Title: Exploring retinal and functional markers of diabetic neuropathy

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Abstract

Diabetic peripheral neuropathy (DPN) is one of the most debilitating complications of diabetes. DPN is a major cause of foot ulceration and lower limb amputation. Early diagnosis and management is a key factor in reducing morbidity and mortality. Current techniques for clinical assessment of DPN are relatively insensitive for detecting early disease or involve invasive procedures such as skin biopsies. There is a need for less painful, non-invasive and safe evaluation methods. Eye care professionals already play an important role in the management of diabetic retinopathy; however recent studies have indicated that the eye may also be an important site for the diagnosis and monitoring of neuropathy. Corneal nerve morphology has been shown to be a promising marker of diabetic neuropathy occurring elsewhere in the body, and emerging evidence tentatively suggests that retinal anatomical markers and a range of functional visual indicators could similarly provide useful information regarding neural damage in diabetes – although this line of research is, as yet, less well established. This review outlines the growing body of evidence supporting a potential diagnostic role for retinal structure and visual functional markers in the diagnosis and monitoring of peripheral neuropathy in diabetes.

Key words: diabetes mellitus, peripheral neuropathy, retina, visual function, optical coherence tomography, visual fields

Diabetes mellitus is an increasingly prevalent chronic disease, with a high rate of morbidity and mortality worldwide. Reducing diabetes-related morbidity and mortality and improving quality of life are major public health goals, which could be assisted by earlier disease diagnosis and improved screening protocols for associated complications. This could ultimately lead to earlier and more effective management of risk factors. Complications can include myocardial infarction, stroke and nephropathy as well as retinopathy – a ramification highly familiar to eye-care professionals. Another major complication of diabetes is neuropathy – pathological changes affecting the neural system throughout the body, which can lead to devastating consequences such as lower limb amputation. Neuropathy can also affect the eyes; however its impact in this regard is less familiar to health professionals. Although changes to corneal structure in diabetes are relatively better characterised,[1, 2] this review will outline what is known about alterations in retinal structure and visual function, which may ultimately manifest as a consequence of neural pathology. Conversely, it will explore how retinal structure and visual function may hypothetically provide valuable and readily accessible early indicators of neuropathy elsewhere in the body.

Diabetes mellitus is defined by the level of hyperglycaemia.[3] There are two main types of diabetes. Type 1 diabetes mellitus, formerly known as insulin-dependant diabetes, is characterized by auto-immune destruction of pancreatic beta-cells, leading to a loss of insulin secretion. Type 2 diabetes mellitus is the most common form of the disease, accounting for over 90 percent of diabetes in Australia.[4] It involves a combination of insulin resistance and impaired insulin secretion. Prevalence increases with age and it is strongly associated with obesity. Gestational diabetes is a less common form that occurs

in approximately 7% of all pregnancies and is a major risk factor for later development of Type 2 diabetes.[5]

The prevalence of diabetes increases with age and with population growth[6] and is higher amongst certain racial minorities.[7] In 2005, over 600,000 Australians (more than 3% of the population) were estimated to have established diabetes, with many more cases remaining undiagnosed.[8] Trends for international prevalence of the disease mirror this profile.[6] Diabetes has multiple and wide-ranging health ramifications – however, diabetes-related mortality is predominantly caused by cardiovascular disease. Increased morbidity is driven by long term microvascular complications; retinopathy is the leading cause of premature blindness worldwide,[9] nephropathy is the most common cause of end stage renal failure [10] and neuropathy is the leading cause of non-traumatic lower limb amputation.[11]

Complications of diabetes

The incidence of micro-vascular problems such as retinopathy and nephropathy, as well as neuropathy, is tightly linked to glycaemic control and to disease duration in both Type 1 and Type 2 forms.[12] Macro-vascular complications of diabetes such as coronary artery disease and myocardial infarction are also frequent in this population and risk factors like hyperglycaemia, hypertension and dyslipidaemia are thought to play a strong role in their incidence.[3]

Peripheral neuropathy – one of the most common complications in the diabetic population worldwide[13] – is characterised by pathology of sensory, motor and

autonomic nerves and it has both anatomical and functional repercussions. Its effects are primarily evident in the feet and legs where it can result in gait disturbances, neuropathic pain, and an increased risk of foot ulceration and subsequent lower limb amputation. Although the cardiac manifestations of diabetes are primarily vascular in origin, a subset of people with diabetes suffer from autonomic neuropathy, [14] which can affect heart rate and rhythm, potentially resulting in exercise intolerance and postural hypotension. This review focuses primarily on peripheral sensory neuropathy, the most common form of neuropathy in diabetes.[15].

