a lack of stimulus localization, but produces an exquisite level of sensitivity to external stimuli. The nerves also perform a role in the maintenance of a healthy epithelium,[13] and any alteration in their function may have a detrimental effect on the eye.

The sensitivity of the corneal epithelial nerves to external stimulation is known to vary with several physiological factors. Previous studies have shown an effect in nondiabetic subjects of diurnal variation, environment, iris color, and corneal test location.[14–18] The effect of age and gender in nondiabetic subjects has also been investigated. Millodot[19] found only a slight decline in corneal sensitivity up to the age of 50 years, and a more significant reduction to half that level by 65 years. Age-related changes are also observed in other aspects of ocular health and vision. The most obvious effects are the development of arcus senilis in the cornea, cataract formation, and the onset of presbyopia. In sensory terms, there is a general reduction in sensitivity for hearing, taste, and smell with age. More relevant for this study is the reduced cutaneous sensitivity to tactile and vibrotactile stimuli in the elderly.[20,21] For

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example, tactile thresholds on the palmar surface of the finger are two to three times higher in older subjects than in younger subjects.[22]

Diabetic retinopathy keratoepitheliopathy and can develop in patients with diabetes mellitus. It can manifest as superficial epithelial defects, erosion, or endothelial abnormalities.[23–25] A decrease in corneal sensitivity,[26–32] in association with reduced tear secretion,[33,34] increased fragility,[35,36] and irregular junctions between corneal epithelial cells,[37] is a possible change that leads to keratoepitheliopathy. Reviewing the literature, we found only one report that contradicts the popular view that corneal sensitivity is reduced in diabetes mellitus. O'Donnell et al.[38] did not find a significant difference in corneal sensitivity between diabetic and nondiabetic subjects. Could any change in corneal sensitivity in diabetes mellitus be related to the duration of the disease or extent of any accompanying retinopathy? The effort to answer this question has been fraught with uncertainty. According to Saini and Khandalavla,[35] corneal sensitivity is up to three times greater in diabetic subjects without retinopathy than in those with proliferative retinopathy. Complications of the cornea, including reduced sensitivity, are not uncommon after vitrectomy in advanced stages of retinopathy.[39,40] However, Inoue et al.[32] did not uncover any association between corneal sensitivity and the extent of retinopathy. Furthermore, they found no correlation between corneal sensitivity and age in their diabetic subjects.

Most studies on corneal sensation in nondiabetic subjects and diabetic subjects have used the Cochet-Bonnet aesthesiometer, or a similar instrument, to stimulate the corneal nerves by direct mechanical contact (A∂ fibers). This method has several drawbacks, primarily caused by a restricted stimulus intensity range and a tendency to produce epithelial cell damage as a result of the invasive stimulus thread. The lack of a low-intensity stimulus has a major detrimental effect on the ability of the instrument to detect subtle changes.

Millodot,[19] using the Cochet-Bonnet aesthesiometer, was unable to locate the true mechanical sensation threshold baseline in his younger subjects and so masked any gradual change in sensitivity that might occur through these earlier years. Clearly, it is important to establish whether there is significant variation in corneal sensitivity with age when selecting subjects for comparative studies.

The noncontact corneal aesthesiometer (NCCA) is a recently developed alternative to the Cochet-Bonnet aesthesiometer.[41] This instrument uses a controlled pulse of air to stimulate the corneal nerves, and so its mode of action is very different from the invasive mechanical stimulus of the Cochet-Bonnet aesthesiometer. The NCCA does not directly touch the cornea; rather, the air pulse produces a localized cooling of the precorneal tear film, by evaporative heat loss, and this change in surface temperature is transferred by conduction to the corneal epithelium. The ocular surface cooling produced by the air pulse has been visualized using thermal imaging.[42,43] The temperature-sensitive C-fibers within the epithelium detect this change, and the subject experiences only a cool sensation. The sensation experienced by the subject also lends support to the temperature change's being the stimulus modality for the NCCA air pulse. If there were a mechanical component, the subject would experience irritation or discomfort. The instrument has been used previously in other studies of corneal sensitivity after anesthesia, contact lens wear, and refractive surgery, and, unlike the Cochet-Bonnet, has a full range of stimulus intensities.[44–51]

The NCCA is capable of measuring corneal sensitivity beyond the limits of the Cochet-Bonnet aesthesiometer. We found that the NCCA was capable of detecting minor differences between hypersensitive subjects that were missed by the Cochet-Bonnet device.[51] The age effect on corneal sensitivity and the decrease associated with diabetes could be manifestations of changes in A∂, C, or both types of fiber activity. Because the Cochet-Bonnet device quantifies A∂ function, we can say, aging and diabetes affect A∂ fibers. However, we cannot be certain whether C fiber activity is similarly affected. Using the NCCA we should be able to determine whether age and/or diabetes affects C fiber function.

