Corneal Sensitivity in Diabetic Patients Subjected to Retinal Laser Photocoagulation

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PURPOSE. To determine the changes in corneal sensitivity to different stimulus modalities in diabetes mellitus (DM)1 and DM2 patients with retinopathy, and to explore whether argon laser photocoagulation exacerbates sensitivity loss in diabetic patients.

METHODS. Corneal sensitivity to different modalities of stimulus was determined in one randomized eye in 52 patients with DM1 ($n = 35$) or DM2 ($n = 17$), and in 27 healthy subjects. Medical history was obtained from all the patients, including age, sex, time from DM diagnosis, type of diabetes, time from onset of retinopathy, type of diabetic retinopathy, and type of argon laser treatment. Corneal sensitivity was determined using a gas esthesiometer. Mechanical, chemical, and thermal (heat and cold) stimuli were applied on the central cornea.

RESULTS. Sensitivity thresholds to selective mechanical, chemical, and cold stimulation were significantly higher in DM patients compared to controls. Sensitivity threshold to mechanical and chemical stimuli was higher in DM2 than in DM1 patients. In DM1 patients, mechanical threshold increased with time after DM diagnosis. No correlation was found between sensitivity thresholds to chemical or thermal stimulation and the age of the patient, type of retinopathy, or time from its diagnosis. Laser treatment generated a further impairment of corneal sensitivity.

CONCLUSIONS. Corneal sensitivity to mechanical, chemical, and thermal stimulation is decreased in DM patients, suggesting that diabetes affects homogeneously the different types of sensory neurons innervating the cornea. Corneal sensitivity appears to be more disturbed in DM2 than in DM1. Laser treatment of DM patients generates a further impairment in corneal sensitivity, probably as the result of physical damage to ciliary nerves. (*Invest Ophthalmol Vis Sci.* 2011;52:6043–6049) DOI:10.1167/iovs.10-7054

The sensory innervation of the cornea travels primarily with the ophthalmic division of the trigeminal ganglion.[1] Nerve axons travel into the nasociliary branch of the ophthalmic nerve and enter the posterior pole of the eyeball, forming the long and short ciliary nerves, which contain a mixture of sensory, sympathetic, and parasympathetic fibers. After piercing the sclera, ciliary nerve bundles divide profusely during their trajectory toward the front of the eye within the suprachoroidal space, finally entering the corneal stroma in a radial manner.[2] Corneal stromal nerves run parallel to the corneal surface in the upper third of the stroma, dividing extensively into smaller branches that bend 90° and penetrate the epithelium to form the sub-basal nerve plexus, located between the Bowman's layer and the basal epithelial cells.[3] Nerve fibers of this plexus run upward between epithelial cells and terminate as free nerve endings in the uppermost superficial layers of the corneal epithelium.[1,3]

Based on their response to specific stimuli, different functional types of sensory nerve fibers have been identified in the cornea: mechanonociceptors (~20% of fibers) that react only to mechanical forces; polymodal nociceptors (~70% of fibers) that respond to mechanical forces but also to heat, exogenous chemical irritants, and endogenous inflammatory mediators; and cold-sensitive receptors (~10%–15% of fibers) that display an ongoing impulse activity at basal corneal temperatures and increase markedly their firing frequency with moderate cooling (see Ref.4 for a review). Selective activation of each of these functional types of corneal sensory fibers leads to conscious sensations of different quality.[5]

Corneal sensitivity is variably impaired in a number of ocular and systemic diseases.[6,7] One of them is diabetes mellitus (DM), in which corneal sensitivity appears reduced in approximately 20% of patients.[8,9] Accordingly, a high mechanical threshold, measured using the Cochet-Bonnet esthesiometer, was reported in DM patients.[8,10–13] Low corneal sensitivity in DM patients has been associated to the degree of sensory neuropathy and retinopathy, age, and duration of the disease.[8,9,12–14] Corneal confocal microscopy in DM patients demonstrated a reduction in the number of sub-basal nerve fibers bundles[8,15] which could explain in part the impairment of corneal sensation in these patients. The sensitivity loss is expected to be aggravated by retinal laser treatments that have been increasingly used in DM during the past decades.[16] Although Ruben et al.[11] reported no changes in corneal sensitivity measured with the Cochet-Bonnet esthesiometer after application of panretinal photocoagulation to DM patients, laser photocoagulation in diabetic retinopathy is likely to cause iatrogenic lesion of ocular nerves[12,17,18] and additional loss of corneal sensitivity, which may not be detected with an instrument with limited accuracy such as the Cochet-Bonnet esthe-

