Current Diabetes Reports (2019) 19: 17
https://doi.org/10.1007/s11892-019-1134-5

MICROVASCULAR COMPLICATIONS—RETINOPATHY (DL CHAO AND G YIU, SECTION EDITORS)

Retinal Neurodegeneration as an Early Manifestation of Diabetic Eye Disease and Potential Neuroprotective Therapies

Sidra Zafar[1] · Mira Sachdeva[1] · Benjamin J. Frankfort[2] · Roomasa Channa[1,2]

Published online: 26 February 2019

Abstract

Purpose of Review Diabetic retinopathy (DR) is a major cause of visual impairment and blindness throughout the world. Microvascular changes have long been regarded central to disease pathogenesis. In recent years, however, retinal neurodegeneration is increasingly being hypothesized to occur prior to the vascular changes classically associated with DR and contribute to disease pathogenesis.

Recent Findings There is growing structural and functional evidence from human and animal studies that suggests retinal neurodegeneration to be an early component of DR. Identification of new therapeutic targets is an ongoing area of research with several different molecules undergoing testing in animal models for their neuroprotective properties and for possible use in humans.

Summary Retinal neurodegeneration may play a central role in DR pathogenesis. As new therapies are developed, it will be important to develop criteria for clinically defining retinal neurodegeneration. A standardization of the methods for monitoring neurodegeneration along with more sensitive means of detecting preclinical damage is also needed.

Keywords Diabetes · Diabetic retinopathy · Neurodegeneration · Neuroprotection

Introduction

Diabetic retinopathy (DR) remains the most common cause of visual impairment in the working age population. It is also a leading global cause of blindness. As of 2010, an estimated 95 million people are believed to have signs of DR, and third of these develop vision-threatening disease. These numbers are expected to increase in the coming years because of the diabetes mellitus (DM) pandemic and due to increasing life expectancies [1, 2]. DR is diagnosed and classified by its vascular features, including microaneurysms, dot blot hemorrhages, and hard exudates in early forms, with neovascularization of the retina and vitreous hemorrhage in the more proliferative forms. Current treatments for DR involve treating these vascular phenotypes, through injection of medications such as anti-vascular endothelial growth factors (VEGF) or steroids and panretinal photocoagulation. However, in recent years, there has been a growing interest in the contribution of neuroretinal degeneration to the pathogenesis of diabetic retinopathy, termed diabetic retinal neurodegeneration (DRN). This is primarily based on a large body of literature suggesting that there is decrease in visual function as measured through electrophysiology and visual fields as well as loss of retinal cells through optical coherence tomography (OCT) imaging which precedes the vascular phenotypes of diabetic retinopathy. However, whether neuropathy or vasculopathy is the primary initiating event and the relative contribution of neuropathy versus vasculopathy to overall visual disability remains to be

This article is part of the Topical Collection on *Microvascular Complications—Retinopathy*

✉ Roomasa Channa
rchanna@bcm.edu

Sidra Zafar
sidrazafariqbal@gmail.com

Mira Sachdeva
msache4@jhmi.edu

Benjamin J. Frankfort
Benjamin.frankfort@bcm.edu

[1] Wilmer Eye Institute, Johns Hopkins Hospital, Baltimore, MD 21287, USA

[2] Department of Ophthalmology, Baylor College of Medicine, Houston, TX, USA

elucidated. In this review, we discuss the evidence of visual dysfunction and cell loss prior to the vascular manifestations of diabetic retinopathy and potential neuroprotective strategies to treat the neurodegeneration found in diabetic retinopathy.

"Neurovascular Unit" of the Retina

The term '"neurovascular unit" was first applied to the blood–brain barrier [3] and then to the retina [4]. The term encompasses the intricate functional coupling and interdependency between neurons, glial cells, the basement membrane, and the retinal vascular elements (endothelial cells, pericytes) that is essential to maintaining the integrity of the inner blood–retinal barrier (iBRB), while dynamically regulating blood flow in response to metabolic demands [5]. The iBRB, which consists of tight junction complexes, is central to regulating the exchange of metabolites between the vascular lumen and the neural retina, thereby maintaining the appropriate environment necessary for neuronal function. Studies involving human retinas have shown organization of the neuronal and vascular networks in a highly stratified and functional architecture, with the superficial capillary plexus (SCP) present at the level of retinal nerve fibers, the middle capillary plexus present at the inner boundary of the inner nuclear layer, while the deep capillary plexus (DCP) lies in the boundary between the inner nuclear layer and outer plexiform layer [6, 7]. Functional hyperemia or retinal vascular flow is mediated, either directly or indirectly, by neuronal signaling to blood vessels. Neuronal activity stimulates glial cells to raise their intracellular calcium levels and, in turn, initiates physiological responses through the release of additional transmitters and produce vasoactive mediators [4]. Thus, under normal circumstances, flickering light stimulation selectively elevates the metabolic demand of cells in the inner retinal layers [8] causing retinal blood vessels to dilate, whereas breathing 100% oxygen causes them to constrict [9]. In DM, early activation of the innate immune system, the complement system and microglia is believed to play a role in damage of the retinal neurovascular unit [10] causing altered neuronal glutamatergic and dopaminergic neurotransmitter signaling [11], reduced synaptic protein expression [12], and altered glial function [13]. The normal function of the neurovascular unit is therefore diminished in patients with DM resulting in impaired physiologic responses early in the course of the disease, and even among patients with no or only mild retinopathy changes [14–16]. Progressive disintegration of the retinal neurovascular unit eventually manifests as signs of clinically recognizable retinopathy, with pericyte loss particularly, compromising capillary integrity and leading to weakening of the iBRB and vascular leakage [17, 18]. DR may therefore be considered as a sensory neuropathy or neurovascular degeneration, and not solely a microvascular disease [17].