Peripheral nervous system

The peripheral nervous system (PNS) in humans comprises a range of sensory and motor nerve bundles with differing characteristics. In a simplified model, afferent (sensory) neurons originate at sensory receptors and serve to inform the central nervous system (CNS) of the presence of relevant stimuli, whilst efferent (predominantly motor) neurons connect the CNS to muscles to precipitate movement. The somatic subdivision of the PNS primarily consists of efferent nerves innervating voluntary skeletal muscle but also includes afferent components from the skin, whereas the autonomic subdivision innervates involuntary muscles as well as having visceral sensory components (Fig. 1). The autonomic system has further sympathetic, parasympathetic and enteric subdivisions.[16]

Axon diameter plays an important role in the classification of nerve fibres in the PNS, with the thicker myelinated fibres having a faster conduction velocity. Based on this

characteristic, fibres can be categorized into three broad groups. The largest myelinated axons belong to group A and these are further delineated into α, β, δ and γ according to their function. Group B fibres are primarily myelinated axons of autonomic pre-ganglionic neurons and they are less well characterized than those in the other groups. Group C consists of sensory nerves with relatively small un-myelinated axons.[16] A classification summary of peripheral nerve fibres is provided in Table 1. Diabetic peripheral neuropathy can potentially affect nerves from all of these classes.

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Diabetic peripheral neuropathy

Neuropathy is an important cause of lower limb pathology in diabetes. Pain is a frequent and critical end point of peripheral sensory nerve damage and can eventually result in depression and other negative psychological outcomes in affected individuals.[17] Loss of sensation is another outcome of advanced neuropathy and contributes significantly to the pathogenesis of diabetic foot complications such as ulceration and amputation.[17]

The true prevalence of diabetic peripheral neuropathy (DPN) is uncertain as reported data have been derived from varied study designs.[18] Additionally, epidemiological

reports primarily focus on people who have sought medical care for their condition, leaving open the possibility of significant numbers of additional sufferers who have not attended for assessment and care. This potential incongruity is highlighted by one epidemiological report that indicated DPN exists in 20% of the community in general, but in 30% of patients who attend hospitals.[19] Other studies, however, have reported prevalences as low as 14% or as high as 54%, with the discrepancy explained by differences in study populations and the tests used to evaluate neuropathy.[20, 21]

Several factors are involved in the pathophysiology of diabetic neuropathy. Hyperglycaemia, however, is clearly important and is involved at a very early stages of diabetes.[22] At a simplistic level, increased glucose flux through the polyol pathways can lead to peripheral nerve damage; the same mechanism can also produce crystalline lens changes in the eye.[23] Oxidative stress and vascular compromise have been postulated as further factors potentially underlying DPN.[15]

There are a number of classification systems for diabetic neuropathy, which are variously based on anatomical, pathological and pathogenetic features. However, those describing clinical manifestations are the most widely used. Thomas[24] proposed a classification system based on a combination of anatomical site and clinical findings. According to this classification, diabetic neuropathy is not a sole condition but collectively describes a number of disorders affecting peripheral nerves. Chronic sensori-motor neuropathies are the most common neuropathy types encountered in diabetes.[15]

Methods of assessing diabetic peripheral neuropathy

Examination of DPN can be performed using a range of methods, incorporating tools as simple and inexpensive as a tuning fork, through to sophisticated electrophysiological nerve conduction equipment and associated techniques. Symptoms of pain and sensation loss can be assessed using a range of questionnaires. [25] [26] One clinical measurement of neuropathy utilises a relatively simple protocol for evaluating sensory deficits. This procedure is a composite known as the Neuropathy Deficiency Score (NDS); it assesses a number of sensation modalities such as temperature, vibration and touch sensitivity as well as looking at distal tendon reflexes.[25] The Semmes-Weinstein monofilament also investigates touch sensitivity and is another simple screening device designed to detect advanced neuropathy and hence those at risk of ulceration.[15] Quantitative sensory testing (QST) is an established, less subjective method of evaluating responses to vibrating and thermal stimuli and for determining sensation and pain thresholds; it can be applied at a number of anatomical sites but is commonly used on the feet in people with suspected DPN (Fig. 2).[11] Electrophysiological nerve conduction studies evaluate factors such as conduction velocity, response amplitudes, and latencies for major peripheral sensory and motor nerves in order to define damage and the rate of progression of neuropathy (Fig. 3).[27] Electrophysiological assessment is considered precise and objective; however it does not assess damage to small fibres.[28] Additionally, the procedure is uncomfortable to undergo. Skin and nerve biopsies are considered to be an accurate and early diagnostic tool for peripheral neuropathy. These procedures, however, are invasive, painful, and may be associated with subsequent infection in the sampled area.[11]