siometer. To explore the alteration of the different functional types of corneal afferent fibers in diabetic patients, in the present work we examined in detail the characteristics of sensations evoked by selective mechanical, chemical, and thermal stimulation of the cornea in DM types 1 (DM1) and 2 (DM2) with retinopathy, comparing also threshold values to the different stimulus types between patients treated or not with argon laser photocoagulation.

PATIENTS AND METHODS

Patients

Fifty-two patients (22 women and 30 men; mean age, 48.2 ± 1.9 years; range, 22-70) with type 1 ($n = 35$; 21 women, 14 men; mean age, 43.4 ± 2.1 years; range, 22-65) or type 2 ($n = 17$; 8 women, 9 men; mean age, 57.9 ± 2.6 years; range, 32-70) diabetes were recruited at the Department of Ophthalmology at the Helsinki University Eye and Ear Hospital, and participated voluntarily in the study. A group of 27 age-matched healthy subjects (mean age, 49.1 ± 2.2 years; range, 26-67; 19 female and 8 male) served as a control group. The study protocol was approved by the Ethical Review Committees of Helsinki University Eye and Ear Hospital and the University Miguel Hernandez, and followed the tenets of the Declaration of Helsinki and the legal European Union regulations. A medical history was obtained from all of the patients, including age, sex, time from DM diagnosis (15.1 ± 1.6 years; range, 0.5-38), type of diabetes (DM1 or DM2), time from the onset of retinopathy (1.3 ± 0.2 years; range, 0.5-6), type of diabetic retinopathy (proliferative diabetic retinopathy, $n = 21$, or nonproliferative diabetic retinopathy, $n = 31$), type of argon laser treatment received by the patient (none, $n = 19$; focal photocoagulation, $n = 12$; panretinal photocoagulation, $n = 21$). Before sensitivity testing, the ocular surface of each patient was examined by biomicroscopy. All corneas were characterized by the absence of clinical signs of keratopathy and/or epithelial defects and corneal opacities.

Corneal Esthesiometry

Sensitivity of the cornea to selective mechanical, thermal (heat and cold), and chemical stimulation was evaluated using a commercial design of a gas esthesiometer (Belmonte OPM, Deriva Global S.L., Valencia, Spain). The standardized procedure to measure the sensitivity thresholds for each modality of stimulus have been described in detail in Gallar.[6] In brief, gas jets of 3 seconds duration separated by 2-minute pauses were applied at random to the center of the cornea. Selective mechanical stimulation consisted of jets of warmed air of increasing flow rates (0-200 mL/min); chemical stimulation was performed with gas pulses containing a mixture of warmed air and CO_2 at variable concentrations (0%-80% CO_2), applied at a subthreshold flow value. Selective heat and cold thermal stimulation was obtained with pulses of air at different temperatures (-10° to +75°C at the tip of the probe) applied at a subthreshold flow, which induced corneal surface temperature changes between -4.5° and +2.1°C from the basal temperature value (34.2 ± 0.1°C).

Immediately after each stimulating pulse, the subject was asked to respond verbally whether he or she had felt the stimulus. The sensation threshold was assessed, taking as threshold the lowest stimulus intensity that evoked a positive response. All of the measurements were performed in the same room, where environmental temperature and humidity were maintained in the comfort range (23°C and 40%, respectively).

Statistical Analysis

Data were collected and processed for statistical analysis using commercial software (SigmaStat; Systat Software Inc., Richmond, CA), and the appropriate parametric or nonparametric statistical test to compare the differences between groups, as indicated. All data are expressed as mean ± SEM. Correlation analysis was performed to predict the association between sensation threshold values and several variables: age of subject, type of DM, time from DM diagnosis, type of retinopathy, and time from its diagnosis.

