Journal of Clinical & Experimental Ophthalmology

Nasralah et al., J Clin Exp Ophthalmol 2013, 4:6
DOI: 10.4172/2155-9570.1000306

Research article Open Access

Measuring Visual Function in Diabetic Retinopathy: Progress in Basic and Clinical Research

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Abstract

Diabetic retinopathy is a common consequence of diabetes mellitus and the leading cause of vision loss in working-age people in the United States. The pathology of this disease is well characterized by microvascular lesions but also includes deficits in visual function, possibly as a consequence of retinal neurodegeneration. Microvasculature changes are clinically detected by fundus examination and used as the primary method of diagnosis, but functional tests may represent alternative endpoints that may be useful in translational research. Components of visual function can be characterized in a variety of different ways including measures of acuity, contrast sensitivity, dark adaptation and a number of electrophysiological parameters of the retina. This review discusses loss of function as measured both in human and animal models of diabetic retinopathy.

Keywords: Diabetes; Spatial frequency threshold; Dark adaptation; Contrast sensitivity; Electroretinogram; Visual evoked potential

Abbreviations: DR: Diabetic Retinopathy; NPDR: Non-Proliferative Diabetic Retinopathy; STZ: Streptozotocin; ERG: Electroretinogram; CS: Contrast Sensitivity; SFT: Spatial Frequency Threshold; VA: Visual Acuity

Introduction

Diabetic retinopathy (DR) is one of the more common complications of diabetes, and is the leading cause of vision loss in working-age adults. Retinopathy remains a serious health problem, accounting for 8% of all cases of blindness in the United States [1]. In 2008 the CDC reported that 3.6 million Americans aged 18 years or older were visually impaired due to diabetes, which was dramatically elevated from 2.6 million in 1998 due to the increasing incidence of diabetes. According to American Diabetes Association statistics, between 12,000 and 24,000 people lose their vision each year because of diabetic retinopathy. Due to the increasing incidence of diabetes it is predicted that by the year 2050 the number of Americans over the age of 40 with diabetic retinopathy will be more than 19 million [2,3]. The yearly economic cost of treating retinopathy and other ophthalmic complications of diabetes was estimated at $422 million in 2002 and the present cost is likely to be even higher [4,5]. With an estimated seven million undiagnosed cases of diabetes mellitus in the United States [5,6], the number of individuals affected by diabetic retinopathy is likely to be higher than recorded. Additionally, symptoms often do not appear in the early stages of DR, making prevention and early treatment more challenging, especially in the population with undiagnosed diabetes. Current therapies aimed to ameliorate the vascular pathology in diabetes can only be instituted after vascular lesions have become evident. The degree of human suffering caused by diabetic retinopathy strongly mandates further research.

DR can be separated into two sub-classifications based on vascular pathology detected by fundus exam: non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR). NPDR occurs in the early stage of the disease and is characterized by microaneurysms, macular edema and other vascular lesions [7]. In addition, lipid exudates, hemorrhages, cotton wool spots, and basement membrane thickening are also present in NPDR. Proliferative diabetic retinopathy is the advanced stage of DR and is characterized by highly permeable neovascularization, hemorrhages, and retinal detachment [8-10].

In addition to microvascular changes, retinal neurodegeneration is now thought to be a component of DR [11-13]. Studies of diabetic rats and mice have established that diabetes leads to a significant reduction in the thicknesses of the inner plexiform layer, inner nuclear layer, and overall retina in diabetic animals compared to age-matched controls [11,14]. Terminal dUTP nick end labeling (TUNEL) and other histological markers revealed apoptotic markers in the neural cells of the retina, especially in the retinal ganglion cell layer [13]. These histological studies showed that diabetes increases the rate of apoptosis of several types of cells in the retina, including neurons, and lead to the suggestion that DR can be considered a chronic neurodegenerative disease of the retina [12].

