The Glenn A. Fry Award Lecture 2010:
Ophthalmic Markers of Diabetic Neuropathy

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ABSTRACT
Diabetic peripheral neuropathy (DPN) is a debilitating condition that affects about 50% of diabetic patients. The symptoms of DPN include numbness, tingling, or pain in the arms and legs. Patients with numbness may be unaware of foot trauma, which could develop into a foot ulcer. If left untreated, this may ultimately require amputation. Currently, the only method of directly examining peripheral nerves is to conduct skin punch or sural/peroneal nerve biopsies, which are uncomfortable and invasive. Indirect methods include quantitative sensory testing (assessing responses to heat, cold, and vibration) and nerve electrophysiology. Here, I describe research undertaken in my laboratory, investigating the possibility of using a range of ophthalmic markers to assess DPN. Corneal nerve structure and function can be assessed using corneal confocal microscopy and non-contact corneal esthesiometry, respectively. Retinal nerve structure and visual function can be evaluated using optical coherence tomography and perimetry, respectively. These techniques have been used to demonstrate that DPN is associated with morphological degradation of corneal nerves, reduced corneal sensitivity, retinal nerve fiber layer thinning, and peripheral visual field loss. With further validation, these ophthalmic markers could become established as rapid, painless, non-invasive, sensitive, reiterative, cost-effective, and clinically accessible means of screening for early detection, diagnosis, staging severity, and monitoring progression of DPN, as well as assessing the effectiveness of possible therapeutic interventions. Looking to the future, this research may pave the way for an expanded role for the ophthalmic professions in diabetes management.

Key Words: diabetic peripheral neuropathy, ophthalmic markers, corneal confocal microscopy, non-contact corneal esthesiometry, optical coherence tomography, perimetry

The subject I have chosen for my Glenn A. Fry Award Lecture—and therefore the topic of this article—relates to research that I have conducted over the past decade, investigating potential ocular tests for diabetic peripheral neuropathy (DPN)—one of the most common and debilitating complications of diabetes. Those who are familiar with my scientific writings over the past 33 years may be somewhat confused by this choice of topic, given that the vast majority of my published works have been in the field of cornea and contact lenses.

A Recurring Dream

The aspect of contact lens research that fascinated me most of all was the ocular response to contact lens wear. Throughout the 20th century, the principle clinical tool for evaluating the various metabolic, mechanical, toxic, and immunological reactions of the anterior eye to contact lens wear was the slitlamp biomicroscope. This instrument, which enables examination of the cornea up to a magnification of ×40, allows subtle changes to be observed such as epithelial staining and microcysts, stromal infiltrates, folds and striae, and gross endothelial irregularities. My interest in this field led to the development of grading scales for contact lens complications,1,2 which are now used extensively by contact lens practi-
tioners around the world, and resulted in numerous publications including a book devoted exclusively to this topic.3

Although it has long been possible to inspect the endothelium at a cellular level using a specular microscope (capable of \( \times 200 \) magnification), examination of the remainder of the cornea at this or higher levels of magnification was not possible for most of the 20th century, and I used to dream of the day when such technology might be developed. This dream turned to reality with the introduction of the corneal confocal microscope (CCM) for clinical use in 1990,4 and I recall being mesmerised by a presentation given by Dwight Cavanagh at the sixth scientific meeting of the International Society of Contact Lens Research in Monte Carlo on my 36th birthday—September 3, 1990—in which he showed the first grainy images obtained with a prototype, laboratory-built CCM, at a magnification of around \( \times 600 \).

I remember seeing individual corneal epithelial cells and stromal keratocytes. The potential for this technology to solve the long list of unanswered questions relating to the cellular basis of corneal complications of contact lens wear was obvious, but I had to wait another 8 years until the first CCM became commercially available—the Tomey Confoscan P4 (Tomey, Erlangen, Germany). This was a precursor instrument to one of the two currently available CCMs—the Nidek ConfoScan 4—which I shall describe in more detail below.

**Initial Observations with the CCM**

I acquired a Tomey CCM in 1998 and immediately initiated a series of studies, beginning with a detailed documentation of the normal human cornea, stratified by decade of life.5,6 While undertaking my first scans through the cornea, a particular feature at the base of the epithelium caught my attention—a rich plexus of well defined, often beaded and extensively anastomosing nerve fibers, apparently located in Bowman layer, immediately posterior to the epithelial basal lamina (Fig. 1). This feature, which has become known as the corneal sub-basal nerve plexus, had been only loosely referred to in the previous literature.7 The reason for this is that these nerves are very fine and beyond the resolution of a slitlamp biomicroscope (which can only be used to visualize thicker stromal nerves).

Previous histological studies on cadaver eyes have provided only scant insights into the existence of the corneal sub-basal nerve plexus because these nerves begin disappearing immediately post-mortem and are almost completely obliterated after about 13.5 h postmortem.8,9 It is logistically very difficult to obtain cadaver specimens more quickly than this. As I shall describe later, modern immunohistochemical techniques and refined methodologies for preserving postmortem specimens have facilitated generation of fantastic in vitro image maps of the corneal sub-basal nerve plexus; these highly detailed nerve maps were published for the first time in 2010 by three independent research groups.10–13

Recognizing the novel aspect of this hitherto poorly described but potentially significant feature of corneal anatomy, I worked with my then Masters student, Laura Oliveira-Soto, to document the morphology of all corneal nerves visible with the CCM. Our article on this topic,14 which was published in 2001, provided the first detailed account of the corneal sub-basal nerve plexus. This article has turned out to be of considerable utility to researchers in the field, as indicated by the high number of citations it has attracted (82 as of March 2011 according to Web of Science).

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Initial studies with the CCM allowed me to realize my dream of unraveling some of the mysteries of the ocular response to contact lens wear at a cellular level. These included studies of the impact of rigid
lenses on the cornea, observations of dark orthogonal stromal lines resulting from corneal edema induced by overnight lens wear, stromal microdots, keratocyte apoptosis, endothelial blebs, acanthamoeba keratitis, chronic morbidity of corneal infiltrative events, and changes in conjunctival morphology. I shall not provide a detailed account of these observations; those who are interested can read an extensive review of this work which I have published elsewhere.

It was, however, images of the corneal sub-basal nerve plexus that continued to play on my mind. After learning that corneal nerves—as imaged with the CCM—are apparently unaltered by contact lens wear, I started to consider alternative applications for evaluating this substructure in other areas of ophthalmic science, or indeed general medial science, and it is here that my foray into diabetes research began.

Diabetes from a Personal Perspective

Having had type 2 diabetes since the age of 34, I had developed a good understanding of this condition. At the time I acquired the Tomey CCM, I had noticed occasional mild numbness in my fingers after extended periods of typing on my computer (for example, when writing long articles such as this). I reported these symptoms to my personal diabetic physician, Professor Andrew Boulton, at the Manchester Diabetes Center, early in 2000. Andrew informed me that my symptoms were due to a compression neuropathy (also known as “nerve compression syndrome”), a condition that has a higher prevalence in patients with diabetes. In my case, this compression neuropathy was apparently caused by leaning against the arm rests of my office chair while typing, and exacerbated by my diabetic condition. I took this opportunity to tell Andrew about a study I had a recently completed, documenting human nerves with a CCM, and handed him a draft copy of my article.

Andrew was intrigued by this, but he was about to head off overseas on an extended sabbatical. So, he urged me to discuss my work further with his research associate, Professor Rayaz Malik, who was also interested in this idea. I soon learned why these clinical endocrinologists were so keen to consider this new technique of examining nerve fibers in vivo: as it turns out, both have a strong research interest in DPN and were so keen to consider this new technique of examining nerve fibers in vivo. As it turns out, both have a strong research interest in DPN and were so keen to consider this new technique of examining nerve fibers in vivo: as it turns out, both have a strong research interest in DPN and were so keen to consider this new technique of examining nerve fibers in vivo.