RESULTS

Control Subjects versus Diabetic Patients

Differences in Sensitivity to Selective Stimulation. Selective mechanical, chemical, and thermal stimulation of the central cornea evoked sensations in all control subjects (Table 1). Frequency histograms showed differences in the probability distribution of mechanical, chemical, and heat and cold sensitivity thresholds between control and DM patients (Fig. 1), reflecting the augmented probability of a higher threshold value for all the stimulus qualities in DM. All DM patients responded to mechanical and chemical stimulation of the cornea, but notably 44% (23/52) of DM patients were insensitive to heat stimulation of the cornea up to +2.1°C over the basal temperature value; one of them also lacked sensitivity to cold stimuli in the explored range (-0.1° to -4.5°C) (Table 1, Fig. 1). The reduction in the proportion of DM patients responding to heat stimulation was significant ($P < 0.001$, z-test). Intriguingly, the sensation threshold for heat stimulation measured in the group of DM patients who responded to heat was significantly lower than in control subjects ($P < 0.001$, Mann-Whitney Rank Sum test; Table 1). Thresholds of DM patients were significantly higher for mechanical ($P = 0.001$, Mann-Whitney test), chemical ($P < 0.001$), and cold ($P < 0.001$) stimulation in comparison to controls (Table 1).

Relationship between Sensation Thresholds and Age. Sensation thresholds to mechanical, chemical, and heat and cold stimulation became higher proportionally with age in
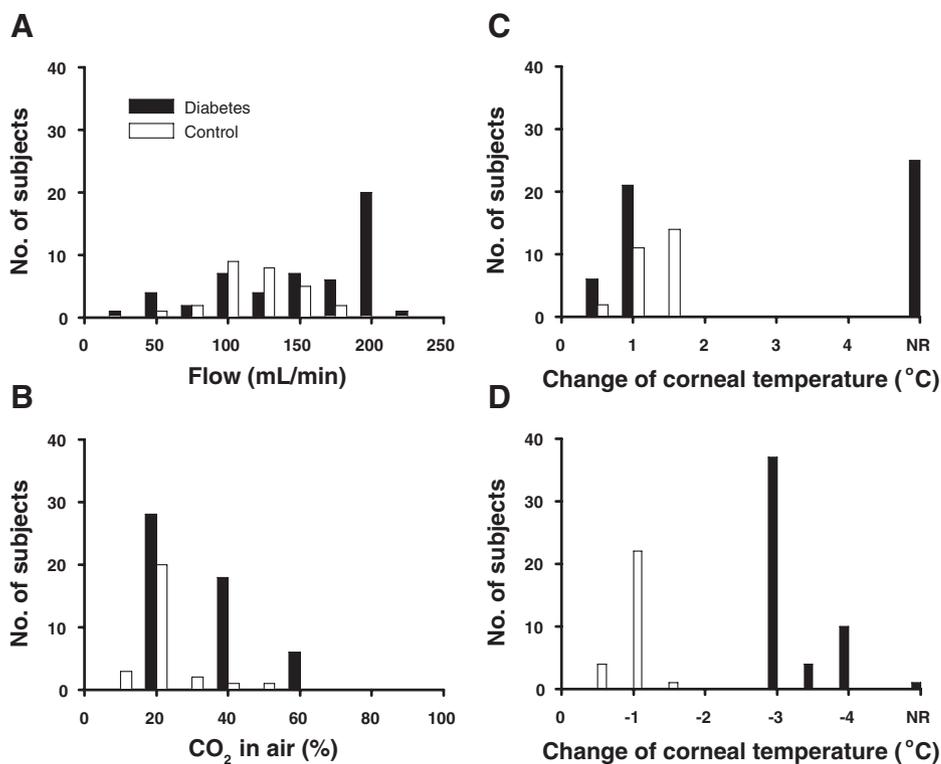
TABLE 1. Sensation Thresholds to Selective Stimulation of the Cornea