Diabetes and Neural Structural Alterations

The detrimental effects of DM-associated hyperglycemia on neural retina have been known for more than five decades [19]. Cellular dysfunction combined with biochemical alterations such as hyperglycemic pseudohypoxia, activation of protein kinase C pathway, oxidative stress, and increased generation of advanced glycation end products (AGEs) is believed to trigger an inflammatory cascade, causing retinal damage [20]. Glutamate, one of the primary excitatory amino acid neurotransmitters in the retina [21], is also believed to play a role in diabetic retinal neurodegeneration. Animal-based studies have shown an association between excess glutamate levels and neurotoxicity in both the central nervous system [22] and the retina [23, 24]. Toxic effects are believed to be mediated via excessive activation of the glutamate NMDA receptor, which allows intracellular calcium influx. Increase in intracellular calcium levels, in turn, starts a cascade leading to eventual cell death, with retinal ganglion cells being susceptible to glutamate excitotoxicity [25]. The hypothesis that glutamate may have a role to play in DR is based in part on measurements of elevated glutamate levels in vitreous of diabetic subjects [26, 27]. Ambati et al. demonstrated a two-fold elevation in glutamate levels in the vitreous of proliferative DR patients compared with healthy controls [26]. Diabetes has also been shown to induce alterations in glutamate receptor subunits of human retinas [26]. Santiago et al. demonstrated increased immunostaining of the glutamate receptor subunit, GluR2, in the inner plexiform (IPL) layer, and outer plexiform layers (OPL) of post-mortem human eyes, indicating elevated tissue levels of these proteins [28]. However, its specific role in DRN is yet to be established.

Accelerated neuronal loss from apoptosis is one of the most recognized features in neurodegenerative diseases and has increasingly been demonstrated in DR as well. Bloodworth et al., in a histopathological analysis of 295 post-mortem eyes, observed degeneration of the ganglion cells and IPL [29]. Immunohistochemical studies involving diabetic retinas (of donor eyes or from animal models) have reported apoptosis of amacrine and Müller cells, microglial activation, and dysregulation of neurotrophic factors [30–33]. Barber et al. noted a 10% decrease of ganglion cell density with concurrent reductions of the IPL (by 22%) and inner nuclear layer (INL) in the streptozotocin (STZ)-induced type 1 diabetic rat model. These changes were evident after 7.5 months of DM onset and insulin therapy partially reduced the burden of apoptotic cells [34]. While the rate of progression has varied, occurrence of neurodegeneration has been validated several times in diabetic animal models [35–37]. Overexpression of pro-apoptotic molecules such as BAX, Fas, and caspase-3 has been demonstrated in post-mortem human diabetic retinas as well, particularly ganglion cells in the inner retina [38–40], thus confirming the results from animal studies. Valverde et al. linked cell damage

and apoptosis in human diabetic neuroretinas to increased expression of pro-apoptotic molecules causing activation of the death receptors and mitochondrial injury [41].