DR is primarily diagnosed and monitored by fundus exam, which allows for visual detection of the vascular lesions and macular edema. Less attention is usually paid to visual function, despite the evidence that a variety of measurable defects in vision occur during the early course of the disease [15]. The vision loss encountered in DR is often characterized as a simple loss of acuity; however, it is clear that many different functional components of vision are altered in measurable ways and include blurred or fluctuating vision, dark areas in vision,

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Received September 12, 2013; **Accepted** November 16, 2013; **Published** November 23, 2013

Citation: Nasralah Z, Robinson W, Jackson GR, Barber AJ (2013) Measuring Visual Function in Diabetic Retinopathy: Progress in Basic and Clinical Research. J Clin Exp Ophthalmol 4: 306. doi: 10.4172/2155-9570.1000306

reduced nighttime vision, impaired color perception, as well as significant changes in the electrophysiological characteristics of the retinal response to light. Innovations in methods to measure visual function in animal models are beginning to allow a better comparison between human and animal studies, and the results generally confirm that diabetes in animals is accompanied by the same types of functional deficits as those noted in humans, suggesting common mechanisms of vision loss. In this brief review we examine some of the established changes in visual function in diabetes and discuss their common mechanisms in animal models and humans.

Visual function is a broad term that can be broken down into several more specific parameters of retinal performance such as acuity, dark adaptation, contrast sensitivity, and color contrast. There are data suggesting that each of these functional parameters is compromised in people with diabetes, suggesting that there are underlying changes in the mechanisms of vision that have not been fully examined.

Visual acuity

Visual acuity (VA) is the most widely used non-invasive indicator of visual function. VA testing usually involves the use of a chart containing rows of letters of decreasing sizes within each row. Scores reflect the ability to discern individual letters of various sizes on the charts, reflecting the spatial resolution of the retina. It is widely recognized that VA is compromised by diabetic retinopathy and doubling of the visual angle is associated with age, duration of diabetes, severity of diabetic retinopathy, and presence of macular edema [16]. Common VA tests include the Snellen chart and the Early Treatment for Diabetic Retinopathy Study chart. VA measured by a similar test, the Bailey-Lovie chart, revealed lower VA scores for diabetic patients compared to controls, even in a subgroup of individuals with minimal clinical features of retinopathy and no macular edema [17]. VA scores may not, however, be a particularly sensitive measure of function. The measure of VA was not sensitive enough to distinguished between groups with diabetes but no retinopathy, early DR and non-diabetic controls [18]. This indicates that VA scores are not sensitive enough to distinguish between diabetic subgroups during the earlier stages of DR. In agreement with these findings, Ismail et al. [19] reported a significant difference between VA scores in individuals with progressive DR and controls, and when comparing them to groups with diabetes and early DR. No significant difference was found when comparing the early DR group with non-diabetic control groups. Therefore, VA is significantly reduced as DR progresses, but may not be a sensitive enough approach to detect the early stages of DR.

Despite the lack of sensitivity of VA, it has been used as a simple test of function in large clinical drug trials, such as the ruboxistaurin studies. In the original Phase III trial on the PKC beta inhibitor, ruboxistaurin, there was a reduction in the risk occurrence of sustained moderate vision loss, assessed by VA [20]. In a follow up study, sustained moderate vision loss was examined as a primary endpoint and extended use of ruboxistaurin was found to slow the reduction in VA [21].

Contrast sensitivity

There are several components of vision that appear to be altered by diabetes. One such defect that is widely recognized is in contrast sensitivity (CS), which is a measure of the ability of the retina to discriminate between different shades of gray. Reductions in CS have been noted in humans with diabetes by numerous studies [22-27]. While not all findings are consistent, CS appears to be a more sensitive test of retinal function compared to VA. Although some findings reveal that impaired CS is characteristic of both early and progressed DR, others report differences in CS only when all diabetic subjects are compared to controls and no significant difference was found between the subgroups of diabetic retinopathy [19].