	Control Subjects	DM Patients
Mechanical (air flow, mL/min)	108.1 ± 5.7 (33 to 170) 27/27	142.3 ± 7.6* (20 to 200) 52/52
Chemical (CO_2 in air, %)	18.3 ± 1.7 (10 to 50) 27/27	31.5 ± 1.9* (20 to 60) 52/52
Heat (temperature change, °C)	+0.9 ± 0.1 (0.3 to 1.2) 27/27	+0.4 ± 0.1* (0.1 to 0.5) 29/52†
Cold (temperature change, °C)	-0.8 ± 0.1 (-0.4 to -1.4) 27/27	-3.0 ± 0.1* (-2.7 to -3.9) 51/52

Data are shown as mean ± SEM, the data range (in parentheses) and the number of subjects who responded out of the total number of explored individuals.

* $P < 0.001$, Mann-Whitney test; difference between control and DM groups.

† $P < 0.001$, z-test, difference between DM and control subjects in the proportion of responding versus explored individuals.

Figure 1. Distribution of sensitivity thresholds to selective mechanical (**A**), chemical (**B**), heat (**C**), and cold stimulation (**D**) of the central cornea in DM patients and control subjects. NR, no response.

control subjects (Table 2, Fig. 2). In contrast, sensation thresholds for the different stimulus modalities in DM patients were independent of age (Table 2, Fig. 2). DM2 patients were older than DM1 patients (57.9 ± 2.6 years vs. 43.4 ± 2.1 years, respectively; $P < 0.001$, Student's t-test), although group sensitivity threshold and ages did not correlate in either group (data not shown).

DM1 versus DM2 Patients

Sensitivity Threshold. Mechanical ($P = 0.019$, Mann-Whitney test) and chemical ($P = 0.035$) sensitivity thresholds were higher in DM2 than in DM1 patients (Table 3). No significant differences in heat and cold sensitivity thresholds were found between DM1 and DM2 patients ($P = 0.972$ and 0.271, respectively; Table 3).

Relationship between Sensitivity Thresholds and Characteristics of the Disease. No correlation was found between sensitivity threshold values and the time from DM diagnosis, type of retinopathy, or time from its diagnosis for the complete group of diabetic patients or the DM1 and DM2 subgroups (data not shown), except for mechanical threshold

TABLE 2. Correlation between Sensation Thresholds and Age

	Control Subjects	DM Patients
Mechanical stimulation	CC = 0.512	CC = 0.007
	$P = 0.006$	$P = 0.961$
Chemical stimulation	CC = 0.382	CC = 0.036
	$P = 0.049$	$P = 0.801$
Heat stimulation	CC = 0.692	CC = 0.436
	$P < 0.001$	$P = 0.228$
Cold stimulation	CC = 0.647	CC = 0.108
	$P < 0.001$	$P = 0.451$

Correlation coefficients (CC) and P values obtained with Pearson correlation test to analyze the relationship between threshold value and age.

in DM1 patients, which increased proportionally with the time elapsed since the DM was diagnosed (correlation coefficient [CC] = 0.345, $P = 0.042$).

DM Patients with or without Retinal Photocoagulation

Multiple-group comparison tests showed significant differences between all three groups (control subjects, DM patients without laser treatment, and DM with laser treatment), for mechanical ($P = 0.005$, Kruskal-Wallis ANOVA on Ranks), chemical ($P < 0.001$), heat ($P < 0.001$) and cold ($P < 0.001$) thresholds (Fig. 3).

Three-way ANOVA showed that the increased threshold observed in laser-treated patients was not associated to other factors such as the age, type of DM, time from DM diagnosis, type of retinopathy, or time from its diagnosis (data not shown). In the same way, no differences were found between selective sensitivity thresholds of DM patients who received focal photocoagulation or panretinal photocoagulation (data not shown). The sensitivity loss appeared even after a single retinal laser session and was independent of the number of laser sessions and the energy, size, and number of fires received by the patient (data not shown).