Various preclinical studies have additionally attempted to better understand, which specific cell types are most affected by the apoptotic process early on in DM. Fu et al. demonstrated a 53% decrease in Muller cell density, 10 months after induction of DM in mice, which also coincided with thickness reductions in the INL, ganglion cell layer (GCL), and ONL [42]. Gastinger et al. reported a 20% and a 16% reduction in cholinergic and dopaminergic amacrine cells, respectively in diabetic mouse retinas compared with non-diabetic rodents [43]. Photoreceptor cells have been observed to undergo apoptotic death as early as within 4 weeks of DM onset in animal models, with significant reductions in ONL thickness by 24 weeks [44]. Accumulating data from more recent studies however seems to suggest that photoreceptor cells might play a critical role in the development of DR. Increased oxidative stress from hyperglycemia-associated metabolic abnormalities is involved in the development of diabetic retinal microvascular changes [45]. Of all retinal cells, photoreceptor cells contribute most to DM-induced retinal oxidative stress and were shown as the major site of superoxide generation in diabetic mice [46]. Photoreceptor cells have additionally been demonstrated to produce inflammatory proteins that contribute directly to increased retinal endothelial cell permeability and cell death in diabetic mouse models [47, 48]. Indeed, there is evidence that seems to suggest that loss of photoreceptors in the outer retina may be associated with reduced severity of DM-induced vascular degeneration. In a study by Gooyer et al., loss of the outer retina was found to be associated with decreased retinal hypoxia and reduced DR severity in rhodopsin knockout mice ($Rho^{-/-}$) models [49]. Neural alterations have preceded vasculopathy in certain cases [32, 50]. Studies with STZ-induced diabetic rodents have demonstrated neural structural deficits as early as 6 to 12 weeks of DM onset [51••, 52]. Significant reductions in ganglion cell density were noted by Sohn et al. 20 weeks after DM induction with no concurrent changes in pericyte density, classically defined as the earliest detectable histological marker of DR in retinal vessels [53]. Another manifestation of preclinical DR, the number of acellular capillaries [54] did not significantly change either. The same study also reported thinning of the nerve fiber layer (NFL) and GCL in *db/db* type 2 diabetic (T2DM) rodent models [51••]. Presence of certain ocular comorbidities can further accelerate this observed neuronal loss. Chronically elevated intraocular pressure for instance has been associated with an increase in the number of DM-induced apoptotic cells by an estimated factor of eight, suggesting that a combination of glaucoma and DM has an additive effect in promoting retinal neuronal damage [55].

Retinal Assessment Via Optical Coherence Tomography

The advent of optical coherence tomography (OCT) has allowed microstructural analyses of the retina to become part of routine clinical practice, with automated segmentation, non-invasive, and high resolution in vivo visualization of retinal layers can be performed [56], resembling that of histological specimens [57]. Measurements are precise and highly reproducible (reproducibility of retinal thickness measurements between ± 5% and ± 6%) [58, 59]. Multiple cross-sectional studies have evaluated neural anatomical alterations in diabetic retinas using OCT imaging. Studies using OCT for evaluation of DRN have reported variable thickness measurements of patients with DM compared to controls. While most studies have reported reduced thickness measurements, particularly of NFL and ganglion cell-inner plexiform complex (GCL-IPL) across various cohorts of both type 1 and type 2 DM (T1DM and T2DM respectively) [60–74] (Table 1), including those with no clinically visible retinopathy or minimal retinopathy, other studies have shown increased thickness or no changes at all. Gundogan et al. and Carpineto et al. analyzed thickness changes of individual retinal layers in 190 and 200 participants, respectively. Both authors reported significant reductions in RNFL and GCC in participants with T1DM or T2DM and no or minimal retinal vascular changes when compared with healthy controls [62, 65]. Gundogan et al. also found significant negative correlations of GCC thickness and RNFL thickness with duration of T1DM and hemoglobin A1c levels, respectively. OCT-derived thickness parameters may additionally vary with duration of DM [69, 78, 82].

Sohn et al. evaluated DM-associated neurodegenerative effects on the retina using OCT imaging. Over a 4-year period, they found progressive, gradual thinning of the NFL (0.25 μm/year) and of the GCL-IPL (at 0.29 μm/year), significantly higher than age-related decline in thickness which averages around 0.1 μm/year per layer [83]. The authors also stated if the current rate of degeneration was extrapolated for the next 10 to 20 years, the magnitude of neural loss from DRN would be comparable to that caused by severe glaucoma. However, compared with glaucomatous damage which is classically associated with scotoma, it was suggested by the authors that diabetic DRN-associated loss may more likely be diffuse. The authors noted that NFL loss and GCL-IPL loss was independent of age, gender, presence, or progression of DR and hemoglobin A1c. This study concluded that neuroretinal loss progresses with increasing duration of DM and that DRN precedes microvascular manifestations of DM [51].

On the other hand, there have also been studies which reported an increase in thickness of retinal layers in diabetic patients [75, 78], findings consistent with similarly reported transient increases in thickness of inner retinal layers in histological studies of diabetic rat retinas. It was postulated that loss

Table 1 Overview of studies that have used optical coherence tomography (OCT) for assessing structural changes in the retina of patients with no or minimal diabetic retinopathy