CS can be measured by the Pelli-Robson chart, which has revealed reduced performance in diabetic subjects without DR and subjects with early DR when compared to controls in several studies [17-19]. Because these differences were detected by CS and not by VA, CS is considered to be a more sensitive detector of early retinal changes. Ismail et al. reported that while CS testing revealed differences between healthy controls and those with diabetes, diabetes with early DR, and diabetes with more progressive DR, the test did not reveal differences between these diabetic sub-groups and therefore may not predict DR progression [19]. In agreement with this conclusion, Misra et al. attributed CS decline in DR to levels of glycosylated hemoglobin, and not to retinal microvascular changes [17]. They reported that there was no significant CS difference between diabetics with and without vascular lesions, indicating that microvascular changes may not be a significant contributing factor to CS decline [17]. Stavrou et al. also demonstrated the sensitivity of CS testing by comparing scores of non-macular edema participants and controls. They reported that participants without macular edema had significantly reduced CS scores compared to controls [18].

Unlike VA testing, CS differences were detected in the absence of obvious signs of DR, suggesting greater sensitivity of this test. The causal relationship between this change in function and the vascular lesions of DR is less clear. Since CS is thought to be derived from neural processing within the inner retina, the data may suggest a failure in neural or synaptic information processing due to structural and biochemical mechanisms that may only be indirectly related to vascular dysfunction.

Color contrast

Color vision, popularly tested with the 100-Hue test, has been found to decline in people with diabetes. Early studies showed, however, that the deficits in color vision did not correlate with the duration of diabetes or severity of DR. Patients with non-proliferative diabetic retinopathy were found to have significant deficits in blue-yellow color contrast [28]. Other similar studies also showed reduced discrimination of colors along the blue-yellow and green-blue axes in diabetes. A later study also confirmed a significant deficit in color vision, especially blue-yellow discrimination, but in this case the degree of retinopathy predicted greater error scores, reflecting poorer color discrimination with progression of diabetes [19]. Another study found that the tritan contrast threshold correlated with the presence of sight-threatening DR as well as the presence of retinopathy assessed by fundus photography, suggesting that this level of functional testing could be included in screening for DR [29]. The FM-Hue test was used to determine color and brightness discrimination in a longitudinal study of ten patients with juvenile onset diabetes and found that discrimination of long wave light was unaffected while the short wave light discrimination was significantly reduced, again suggesting a preferential deficit in the S-cone pathway [30].

Color sensitivity has also been examined using clinical electrophysiology. A flash-on-flash protocol was used in a group of patients with diabetes but no clinically evident DR, in which a blue spot was paired with a short blue flash on a yellow background to isolate the S-cone pathway. The patients with pre-proliferative DR had a reduced response to S-cone stimulation compared to age-matched controls [31].

Another approach using different colors of flash stimuli found that the amplitude of the ERG b-wave of S-cones was significantly reduced in patients with diabetes but was not correlated with blood glucose or presence of DR, again suggesting a lack of complete dependence between vascular pathology and deficits in visual function [32].

The mechanisms of the deficit in color contrast sensitivity are difficult to study because this subtle alteration in visual function is likely to involve features that may not be possible to model in animals. A histological study on human tissue revealed, however, an elevated cell loss specifically in the S-cone population, accompanied by an increase in TUNEL-positive S-cones, suggesting accelerated apoptosis specifically within the blue-cone population. The selective reduction in abundance of S-cones compared to M- and L-cones may go some way towards explaining the deficits in tritan color contrast observed in the diabetic population [33].