DISCUSSION

The present work confirms that corneal sensitivity is decreased in DM patients compared to control individuals and that sensation thresholds for mechanical, chemical, and cold selective stimulation are significantly higher in DM patients. For heat stimulation, sensation threshold could be measured only within a small range of stimulus temperatures (+0.1°C to +2.1°C). Almost 50% of the patients did not detect the maximal stimulus temperature applied, indicating that their heat threshold was above this value. Thus, mean threshold to heat stimulation of the entire group of DM patients was higher than in control individuals, in spite of the fact that the fraction of
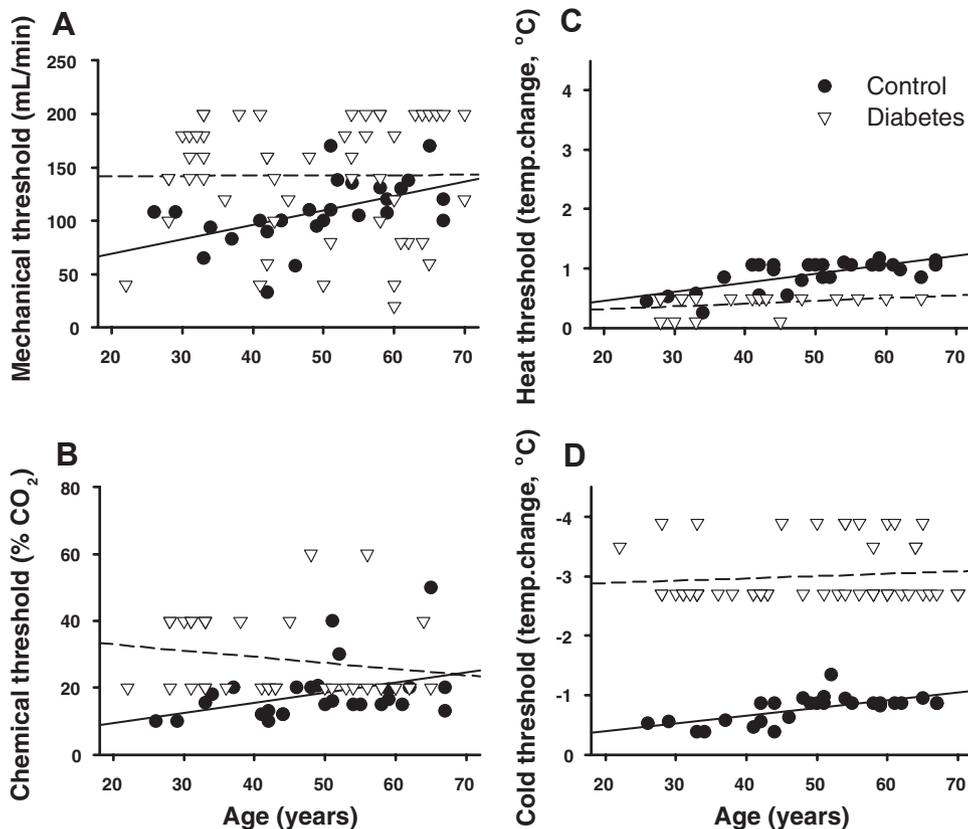
FIGURE 2. Relationship between age and corneal sensitivity threshold values to mechanical (**A**), chemical (**B**), heat (**C**), and cold (**D**) stimulation in DM patients and in control subjects. Linear regression analysis showed significant correlation between sensitivity threshold and age in control subjects (*solid line*) but not in DM patients (*dashed lines*). (See Table 2 for details.)

DM patients who responded to heat had a mean threshold lower than that of control individuals. Corneal surface temperature is lower in diabetic patients than in age- and sex-matched healthy subjects.[19] Hence, heat stimuli applied to colder corneas are expected to induce corneal surface temperature rises that may be below the activation threshold of polymodal nociceptors. This could explain in part why approximately 50% of the DM patients did not respond to heat in the explored temperature range. However, differences in corneal temperature between diabetic and normal subjects cannot account for the lower sensitivity to cold observed in DM patients because the contrary argument applies for cold stimuli, which in cooler corneas are expected to produce more prominent temperature reductions and stronger activation of cold thermoreceptors. Altogether, our results indicate that all modalities of corneal sensation are impaired in DM patients.