"Insert Figure 2 here"

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A novel ophthalmic marker of diabetic neuropathy

The cornea is the most densely innervated tissue in the human body.[1] It contains C and Aδ sensory fibres arising from branches of the trigeminal nerve, and it has recently become a focus in neuropathy studies as a prospective ophthalmic marker of DPN. Corneal confocal microscopy (CCM) is a recently developed, sophisticated method of imaging the corneal sub-basal nerve plexus, which occurs as a monolayer at the level of Bowman's membrane.[29] CCM is capable of detecting changes in nerve fibre density and branching at early stages of diabetic neuropathy in a non-invasive manner.[1, 11] It has been demonstrated that corneal nerve fibre density is reduced in people with diabetes when compared with healthy controls, and this nerve density reduction is associated with the severity of neuropathy.[1] One CCM study has shown that pancreas transplantation can lead to evidence of small fibre repair in people with DPN, suggesting that neuropathy may be reversible with improvement in blood glucose status.[30] Corneal sensitivity has also been explored as a means of assessing corneal structure-function relationships; reduced corneal sensitivity also appears to be related to the severity of diabetic neuropathy.[31] An example of a CCM image is shown in figure 4.

"Insert Figure 4 here"

The lower limbs are the most evident focus for DPN, particularly given the fibre-length dependant nature of neuropathy, but nerves anywhere in the body can theoretically be damaged by an underlying metabolic abnormality. However, it is not well understood whether nerve changes in one part of the body accurately predict what is happening elsewhere. In the eye, the cornea and the retina are richly endowed with sensory nerve cells and their axons, and are therefore potential sites for neuropathy-related damage. This review is primarily concerned with changes to visual function and associated anatomical structures, and, as such will concentrate predominantly on putative effects on nerves comprising the retina and visual pathways.

Anatomy of the retina

The retina is a ten-layered sensory tissue forming the internal layer of the posterior eye.[32] It is the entry point for processing of visual information and, as such, much of its volume consists of neural elements, although it also contains glial and vascular components. The retina is extremely metabolically active and consumes the highest percentage of oxygen per weight of any human tissue.[33] Figure 5 shows a schematic of the retina.

“Insert Figure 5 here”

The retina houses complex connectivity networks but, in a conveniently over-simplified model, its neural components can be categorized into three broad groupings. Firstly, photoreceptors comprise the neural elements of the outer retina. These cells are specialized for converting light energy to nerve impulses. The two photoreceptor types are rods and cones, which have an approximate population ratio of 20:1.[32] Secondly, the centrally located plexiform and nuclear layers comprise several distinct neuronal cell types. Bipolar cells in these layers act as afferent connectors between the photoreceptors and the ganglion cells of the inner retina.[34] Horizontal cells are inter-neurons that run parallel with the retinal surface. They are associated with rods and cones via their long and short processes respectively. Amacrine cells are also inter-neurons that play an integrative role in retinal circuitry, synapsing with bipolar and ganglion cells. They have large cell bodies and no true axons. Finally, the third grouping comprises retinal ganglion cells (RGC) and their long axons, which form the retinal nerve fibre layer (RNFL) and eventually the optic nerve; these are the neural components of the inner retinal layers. Ganglion cells relay partially processed visual information from the retina to the lateral geniculate nucleus (LGN). The RNFL is discussed in more detail below.

In addition to these neural cells, the retina contains glial and vascular tissue. Müller cells are the principal glial cells of the retina and they serve a role similar to that of astrocytes in the CNS. They span the entire retina radially and their functions include structurally supporting the retina, providing nourishment for neural cells and assisting regulation of retinal blood flow.[35] The central retinal artery (CRA), which arises from the ophthalmic artery, gives rise to the inner retinal vascular network that nourishes the anterior two-thirds of the retina. The choriocapillaris, located outside the retinal pigment

epithelium (RPE), provides a blood supply for the remaining third.[32] The blood-retinal barrier, which is jointly achieved by the RPE and by tight junctions between retinal vascular endothelial cells, protects the neural retina from large molecules and toxic substances while permitting diffusion of essential nutrients.