This article reports on the results of two studies that assessed the effect of age and diabetes on corneal sensitivity using the NCCA. These studies will benefit from the full stimulus intensity range of the NCCA to clarify any variation in corneal sensitivity that occurs from these variables.

Methods

Procedure

The corneal cooling sensation threshold was measured with the NCCA on one eye of each subject, by using the previously described yes-no response, double-staircase procedure at the center of the cornea.[41] Subjects were presented with a stimulus and asked whether they felt it. Subjects were required to give an answer for each presentation, and if they were unsure, they were instructed to answer no (not felt). Sham presentations were used to monitor subject responses. An initial stimulus above threshold was presented to the subject to demonstrate the sensation experienced, and then sufficient stimuli of descending and ascending intensity were presented to locate the sensation threshold. Initial stimulus intensity steps were 0.10 millibars, and this was refined to 0.05-millibar steps, once the threshold had been located, to increase refinement. All measurements were taken in the afternoon at approximately the same time, to avoid any diurnal bias. One investigator measured corneal sensitivity within the nondiabetic group at one site, and another investigator measured corneal sensitivity within the diabetic group at another site.

Subjects

Nondiabetic Group. A total of 116 healthy, non–contact lens–wearing subjects were recruited and divided into three broad groups according to age: Young (≤29 years), Middle (30–59 years), and Older (≥60 years). The Young group had 69 subjects (mean age, 22.7 years; range, 19–29), the Middle group had 30 subjects (mean age, 41.8 years; range, 30–59), and the Older group had 20 subjects (mean age, 68.9 years; range, 60–80).

Diabetic Group. A total of 111 diabetic subjects were recruited (mean age 55.8 years, range 22–78) consisting of 51 women (mean age, 54.8 years; range, 24–78) and 60 males (mean age, 55.6 years; range, 22–76). Thirty-three were type I diabetic subjects, the remainder were type II.

All subjects were patients attending a routine multidisciplinary diabetes outpatient clinic. Corneal sensitivity was measured only after the procedure and the reason for the study were fully explained and a signed consent obtained in accordance with Ethics Committee requirements. The study protocol complied with the tenets of the Declaration of Helsinki. The exclusion criteria included severe diabetic retinopathy requiring treatment, any previous history of invasive ocular surgery, or a history of conditions that could affect corneal sensitivity (e.g., corneal trauma).

Results

Effect of Age

The mean (± SD) corneal cooling sensation thresholds for each subject group, and each age group, are given in Table 1. Using the Shapiro-Wilk test for normality, we found the distributions

TABLE 1. Corneal Cooling Sensation Thresholds (Mean ± SD: Millibars) for Each Age Group, at the Center of the Cornea

	Central Corneal Cooling Sensation Threshold		Log Central Corneal Cooling Sensation Threshold	
Age Group	**Nondiabetics**	**Diabetics**	**Nondiabetics**	**Diabetics**
Young	0.58 ± 0.25 (n = 69)	0.65 ± 0.38 (n = 5)	−0.46 ± 0.13 (n = 69)	−0.24 ± 0.24 (n = 5)
Middle	0.81 ± 0.56 (n = 30)	0.75 ± 0.42 (n = 54)	−0.15 ± 0.21 (n = 30)	−0.19 ± 0.24 (n = 54)
Older	1.53 ± 0.80 (n = 20)	0.99 ± 0.45 (n = 52)	0.13 ± 0.24 (n = 20)	−0.05 ± 0.21 (n = 52)

of the corneal sensitivity measurements to be skewed. However, a natural log transformation of the data was normally distributed, and so all sensitivity data were transformed in this manner to allow the use of parametric statistical analysis.[52,53] The mean (± SD) log-transformed corneal cooling sensation thresholds are therefore included in Table 1. Figures 1 and 2 show the scatterplots of the transformed data for the nondiabetic subjects and diabetic subjects, respectively.