In the course of planning some pilot studies on patients with DPN in late 2000, an article was published by Rosenberg et al. documenting a reduction in corneal sub-basal nerve density in diabetic patients with mild to moderate neuropathy. Thus, despite having thought of this idea independently, credit must be accorded to Rosenberg et al. for having first described the link between corneal nerve morphology and severity of DPN. The article of Rosenberg et al. further fuelled our enthusiasm for persuading this line of research, and the challenge now was to confirm and expand on their initial observations and to explore other possible ophthalmic markers of DPN.

Diabetic Peripheral Neuropathy

Recently, an international consensus group agreed to the following definition of confirmed diabetic sensorimotor polyneuropathy: “The presence of an abnormality of nerve conduction and a symptom or symptoms or a sign or signs of neuropathy.” Common symptoms, usually in the feet or legs, include tingling, numbness, extreme sensitivity to touch, prickling, burning, and pain (Fig. 2). These symptoms are usually worse at night.

According to the American Diabetes Association, mild to severe forms of nervous system damage occur in 60 to 70% of patients with diabetes. This condition affects sensory, autonomic, and motor neurons of the peripheral nervous system. In advanced cases, it can lead to foot ulceration (Fig. 2) and lower limb amputation. About 71,000 non-traumatic lower-limb amputations were performed in the United States in 2004, with significant attributable health care costs, and the vast majority of these were due to late complications of DPN. The accurate detection, characterization, and quantification of this condition are important to define at-risk patients, anticipate deterioration, monitor progression, and assess new therapies.

Using a specific definition of neuropathy (symptoms plus one abnormal physical finding, or two abnormal physical findings), Walters et al. reported that the prevalence of neuropathy was 16.3% [95% confidence interval (CI), 14.6 to 19.0%] in diabetic patients and 2.9% (95% CI, 1.4 to 4.4%) in non-diabetic subjects, yielding a prevalence odds ratio of 6.75 (95% CI, 3.87 to 11.79) (p < 0.001). In type 1 diabetes, the prevalence was 12.7% (95% CI, 8.0 to 17.6%) and in type 2 diabetes 17.2% (95% CI, 15.9 to 18.5%). After adjusting for age, this difference was not significant (odds ratio, 1.60; 95% CI, 0.95 to 2.76). The prevalence of neuropathy increased with age in diabetic and non-diabetic subjects. Patients with painful DPN have a reduced quality of life and incur increased long-term health costs compared to those with non-painful DPN.

Foot ulceration in patients with neuropathy has an annual incidence in excess of 7%, compared with an incidence of <1% in those without neuropathy. Because foot amputations are preceded by foot ulceration in 80% of cases, early detection or prediction of foot ulceration is vital. Ollendorf et al., using a model based on the incidence and cost of lower extremity amputations in diabetes, predicted potential savings of up to $3 million over 3 years if foot ulcerations could be prevented. Ramsey et al. estimated the attributable costs for a middle-aged diabetic male patient to be $28,000 two years after a new foot ulcer.

Risk factors associated with the development of neuropathy in diabetes include increased age, height and body mass index, duration of diabetes, hypertension, smoking, poor glycemic control, and abnormal lipid profile and albumin level. Although these studies provide valuable information relating to factors associated with the development of DPN, they only define neuropathy using clinical markers. Large nerve fiber dysfunction and shed no light on the early pathological changes taking place in small nerve fibers or the degree to which changes in symptoms and signs correlate with small nerve fiber degeneration.

The Basis of Neural Loss in DPN

A wide range of metabolic and ischemic sources have been ascribed as being of etiological significance in DPN, and these are detailed in the following sections.

Hyperglycemia. The duration and severity of exposure to hyperglycemia can influence the severity of DPN. Severity of
neuropathy in patients with impaired glucose tolerance has been shown to be more mild than in newly diagnosed patients. This suggests that nerve damage caused by hyperglycemia can happen at a very early stage of diabetes. As such, insulin therapy and/or pancreas implant have been suggested as potential methods for improvement of impaired glycemic control and consequently DPN.

**Polyol Pathway.** The aldose reductase enzyme in the polyol pathway is known to have a role in reforming glucose to sorbitol. Complications of diabetes have been hypothesized to be related to sorbitol accumulation in tissue. Moreover, animal studies have shown that greatest risk of developing DPN is involved with aldose reductase over-expression. Increased glucose flux through the polyol pathways can lead to peripheral nerve damage, and a similar mechanism can cause changes to the crystalline lens in the eye.

**Oxidative Stress.** Diabetes can cause an increase in the concentration of intracellular glucose content. Glycol-oxidation or lipoxidation compounds, as two end-points of interaction between glucose and reactive oxygen species, increase extracellular osmotic stress. This ultimately leads to aggregation of protein kinase C and a reduction in antioxidant cell defense. The increased protein kinase C gives rise to microvascular permeability, which can lead to damage of associated nerves.

**Vascular Factors.** Neuropathy in diabetes has been shown to be associated with microvascular complications, and there is evidence of improvements in neuropathic condition as a consequence of improved tissue blood flow. The importance of vascular factors in the pathogenesis of DPN has been highlighted in focal ischemic nerve lesions in association with severe blood vessels damage. This has been shown to mainly occur in diabetic focal neuropathies.

**Other Factors.** There are other factors involved in pathogenesis of DPN including insulin-like growth factors, vascular endothelium growth factors, and immune factors.

**Traditional Tests for DPN**

Many clinical tests are available to diagnose DPN, assess the severity of the condition, and monitor progression. Conventional techniques such as nerve electrophysiology and quantitative sensory testing (QST), along with an assessment of neurological disability, offer a relatively robust means of defining neuropathic severity. However, these procedures have potential shortcomings when they are used to define therapeutic efficacy in clinical intervention trials. These shortcomings relate to an inability to target the small fiber types demonstrating regeneration and repair.

To assess DPN, the American Diabetes Association recommends one measure from each of the following categories: clinical symptoms, clinical examination, electrdiagnostic studies, QST, and autonomic function testing.
Towards the Development of a Comprehensive Model of DPN Assessment Using Ophthalmic Structure/Function Markers

A primary role of corneal nerves is to afford a protective function via an aversion response to traumatic, toxic, infectious, and other noxious insult. Any compromise of the structural integrity of the nerve plexus that mediates this response may result in a functional deficit. It was therefore decided to assess both corneal nerve morphology (structural measure) and sensitivity (functional measure) in patients with DPN with the aim of developing a corneal structure/function model of this condition.

The idea of modeling a relationship between structure and function in ophthalmic disease is not new. Back in 1989, Caprioli suggested a link between changes in the optic disc and surrounding nerve fibers observed ophthalmoscopically with deficits in the visual field. With the recent advent of optical coherence tomography (OCT) and more sophisticated paradigms of visual field assessment, the search for such relationships—especially in the field of glaucoma—has gained renewed momentum.

Applying these concepts to the field of DPN, it could be hypothesized that a drop-out of nerves may be occurring in the retina, which could be mirrored by a loss of visual function. That is, it may be possible to develop both corneal and retinal structure/function models of DPN.

Corneal Structure as a Marker of DPN

Corneal Confocal Microscopy

Optical Principle. The principle of operation of the CCM is that a single point of tissue is illuminated and simultaneously imaged by a camera in the same plane. Structural elements above or below the focal plane are thus out of focus and eliminated. The resultant image has a very high resolution but very narrow field of view. The CCM overcomes this problem by instantaneously illuminating, and synchronously imaging, a small region of the cornea with thousands of tiny spots of light each second. The spot images are reconstructed to create a usable field of view offering high resolution and magnification. A similar result can be achieved using a scanning slit beam of light.