To the best of our knowledge, this is the first work in which a new commercial version of the Belmonte esthesiometer has been used to study corneal sensitivity in normal and disease conditions. The relatively short range of heat stimulus temperatures offered by this instrument represents a limitation, because increases of corneal surface temperature to or above $+2.1°C$ are often needed to evoke a sensation in diseases in which corneal sensitivity is reduced,[6,20] including DM. Nonetheless, heat and CO_2 stimuli are known to activate the same class of sensory afferents, namely the polymodal nociceptors. The esthesiometer used here provides a wide range of CO_2 concentrations, thus allowing one to explore accurately the sensitivity of polymodal nociceptors to acid within an ample range of stimulus intensities.

Spontaneous pain, hyperalgesia, and allodynia are common in human diabetic neuropathy and in diabetic rodents. Pain associated with diabetic neuropathy has been linked to hyperexcitability of damaged primary sensory neurons and their peripheral axons, and to aberrant spontaneous impulse generation. However, in animal models of diabetes, peripheral sensory nerves rarely exhibit spontaneous activity or increased responsiveness to peripheral stimuli. In fact, sensory input to

TABLE 3. Corneal Sensation Thresholds in Different Types of Diabetes

	DM1	DM2
Mechanical (air flow, mL/min)	132.6 ± 8.7 (20 to 200) 35/35	162.3 ± 13.8* (40 to 200) 17/17
Chemical (CO_2 in air, %)	28.6 ± 2.1 (20 to 60) 35/35	37.6 ± 3.8* (20 to 60) 17/17
Heat (temperature change, °C)	+0.4 ± 0.1 (0.1 to 0.5) 20/35	+0.4 ± 0.1 (0.1 to 0.5) 9/17
Cold (temperature change, °C)	−3.1 ± 0.1 (−2.7 to −3.9) 34/35	−2.9 ± 0.1 (−2.7 to −3.9) 17/17

Data are shown as mean ± SEM, the data range (in parentheses) and the number of responding subjects out of the explored individuals.

* $P < 0.05$, Mann-Whitney test, difference between DM1 and DM2 groups.
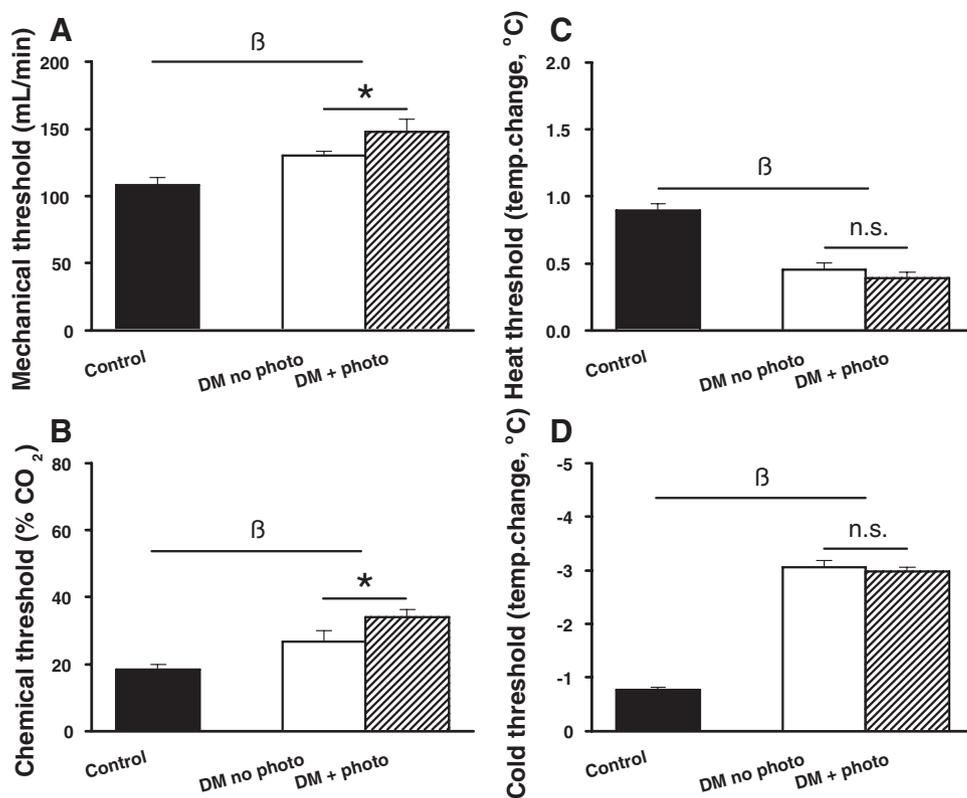
FIGURE 3. Sensitivity thresholds for selective mechanical (**A**), chemical (**B**), heat (**C**), and cold (**D**) stimulation determined in control subjects ($n = 27$) and in DM patients with ($n = 33$) or without ($n = 19$) retinal photocoagulation. $\beta P = 0.005$, Kruskal-Wallis ANOVA on ranks, difference between control, DM without laser treatment, and DM with laser treatment groups; *$P < 0.05$, Student's *t*-test, difference between DM patients with and without retinal photocoagulation treatment). n.s., not significant.