Author[a]	Diabetes status	Sample size (*N*) (patients, controls)	Thickness change on OCT compared with non-diabetic controls
Scarinci [60]	Type 1 diabetes (T1DM)	51 (38, 13)	*Decreased* RNFL and GCL *Increased* INL
Ferreira [75]	Type 2 diabetes (T2DM)	175 (125, 50)	*Decreased* Photoreceptor layer *Increased* RNFL, INL, RPE, GCL, and OPL/ONL
Sohn [51]	T1DM	45 diabetic cases	*Decreased* RNFL, GCL, and IPL
Gundogan [62]	T1DM	190 (90, 100)	*Decreased* RNFL and GCC
El-Fayoumi [63]	T1DM	96 (46, 50)	*Decreased* RNFL and GCC
Chen [70]	T1DM and T2DM	121 (60, 61)	*Decreased* NFL (nasally in both T1DM and T2DM), GCL-IPL, and INL/ONL (T1DM only) *Increased* INL and ONL (in T2DM cohort only) *Similar* GC-IPL (in T2DM cohort only)
Carpineto [65]	T2DM	200 (150, 50)	*Decreased* RNFL and GC-IPL
Tie Pei [76]	T2DM	299 (141, 158)	*Decreased* GCC (superiorly) *Similar* RNFL (peripapillary)
Rodrigues [66]	T2DM	102 (74, 28)	*Decreased* RNFL and GCC
Vujosevic [77]	T1DM and T2DM	124 (74, 50)	*Decreased* RNFL and ILM *Increased* INL and OPL *Similar* GC-IPL
Verma [72]	T2DM	110 (70, 40)	*Decreased* RNFL
Van Dijk [68]	T2DM	121 (64, 57)	*Decreased* RNFL and GCL-IPL (pericentral macula) RNFL and IPL (peripheral macula) (Changes in T2DM with minimal DR only) *Similar* RNFL and GC-IPL (in T2DM cohort without DR)
Araszkiewi [78]	T1DM	108 (77, 31)	*Increased* RNFL and GCL
Van Dijk [79]	T1DM	70 (32, 38)	*Decreased* GCL and INL (pericentral)
Van Dijk [69]	T1DM	80 (40, 40)	*Decreased* RNFL (peripheral macula) and GCL (pericentral macula) (Changes in T1DM with minimal DR only)
Van Dijk [69, 74]	T1DM	196 (97, 99)	*Similar* RNFL, GCL-IPL, INL, ONL/OPL, and PR (in T1DM cohort without DR)
Van Dijk [74]	T1DM	116 (57, 59)	*Decreased*

Table 1 (continued)

Author[a]	Diabetes status	Sample size (*N*) (patients, controls)	Thickness change on OCT compared with non-diabetic controls
			GC-IPL and INL (pericentral macula only) (Changes in T1DM with minimal DR only)
Verma [80]	N/A	78 (39, 39)	*Decreased* Photoreceptor layer
Oshitari [81]	N/A	100 (69, 31)	*Decreased* RNFL

[a] Table arranged chronologically with most recent studies listed first

of blood–retinal barrier integrity, especially the inner barrier, followed by increased vascular permeability and edematous swelling, was the underlying reason for this observed change [44, 84].

We believe that the thickness variations observed across these studies might in part be attributable to patient-related differences in the type and duration of DM, the level of glycemic control and the degree of DR as well as the patients' age and sex. There may also be individual-level variations in baseline ocular measurements related to intraocular pressure [85] and refractive error [86], factors which are known to affect RNFL thickness. Furthermore, given the dynamic nature of DM, establishing a consistent time point of study is often difficult. This lack of temporal fidelity can therefore make evaluation problematic and may also explain why some studies showed thinning, others thickening, and why in some studies no changes in thickness were observed. In summary, neural structural alterations on OCT seem to occur early in the disease course. However, it is yet to be established whether NFL changes [77, 81] or GCL changes [76, 87] are a more sensitive indicator of diabetic neurodegeneration. Van Dijk et al. suggested that the observed decrease in inner retinal thickness might initially be due to pericentral ganglion cell loss followed by peripheral RNFL thinning in the macular region.