Retinal nerve fibre layer

The retinal nerve fibre layer (RNFL) comprises unmyelinated axons of ganglion cells as well as astrocytes, Muller cell end feet and vasculature. [36] The fibres converge in a unique pattern to eventually form the optic nerve,[32] where they exit the eye. The RNFL is responsible for carrying visual information directly to the lateral geniculate nucleus (LGN) where the majority of axons first synapse. These axons are myelinated posterior to the lamina cribrosa. The fibres originate from a range of locations in the retina and their spread-pattern is specifically dependant on this location.[37] For example, fibres that originate from the foveal area find a relatively direct path to the optic nerve head, while those arising temporal to this need to execute a path around the putatively earlier-developing ones (Fig. 6). This model neatly accounts for the well-described arcuate conformation of the RNFL in this region.[38] A number of glaucoma studies have pointed out the utility of modelling axon growth as a means of describing the relationship between nerve fibre location and the function these fibres subserve.[39-41] Clinical evaluation of RNFL patency and determination of RNFL thickness is of great importance in glaucoma and may yet prove to be of interest in diabetes, even though the patterns of change and the aetiology may in fact be substantially different between the two diseases.

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There is substantial variation in RNFL thickness in a normal, healthy population[42] and a number of factors have been identified that appear to account for at least some of this variance. Several studies have shown a relationship between RNFL thickness and age.[43-45] Histological counts of optic nerve fibres in post-mortem eyes have shown a loss of 4000-5000 fibres per year.[46] Other studies have reported an age-related decline in RNFL thickness using scanning laser polarimetry (SLP) with one reporting a loss of 7.6% per decade[42] and another reporting 0.39 µm per year.[43] Optical coherence tomography (OCT) has been used to show thinning of the RNFL with increasing age, particularly in the temporal area around the optic nerve head.[47-49] One OCT study reported that RNFL thickness decreased globally about 2.6 – 2.9 µm per decade[50] while another showed that thickness of the superior RNFL quadrant may be selectively reduced with age.[51] Optic nerve head size also has an apparent correlation with RNFL thickness (albeit at a fixed spatial location) with most studies finding larger optic nerve heads in conjunction with thicker RNFL,[48, 52] although one study appeared to find the opposite, which may be due to employment of disparate techniques.[53] RNFL thickness has been shown to have an inverse correlation with axial length.[48, 50] It can also vary with ethnicity [43, 48] but there is minimal evidence of a gender difference.[48, 54] These factors need to be considered when investigating the effect of diabetes on retinal morphology in general, and on RNFL thickness in particular.

Diabetes related retinal pathophysiology

Apoptosis is a series of biochemical changes which leads to regulated cell death subsequent to internal cell changes that include DNA fragmentation and cell shrinkage.[55] Apoptosis is believed to mediate the pathogenesis of diseases of the retina such as glaucoma.[56] Neuro-degeneration in diabetes has been proposed as an underlying cause of retinal vascular changes, and apoptosis of retinal ganglion cells has been reported in post-mortem human studies and in animal models of diabetes.[57, 58] RNFL thinning 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,[59] and blood-barrier impairments. 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.[60] 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 retina can cause oxidative stress,[61] which is known to be a contributing factor to DPN.

Clinical assessment of retinal nerve fibre layer integrity

A number of different procedures allow clinical assessment of the RNFL. These have historically been used to assess glaucoma risk, but are nonetheless applicable to other disease models. Various ophthalmoscopy techniques can be used to assess RNFL integrity, although decisions regarding the existence of and, in particular, the progression of suspected pathological damage based on ophthalmoscopy alone are highly subjective. The development of ocular fundus photographic techniques improved this situation by allowing a permanent objective record for future comparison. However, photographic assessment also largely relies on qualitative judgement. Hoyt et al[62] used red-free photography to evaluate diffuse and local loss in the peripapillary retina and described the fundoscopic signs of early RNFL loss in glaucoma. This method of photography was further developed using black and white negatives.[63] Other groups enhanced red-free photographs of the RNFL using computer programmes [64] or used an image analyser to measure grey levels in red-free photographs for normal and glaucomatous eyes.[65] Yamazaki et al[66] eventually developed an analysis programme to detect changes of RNFL at early stages of glaucoma. Photographic assessment techniques can be limited by pupil size and by the presence of media opacities, as well as by the contrast-processing capability of the camera and associated software.