A power analysis was completed assuming unequal sample sizes, equal variance: $\alpha \leq 0.05$, $\beta \leq 0.20$. The power results were nondiabetic subjects/diabetic subjects, 0.84; nondiabetic subjects: Young/Middle, 0.94; Middle/Older, 0.93; Young/Older, 1.00; and diabetic subjects: Young/Middle, no power; Middle/Older, 0.82; Young/Older, no power. The low sample size in the Young diabetic group limits the conclusions that can be drawn from this group.

Analysis of the scatterplot of each subject's logged central cooling sensation threshold revealed a gradual loss of sensitivity, with increasing age (nondiabetic, $r^2 = 0.35$; diabetic subjects, $r^2 = 0.145$; Figs. 1, 2). Within the nondiabetic group, statistical analysis of the central corneal cooling sensation thresholds for the three groups found significant differences between each of them: Young/Middle comparison, two-way t-test, $P < 0.01$; Young/Older comparison, two-way t-test, $P < 0.01$; Middle/Older comparison, two-way t-test, $P < 0.01$. Within the diabetic group, statistical analysis of the central corneal cooling sensation thresholds revealed a significant difference between the Middle and Older categories (two-way t-test, $P < 0.05$). These differences in sensitivity are seen most readily in the raw data in Figure 3. The mean log-transformed sensation thresholds for each age group are given in Figure 4. In summary, the Young group is more sensitive than the Middle group, which is more sensitive than the Older group.

Statistical analysis of the results indicated no significant differences between male and female corneal cooling sensation thresholds in both groups (two-way t-test, $P > 0.05$). No significant difference was found between the diabetic and nondiabetic subjects when the Young and Middle groups were compared (two-way t-test, $P > 0.05$), but a significant difference was noted between the Older groups (two-way t-test, $P < 0.05$). However, the low sample size in the Young diabetic group limits the conclusions that can be drawn from this group.

Duration and Type of Diabetes

Mean duration of diabetes within type I patients was 11.7 ± 9.6 years (SD; n = 33), and for type II it was 8.2 ± 5.2 years (n = 78). In terms of duration, the difference between the two groups was significant ($P < 0.05$). However, there was no significant difference between the two groups in terms of average corneal sensitivity threshold values (type I, 0.87 ± 0.46; type II, 0.84 ± 0.46). Within both type I and type II patients, there was neither a significant relationship between duration of the disease and corneal sensitivity ($P > 0.05$) nor a gender-based difference ($P > 0.05$).

DISCUSSION

Peripheral sensory nervous systems experience a gradual deterioration in their performance with increasing age.[20] The number of functional nerves in the system decreases, and those remaining become less efficient at transmitting signals to the central nervous system. Thus, the extent to which corneal sensitivity changes in an older subject is due to the subject's

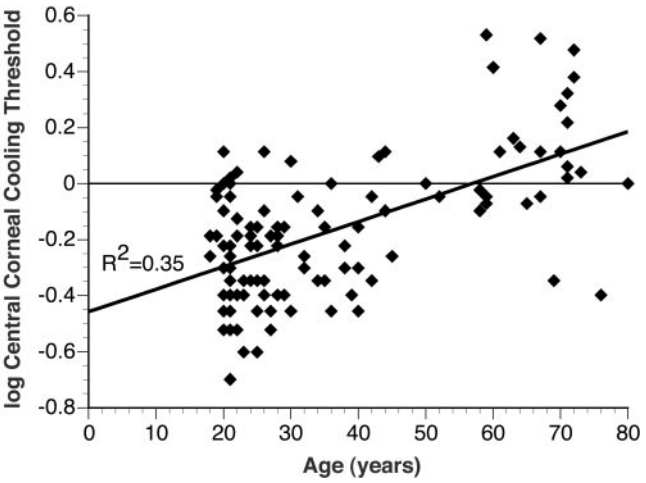

FIGURE 1. Log-transformed central corneal cooling sensation thresholds (millibars) for each nondiabetic subject, plotted against their age.