Visual Function Assessment

Visual function has been assessed among diabetic patients, both with and without clinically established DR, using electrophysiological tests such as pattern electroretinogram (pERG) which assesses ganglion cell activity, multifocal ERG (mfERG), visual evoked potentials (VEP), and microperimetry. Reductions in pERG amplitude have been reported by multiple authors among diabetic patients, with minimal or no microvascular changes on fluorescein angiography [88–90]. mfERG studies that have analyzed neuroretinal response in diabetic subjects have shown reduced amplitudes and/or implicit time delays, both indicators of retinal dysfunction [91–95]. In a study by Bronson-Castain et al., 28% of patients with T1DM and 40% of patients with T2DM had significant mfERG implicit time delays, with concurrent retinal thinning on OCT [96]. Delayed implicit times in adults have been associated with increased risk of developing retinopathy and even predictive of development of future retinopathy in corresponding retinal locations. Han et al. [93] followed 22 diabetic eyes, both with and without retinopathy over 1 year. Although non-DR eyes did not develop any vascular changes, they found that for eyes with pre-existing vasculopathy, relative risk for developing new retinopathy was 21 times greater in areas with abnormal baseline mfERG implicit times. mfERG-based prediction models have in fact shown excellent sensitivity and specificity [97, 98] for future DR risk prediction in given retinal regions. More recently, the European Consortium for the Early Treatment of Diabetic Retinopathy (EUROCONDOR) reported the baseline data of their study cohort. The EUROCONDOR was created to assess the effectiveness of eye drops containing neuroprotective agents (brimonidine or somatostatin) among 449 patients with T2DM with no visible DR (Early Treatment of Diabetic Retinopathy Study [ETDRS] level < 20) or with early diabetic retinal disease (ETDRS level 20–35) (NCT01726075). mfERG abnormalities were found in almost 60% of the patients with no apparent fundus abnormalities. An association was also observed by the authors between SD-OCT thinning and mfERG abnormalities in 67% of the eyes with ETDRS < 20 and in 83% of the eyes with ETDRS level of 20–35. However, about a third of patients with early diabetic retinal disease (ETDRS 20–35) did not have any abnormalities, either on the mfERG or SD-OCT. The authors concluded that, while there seemed to be a link between mfERG and SD-OCT measurements that increased with the presence of microvascular impairment, retinal neurodegeneration may not play a role in DR pathogenesis for all patients with T2DM [99•].

Rarebit fovea and Rarebit perimetry tests can detect subtle changes in the integrity of the retinocortical detector matrix. This detector matrix consists of foveal photoreceptor density

and retinal ganglion cell density peripherally [100]. In one of the first studies to correlate structure with function, Van Dijk found decreased macular GCL thickness to be the only significant predictor of abnormal mean hit rate (MHR) [79] on perimetry. Similar findings were also seen by Verma et al., who reported an association between decreased retinal sensitivity on fundus-related microperimetry and reduced retinal thickness on OCT [80].

VEPs assess integrity of the visual pathway, from the optic nerve up to the occipital cortex. Balta et al. found pattern VEP latencies were significantly prolonged in patients with T2DM who did not have evidence of retinopathy when compared with non-diabetic controls [101]. These findings have been validated in multiple studies involving both type 1 and type 2 diabetic cohorts [102–104]. Positive correlations have additionally been reported between altered VEP parameters and DM duration [101, 105] as well as glycemic control [105].

Alteration of visual function has also been tested through oscillatory potentials [106–111], color vision [112, 113], contrast sensitivity [114, 115], and dark adaptation [116–118]. In a cross-sectional analysis of 141 patients with T2DM with no DR, contrast sensitivities were found reduced at all spatial frequencies and correlated with GCL thickness [76]. Oscillatory potentials are considered a good indicator of early neural dysfunction and altered parameters can be appreciated in the absence of photographic evidence of vasculopathy [111]. Changes in oscillatory potentials have also been shown to correlate with retinopathy severity [110, 119]. A study by Bresnick et al. involving 85 participants with DM found that reduced oscillatory potential amplitudes predicted severe proliferative DR onset more accurately than non-perfusion on fluorescein angiography over a follow-up duration of approximately 2 years. The risk of progression to severe proliferative DR was 35% for eyes with low amplitudes (≤ 100 μ) compared with 11% for eyes with higher amplitudes. Capillary non-perfusion, on the other hand, did not contribute independently to the risk prediction model [120]. Reported prevalence [121, 122] of impaired color vision in subjects with DM but no retinopathy has varied from 3.5% [123] to as high as 39.5% [124]. Color vision impairment is known to worsen with increasing retinopathy severity [121] and with macular edema [125].

Flicker-induced retinal diameter change, a function of neurovascular coupling with modulation by nitric oxide (NO), has been shown to deteriorate early in diabetic patients [15, 126]. In a study by Pemp et al., arteriolar response to flicker light was 2.9% in patients with DM versus 7% in healthy controls, whereas venular change was 4.6% in DM and 6.8% in controls. Interestingly, however, response to exogenous NO was preserved in both cohorts suggesting that abnormal flicker-induced vasodilatation in DM was not the result of generally reduced retinal vascular reactivity and might be secondary to impaired neural regulation [14].

To summarize, the various studies evaluating visual function in DM seem to suggest that damage to neural elements occurs in the early stages of DR or it may develop independent of the retinal vascular changes.

Neuroprotective Strategies for Treating Diabetic Neurodegeneration

The impact of DRN on visual function and whether DRN is an independent pathway of vision or intertwined with microvascular abnormalities still needs to be established. However, a growing body of evidence supports the role of neurodegeneration in DR pathogenesis, and the role of neuroprotective agents in DRN is being actively evaluated. Here, we will briefly discuss some of the experimental molecular targets and treatment strategies for preserving neural retina.