Available Instruments. There are currently two CCMs on the market. The Nidek ConfoScan 4 (Nidek Technologies, Padova, Italy) is a fully automated white-light slit-scanning instrument. The Heidelberg Retina Tomograph 3 with Rostock Corneal Module (Heidelberg, Germany) is a semiautomated laser scanning instrument. Laser light has the advantage of producing very high contrast images of thin layers from the transparent cornea and semitransparent conjunctiva. The Heidelberg CCM produces excellent high-contrast images of the corneal sub-basal nerve plexus and is thus the preferred instrument for morphological assessment of this tissue layer (Fig. 3).

Examination Technique. For examination of corneal nerve structure using the Heidelberg CCM, a drop of topical anesthetic (benoxinate hydrochloride 0.4%) is instilled in the eye to be examined. We have established that nerve morphology as imaged with the CCM is unaffected by the use of topical anesthetic. The patient is instructed to fixate a target with the contralateral eye. The objective lens of the laser microscope is housed within a sterile disposable Perspex cap. A drop of viscoelastic gel (GenTealEyes; Novartis, North Ryde, NSW, Australia) is placed on the tip of the objective lens before the cap is mounted on top. The gel optically couples the objective lens to the cap. The surface of the cap is brought gently into contact with the cornea; this procedure is facilitated by a side-mounted CCD camera, which displays a magnified, real time image of the cap as it makes contact with the cornea. Placing a drop of viscoelastic gel on the front of the cap further assists optical coupling with the cornea and aids patient comfort.

Image Acquisition. Images are obtained using one of three possible examination modes. Section mode enables manual acquisition and storage of a single image at a time. The cornea is scanned manually in x, y, and z axes, and image capture is effected with the aid of a foot pedal. Volume scan mode allows automatic acquisition of up to 40 images, ~2 μm apart, in the z axis. Thus, a section of cornea 80 μm in depth can be scanned (using the 400 μm field lens). Sequence scan mode allows acquisition of up to 100 images at capture rates from 1 to 30 frames/s, a feature that facilitates nerve mapping (discussed below).

Morphometry of Corneal Nerves

Image Analysis. Various indices of corneal nerve fiber morphology have been investigated, including nerve fiber count, length, branching, beading, width, tortuosity, orientation, and reflectivity. Discrepancies in absolute values reported by different research groups can be attributed to the use of different instruments and different approaches to stereological analysis of confocal images. Corneal nerve fiber length is perhaps cited most often; this parameter is defined as the total length of nerve fibers visible per unit area of cornea. Corneal nerve fiber length per unit area in healthy subjects has been reported as being 20 to 22 mm/mm² in the central cornea with use of the Heidelberg instrument.

We have developed a custom-designed semiautomated nerve analysis software package (CCMetrics; University of Manchester, UK) that involves tracing the nerves on a Wacom Graphics Tablet (Wacom Co., Saitama, Japan) using a grip pen. This procedure results in an intraclass correlation coefficient of 0.95 for repeated measures by an individual subject and 0.95 for measurements by two observers.

A number of fully automated systems for assessing nerve morphology have been developed. For determining corneal nerve fiber length, Scarpa et al. demonstrated that 94% of nerves identified manually were correctly identified with an fully automated algorithm. Dabbah et al. demonstrated a strong correlation (r = 0.92) between manual and fully automated methods.

Nerve Mapping. Patel and McGhee used the CCM to capture a large number of images across the cornea, which were...
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<th>Test</th>
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<tr>
<td><strong>Subjective tests (require a response from the subject under examination)</strong></td>
<td>Automated quantitative determination of thresholds for heat, cold, heat pain, cold pain, and vibration.</td>
<td>CASE IV (WR Medical Electronics Co, MN). Medoc Quantitative Sensory Analyzer (Medoc Advanced Medical Systems, Ramat-Yishai, Israel).</td>
<td>Allows response quantification on a continuous scale; expensive apparatus.</td>
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<td>QST(^54)</td>
<td>Manual scoring method for determining hot and cold sensations, vibration and Achilles tendon reflex. Scoring system: 0–2—no neuropathy 3–5—mild neuropathy 6–8—moderate neuropathy 9–10—severe neuropathy</td>
<td>Metal rods immersed in beakers with hot and cold water, Neurep (sharp/blunt stimulus), tuning fork (vibration) and tendon hammer.</td>
<td>Simple and inexpensive; easy to apply clinically; allows general response categorization.</td>
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<td>NDS(^55)</td>
<td>10 g nylon filament applied to sole of foot; patient reports if they can feel it.</td>
<td>Monofilament pen.</td>
<td>Rapid; inexpensive; non-quantitative.</td>
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<td>Monofilament test(^56, 57)</td>
<td>A test for scoring symptoms of unsteadiness in walking, neuropathic pain, paraesthesia, and numbness. Scoring system: the presence of one symptom is scored as 1 point; (maximum score is 4 points). A score of 1–4 indicates DPN.</td>
<td>Questionnaire.</td>
<td>Validated symptom assessment; high predictive value to screen for DPN.</td>
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<td>DNS score(^58)</td>
<td>Properties of nerve conduction (latency, amplitude and velocity) assessed with skin-attached stimulus and recording electrodes.(^59)</td>
<td>Computer-assisted electromyography equipment.</td>
<td>Direct assessment of nerve function; uncomfortable for subject; requires trained technician or specialist; primarily measures large fibers.</td>
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<td>Nerve conduction studies</td>
<td>Immunohistochemical examination of fine nerves obtained via a 3 mm skin punch biopsy or excised sural or peroneal nerve tissue.</td>
<td>Dermal skin punch; surgical tools; pathology laboratory with appropriate immunohistochemical staining agents.</td>
<td>Direct observation of small dermal nerves; painful; invasive; non-reiterative; small risk of complications.</td>
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<tr>
<td>Skin(^60) and nerve(^61) biopsy</td>
<td>Quantification of spinal cross-sectional area.</td>
<td>MRI scanner.</td>
<td>Non-invasive; very expensive; not suitable for routine clinical use.</td>
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<td>MRI(^62)</td>
<td>Adhesive pad containing cobalt salts is attached to plantar aspect of foot; pad changes color from blue to pink within 10 min if cholinergic sympathetic function is normal.</td>
<td>Neurep (Micro, Drabenderhöhe, Germany).</td>
<td>Tests autonomic neuropathy; rapid; simple; inexpensive; assesses small fiber dysfunction.</td>
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<tr>
<td>Neuropad(^63)</td>
<td>Assessment of the beat-to-beat alterations in heart rate during deep breathing.</td>
<td>Electrocardiograph equipment.</td>
<td>Tests autonomic neuropathy; expensive apparatus.</td>
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<td>Heart rate variability(^64)</td>
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DNS, Diabetic Neuropathy Symptom; MRI, magnetic resonance imaging.
then mapped together to form a montage. This allows the pattern of the sub-basal nerve plexus to be appreciated across the central 3 to 4 mm of the cornea. Using this technique, the authors also demonstrated that the sub-basal nerve plexus radiates from the limbus toward a whorl-like complex located 1 to 2 mm below the corneal apex. Clockwise\(^1\)\(^2\)\(^5\) and counterclockwise\(^1\)\(^2\) convergence of nerves has been noted in this region.

The pioneering technique devised by Patel and McGhee\(^8\) is tedious, requiring long sessions of image capture and many hours to form a montage of the nerve plexus. We have developed a novel, faster, and semiautomated technique for mapping the corneal sub-basal nerve plexus using the video capture facility of the CCM. While the subject tracks a moving target on a large computer screen, images are captured, using sequence mode on the CCM, from the yoked contralateral eye as it moves in the same direction. This procedure, which takes about 20 s and captures 100 contiguous images, is repeated along 13 radial meridians. The second stage of montaging is performed with Image-Pro Plus 7 software (MediaCybernetics, Bethesda, MD) to align and blend the radial image strips together. A map of equal or superior quality is generated in approximately one fifth of the time compared with the technique of Patel and McGhee (Fig. 4).\(^8\)

As can be seen from the map of a healthy non-diabetic subject (Fig. 4C) and the corresponding nerve tracing (Fig. 4D), there is significant regional variation of nerve density across the cornea; morphometric studies have shown this variance to be statistically significant (\(p < 0.001\)).\(^7\)\(^8\) These nerves emanate from neural nodes in the corneal mid periphery;\(^1\)\(^0\)\(^1\)\(^1\) as they traverse toward central cornea, they increase in density. Sub-basal nerve density is highest at the corneal center (14,731 ± 6,056 \(\mu m/mm^2\)) and lowest at the nasal midperiphery (7,850 ± 4,947 \(\mu m/mm^2\)).\(^7\)\(^8\) This variance highlights the importance of obtaining CCM images from a constant location when looking for changes over time or differences between subjects or groups. The general approach adopted by most researchers at the present time is to obtain images from the central cornea.