The application of laser-based techniques for imaging retinal tissue commenced with the development of scanning laser ophthalmoscopy. This instrument afforded advantages over photography such as improved resolution, the ability to be applied in the presence of media opacities,[67] and accompanying quantitative software analysis

packages.[68, 69] However, it also introduced relative disadvantages such as the inability to represent true fundus colour information. More recently evolved confocal scanning laser ophthalmoscopes (CSLO) and associated software have allowed an indirect measure of RNFL thickness at the optic nerve head margin, and generated a range of two and three-dimensional topographic features, many of which have demonstrated commendable reproducibility.[70] Scanning laser polarimetry (SLP) is another imaging technique that has been shown to successfully use RNFL information to discriminate between healthy and glaucomatous eyes.[71, 72] SLP employed a novel imaging principle to generate quantitative information regarding RNFL integrity. This method was based on an assumption that the RNFL is a birefringent (referring to multiple direction-dependent refractive indices) medium; this property affects the polarisation characteristics of a laser beam that is reflected from the retina.[71]

Optical coherence tomography (OCT) arguably provides the most direct measure of RNFL thickness of the established retinal imaging techniques. It is a non-invasive and reliable technique for quantitative analysis of axial retinal morphology in multiple planes.[73] OCT uses low-coherence interferometry and is comparable to ultrasonic echo techniques, except that light is used instead of sound. The latest generation OCT techniques (fourier-domain technology) have shown improved diagnostic capacity for monitoring retinal pathologies by capturing higher resolution images (5 μm axially) with a more rapid acquisition time (65 times faster than time-domain technology) compared with their predecessors.[74, 75] This has enabled improved identification of individual retinal layers, including the RNFL, with minimal intrusion of eye movements

on accuracy and repeatability.[76] The precise optical principles of OCT are outside the scope of this review; they have been described in detail elsewhere.[77, 78]

OCT has been used widely in identifying pathologies that are known to impact on retinal morphology. OCT algorithms calculate retinal thickness based on the reflectivity of each individual layer.[77] A standard circumpapillary OCT image, available on most commercially available instruments, is acquired using a cylindrical scan pattern of 3.4 mm diameter around the optic nerve head, and the analysis is displayed as 12 position sectors around the disc in a manner resembling a clock. The graphical figure of RNFL thickness is often referred to as temporal-superior-nasal-inferior-temporal (TSNIT) or a "double-hump pattern"[79] with the inferior quadrant having, on average, the greatest thickness followed by superior, nasal and temporal quadrants (Fig. 7).

"Insert Figure 7 here"

Evaluation of retinal nerve fibre layer in diabetes

Examination of the retinal nerve fibre layer (RNFL) has proven useful in identifying axonal loss prior to visual field abnormalities becoming evident.[48] The RNFL has been the focus of many glaucoma studies, given that changes in the thickness of this layer has become an important factor in diagnosis of the pathology.[49] However, RNFL morphology and its relationship to DPN may also be of interest. Although a potential relationship between the presence of retinal microvasculopathy in diabetes and severity

of DPN has been suggested,[80] a clear link between retinopathy and DPN has not yet been established and only a limited number of studies have investigated this relationship.[80, 81] Given that nerve damage in diabetes can be caused by a variety of mechanisms, including hypoxia, oxidative stress and changes to the polyol pathways,[15] it is reasonable to question whether such mechanisms can also damage the RFNL and, if so, whether RNFL changes can predict distal neuropathy elsewhere in the body.

Chihara et al[82] measured RNFL thickness in 137 patients with Type 2 diabetes. They used green filtered achromatic photographic negatives to assess the nerve layer, and they classified retinopathy into 4 groups with level 1 showing no vascular pathology, and other levels representing increasing stages of retinopathy. Their results showed that 20% of patients classified as having no retinopathy had RNFL defects and they suggested that the severity of retinopathy was a potential risk factor for a RNFL defect. They also suggested that cotton-wool spots, which generally occur in more advanced stages of retinopathy, are likely to cause retinal nerve fibre layer defects.

Lopes de-Faria et al[83] conducted quantitative assessment of RNFL thickness, using SLP in a small sample (N=10) with Type 1 diabetes. Their results showed, when compared with controls, a significant reduction in mean RNFL thickness in the superior quadrant for a cohort with diabetes who had no ophthalmoscopically evident retinopathy.[83] Another study by Skarf et al[84] found similarly. Sugimoto et al[85] were one of the first groups to evaluate RNFL thickness in people with diabetes using OCT. Their results showed a general reduction in RNFL thickness in all quadrants for a diabetic cohort

without evident retinopathy but the reduction was more significant superiorly, in agreement with earlier SLP findings.[83] Each of these studies suggested that impaired vasoconstriction processes in the superior quadrant, secondary to diabetes, may best explain the RNFL loss in this region. In contrast, a recent OCT study failed to find a significant difference in RNFL thickness between a diabetic group without retinopathy and controls, even though the diabetic group demonstrated comparatively reduced RNFL thickness globally and in all quadrants.[86] Their findings did, however, show that reduction in RNFL thickness was more evident in proliferative rather than earlier stages of retinopathy, and that RNFL thickness changes correlated with longer duration of diabetes. The authors also suggested that diabetes-related RNFL damage may develop more rapidly in men than in women, but they did not propose a specific mechanism for this finding.