Acuity and contrast sensitivity in animal models of diabetes

Recent experimental approaches have made it possible to measure acuity and contrast sensitivity in animal models of diabetes. The optokinetic reflex, which elicits head tracking movements in rodents in response to a moving visual target such as a vertical grating can be used to assess visual acuity (or the spatial resolution of the retina, known as the spatial frequency threshold in these animal studies) as well as contrast sensitivity [34] (Prusky et al. 2004). This approach has been used to determine visual function defects in STZ-diabetic rodents. The optokinetic response was used to assess the impact of diabetes on visual function in a microarray study that determined diabetes-related changes. Molecular changes in a number of groups of genes indicating glial and neural dysfunction were identified within the first month of diabetes and included genes associated with the visual cycle. The gene expression changes occurred simultaneously with deficits in the spatial frequency threshold assessed by optokinetic reflex [35]. More recently it was shown that the reduction in acuity in the STZ-diabetic Long-Evans rat correlates with the delays in the ERG implicit time, suggesting that the behavioral and electrophysiological measures of function may be altered by common mechanisms of dysfunction in diabetes [36].

Reductions in the spatial frequency threshold and contrast sensitivity have also been identified in the spontaneously diabetic $Ins2^{Akita}$ mice, although the rate of development of visual deficits may be different compared to rats [37]. Another recent study using $Ins2^{Akita}$ mice also reported deficits in the scotopic optokinetic response, suggesting that the impact on visual behavior is not limited only to the light-adapted retina but also affects the rod-mediated visual pathway [38]. The $Ins2^{Akita}$ mouse was also used in a recent MRI study that identified reductions in choroidal blood flow which did not correlate with the deficits in spatial frequency threshold and contrast sensitivity, suggesting that separate mechanisms may be responsible for functional changes in vision and choroidal blood flow [39].

While it has been difficult to examine acuity and contrast sensitivity in awake rodent models of diabetes, improvements in behavioral testing [34] have enabled confirmation that the animal models rapidly develop visual function defects that closely model the defects previously established in humans with diabetes.

Clinical studies on dark adaptation in diabetes

Dark adaptation can be defined as the change in sensitivity of the retina when moving from bright light to low illumination conditions. At low levels of light, the rod photoreceptors are primarily responsible for vision, while the cones become less active; this is referred to as scotopic vision. Therefore, dark adaptation refers to the adjustments made within the retina to allow for scotopic vision to occur. Dark adaptation can be measured in humans by first allowing the retina to adapt to total darkness and then measuring the time taken for the retina to return to a specific threshold of sensitivity after photobleaching with a bright flash of light. It has been shown that humans with the early stages of DR have a significantly extended time of dark adaptation compared to non-diabetic subjects, so this may be a sensitive marker of early DR [40].

For some time there has been clinical evidence to suggest that the rate of dark adaptation is reduced in some retinal diseases including DR [41]. Using a dark adaptometer to measure dark adaptation in subjects with diabetes ranging from 3 months to 51 years duration, patients with diabetes not only had delayed adaptation to scotopic conditions, but they also had elevated thresholds indicating reduced sensitivity to light. Some subjects with a short duration of diabetes had increased threshold elevation while others with longer disease duration did not show significant threshold elevation. Another study compared individuals with diabetes and either no or early retinopathy to subjects without diabetes and reported significant elevations in thresholds in cases of diabetes. Unlike other studies, however, a delay in the time course of dark adaptation was not reported. Similarly, Holopigian et al. reported an elevated threshold of dark adaptation in subjects with DR [42].

A study testing the effect of photocoagulation on dark adaptation similarly reported elevated thresholds in diabetic subjects [43]. More recently Jackson et al. described significantly impaired dark adaptation in subjects with non-proliferative DR compared to controls, verifying that dark adaptation deficits occur in the early stages of disease [40]. An additional study that aimed to determine the effect of hyperglycemia on dark adaptation in subjects with type II diabetes and mild DR reported that dark adaptation was impaired in cases of diabetes and mild retinopathy. This study further reported that the impairment is reversible during transient hyperglycemia compared to conditions of fasting [44].