Recent advances in fluorescent immunohistochemistry and postmortem specimen preservation have allowed researchers to obtain high-quality wide-field images of the human corneal sub-basal layer in vitro. Marfurt et al.\(^1\)\(^3\) stained the nerve plexus with a primary antibody against beta neurotubulin (Fig. 4). Al-Aqaba et al.\(^1\)\(^0\)\(^1\) stained corneal whole mounts for cholinesterase enzyme. He et al.\(^1\)\(^2\) used a fluorescent microscope to image the cornea after staining with a monoclonal antibody specific for neuronal class \(\beta III\) tubulin.

Maps of the corneal sub-basal nerve plexus generated in vitro by these authors are strikingly similar, and the general form of the nerve plexus is virtually identical, to those generated in vivo using CCM. These similarities essentially cross-validate the in vitro and in vivo approaches. It should be noted, however, that all nerve maps displayed in Fig. 4 under-represent the full extent of the nerve plexus. Marfurt et al.\(^1\)\(^3\) point out that, for clarity, only the largest diameter sub-basal nerve fibers were illustrated in Fig. 4A and they demonstrate the full extent of sub-basal nerve density in their article. The CCM is only capable of resolving nerves greater than about 0.5 \(\mu m\) in diameter,\(^1\)\(^2\) so thinner nerves can not be imaged.

### Assessing Corneal Sub-Basal Nerve Fiber Loss in DPN

In a preliminary pilot study using a Tomey P4 CCM, we obtained images of the corneal sub-basal nerve plexus from 18 diabetic patients and 18 age-matched control subjects. Corneal nerve fiber density (\(F_3 = 9.6, p < 0.0001\)), length (\(F_3 = 23.8, p <\)
FIGURE 4.
0.0001), and branch density ($F_3 = 13.9, p < 0.0001$) were reduced in diabetic patients compared with healthy control subjects, with a tendency for greater reduction in these measures with increasing severity of neuropathy. These results confirmed the earlier findings of Rosenberg et al. and suggested that CCM could serve as a rapid, non-invasive in vivo clinical examination technique capable of accurately defining the extent of corneal nerve damage and repair and acting as a surrogate measure of DPN.

We subsequently performed a more comprehensive study on 101 diabetic patients and 17 age-matched healthy control subjects. These study participants underwent neurological evaluation [to determine the Neuropathy Disability Score (NDS)], electrophysiology tests, QST, and evaluation of corneal nerve morphology using the Heidelberg CCM. Again, corneal nerve fiber density, length, and branch density correlated strongly with neuropathic severity (Fig. 5). Receiver operating characteristic curve analysis for the diagnosis of neuropathy (NDS >2) established that, for a nerve fiber density of <27.8 no./mm², CCM had a sensitivity of 0.82 and specificity of 0.52; for a nerve branch density of <13.9 no./mm², CCM had a sensitivity of 0.91 and specificity of 0.45, and for a nerve fiber length of <3.4 mm/mm², CCM had a sensitivity of 0.64 and specificity of 0.79.

In relation to detecting patients at risk of foot ulceration (NDS >5), CCM had sensitivity of 0.71 and specificity of 0.64 for a nerve fiber density cut-off of <20.8 no./mm²; a sensitivity of 0.71 and specificity of 0.71 for a nerve branch density cut-off of <6.9 no./mm²; and a sensitivity of 0.64 and specificity of 0.71 for a nerve fiber length cut-off of <3.3 mm/mm². The appropriate combination of nerve parameter and criterion can be selected to optimize the capability of CCM for diagnosing DPN.

Subsequent studies by numerous other authors have confirmed a general association between diabetes and morphological deficits in corneal nerves using CCM. Frueh et al. and Mocan et al. have described changes in stromal nerves in diabetes; however, Patel and McGhee urged caution in interpreting the results of these studies, because, unlike corneal sub-basal nerves which essentially run in a single plane, stromal nerves run through the cornea in three dimensions, leading to errors in morphometric sampling by CCM. Accordingly, most attention has focused on changes observed in the sub-basal nerveplexus. However, none of these studies stratified the diabetic cohort by neuropathic severity.

Although duration of diabetes is a risk factor for DPN, corneal nerve damage does not appear to be directly correlated with the duration of diabetes. Messmer et al. reported that there was no difference in corneal neuropathy between patients with type 1 vs. type 2 diabetes.

Notwithstanding the demonstrated capacity of CCM to assess DPN as outlined above, the inability of this instrument to image very fine sub-basal nerves may, to some extent, limit its sensitivity to detect very early nerve degeneration because the more distal, thinner nerves are likely to be affected first. Evidence of distal nerve degeneration in DPN can be seen from inspection of the nerve maps in Fig. 4. In the maps obtained from a patient with severe DPN (Fig. 4E, F), the most distal element of the map—the central whorl—has been virtually obliterated. This becomes evident when comparing these maps with those of a healthy control subject (Fig. 4C, D).

**Dermal Intraepithelial vs. Corneal Sub-Basal Nerves as Markers of DPN**

Before the advent of CCM, skin punch and sural/peroneal nerve biopsy were the only techniques available for observing peripheral nerve fibers, albeit indirectly in vitro via the examination of excised tissue. This approach is invasive, expensive, time consuming and requires medical expertise to perform the biopsy and pathological expertise to investigate the tissue sample. It is also uncomfortable for the patient, subsequent biopsies can not be performed at the same site, and there is a small risk of complications.

To compare the utility of CCM vs. skin punch biopsy for assessing DPN, we performed both techniques on 54 diabetic patients stratified for neuropathy and 15 control subjects (Fig. 6). Dermal intraepithelial nerve fiber density ($r = -0.43, p < 0.001$), nerve branch density ($r = -0.38, p < 0.006$), and nerve fiber length ($r = -0.34, p = 0.012$) showed a progressive reduction with increasing severity of neuropathy; this relationship is shown in Fig. 7 for intraepithelial nerve fiber length. A progressive reduction in corneal nerve fiber density and branch density was also demonstrated with CCM. These results demonstrated that both skin punch biopsy and CCM assessment can accurately quantify small nerve fiber damage in diabetic patients. However, because CCM quantifies small fiber damage rapidly and non-invasively and detects earlier stages of nerve damage compared with intraepithelial nerve fiber pathology, it could be considered to be the preferred technique.

**Monitoring Recovery of DPN**

We have previously established that corneal nerves can regenerate after surgically induced trauma, such as penetrating keratoplasty or various forms of refractive surgery. Sub-basal corneal nerves

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**FIGURE 5.**

Corneal nerve fiber length in control subjects (no diabetes or neuropathy) and diabetic patients with no, mild, moderate, and severe neuropathy. Nerve fiber length is significantly reduced as severity of neuropathy increases among diabetic patients (analysis of variance, $p < 0.0001$). Mean ± standard error. Significant differences from control subjects indicated by * ($p < 0.0001$). After Tavakoli et al.
within the donor cornea or perimeter of the flap are totally obliterated in the first few weeks after penetrating keratoplasty or refractive surgery, respectively. Significant, albeit incomplete, recovery of the nerve plexus can be observed over the next few months. These examples serve as a useful model for examining the potential for CCM to record nerve recovery in patients with DPN.