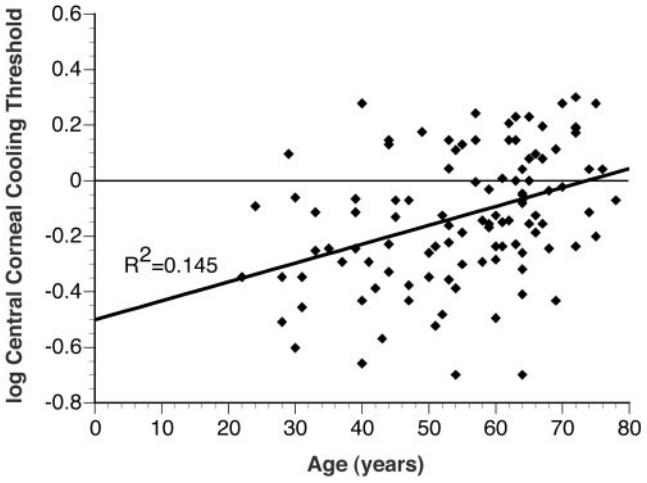

FIGURE 2. Log-transformed central corneal cooling sensation thresholds (millibars) for each diabetic subject, plotted against their age.

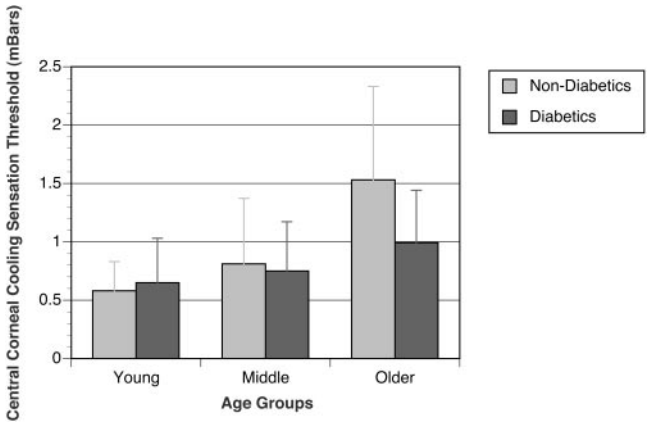

FIGURE 3. Mean (± SD) central cooling corneal sensation thresholds (millibars) for nondiabetic and diabetic groups, for each age group.

natural variation in corneal nerve function loss and level of alertness.[20,54]

The results of this study demonstrate a gradual reduction in corneal sensation as the age of the subjects increases. Figures 1 and 2 indicate an increasing dispersion in the cooling sensation threshold with age, but no matching increase in data variance (F-test Middle/Older: nondiabetic subjects, F = 0.786, $P = 0.274$; diabetics, F = 1.245, $P = 0.217$). However, the results also differ from those reported by Millodot[19] of a relatively even level of sensitivity up to the age of 50 years, with an increasing reduction in sensitivity to twice that level at 65 years. Figures 1 and 2 of our study indicate that a gradual reduction in sensation occurs with increasing age, with the measured mean cooling sensation threshold doubling between the ages of 20 and 50 years. This difference in results may be attributed to the inability of the Cochet-Bonnet aesthesiometer, used by Millodot to measure mechanical sensation thresholds that are found beyond its restricted stimulus intensity range. However, once the sensation of the subject has declined sufficiently, the Cochet-Bonnet is able to assess changes in sensitivity, and so reveals the increasing loss of sensitivity measured over the age of 50 years.