The retina is more metabolically active in the dark than the light, due to the greater energy consumption and neurotransmitter release from photoreceptors, so it has even been suggested that DR is exacerbated during dark adaptation because of the increased energy demand of photoreceptors [45,46]. A recent functional MRI study confirmed that a greater metabolic activity is localized to the outer retina in dark-adapted rats, compared to the inner retina [47]. The impact of diabetes on the differential neural activity in the dark-adapted retina is an important aspect of visual function that has been overlooked by many mechanistic studies in DR research.

Dark adaptation in animal models

There is evidence that dark adaptation deficits occur in diabetic rodents. An electrophysiological approach can also be used to test dark adaptation by using a flicker paradigm. In this case a rapidly repeated flash stimulation elicits a repeated retinal response with a magnitude determined by the rate of recovery of the photoreceptors after each flash of light. Thus the slowed photoreceptor recovery observed in diabetes can be detected as a reduction in the amplitude of the flicker response, and is essentially an index of dark adaptation [48].

Similar to the lack of understanding of the mechanism of compromised contrast sensitivity, the reduction in dark adaptation is likely to have an underlying biochemical mechanism but this has not been explored to any great extent. The biochemical mechanisms of dark adaptation have not been considered in animal studies of DR in great

detail. Dark-adapted mice had more manganese uptake, measured by MRI in the outer segments of retina, suggesting greater divalent cation transport in photoreceptors in the dark [49]. The compensatory increase in cation transport appeared to be diminished in diabetic mice in this study. The abnormal manganese uptake in the outer retina of dark-adapted diabetic mice was partially corrected by systemic treatment with 11-cis-retinal, suggesting that the deficit in outer retina cation uptake was due to a rhodopsin-mediated mechanism or possibly a defect in vitamin A availability [50]. Ostroy and colleagues also demonstrated significant reduction in rhodopsin regeneration of diabetic mouse retinas and suggested that the elevated threshold observed in subjects with diabetes may be attributed to the reduction in rhodopsin regeneration [51]. Other components of photoreceptor function also appear to be impacted in diabetic rats. Expression levels of rhodopsin kinase and recoverin were increased and decreased, respectively, in retinas of diabetic rats [52]. An earlier study also reported an impaired vitamin A uptake in retinas of diabetic rats [53]. Therefore several biochemical components of phototransduction are affected by diabetes, which may account for the deficits in dark adaptation.

Electrophysiology studies

The electroretinogram has been used countless times as a method to determine changes in retinal function due to diabetes, and has been suggested as a predictor of the progression of DR [54-56]. Traditional ERG measurements have recorded reductions in the amplitude of the b-wave and oscillatory potentials, as well as delays in the implicit times of the ERG waveform. These observations have been made in both humans and animal models with diabetes. Scotopic full-field ERG a-wave, b-wave, and oscillatory potential wave amplitudes and implicit times were also significantly impaired in a study analyzing differences between diabetic and NPDR subjects compared to controls [57,58].

The electroretinogram (ERG) is a noninvasive electrophysiological method that can be used to detect functional changes in the retina [59]. Most commonly, investigators record the standard ERG in which a corneal electrode is used to detect the electrophysiological signal of the retina in response to flashes of light. Reductions in amplitude of the b-wave and oscillatory potentials of the ERG signal in diabetic animals and humans are well documented [60-63]. There is good evidence that the amplitude of the scotopic threshold response is reduced in both humans and animals with diabetes [63-65]. Some reports of ERG recordings also suggest a decrease in the a-wave amplitude and delayed implicit times in diabetic rats, indicating a possible decrease in the magnitude of the photoreceptor response to light [66].

Reduction in the amplitude and implicit times of the oscillatory potentials (OPs) are the most consistent ERG changes associated with DR. The OPs are small wavelets superimposed along the ascending phase of the b-wave, thought to be generated by horizontal and amacrine cell activity and this measure has been suggested as a predictor of the onset and progression of DR [66-69]. A decrease in both the frequency and amplitude of the OPs, as well as an increase in OPs latency, has been suggested to indicate inner retinal dysfunction [66,70].