We used CCM to assess the neurological benefits of two methods of improving DPN—a simple clinical approach, and a radical surgical intervention. In the first study, a group of 25 diabetic patients were put on a strict diet, exercise, and diabetes medication regimen to improve glycemic control. After 24 months on the “strict regimen,” there was significant improvement in corneal nerve fiber density, which correlated with the improvement in HbA1c ($r = -0.52$, $p = 0.008$).

In the second study, CCM was performed in 15 patients with type 1 diabetes before and after undergoing simultaneous pancreas and kidney transplantation. Before surgery, nerve fiber length was significantly compromised when compared with healthy age- and sex-matched controls. Six months after surgery, nerve fiber length had recovered significantly (Fig. 8). The above findings established the potential for CCM to monitor nerve repair in DPN after clinical or surgical intervention.

Assessing Other Systemic Neurological Disorders

CCM has the potential for assessing nerve loss in neurological disorders unrelated to diabetes. For example, we have demon-
Corneal Function as a Marker of DPN

**Corneal Esthesiometry.** The Cochet-Bonnet Esthesiometer (C-BE) (Luneau Ophthalmologie, Chartres, France) has been the standard clinical method for measuring corneal sensitivity since being introduced by its eponymous inventors half a century ago. The instrument consists of a nylon monofilament of constant diameter which, depending on its length, can exert more or less pressure. The length of filament at which the subject reports noticing a sensation, which defines the pressure exerted, represents the corneal touch threshold. The primary disadvantages of this technique are (1) it is mildly invasive, causing subclinical microtrauma to the region of cornea touched and (2) patients undergoing this procedure are often apprehensive when visualizing the fine filament approaching their eye.

**Non-Contact “Air Puff” Corneal Esthesiometer.** Murphy et al. have described how corneal sensitivity can be measured using this novel non-invasive method. The non-contact corneal esthesiometer (NCCE) uses controlled pulses of air of varying pressures to stimulate the cornea. It measures the threshold sensitivity to a composite stimulus consisting of air pressure along with tear film evaporation and disruption.

The advantage of NCCE over the CB-E is that a large, continuous range of stimulus intensities can be produced. Furthermore, the NCCE stimulus is more precise and sensory-specific, testing is less variable, there is no corneal microtrauma and patient apprehension is minimized. The NCCE can assess the corneal sensitivity threshold in an accurate and repeatable manner, and it has been shown to be better at measuring lower stimulus thresholds than with the C-BE. The NCCE is not commercially available, is expensive to make and is not as portable as the CB-E.

**Reduced Corneal Sensitivity in Diabetes**

Using the CB-E, reduced corneal sensitivity in diabetic patients was established almost 40 years ago. Murphy et al. confirmed a loss of corneal sensitivity in diabetic patients with the NCCE compared with age-matched control subjects.

**Reduced Corneal Sensitivity in DPN**

Nielsen and Lund provided preliminary evidence of an association between reduced corneal sensitivity and DPN in 1978, which was confirmed over 20 years later by Rosenberg et al. We have extended this work by demonstrating that corneal sensitivity, measured with the CB-E and NCCE, is significantly reduced in patients with DPN compared to healthy controls.

The results obtained with the two instruments were significantly correlated (r = 0.42, p < 0.0001). As well, there was a significant correlation between neuropathic severity, assessed using NDS, with the CB-E (r = 0.62, p < 0.0001; Fig. 9) and NCCE (r = 0.35, p < 0.0001; Fig. 10).

We have assessed the discriminative capacity of NCCE as a test for DPN in separate studies in the United Kingdom and Australia. In a group of 101 patients with diabetes in Manchester, a sensitivity of 0.60 for a corresponding specificity of 0.61 for the presence of neuropathy (defined as NDS >2) was reported, for a threshold of 1.12 mbar. A sensitivity of 0.70 and specificity of 0.75 was revealed, for a threshold of 0.66 mbar, in a Brisbane population of 81 patients with type 2 diabetes. Both studies failed to show that NCCE is able to differentiate those at risk of foot ulceration (NDS >5).

**Searching for Evidence of a Link Between Corneal Structure and Function in DPN**

One would expect fewer corneal nerves to be associated with reduced corneal sensitivity; however, evidence for such an association is not strong. Using CCM and NCCE, Patel et al. reported a significant correlation of 0.31 (p = 0.001) between the number of visible nerves and corneal sensitivity in patients with keratoconus. Benitez del Castillo et al. demonstrated a significant association (p < 0.0001) between CCM and CB-E measures in individuals with dry eye; however, the correlation coefficient was not reported. Patel et al. examined one eye of each of 60 healthy subjects of varying age using CCM and NCCE and reported a significant correlation (r = 0.18, p = 0.05).
between nerve fiber density and corneal sensitivity threshold in the temporal cornea.

Using the CCM and CB-E, Rosenberg et al.\textsuperscript{27} showed that the number of long nerve fiber bundles was positively correlated with corneal sensitivity ($r = 0.417, p = 0.048$) in a group of 23 patients with type 1 diabetes and varying degrees of neuropathic severity. These results provide some support for a direct link between corneal structure and function; however, further validation of this association is required.

**Retinal Structure as a Marker of DPN**

Neurodegeneration in diabetes has been proposed as an underlying cause of retinal vascular changes, and apoptosis of retinal ganglion cells has been reported in postmortem human studies and in animal models of diabetes.\textsuperscript{125,126} Thinning of the retinal nerve fiber layer (RNFL) is a potential by-product of retinal ganglion cell apoptosis and consequent axonal loss.

Müller cells are another potential target for apoptosis in the retina. One of the main functions of these cells is to biochemically support the vascular endothelial cells that form the inner blood-retinal barrier. Apoptosis of retinal glial cells, including Müller cells, can thus potentially contribute to microangiopathy, or dysfunction of small blood vessels, that is closely related to complications of diabetes—including retinopathy, neuropathy,\textsuperscript{127} and blood-barrier impairments.\textsuperscript{126} High concentration of glucose in neural tissue, as a consequence of high blood-retinal barrier permeability, leads to impairment of some glial and neural cell function and hence may interrupt glucose uptake from retinal circulation.\textsuperscript{128} Müller cells also act as a transporter to remove glutamate, which is highly toxic to retinal neurons. There is a likelihood that impaired function of Müller cells in diabetic retinae can cause oxidative stress,\textsuperscript{129} which is known to be a contributing factor to DPN.

Further to the above considerations, our justification for exploring a link between changes in the neuroretina and DPN was simple. Our studies with the CCM have demonstrated that damage to corneal nerves (derived from the ophthalmic division of the trigeminal, or fifth, cranial nerve) correlates with damage to dermal intraepithelial nerves, which are derived from long peripheral nerves (i.e., spinal nerves). This suggests that nerve pathology associated with DPN may be ubiquitous, affecting both peripheral spinal nerves and central cranial nerves.\textsuperscript{130}

So, if the fifth cranial nerve can be affected as part of this disease process, why not other cranial nerves? Given that I work in an ophthalmic research facility, numerous techniques are readily available for investigating the other cranial nerves that control ocular functions: specifically, the second (optic), third (oculomotor), fourth (trochlear), and sixth (trigeminal) cranial nerves. The second cranial nerve seemed to be a prime candidate for further investigation of markers of DPN for the following reasons.