Neurotrophic Factors

Imbalance in neurotrophic factors is considered one of the major hallmarks of retinal neurodegeneration. These molecules are central for growth, differentiation, and maintenance of various retinal elements as well as for neurovascular coupling. Pigment epithelium-derived factor (PEDF), somatostatin (SST), insulin and insulin-like growth factors (IGF), and brain-derived neurotrophic factor (BDNF) are examples of some peptides important for retinal neural health.

PEDF is endogenous to retinal pigment epithelium and has both anti-angiogenic and neuroprotective properties; in fact, it has been shown to be among the most potent inhibitors of angiogenesis [127]. Decreased PEDF levels have been observed in the aqueous humor and vitreous of patients with proliferative DR [128]. In addition, PEDF is anti-inflammatory and counteracts glutamate excitotoxic damage and oxidative stress [129]. In a study designed to evaluate therapeutic effects of PEDF, PEDF administration in diabetic animals with early retinopathy changes was beneficial in restoring retinal function and reducing the elevated VEGF levels. The severity of retinal neuronal damage was also suppressed, possibly secondary to anti-oxidative actions of PEDF molecule [130].

The trophic support provided to retinal neurons by insulin and its receptor signaling pathway is impaired in DM. Loss of this survival pathway may contribute to early stages of DR [131, 132]. Altered insulin metabolism has been shown to increase retinal neuronal apoptosis which was reversed after insulin administration [34]. Systemic and intravitreal insulin administration was found to improve insulin receptor activity in diabetic rats and improve pro-survival signaling [132].

BDNF is believed to play a critical role in retinal ganglion cell survival. STZ-induced DM mice were found to have reduced BDNF levels in both retinal ganglion cells and Muller cells; this was also accompanied by dopaminergic amacrine cell

degeneration. The neurodegenerative changes improved with administration of BDNF, highlighting the molecule's role in neuronal survival. Interestingly, BDNF administration increased the cell density of dopaminergic neurons in non-diabetic rodents as well [133]. Therapies targeting BDNF receptor activation have also shown positive results in promoting RGC survival in acute and chronic models of glaucoma [134, 135].

Fibroblast growth factor (FGF) 21 is a unique member of the FGF family and plays an important role in regulating metabolic homeostasis in DM [136]. Produced predominantly in the liver, FGF21 has been shown to have protective effects in DM-induced testicular apoptotic cell death [137], renal dysfunction [138], and against diabetic nephropathy [139] through upregulation of nuclear factor erythroid 2–related factor 2 (*NRF2*) [139]. *NRF2* orchestrates transcriptional induction of multiple anti-oxidant enzymes [140] that play a central role in protecting against DM-induced oxidative stress. Most recently in the study by Fu et al., administration of a long-acting FGF21 analog in diabetic mice was found to be associated with improved photoreceptor function and morphology as well as reduced photoreceptor-derived oxidative stress and retinal inflammation [141].

SST and its receptors express anti-angiogenic properties via reducing VEGF expression. Reduced levels of this neuroprotective agent have been demonstrated in diabetic eyes, both with [142] and without retinopathy changes [50]. In human eyes, underproduction of SST was associated with neuronal apoptosis, especially in the GCL and with microglial activation [50]. A multi-center, phase II/III, randomized controlled clinical trial (EUROCONDOR) to evaluate the efficacy of topical SST eye drops in preventing retinal neurodegeneration is ongoing (EudraCT number: 2012-001200-38).

Glucagon-like peptide 1 (GLP-1) and nerve growth factor (NGF) are additional therapeutic targets. Topical administration of GLP-1R agonists (liraglutide) as eye drops in the *db/db* mouse model was found to preserve blood–retinal barrier integrity via attenuating overexpression of VEGF, whereas its systemic administration demonstrated significant neuroprotective benefits. In addition to reducing expression of glial fibrillary acidic protein (GFAP), a marker of glial reactivity, liraglutide-treated mice also showed decreased rate of cell apoptosis [143]. Moreover, Hernandez et al. demonstrated a significant increase in GLP-1 after topical administration of dipeptidyl peptidase IV inhibitor (DPP-IVi) and which was shown to be associated with decreased glial activation, apoptosis, and vascular leakage in *db/db* mice [144]. NGF-containing eye drops have also been shown to protect retinal ganglion cells from damage in experimental models of glaucoma and DR [145].