ERG approaches in animal models

There are numerous studies reporting ERG changes in diabetic animal model studies and this is now often used as a functional endpoint in drug intervention studies [71-73]. The amplitude and frequency of the waves on the ERG are thought to be useful indicators of retinal function. The amplitude of the a-wave, which reflects the hyperpolarizing activity of photoreceptors, is thought to correspond to the magnitude of the photoreceptor response to light. Currently, there are conflicting data about changes in the a-wave. Kohzaki et al. report that in dark-adapted diabetic rats, the a-wave is unaffected [63]. Other investigators have found subtle changes in the amplitude of the a-wave in dark-adapted diabetic rats [66,70,74].

Typically, animal studies focus on the b-wave and OPs and it is generally well established that the amplitudes of these ERG components are reduced in diabetic rodents, while the OPs latencies are increased, indicating loss of function or perhaps cell death in the bipolar and amacrine cells, as well as other neurons of the retina [48,62,63,66,75,76].

Similar to findings in humans, conflicting reports about impairments along the ERG waveform exist; but many studies agree that there are impairments in oscillatory potentials [74], while reports on a-wave and b-wave impairments are contradictory [66]. Scotopic full-field ERGs recorded from diabetic rats revealed significant reductions in a-wave, b-wave, and oscillatory potential wave amplitudes [77]. In addition to reductions in b-wave and oscillatory potential amplitudes [61,76] and increases in b-wave and oscillatory potential implicit times in diabetic rats after scotopic full-field ERGs were recorded [76]. Just as differences in waveform impairments are reported using the same tool, full-field ERG, different forms of ERG also yield variation in retinal activity in the dark.

The multifocal ERG

While the full-field ERG records responses from the entire retina, the multifocal electroretinogram (mfERG) measures the topographical responses of several areas of the cone-driven retina to a standard visual stimulus of hexagons alternating between white and black colors. The levels of light and stimulation rate ensure that the responses generated are from cones and not from rods [78]. This method is valuable due to its ability to detect small localized changes in retinal function that cannot be detected by full-field ERG. The mfERG generates a waveform with three major peaks (PI, N1, and P2), measuring bipolar cell activity. mfERG of diabetic patients with and without retinopathy display increased response delays, indicating dysfunction that can be localized to focal regions of the retina [79,80]. The mfERG has been shown to be abnormal in patients both with and without vascular retinopathy [81].

The most interesting use of mfERG has been in linking the electrophysiological response with the vascular pathology of DR. The increased delay in response implicit times was associated with the presence of non-proliferative DR, and was greatest near vascular lesions [82]. The mfERG has also been used in combination with stereo fundus imaging in patients with non-proliferative retinopathy to show that areas with abnormal ERG responses were contiguous with areas containing vascular lesions [83]. Follow-up studies showed that electrophysiological changes in the retina precede vascular lesions and the onset of retinopathy, and that local mfERG deficits may be predictive of the appearance of new vascular lesions developing within a 2-3 year time period [84,85]. These findings indicate that the abnormal electrophysiological signature of the neural retina may precede the appearance of gross vascular lesions.

The scotopic threshold response

The scotopic threshold response (STR) is an ERG waveform that is rarely used as a measure of retinal function, despite its potential sensitivity to inner retinal disease. The testing protocol requires that subjects undergo a prolonged period of dark adaptation, as the subsequently-measured waveform is the response to very low intensity (scotopic) flash stimulation. The STR is thought to be derived from the

inner retina, probably retinal ganglion cells and amacrine cells [86]. There is some evidence that the amplitude of the STR is reduced in humans with diabetes [64,87]. This result was found to also occur in animals. A study by Kohzaki et al., wherein STR amplitudes in diabetic rats were altered to a greater extent than the other more traditional ERG parameters such as the b-wave amplitude [63]. The deficit in the STR of humans and rodents with diabetes suggested an inner retina dysfunction that most likely involves the ganglion cells. More recently, ganglion cell dysfunction was confirmed in diabetic mice and the STR was used to show that retinal ganglion cells can be rescued by pentazocine, a ligand for the sigma receptor [65]. This suggested that the reduced STR amplitude could be due to retinal ganglion cell apoptosis, and that using a neuroprotective drug can also prevent the reduction in the functional output of inner retina neurons.