the spinal cord is decreased rather than increased in diabetic rodents.[21] Therefore, in the absence of abnormal peripheral nerve activity, hyperalgesia and allodynia have been attributed to aberrant spinal or supraspinal modulation of sensory processing. In experimental models of diabetes, neurons in the ventral posterolateral thalamus become hyperexcitable, firing at abnormally high frequencies and generating aberrant spontaneous activity. Likewise, in humans, central neuropathic mechanisms such as thalamic dysfunction have been suggested to contribute importantly to pain experienced with diabetes.[22] Ocular hyperalgesia is unusual in DM patients, suggesting that the reduced sensitivity to natural stimuli consecutive to peripheral damage of ocular sensory nerves described in the present study, is the main effect of DM on the function of ocular pain pathways.

Previous studies in DM patients, performed primarily with the Cochet-Bonnet esthesiometer, already detected a reduced corneal sensitivity to mechanical stimulation.[8–12] Our results show that the corneas of diabetic patients present as well a lower sensitivity to chemical, heat, and cold stimuli, indicating that the metabolic disease affects all functional classes of corneal trigeminal sensory neurons and/or their peripheral axons. This contrasts with other ocular pathologies, also accompanied by corneal sensory deficits as HSV keratitis, in which responses to selective mechanical, heat, and chemical stimuli but not to cold stimulation were disturbed, suggesting that only polymodal nociceptive neurons were substantially affected.[7]

The broad alteration of all functional classes of corneal sensory afferents seen in DM is in accordance with the suggested mechanisms of pathogenesis of the peripheral neuropathy in diabetes.[23] Failure of the reciprocal dependence between nerves and blood vessels and biochemical changes in enzymatic activity or in intracellular second messengers and metabolites are probably responsible for the neural alterations and lead to the broad nerve damage observed in all types of corneal nerves.[24–26]

In addition to the functional impairment, a decrease in the total number of trigeminal sensory neurons innervating the ocular surface presumably contributes to the reduction of corneal sensitivity in DM patients. There are no available data on the functional condition of corneal trigeminal neurons in human diabetic patients, but several studies using confocal microscopy of the cornea found a significant reduction in the number of nerve bundles innervating this tissue,[8,15] which could explain, at least in part, the reduced corneal sensitivity. Likewise, in other peripheral nerves accessible to biopsy, such as those innervating the forearm skin, a significant reduction of the density of nerve fibers (particularly unmyelinated and small myelinated fibers conveying nociceptive and thermal sensory information) has been described in diabetic patients.[27]