The RNFL makes up the innermost neural layer of the retina. It is composed of large unmyelinated axons of ganglion cells that originate from various locations of the retina and converge to form the optic nerve; therefore, the RNFL forms an anatomical and functional continuum with the second cranial nerve. Our hypothesis is that the thickness of the RNFL in a standardized location, which can be measured using OCT, could serve as an index of the structural integrity of the neuroretina in DPN. Here, I shall present a brief overview of preliminary studies we have conducted in patients with DPN, based on this assumption.
Optical Coherence Tomography

Just as the CCM has revolutionised our basic and clinical understanding of the anterior eye, OCT has done the same for the posterior eye. Several OCT instruments are now available on the market; in our studies, RNFL thickness was measured using the RTvue (Optovue, Freemont, CA). This instrument uses the principle of low-coherence interferometry to produce two-dimensional images of optical back-scattering with an axial resolution of 5 μm.131 Measurement are acquired as follows. Participants are seated comfortably in a darkened room and are directed to fixate on an internal target. The “optic nerve head” protocol is used to generate an RNFL thickness map centered on the optic nerve head. The software algorithm acquires 24 radial scans in 0.37 s and records RNFL thickness around the optic disc at a diameter of 3.45 mm. The rapid rate of data acquisition minimizes possible confounding effects of eye movements.

RNFL thickness measurements represent the retinal layer containing ganglion cell axons, which appears as the highly reflective innermost layer of each scan (Fig. 11). Variation between measurements could be indicative of changes to the number of axons, average axonal thickness, glial cell structure, extracellular fluid volume, retinal vessel diameter,132 or any combination of these. A global mean RNFL thickness (μm) is calculated in addition to temporal, superior, nasal, and inferior quadrant averages (Fig. 11).

RNFL Thinning in Diabetes

Apoptosis of retinal neural cells has been reported in postmortem human studies of diabetes126; RNFL thinning would be expected to occur as a result of such changes. Takahashi et al.133 and DeBuc and Somfai134 found RNFL thinning in diabetic patients, but both groups attributed these changes to the presence of diabetic retinopathy. Sugimoto et al.135 and Lopes de Faria et al.136 reported RNFL thinning in the superior quadrant in diabetic patients without retinopathy. Sugimoto et al.137 also observed recovery of RNFL thickness toward normal levels in a group of diabetic patients who were brought under better blood glucose control. None of these studies, however, stratified their diabetic cohorts according to DPN status.

RNFL Thinning in DPN

We investigated the relationship between RNFL thickness and DPN in 82 patients with type 2 diabetes and 24 healthy control...
Visual Function as a Marker of DPN

Assessing Visual Function in Diabetes

Several studies on diabetic patients have shown visual function deficits in eyes that have normal visual acuity and minimal evidence of diabetic retinopathy. We hypothesize that these changes are attributed to DPN; this possible association has not been assessed in such studies. As shall be discussed below, a variety of methodologies is available to investigate visual function in diabetes.

Electroretinography. The electroretinogram (ERG) has been used to investigate functional and (presumed) biochemical changes at a retinal level. There are different types of ERG. Full-field (flash) ERG is the basic method of recording massed retinal electrical responses to light stimulation and it separates photoreceptor from inner retinal responses by isolating a number of recognisable waveforms. Pattern ERG stimulates the retina using patterned stimuli such as checkerboards, and specifically investigates the activity of ganglion cells and associated structures. Multifocal ERG evaluates small areas of retina individually and is valuable for assessing diabetes-related retinal changes, such as cotton-wool spots, that may affect visual function in spatially localized patches; and the dysfunction is predictive of future diabetic retinopathy.

There is evidence that ERG signals are impaired in diabetes before the onset of clinically evident retinopathy. Di Leo et al. and Caputo et al. found reduced pattern ERG amplitudes in diabetic patients without retinopathy. Early functional changes in diabetes have also been demonstrated using multifocal ERG; in this study, the local predictive power of the neural deficit was demonstrated over 3 years. Significant reductions in the direct response amplitude and implicit times in diabetic patients with no evidence of retinopathy have been reported.Other studies have shown that the onset of oscillatory potentials is delayed in diabetes in the absence of retinopathy. Oscillatory potential wave components are believed to originate from inner retinal layers through the activity of amacrine cells.

Visual-Evoked Potentials. Visual-evoked potentials represent electrical responses to counter-phasing visual stimuli (i.e., checkerboard patterns). They can provide diagnostic information about visual pathway integrity or neurosensory disorders beyond the retina. Visual-evoked potential latencies have been investigated in patients with diabetes and P100 latency (a peak of electrical activity ~100 ms after stimulus onset) as been proposed as a potential method for assessing neuropathy of the central nervous system in diabetic patients. Studies have shown significant increases in P100 latency in diabetic groups compared with controls. A positive relationship between peripheral nerve conduction and P100 latency in the absence of retinopathy has suggested a potential effect of neuropathy on optic pathways.

Perimetry. Visual field results represent the functional status of the visual pathway and not the retina exclusively. Very few studies have investigated the ability of commercially available standard (white on white) visual field tests to detect visual sensitivity changes in diabetic patients. Early investigations related to this relied on manual perimetry techniques. Roth suggested that the existence of a scotoma in the central 20° could be an early indicator of retinal compromise in patients with no visible ophthalmoscopic signs. Using Goldman perimetry, Wisznia et al. showed a partial constriction of the central isopter in diabetic patients with non-proliferative retinopathy. However, there is evidence that manual perimetry does not always effectively detect a visual field deficit, even in the presence of significant loss of neural cells.

The evolution of static automated perimetry enabled quantitative analysis of contrast sensitivity for a well-defined grid of test points, improving the potential for visual field analysis techniques to detect earlier, spatially specific changes in visual sensitivity. Trick et al. used automated visual field assessment to examine visual sensitivity in a cohort of patients with diabetes who had either no vascular changes or had mild background retinopathy only. Their findings showed significantly higher pattern deviation and lower mean deviation values for diabetic participants than for age-matched controls. Subgroup
analysis revealed that the mean deviation in both groups was dependent on the level of retinopathy.

Bell and Feldon\textsuperscript{158} found isolated loss of sensitivity in the central 15° of visual field in a diabetic group with normal retinal perfusion; they suggested that the loss may have been caused by microangiopathy and may further reflect retinal glial deficits. Several studies have compared the efficacy of short-wavelength automated perimetry and standard, white-on-white techniques for the detection of early psychophysical abnormalities in diabetes.\textsuperscript{159} Findings from these studies tentatively suggest that short-wavelength automated perimetry has the better potential to detect early functional changes.

Flicker Sensitivity. Flicker sensitivity describes the ability of an observer to detect intermittent light and dark alternation of a visual stimulus. It has been suggested that rapidly flickering stimuli are preferentially perceived by the magnocellular pathway;\textsuperscript{160} this pathway is characterized by fast conduction velocity, sensitivity to high temporal frequency stimuli, and the ability to detect movement.\textsuperscript{161}

Flicker ERG has been used to demonstrate isolated retinal sensitivity in diabetes.\textsuperscript{162} Lobefalo et al.\textsuperscript{163} reported that mean flicker fusion frequency values in children with type 1 diabetes were significantly lower than age-matched controls and were also highly related to the degree of metabolic control. The authors suggested that the presence of flicker impairment in the absence of clinically detectable retinopathy and media opacities could be a result of diabetes-related RNFL abnormalities.\textsuperscript{163}

Stavrou and Wood\textsuperscript{164} evaluated flicker sensitivity in the central visual field for a group with type 2 diabetes and compared these findings with results obtained from standard, white-on-white perimetry. The majority of defects detected by flicker perimetry appeared in the central 6° of visual field, whereas defects shown by the standard technique were located more toward the periphery.

Zele et al.\textsuperscript{165} found sensitivity losses using red-on-white and white-on-white flickering and static stimuli across the central visual field in a diabetic cohort, compared with age-matched controls. The authors suggested that red-on-white perimetry is more capable of detecting deeper defects than the standard white-on-white technique.

Metabolic control of diabetes is believed to have an impact on flicker perception. It has been demonstrated that a flicker stimulus increases capillary blood flow by 30%. This blood flow increase is maximal in perifoveal areas where ganglion cell density is highest. This could indicate that a tight link exists between the microvascular arrangement and areas in the retina with high metabolic demand.\textsuperscript{166} Mandecka et al.\textsuperscript{167} suggested that a flickering stimulus causes vasodilatation, and that this response is diminished before retinopathy is clinically manifested.