The STR is a weak electrophysiological output in response to low intensity flashes of light and is characterized by two waves: positive STR (pSTR), which is thought to reflect ganglion cell activity, and negative STR (nSTR), which follows after the pSTR and is thought to reflect amacrine cell activity [86,88]. Kohzaki et al. demonstrated a reduction in the amplitude of the positive component of the STR of dark-adapted diabetic rats, but no change in the nSTR was observed, suggesting that ganglion cell output is compromised by diabetes before amacrine cell function is affected [63]. This reduction in pSTR may be due to ganglion cell apoptosis, which is a well-established consequence of diabetes [11,13,14,89].

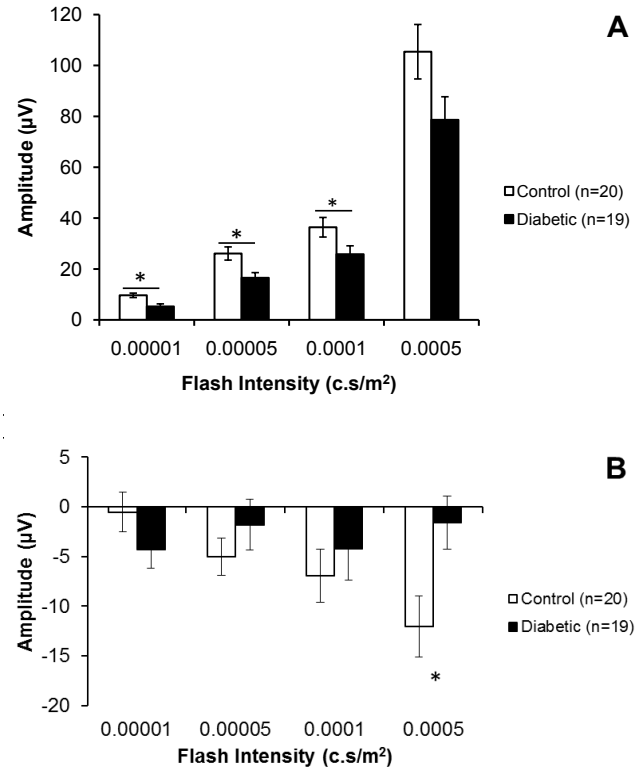

Figure 1: Positive scotopic threshold response for 11 week old Ins2^{Akita} mice. The scotopic threshold response was measured in diabetic Ins2^{Akita} mice after 6-7 weeks of hyperglycemia (n=20), and compared to wild-type litter mates (n=19). Flash intensities of 0.00001 and 0.00005 c.s/m2 are considered to be in the scotopic range. The higher flash intensities presented here mark the first positive peak, but will also include bipolar cell signal from the encroaching b-wave. (A) The positive scotopic threshold response (pSTR) amplitude was smaller in hyperglycemic mice compared to wild-type littermates (*p<0.05). (B) The amplitude of the negative scotopic threshold response (nSTR) was not significantly different in the diabetic Ins2^{Akita} mice compared to wild-type controls. The significantly reduced amplitude at flash intensity of 0.0005 c.s/m^2 is outside the scotopic range and may represent a difference in the regular ERG response (*p<0.05).