Most of the ocular surface alterations reported in DM patients seems to be secondary to a decrease in corneal sensitivity and to the presence of ocular dryness.[8–14] Dry eye can in turn be the consequence of the peripheral neuropathy, which would reduce corneal sensory input to the central nervous system, thereby decreasing basal[28] and reflexly evoked tear secretion,[29] and perhaps also damage autonomic nerve fibers that regulate lachrymal gland secretion.[13,30]

It is well established that corneal sensitivity to selective mechanical, chemical, heat, and cold stimulation in healthy subjects decreases with age.[31,32] Murphy et al.[14] reported that decreases of corneal sensitivity with age, measured with pulses of air at room temperature applied with a noncontact corneal esthesiometer, occur both in nondiabetic and DM patients. In the present study, we did not detect in DM patients the gradual decrease of sensitivity to selective stimulation observed in normal individuals. Conversely, corneal sensitivity appeared evenly reduced in young and old DM patients affected by DM1 or DM2, and was also independent of the time elapsed since the DM diagnosis, the seriousness of the parallel retinopathy, and the time from retinal lesion diagnosis. These findings indicate that the sensory deficit possi-

bly appears at early stages of the disease and suggests that a precise determination of corneal sensitivity disturbances in DM patients could provide useful information about the level of neuropathic damage at the initial stages of DM.

We found that in DM2 patients, the sensitivity to mechanical and chemical stimuli was more severely impaired than in DM1 patients, whereas thermal sensitivity (heat and cold) was affected equally in both types of patients. A second difference between DM1 and DM2 patients was that in DM1 patients, mechanical sensitivity decreased with time after the onset of diabetes whereas in DM2 patients the sensory loss was independent of the time of development of the disease, and was already present shortly after DM diagnosis. A different time course for diabetic peripheral neuropathy in DM1 and DM2 patients has also been described; in the latter, the neuropathy usually develops at early stages and should be screened at the time of diagnosis, whereas in type 1 patients the screening of diabetic peripheral neuropathy is recommended only 5 years after diagnosis.[33] In accordance with our results, corneal sensitivity should indeed be measured in type 2 diabetic patients also at the time of diagnosis to detect an early onset of the neuropathic sensory loss. Likewise, periodical measurements of corneal sensitivity in DM1 patients from the time of diagnosis could serve as a good screening of the evolution of the patient's neuropathic status. This would permit the adoption of preventive actions, which are important considering that DM patients with peripheral neuropathy, symptomatic or not, are at a higher risk of developing corneal trophic alterations.

The main reason for ophthalmologic examination of DM patients is not the detection of disturbances of the ocular surface or of corneal sensitivity but rather the diagnosis and follow-up of diabetic retinopathy (DR), a common complication of the disease resulting from incompetence of retinal microvessels. Panretinal photocoagulation is widely used in DM patients to reduce the vision loss caused by DR, yet the mechanism by which panretinal photocoagulation works remains unknown.[34] This treatment consists in the application with a laser beam of hundreds or up to thousands of burns of defined size and energy, which produce a relatively controlled damage to the retinal tissue. Although the aim of the treatment is the arrest or regression of neovascularization, the procedure unavoidably injures the tissues near the laser burn, including adjacent retinal cells and the underlying ciliary nerve bundles that travel in the suprachoroidal space, as is the case with endolaser treatment of retinal detachment.[35] The same problem seems to occur in DM patients undergoing retinal photocoagulation, and as shown in our work, corneal sensitivity in these patients is further reduced after laser treatment. The impairment of corneal sensitivity after focal photocoagulation treatment is the same as after panretinal photocoagulation, suggesting that the degree of indirect damage of suprachoroidal ciliary nerves is similar under both procedures.

In summary, the present study shows that corneal sensitivity subserved by the different functional types of corneal afferent fibers is homogeneously impaired in DM patients. This occurs already at early stages of the disease except for DM1 patients, in whom sensory loss increased with time after diagnosis of the disease. Thus, a follow-up of corneal sensitivity may serve as a relatively simple, noninvasive means of measuring the evolution of the neuropathic status in diabetic patients. Retinal photocoagulation seems to aggravate corneal sensitivity disturbances already present in DM patients, as a consequence of the physical damage to sensory nerves caused by the laser burns.

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