Evaluation of visual function in diabetes

Diabetic retinopathy is a major cause of blindness in developed countries; as such, diagnosis and intervention prior to the onset of sight threatening vascular complications is crucial.[87] Assessment for retinopathy, in line with Early Treatment for Diabetic Retinopathy Study (ETDRS) recommendations, has focused on the progression of retinal vascular changes, from background through to proliferative stages of the disease.[88] The majority of published work on the retina in diabetes has investigated the effect of vascular changes on visual function; [88-90] however, several studies have shown visual function deficits in eyes that have normal visual acuity and minimal evidence of diabetic retinopathy.[91, 92] Bresnik et al[93] argued strongly that retinopathy should not be

viewed as a vascular pathology in isolation and that a similar argument can be applied to neuropathy; it should not be considered as isolated neural disease. Anatomical and physiological changes to the retina in diabetes highlight the importance of considering neural and vascular complications as potentially linked processes.

The electroretinogram (ERG) has been used to investigate functional and (presumed) biochemical changes at a retinal level.[94, 95] There are different types of ERG. Full-field (flash) ERG is the basic method of recording massed retinal electrical response to light stimulation and it separates photoreceptor from inner retinal responses by isolating a number of recognisable waveforms. Pattern ERG (PERG) stimulates the retina using patterned stimuli such as checkerboards, and specifically investigates the activity of ganglion cells and associated structures. Multifocal ERG (mfERG) evaluates small areas of retina individually and is valuable for assessing diabetic-related retinal changes, such as cotton-wool spots, that may affect visual function in spatially localised patches.[96]

There is evidence that ERG signals are impaired in diabetes prior to the onset of clinically evident retinopathy.[97, 98] Papakostopoulos et al [99] reported decreased ERG b wave amplitude in a Type 1 diabetic cohort without evident retinopathy. Di Leo et al[100] and Caputo et al[101] each found reduced PERG amplitudes in diabetic subjects without retinopathy. Multifocal ERG has also been used to demonstrate early functional changes in diabetes.[102, 103] Significant reductions in the direct response amplitude and implicit times in diabetic patients with no evidence of retinopathy have been reported.[104, 105] Lovasik and Spafford[106] as well as other studies[107, 108] have shown changes to the

amplitude or onset of oscillatory potentials (OP) in diabetes in the absence of retinopathy. OP wave components are believed to originate from inner retinal layers through the activity of amacrine cells.[109, 110]

Visual evoked potentials (VEP) represent electrical responses to counter-phasing visual stimuli (for example, checkerboard patterns). They can provide diagnostic information about visual pathway integrity or neuro-sensory disorders beyond the retina. The most commonly analysed response to visual stimuli is termed the P100, in which a recognisable peak of electrical activity occurs approximately 100 milliseconds after stimulus onset. Alterations to expected latency and waveform of the P100 can be indicative of visual pathway pathology. Non-visual event-related potentials can also be employed to investigate a range of sensory and executive functions. The P300 (also referred to as the cognitive potential) represents an activity peak that occurs approximately 300 milliseconds after the onset of non-specific task-related change.[111]

VEP latencies have been investigated in people with diabetes and P100 latency has been proposed as a potential method for assessing neuropathy of the central nervous system in people with diabetes.[112] Studies have shown significant increases in P100 latency in diabetic groups compared with controls.[113-116] 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.[114] One study using P300 found prolonged latencies related to diabetes in people with normal cognitive function and no retinopathy.[117]

Very few studies have investigated the ability of commercially available standard (white on white) visual field tests to detect contrast sensitivity changes in diabetic individuals. Early investigations related to this relied on manual perimetry techniques. Roth et al[118] examined the effect of diabetes on visual fields using a home designed "scotometer". Their findings suggested that the existence of a scotoma in the central 20 degrees could be an early indicator of retinal compromise in patients with no visible ophthalmoscopic signs. Wisznia et al[119] studied visual field defects at various stages of retinopathy using Goldman perimetry. They 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.[120]

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.[120] Trick et al [121] used automated visual field assessment to examine visual sensitivity in a cohort of people with diabetes who had either no vascular changes, or had mild back ground 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 et al[122] found isolated loss of sensitivity in the central 15 degrees 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 (SWAP) and standard, white-on-white techniques for the detection of early psychophysical abnormalities in diabetes.[123, 124] Findings from these studies tentatively suggest that SWAP has the better potential to detect early functional changes.