Corneal sensitivity measured using a mechanical stimulus is depressed in diabetic subjects and the extent of any depressed sensitivity is related to the duration of the disease process according to Saini and Khandalavla.[35] This result conflicts with the conclusion reached by Inoue et al.[34] We did not find any association between corneal sensitivity and time since diagnosis of diabetes thus, confirming the latter study. We also agree with the results of O'Donnell et al.[38] in not finding any difference in corneal sensitivity between the diabetic subjects and the nondiabetic subjects. It could be argued that we did not detect a reduced sensitivity using NCCA, because the diabetes in our subjects was of relatively short duration. On average, the diabetes in our subjects had been confirmed 9.3 ± 7.1 years (range, 1–43 years) earlier. We would expect a change in sensitivity subsequent to diagnosis of the disease, but this was not the case. For our protocol, we purposefully excluded subjects with more recently diagnosed disease and those who had any history of invasive ocular surgery. The study by Inoue et al.[32] included subjects with an average age of 63.8 years. Of the 114 eyes that they investigated, 13.2% had a history of cataract surgery. Our diabetic subjects had an average age of 55.8 years, and none of the eyes measured had cataract surgery. It should not be forgotten that previous studies relied on an invasive contact device to stimulate the cornea. The Cochet-Bonnet aesthesiometer assesses corneal sensitivity over a test area of 0.011 mm^2 and the NCCA assesses corneal sensitivity over 0.196 mm^2. Lateral inhibition, if it is present, could influence the sensation experienced by the subject when the stimulus is directed over relatively larger receptive area. However, the NCCA is capable of measuring and differentiating corneal sensitivity between subjects beyond the limits of the Cochet-Bonnet aesthesiometer.[51] This is the opposite of what we would expect if lateral inhibition were a key factor controlling the sensation experienced by the subject when a relative large area of the cornea is stimulated. A review of the literature shows that most previous investigations on corneal sensitivity in diabetic subjects lack any masking techniques. Our study was a single-masked, randomized trial in which one operator measured corneal sensitivity within the nondiabetic subjects and another measured the diabetic subjects. The NCCA results were shared between the investigators after data collection was completed. Hence, we believe our investigation was a more objective and less biased study than previous studies investigating corneal sensitivity in diabetes mellitus.

Corneal sensitivity is lowered during contact lens wear and, using the NCCA device, it is postulated that this lowering of sensitivity is driven by respiratory factors and not mere adaptation to touch.[45] The lack of a detectable difference between diabetic subjects and nondiabetic corneal sensitivity according to the NCCA suggests diabetic corneal function is not compromised by the same factors that reduce corneal sensitivity in contact lens wearers. Abnormal glucose metabolism may be the root cause of the diabetic neuropathy that leads to a true loss of corneal function.[25,55] Blockage of biochemical pathways reducing the efficiency of corneal nerves would lead to reduced corneal sensitivity. This would explain the loss of corneal sensitivity encountered using mechanical touch devices such as the Cochet-Bonnet aesthesiometer. However, this does not account for the results according to the NCCA. If there is a genuine difference in neurologic metabolism within the cornea between diabetic subjects and nondiabetic subjects, then we speculate that, in diabetic subjects, A∂ fiber function is targeted over C fiber function, and the reduction in C fiber activity with advancing years is independent of changes in glucose metabolism.

The gradual reduction in sensitivity also has clinical implications for older nondiabetic and diabetic subjects who may require cataract surgery. Any surgery that involves the cornea or limbus has an impact on corneal innervation. The effect depends on the location, depth, and extent of any

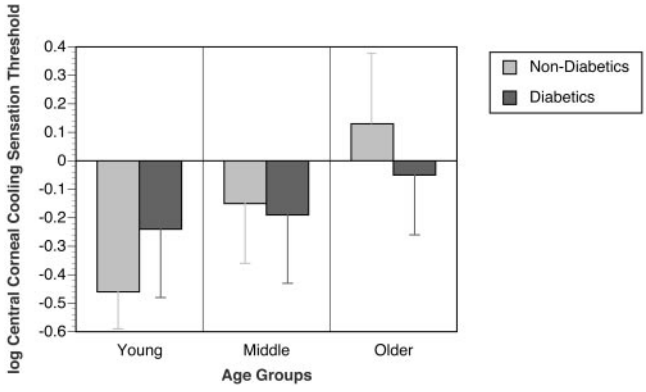

FIGURE 4. Mean (± SD) log central cooling corneal sensation thresholds (millibars) for nondiabetic and diabetic groups, for each age group.

incision, but since the corneal innervation is arranged in a radial fashion from the limbus to the center of the cornea, the arcuate incision used for cataract surgery cuts through many corneal nerves. This type of incision produces a segmental type of sensitivity loss across the cornea. Sensitivity recovers from this type of surgery very slowly, and sensitivity is still much below normal levels 24 months after surgery. In a few patients, cataract surgery does not include an intraocular lens implant, and they must be corrected using a contact lens. These patients are therefore exposed to a triple effect on their sensitivity from ocular surgery, contact lens wear, and age. Because corneal innervation also has a beneficial role in the maintenance and health of the corneal epithelium, these patients may become predisposed to corneal erosions or other associated complications.

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