A recent study by the authors also established that the positive STR was significantly reduced in the hyperglycemic Ins2^{Akita} mice (Figure 1). In this study 11-week-old dark-adapted Ins2^{Akita} mice exposed to very dim (scotopic) flash intensities had significantly diminished amplitude response compared to age-matched control mice. Due to the fact that Ins2^{Akita} mice develop hyperglycemia between 4-5 weeks of age, the diminished pSTR amplitude abnormality was measured after only 7 weeks of hyperglycemia. At flash intensities of 0.00001 c.s/m2 and 0.00005 c.s/m^2, the average amplitudes were 5.28 (±1.00) µV and 16.52 (±2.05) µV, respectively, in diabetic mice; compared to 9.64 (±0.87) µV and 26.08 (±2.59) µV in controls. There were significant differences in the results at 0.0001 c.s/m2 and 0.0005 c.s/m^2 as well, although at these brighter flash intensities, the bipolar cell response was encroaching so that the ganglion cell dominant pSTR became lost in the emerging b-wave (Figure 1A). There were no significant differences in the amplitudes of the nSTR measurements in these mice (Figure 1B). These data show that, as in other models, the diabetic Ins2Akita mice develop an abnormal inner retina electrophysiological response within the first two months of hyperglycemia.

Electrophysiological approaches to study dark adaptation

Electrophysiological approaches can also be used to examine dark adaptation in diabetic animals [49,90]. Tyrberg and colleagues reported an increased flicker response implicit time in dark-adapted diabetic individuals, demonstrating the clinically detectable delay in dark adaptation [91]. Flicker ERG recorded under scotopic conditions also revealed amplitude reductions in recordings from diabetic rats, indicating reduced retinal sensitivity to light [48]. Paired-flash ERG recording also revealed amplitude reductions, suggesting loss of rod function [70].

Visual evoked potentials

The visual evoked potential (VEP) is an electrical signal that can be recorded through the skull over the occipital cortex in response to a bright flash of light. Changes in the VEP are thought to reflect ganglion cell and optic nerve dysfunction and several clinical studies show deficits in humans with diabetes. While this approach is not often used, several studies have demonstrated that deficits in the VEP exist in humans with diabetes. A small clinical study showed that there were functional VEP defects in all patients with diabetes in the study, regardless of the presence of DR [92]. A larger study on juvenile patients with type 1 diabetes also showed that the VEP amplitude was reduced while latency was increased, again in the absence of vascular retinopathy, and the magnitude of the deficits in VEP increased with longer durations of diabetes [93]. These data suggest that damage to optic nerve function may occur very early in the disease or may develop independently from the retinal vascular pathology.

Studies with diabetic animals also indicate that neuropathy develops in the optic nerve as well as the retina. A reduction in the VEP latency was accompanied by significant reduction in the size of myelinated fibers in the optic nerve in diabetic BB rats [94]. There may also be changes in the amplitude of the VEP, limited to specific spectral frequencies [95]. The VEP has been used as a measure of function in a small number of drug studies in diabetic rodents [95-97], and one

unusual study showed that physical exercise improved the VEP deficit in rodents [98].

Summary and Conclusions

There is a wealth of data showing that diabetes leads to a variety of changes in visual function. While it is obvious that DR causes loss of vision, defining the actual deficits in empirical terms is difficult due to the nature of our understanding of vision and the limits to measures of function. Despite the current limitations there is evidence that components of vision, such as acuity, dark adaptation, contrast sensitivity, and various electrophysiological parameters are altered by diabetes. This appears to be generally true in animal models of diabetes as well as humans. The relationship between the different measures of visual function, especially the significance of electrophysiological measures compared to psychophysical ones, is still an area that needs more attention. Correlational studies comparing contrast sensitivity with the ERG, for instance, have not been explored.

Understanding the impact of disease on function is an imperative goal in any field of research. It seems that this approach has been neglected somewhat in the DR field. The difficulty in accurately measuring visual function, or even in defining function empirically, is one reason for this gap in our knowledge. It is hoped that recent refinements in electrophysiological and psychophysiological technology for both clinical and basic research will help to advance the field, providing a better understanding of the mechanisms and treatments for vision loss in DR.

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