Flicker sensitivity describes an observer's ability 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;[125, 126] this pathway is characterized by fast conduction velocity, sensitivity to high temporal frequency stimuli, and the ability to detect movement.[127] Flicker ERG has been used to demonstrate impaired retinal sensitivity in diabetes.[128] Lobefalo et al[129] investigated flicker sensitivity in the central 30 degrees of visual field in a cohort with diabetes who had no clinical signs of retinopathy. They examined a group of children with Type 1 diabetes divided in two groups according to their metabolic control (good and poor). Results indicated that mean flicker fusion frequency values for both diabetic groups 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.[129]

Stavrou and Wood[130] 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 degrees of visual field, while defects shown by the standard technique were located more towards the periphery. In another recent study, Zele et al[131] 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 peri-foveal areas where ganglion cell density is highest. This could indicate that a tight link exists between the microvasculature arrangement and areas in the retina with high metabolic demand.[132] Mandecka et al[133] suggested that a flickering stimulus causes vasodilatation and showed that the vasodilatation response to flicker is diminished before retinopathy is clinically manifested. It has been shown that retinal blood vessels do not have a conventional autonomic innervation despite relevant receptors in the walls of these vessels.[134]

Frequency doubling technology (FDT) perimetry has been shown to be a useful predictor of early visual loss in glaucoma.[135] 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.[136] The perception of this phenomenon is thought to be mediated primarily by the magnocellular visual pathway.[136] Parikh et al[137] demonstrated that FDT could

differentiate between diabetic patients with and without retinopathy, but it demonstrated poor predictive capability for macular oedema. Parravano et al [138] also examined the role of FDT in eliciting early field defects in people with Type 1 diabetes. They suggested that reductions in retinal sensitivity in people with diabetes might relate to magnocellular pathway dysfunction, indicating that this pathway may be more susceptible to damage under hyperglycemic conditions. However, they acknowledged that this may alternately represent the capacity of the less populated parallel pathway to detect earlier functional loss. The authors further suggested that these visual function changes may be a result of neural loss, implying that neuropathy, rather than vasculopathy, is the primary underlying mechanism. Table 2 represents a summary of the findings of key retinal and functional studies with potential neuropathy-related changes.

Other measurements of visual function

Impaired colour vision has been reported to be an early sign of visual function loss in diabetes.[139, 140] Acquired blue-yellow losses using the Farnsworth-Munsell 100 Hue (FM100) test in a diabetic cohort have been reported to occur before the onset of retinopathy.[141, 142] Hardy et al[143] found abnormal colour vision using FM100 in 57% of a cohort who had no evidence of microvascular disease of the retina. Roy et al [144] also reported colour vision losses in a group of people with diabetes who had minimal retinopathy. These findings suggest that colour discrimination losses in diabetes may not necessarily be of vascular aetiology.

Contrast sensitivity measurements can elicit defects that are not readily detectable by commonly employed conventional clinical techniques, such as visual acuity.[91] 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 [91] demonstrated contrast sensitivity changes up to two standard deviations below normal values in a diabetic cohort, compared with age-matched controls. Ghafour et al[145] reported increased contrast thresholds at high spatial frequencies in people with diabetes without clinically evident retinal vascular changes. Another group also found a reduction in contrast sensitivity in patients with early diabetic retinopathy.[146] The impact of metabolic control of diabetes on contrast sensitivity has also been investigated. Di Leo et al[147] suggested that, rather than hyperglycaemia, repeated hypoglycaemia events may be more important factors in the pathogenesis of neuronal damage. Ewing et al[148] also found contrast sensitivity losses during hypoglycaemic events in subjects with Type 1 diabetes who had no evidence of retinopathy. The authors suggested that hypoglycaemic related neural damage may be associated with increased "neural noise" at retinal and brain levels.[149]

Other psychophysical measurements such as dark adaptation have also been investigated in diabetes,[150-152] although a number of groups have focused primarily on post-photocoagulation outcomes.[153, 154] Some findings however, have suggested that longer dark adaption times occur in diabetes and that final adaptation thresholds are higher than age-matched norms.[152] A recent study reported that adaptation changes are related to retinopathy levels but could be observed before the onset of vascular changes.[155] This provides further evidence that changes to visual sensitivity in diabetes, prior to clinical manifestation of retinopathy, may relate to neuropathy. Other studies have used techniques such as flavoprotein autofluorescence, macular photostress test and electroocculogram to demonstrate changes to visual function related to diabetes or

to sudden blood glucose spikes in healthy individuals. [156-158] However these outcomes have been attributed to retinal pigment epithelium function or metabolic tissue stress and are less likely to be strictly aligned with peripheral neuropathy.