Renin-Angiotensin System

Recent studies have pointed towards renin-angiotensin system (RAS) playing a causative role in retinal neurodegeneration. RAS is upregulated in DR and angiotensin II (ATII) is known to mediate oxidative stress, promote angiogenesis, and cause retinal damage [146]. Ola et al. in an experimental study involving diabetic rats demonstrated beneficial effects of telmisartan, an ATII type 1 receptor blocker (AT1R). Compared with diabetic rats that were not administered the drug, telmisartan-treated group exhibited significantly higher levels of BDNF and ciliary neurotrophic factor (CNF). Treatment was also associated with a marked increase of serum and retinal glutathione (GSH) concentration. Telmisartan, through its RAS blocking effects, was thus fundamental in improving neurotrophic support, endogenous anti-oxidant concentration, and decreasing apoptosis in retina of STZ-induced diabetic rats. [147]. In a similar study conducted on diabetic rats, AT1R blockade with either valsartan or telmisartan successfully altered retinal functional parameters assessed via ERG. Improved amplitude and implicit time of oscillatory potentials was hypothesized by the authors to be most likely due to decreased degradation of synaptophysin, a synaptic vesicle protein integral in maintaining neuronal function [148].

Anti-Oxidants

Many studies suggest that oxidative stress plays a major role in the pathogenesis of DR. Anti-oxidants may hence be viewed as potential therapeutic agents in the treatment of DR. Flavonoids are one such example and administration of selected flavonoids has been shown to reduce inflammation and oxidative stress, in addition to preventing neuronal apoptosis and retinal structural degeneration in diabetic mice [149–152]. Administration of the flavonoid, hesperetin in diabetic mice, for example, was shown to be beneficial in counteracting DM-induced effects of Müller cell inflammation and swelling as well as, limiting retinal disorganization from increased intercellular spaces in the INL and ONL. Hesperetin-treated mice also showed reduced expression of AQP4, a water-specific membrane-channel protein, whose overexpression has been implicated in neuroglial swelling. In a crossover randomized clinical trial, flavonoids in *Ginkgo biloba* extract (GBE) improved pre-existing visual field defects in patients with normal tension glaucoma (NTG) [153]. A more recent clinical trial involving NTG patients however found no improvements in contrast sensitivity or visual field damage with GBE administration [154].

Glutamate Blocking Agents

An experimental study conducted with memantine, an NMDA-receptor antagonist showed promising results. Significantly, improved amplitudes of ERG a- and b-waves were observed in STZ-induced diabetic mouse models, 3 weeks after treatment with memantine (MEM). Long-term drug administration was also beneficial in attenuating elevated

vitreo-retinal VEGF levels, improving blood–retinal barrier integrity and increasing RGC count by almost 16% [155].

Despite the promising results shown by these various proposed molecular targets, more studies are needed to further validate their therapeutic effects.

Clinical Translation of Neuroprotective Strategies for Diabetic Retinopathy

Despite the promise shown by neuroprotective agents in preclinical studies, the benefit of these agents in preventing or treating vision loss in DM has not yet been established. Conceptual and methodological problems often make translation from animals to humans difficult, partly because of failure of the animal models to accurately mimic human disease. Furthermore, in most experimental studies, the therapeutic agent is administered at the time of or prior to injury, while in human trials, the intervention is after disease diagnosis. Other barriers include, but are not limited to, challenges associated with participant follow-up, disease heterogeneity, effective drug delivery, and selecting reproducible and clinically important trial endpoints [156]. These challenges explain why several different neuroprotective drug candidates have failed in demonstrating significant patient benefits, despite successful preclinical data. A phase 3 clinical trial examining memantine as a treatment for glaucoma reported that while disease progression was significantly lower in patients receiving a higher dose of memantine than in patients receiving a low dose of memantine, there was no clear therapeutic benefit compared to patients receiving placebo [156]. In the low-pressure glaucoma treatment study, patients treated with brimonidine were found to have a significantly lower rate of visual field defect progression compared to subjects treated with timolol [157]. However, due to issues related to large number of dropouts in the group receiving brimonidine and low amounts of IOP reduction, the study, and hence the neuroprotective efficacy of brimonidine, was recently questioned in two Cochrane reviews [158, 159]. Translational divide exists for ocular diseases other than glaucoma as well, with administration of ciliary neurotrophic factor (CNTF) failing to show any visual acuity benefit in patients with retinitis pigmentosa [160]. CNTF administration however resulted in a dose-dependent increase in retinal thickness, consistent with ancillary studies with adaptive optics scanning laser ophthalmoscopy that showed cone preservation in CTNF-treated eyes compared with the sham-treated fellow eyes in a subset of participants [161, 162]. Most recently, treatment with a surgical implant that released CNTF was found to slow the progression of retinal degeneration in patients with type 2 macular telangiectasia compared with those receiving sham treatments [163]. Multidisciplinary collaborative effort is therefore required to design a set of guidelines for experimental and clinical studies on neuroprotection in ophthalmic disease. A consensus on how to design and execute translational research would optimize the use of resources and facilitate the development of effective neuroprotective agents.