Frequency Doubling Technology Perimetry. Frequency doubling technology (FDT) perimetry has been shown to be a useful predictor of early visual loss in glaucoma.\textsuperscript{168} Frequency doubling occurs when a low spatial frequency sinusoidal grating undergoes a high temporal frequency counter-phase flicker; the theory underlying frequency doubling has been described in detail elsewhere.\textsuperscript{169} The perception of this phenomenon is thought to be mediated primarily by the magnocellular visual pathway.\textsuperscript{169}

Parikh et al.\textsuperscript{170} demonstrated that FDT could differentiate between diabetic patients with and without retinopathy, but it demonstrated poor predictive capability for macular edema. Parravano et al.\textsuperscript{171} also examined the role of FDT in eliciting early field defects in patients with type 1 diabetes. They suggested that these visual function changes may be a result of neural loss, implying that neuropathy, rather than vasculopathy, is the primary underlying mechanism.

Color Vision Testing. Impaired color vision has been reported to be an early sign of visual function loss in diabetes.\textsuperscript{172} Acquired blue-yellow losses using the Farnsworth-Munsell 100 Hue test in a diabetic cohort have been reported to occur before the onset of retinopathy.\textsuperscript{173} Hardy et al.\textsuperscript{174} found abnormal color vision using the Farnsworth-Munsell 100 Hue test in 57% of a cohort who had no evidence of microvascular disease of the retina. Roy et al.\textsuperscript{175} also reported color vision losses in a group of patients with diabetes who had minimal retinopathy. These findings suggest that color discrimination losses in diabetes may not necessarily be of vascular etiology.

Contrast Sensitivity Measurement. Contrast sensitivity measurements can elicit defects that are not readily detectable by commonly used conventional clinical techniques, such as visual acuity.\textsuperscript{176} Changes in contrast sensitivity, some of which are spatial frequency dependent, have been demonstrated in both children and adults with diabetes. Della Sala et al.\textsuperscript{177} demonstrated contrast sensitivity changes up to two standard deviations below normal values in a diabetic cohort, compared with age-matched controls. Ghaour et al.\textsuperscript{178} reported increased contrast thresholds at high spatial frequencies in patients with diabetes without clinically evident retinal vascular changes. Another group also found a reduction in contrast sensitivity in patients with early diabetic retinopathy.\textsuperscript{177}

The impact of metabolic control of diabetes on contrast sensitivity has also been investigated. Di Leo et al.\textsuperscript{179} suggested that, rather than hyperglycaemia, repeated hypoglycemia events may be more important factors in the pathogenesis of neuronal damage. Ewing et al.\textsuperscript{179} also found contrast sensitivity losses during hypoglycaemic events in patients with type 1 diabetes who had no evidence of retinopathy. The authors suggested that hypoglycaemia-related neural damage may be associated with increased “neural noise” at retinal and brain levels.\textsuperscript{180}

Dark Adaptometry. Dark adaptation has been investigated in diabetes.\textsuperscript{181,182} Henson and North\textsuperscript{183} reported that longer dark adaptation times occur in diabetes and that final adaptation thresholds are higher than age-matched norms. A recent study reported that adaptation changes are related to retinopathy levels but could be observed before the onset of vascular changes.\textsuperscript{184} This provides further evidence that changes to visual sensitivity in diabetes, before clinical manifestation of retinopathy, may relate to neuropathy.

Visual Field Loss in DPN

Faced with the broad array of techniques for assessing visual function described above, I had to decide which to use for my own research. I chose to assess visual fields using perimetry, for two reasons: (1) this is a technique used routinely in ophthalmic practice, meaning that my experimental results would be of immediate clinical relevance to eye care practitioners, and (2) unlike many of the other techniques discussed above that offer insights into underlying physiological mechanisms, perimetry provides a direct, subjective measure of vision loss. Results from perimetry studies would therefore allow immediate consideration to be given to implications relating to quality of life issues and any inherent risks that may compromise personal safety due
to, say, a diminished capacity for visual detection and navigation (so as to avert danger).

We conducted a study to test the hypothesis that patients with DPN will exhibit relative contrast sensitivity losses in a characteristic pattern across the visual field, compared to a control group with diabetes but without DPN.184 The Medmont M700 (Medmont International P/L, Vermont, Australia) was used for all visual field assessments. This is an automated static perimeter, which quantifies relative contrast sensitivity by measuring differential light detection thresholds against a uniform background luminance (3.2 cd/m²). The M700 utilizes a test bowl with in-built light-emitting diodes; stimulus presentation is driven by a custom-designed Windows-based software program.

The Medmont perimetry procedure is well validated and has better spatial resolution (i.e., more test points) than other commonly used perimeters.185 All participants underwent perimetry in one eye. The “Central 30” pattern test was used; this test determines a contrast threshold within the central 30 degrees of visual field for each of 106 separate test points.

Two age- and disease-duration-matched groups of patients with type 2 diabetes were recruited, consisting of a cohort with DPN (NDS > 2, n = 40) and a control group without DPN (NDS < 3, n = 30). All participants underwent assessment of visual acuity, slit lamp biomicroscopy, retinal photography, and measurement of intraocular pressure.

Patients with any medical condition (other than diabetes) known to be independently associated with neuropathy or suffering from current foot ulceration or infection were excluded. Ocular exclusion criteria included best-corrected visual acuity worse than 20/30, history of retinal laser photocoagulation, diagnosis or reasonable suspicion of glaucoma, ocular hypertension, clinically significant cataract or maculopathy, or any neurovisual pathology or medication use known to independently affect visual fields. Ocular fundus photography (Visucam Pro NM, Carl Zeiss Meditec, Jena, Germany) was used to grade retinopathy status and any potential participant with retinopathy classed as greater than “mild” by two observers was excluded from analysis (there was only one exclusion on this basis).

Raw data generated by the Medmont M700 represent contrast sensitivity levels (inverse of contrast detection thresholds) in logarithmic decibel (dB) units and these data were used for the primary analysis techniques developed for this study. Four approaches were adopted—an analysis that uses a single average decibel value to represent global sensitivity for each field, a second and third that assess hemisphere and quadrant differences, respectively, between the neuropathy and no neuropathy groups, and a fourth that evaluates three concentric rings (0 to 10°, 11 to 20°, and 21 to 30°).

Global, hemisphere, and quadrant between-group contrast sensitivities differences were constant at −1 dB lower in the diabetic group with DPN vs. the control group of diabetic patients without DPN. Contrast sensitivity deficits in the diabetic group with DPN for global and superior fields were weakly significant (t = 1.84, p = 0.07 and t = 1.94, p = 0.06, respectively). Between-group differences increased from 0.36 dB (p = 0.43) to 1.81 dB (p < 0.05) with increasing eccentricity; these differences were found to be significant beyond 15° after adjustment for age (Fig. 13). Retinopathy levels and disease duration were not significant factors within the model (p = 0.90). We conclude from this study that contrast sensitivity reduces with increasing eccentricity in type 2 diabetic patients with DPN.

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Searching for Evidence of a Link Between Compromised Retinal Structure and Visual Field Loss in DPN

To explore a relationship between compromised retinal structure and visual field loss, a viable technique is required for mapping the visual field onto the optic nerve head, and this is most readily achieved by considering superior and inferior hemifields. All nerve fibers from the superior retina cross the sampled 3.45 mm superior semicircle that the “optic nerve head” protocol uses to generate superior RNFL thickness data, and this corresponds to the inferior hemifield. The converse applies for the inferior retina and superior hemifield. Thus, at a basic level, a structure/function association can be explored by comparing superior/inferior RNFL/visual field deficits. For example, a defect in the inferior neuroretina would be expected to be associated with a defect in the superior visual field.