Summary and conclusion

Neuropathy is a major complication of diabetes, and is known to have widespread impact on many vital organs of the body. Furthermore, at least fifty percent of individuals with diagnosed diabetes will eventually suffer from peripheral neuropathy, which is a major risk factor for foot ulceration and amputation. The establishment of earlier, safe, non-invasive and less painful diagnostic tools to determine the presence and severity of neuropathy needs to be a research priority. Eye-care professionals who provide care for people with diabetes understandably focus on vascular aspects of the disease. However, emerging evidence that corneal nerve structure is a potential marker of early peripheral neuropathy has introduced the prospect of a new and important role for eye care professionals in the management of diabetes. Retinal anatomy and visual function markers similarly have potential to contribute to this process; demonstrated structural and functional changes in diabetes prior to the development of retinopathy can reasonably be presumed to be neuropathic in origin. However, it is yet to be established whether these factors will prove to be clinically useful predictors of diabetic peripheral neuropathy.

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Table 1. Classification of peripheral nerve fibres. † Source of skeletal muscle contraction, ‡ Controlling muscle spindle sensitivity, ¶ visceral sensory fibres, ŧ Less myelinated than A and C fibres. (derived from reference 16 with modification)

Source	Classification	Myelination	Diameter (µm)	Conduction velocity (m/s)
Efferent				
α-motoneurons to muscles †	Aα	Yes	8 - 13	44 -78
γ-motoneurons to muscles ‡	Aγ	Yes	3 - 8	18 - 48
Preganglionic autonomic fibres ¶	B	Yes ŧ	< 3	3- 14
Afferent				
Limb position and motion	Aα	Yes	12 - 20	75 - 120
Touch, pressure, vibration	Aα / Aβ	Yes	6 - 12	30 - 75
Sharp pain, cold sensation	Aδ	Yes	1 - 6	5 - 30
Dull pain, warm sensation	C	No	< 1.5	0.5 - 2

Table 2. Summary of the findings of key retinal and functional studies with potential neuropathy-related changes. T1DM: Type 1 diabetes mellitus, T2DM: Type 2 diabetes mellitus, OCT : optical coherence tomography, SLP: scanning laser polarimetry, ERG: electroretinogram, VEP: visual evoked potential

Method	Study	Finding
Visual field studies	Roth *et al* 1969	Central 20 degree scotoma occurs prior to diabetic retinopathy development
	Trick *et al* 1990	Contrast sensitivity reduction in superior quadrant for T2DM cohort with minimal or no retinopathy
	Afrashi *et al* 2003	Blue on yellow perimetry finds sensitivity reductions not evident in standard perimetry for T1DM cohort without evident retinopathy
	Lobefalo *et al* 1997	Flicker sensitivity reduced in central 30 degrees of visual field for T1DM cohort without angiographic evidence of retinopathy
	Stavrou and Wood 2005	Flicker but not static perimetry thresholds reduced in T2DM cohort with minimal retinopathy
Retinal nerve fibre layer (RNFL) studies	Sugimoto *et al* 2005	Decreased superior RNFL thickness (OCT) for T2DM cohort without evident retinopathy
	Takahashi *et al* 2006 [159]	Decreased RNFL thickness (SLP) for T2DM cohort with mild retinopathy
	Lopes de Faria *et al* 2002	Decreased superior RNFL thickness (SLP) for T1DM cohort without evident retinopathy
Electrophysiological visual function studies	Lovasik and Spafford 1988	ERG b wave and oscillatory potentials altered for T1DM cohort without evident retinopathy
	Papakostopoulos *et al.* 1996	Decreased ERG b wave amplitude in T1DM cohort without evident retinopathy
	Yaltkaya *et al* 1988	VEP P100 latency prolonged in cohort with diabetes but no evident retinopathy

Figure 1. An illustration of interaction between the central nervous system and the peripheral nervous system (courtesy of Kimball's Biology Pages (http://biology-pages.info), February 2010, with modification)

Figure 2. Quantitative sensory testing unit (left) featuring vibration and thermal modalities. Outcome-based colour graphic reports (right) with automated narrative discussion of test results (from Medoc Ltd 2007 website http://www.medoc-web.com/medoc_en_home.aspx)

Figure 3. Electrophysiology test of the peroneal nerve

Figure 4. Corneal confocal microscopy image of a diabetic cornea (right) and normal cornea (left)

Figure 5. Illustration of the retina showing retinal layers (left) and specific neural components (right)

Figure 6. RNFL unique spread pattern

Figure 7. OCT retinal nerve fibre layer histogram illustrating a double hump pattern (courtesy of Optovue RTVue™). TSNIT (temporal-superior-nasal-inferior-temporal)