In separate studies outlined above, we provided evidence for inferior RNFL thinning and reduced contrast sensitivity in the superior field in diabetic patients with DPN. Although this is suggestive of a structure/function association, the evidence we have gathered so far is not strong, especially given that the loss of contrast sensitivity in the superior field was only marginally significant (p = 0.06). Further data, and the employment of sophisticated models such as those being developed in the field of glaucoma, will be required to provide definitive evidence of an RNFL/visual field structure/function association in DPN. As well, a variety of alternative visual function tests such as those discussed above could be deployed to further elucidate this association.

Exploring Evidence for an Association between Corneal vs. Retinal Nerve Dysfunction in DPN

In attempting to develop a unifying model of ophthalmic neuropathy, a good starting point would be to identify a common, clinically verifiable etiological factor that would underpin struc-
tural and functional neuropathic changes that can occur in all ocular tissues. As discussed earlier in this article, peripheral neuropathy in diabetes has been shown to be associated with microvascular complications. Thus, an association between vasculopathy and neuropathy could represent a useful premise for modeling ophthalmic neuropathy.

It is difficult to explore such associations in the cornea, as this is an avascular structure. Nevertheless, abnormalities in the sub-basal nerve fiber layer may be linked to microvascular complications as a result of changes to the blood-aqueous barrier,\(^{186}\) which are known to occur as a result of diabetes.

In the posterior eye, there is evidence that RNFL thinning,\(^{133,135,136}\) visual field defects,\(^{154,157,159,163,164}\) and abnormal visual electrophysiology results\(^{187–189}\) can occur in the absence of clinically evident retinopathy. This suggests that neuropathic changes observed in the eye might be occurring as a direct result of the metabolic compromises of diabetes and not necessarily as a secondary complication of vasculopathy. Certainly, van Dijk et al.\(^{190}\) came to a similar conclusion based on their observations of decreased retinal ganglion cell layer thickness in patients with type 1 diabetes and minimal retinopathy.

Rosenberg et al.\(^{22}\) reported that both corneal sensitivity and nerve fiber density decreased in patients with increasing severity of retinopathy. A number of other studies\(^{90–93,95,97}\) have also shown a direct relationship between abnormal corneal sub-basal nerve morphology and diabetic retinopathy. Again, this association probably reflects the degree of the underlying diabetes-related metabolic derangements that also lead to the observed corneal nerve changes.\(^{93}\)

**Clinical Ramifications**

**Significance for Patients with DPN**

An obvious question to ask in the light of the functional compromises highlighted in this article is the extent to which these defects impact the well-being of patients with DPN. It is my view that these compromises point toward a potential “triple jeopardy” situation faced by those suffering from this condition. This triple jeopardy manifests in different ways in relation to the cornea and compromised visual function.

**The Corneal Triple Jeopardy.** This essentially relates to a series of independent risk factors, which could combine in a number of ways to compromise corneal health in patients with DPN. These risk factors are as follows:

1. The corneal epithelium of diabetic patients is metabolically dysfunctional\(^{191}\) and more fragile.\(^{192,193}\)
2. As demonstrated in studies with NCCE, corneal sensitivity is reduced in diabetes, especially in those with more severe DPN. Accordingly, such patients may be unaware or less aware of corneal trauma or infection.
3. The prognosis for recovery from any corneal trauma or infection in a diabetic patient with neuropathy may be worse than for a diabetic patient without neuropathy or a non-diabetic person.\(^{194,195}\)

Schein et al.\(^{196}\) reported that diabetic patients using contact lenses have an increased risk of developing microbial keratitis; however, the magnitude of the increased risk was not specified. Although diabetes is not generally considered to be a contraindication for contact lens wear,\(^{197,198}\) patients with severe DPN should be advised against wearing contact lenses, or at the very least advised to exercise extreme caution if wearing lenses, in view of the triple jeopardy scenario outlined above.

The **Visual Function Triple Jeopardy.** Visual dysfunction in patients with DPN, along with impaired mobility and slower rates of tissue healing, represent risk factors that may combine to place patients afflicted with these disabilities in jeopardy. These three risk factors are as follows.

1. Mobility is often impaired in patients with DPN as a result of unsteady gait because of compromised joint proprioception or lower limb ulceration/amputation.\(^{199,200}\)
2. As demonstrated in the perimetry studies outlined above, patients with DPN have reduced contrast sensitivity in the peripheral visual field. Accordingly, such patients may experience difficulty seeing stairs/obstacles in their peripheral field of view, especially in low light conditions at low contrast, and therefore may be more prone to accidents and falls.
3. The prognosis for recovery of a patient with DPN from any general trauma or infection resulting from an accident is likely to be worse than for a diabetic patient without neuropathy, or a non-diabetic person, because of the general retardation of wound healing in diabetes.\(^{201,202}\)

**Significance for Health Care Practitioners**

There are a number of implications from these studies that may impact on the health care professions in terms of screening for, and assessment of, DPN.

**Ophthalmic Professions.** Optometrists, ophthalmologists, and allied ophthalmic professionals should anticipate the possibility of observing the following changes in diabetic patients with neuropathy: reduced corneal nerve fiber density (CCM); corneal sensitivity loss (NCCE); RNFL thinning (OCT); and loss of peripheral visual field sensitivity (perimetry). As many of these specialist clinical tests are presently found in ophthalmic clinical practices, the possibility exists for an expanded role for the eye care professions in diabetes screening; i.e., ophthalmic screening for diabetic retinopathy and neuropathy.

As well, there may be a role for optometry/ophthalmology/endocrinology in the ongoing co-management of patients with DPN, in respect of the use of ophthalmic markers to non-invasively and cost-effectively monitor the progression of DPN and the effects of any clinical/therapeutic interventions.

**Endocrinology/Diabetes Specialist Physicians.** Those directly involved in the care of diabetic patients may wish to consider using the various ophthalmic markers of DPN outlined above. Although CCM and NCCE are now well validated as markers of DPN, the tests of retinal structure and function require further validation before they can be considered as viable markers of this disease.

Both endocrinology-diabetes specialist physicians and ophthalmic practitioners should be prepared to counsel patients with DPN on the possible ocular ramifications of their condition, as outlined above in respect of the corneal and retinal triple jeopardy scenarios.
Ongoing Studies

The LANDMARK study (Longitudinal Assessment of Neuropathy in Diabetes using novel ophthalmic MARKers), funded by the Juvenile Diabetes Research Foundation International and the National Health and Medical Research Council (Australia), is currently underway in our two study centers (Brisbane and Manchester). A total of 320 patients with type 1 and type 2 diabetes, with and without neuropathy, and 116 healthy (non-diabetic and non-neuropathic) control subjects, are being followed for 5 years.

A full panel of general health assessments (blood panel, height, weight, waist circumference, blood pressure) neuropathy tests (symptom score, electrophysiology, QST, NDS, Neupadol, monofilament, heart rate variability, skin punch biopsy) and ophthalmic tests (CCM, NCCE, OCT, visual fields, fundus photography, visual acuity, and tonometry) are being performed. Primary aims of this study are to assess the diagnostic capability, and predictive and concurrent validity, of ophthalmic markers for the diagnosis of DPN. We will assess the capability of these ophthalmic tests to detect DPN in the early stages and to monitor disease progression or regression. Differences in ophthalmic manifestations of DPN in type 1 vs. type 2 diabetes are also being investigated.

As proud recipient of the 2010 Glenn A. Fry Lecture Award, I hope that I may be judged to have lived up to expectation enshrined in the motto of my Alma Mater, The University of Melbourne, which is Postera crescam laude ("I shall rise in the esteem of future generations.")

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REFERENCES


*"The Council of the University of Melbourne adopted this Latin motto on August 28, 1854. It is taken from Horace’s Odes III, Carmen XXX (an ode to the poet’s immortal fame): “… usque ego postera crescam laude recens dum Capito- lium scandet cum tactia virgine pontifici …"


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