Conclusion and Future Directions

Retinal neurodegeneration plays an increasingly recognized role in diabetic retinopathy and may occur prior to vascular changes. However, more longitudinal studies are needed to better establish the temporal relationship between vasculopathy and neuropathy, progression of DRN over time, and the impact of neurodegeneration on visual function. The temporal occurrence of DRN with respect to vascular changes should also be re-evaluated using more sensitive measures of detection of vascular pathology such as OCT angiography (OCTA), as OCTA may detect vascular changes not visible on fluorescein angiogram or fundus photos. In addition, greater efforts are required to standardize methods for monitoring neurodegeneration to ensure uniformity across studies. Lastly, while it is important to understand the underlying mechanism(s) of DM-induced neurodegeneration in order to develop neuroprotective agents, researchers must also focus on developing optimal drug delivery methods and defining clinically relevant outcome measures to successfully evaluate the impact of neuroprotective agents in patients with DM.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Lee R, Wong TY, Sabanayagam C. Epidemiology of diabetic retinopathy, diabetic macular edema and related vision loss. Eye Vis. 2015;2:17.
2. Bourne RR, Stevens GA, White RA, Smith JL, Flaxman SR, Price H, et al. Causes of vision loss worldwide, 1990-2010: a systematic analysis. Lancet Glob Health. 2013;1(6):e339–49.

3. Hawkins BT, Davis TP. The blood-brain barrier/neurovascular unit in health and disease. Pharmacol Rev. 2005;57(2):173–85.
4. Metea MR, Newman EA. Signalling within the neurovascular unit in the mammalian retina. Exp Physiol. 2007;92(4):635–40.
5. Simo R, Hernandez C. Neurodegeneration in the diabetic eye: new insights and therapeutic perspectives. Trends Endocrinol Metab. 2014;25(1):23–33.
6. Park JJ, Soetikno BT, Fawzi AA. Characterization of the middle capillary plexus using optical coherence tomography angiography in healthy and diabetic eyes. Retina. 2016;36(11):2039–50.
7. Chan G, Balaratnasingam C, Yu PK, Morgan WH, McAllister IL, Cringle SJ, et al. Quantitative morphometry of perifoveal capillary networks in the human retina. Invest Ophthalmol Vis Sci. 2012;53(9):5502–14.
8. Ames A 3rd, Li YY, Heher EC, Kimble CR. Energy metabolism of rabbit retina as related to function: high cost of Na+ transport. J Neurosci. 1992;12(3):840–53.
9. Langhans M, Michelson G, Groh M. Effect of breathing 100% oxygen on retinal and optic nerve head capillary blood flow in smokers and non-smokers. Br J Ophthalmol. 1997;81(5):365–9.
10. Feng Y, Busch S, Gretz N, Hoffmann S, Hammes HP. Crosstalk in the retinal neurovascular unit – lessons for the diabetic retina. Exp Clin Endocrinol Diabetes. 2012;120(04):199–201.
11. Aung MH, Park HN, Han MK, Obertone TS, Abey J, Aseem F, et al. Dopamine deficiency contributes to early visual dysfunction in a rodent model of type 1 diabetes. J Neurosci. 2014;34(3):726–36.
12. D'Cruz TS, Weibley BN, Kimball SR, Barber AJ. Post-translational processing of synaptophysin in the rat retina is disrupted by diabetes. PLoS One. 2012;7(9):e44711.
13. Lieth E, LaNoue KF, Antonetti DA, Ratz M. Diabetes reduces glutamate oxidation and glutamine synthesis in the retina. The Penn State Retina Research Group. Exp Eye Res. 2000;70(6):723–30.
14. Pemp B, Garhofer G, Weigert G, Karl K, Resch H, Wolzt M, et al. Reduced retinal vessel response to flicker stimulation but not to exogenous nitric oxide in type 1 diabetes. Invest Ophthalmol Vis Sci. 2009;50(9):4029–32.
15. Mandecka A, Dawczynski J, Blum M, Muller N, Kloos C, Wolf G, et al. Influence of flickering light on the retinal vessels in diabetic patients. Diabetes Care. 2007;30(12):3048–52.
16. Garhofer G, Zawinka C, Resch H, Kothy P, Schmetterer L, Dorner GT. Reduced response of retinal vessel diameters to flicker stimulation in patients with diabetes. Br J Ophthalmol. 2004;88(7):887–91.
17. Gardner TW, Davila JR. The neurovascular unit and the pathophysiologic basis of diabetic retinopathy. Graefes Arch Clin Exp Ophthalmol. 2017;255(1):1–6.
18. Simó R, Stitt AW, Gardner TW. Neurodegeneration in diabetic retinopathy: does it really matter? Diabetologia. 2018;61(9):1902–12.
19. Wolter JR. Diabetic retinopathy. Am J Ophthalmol. 1961;51:1123–41.
20. Araszkiewicz A, Zozulinska-Ziolkiewicz D. Retinal neurodegeneration in the course of diabetes-pathogenesis and clinical perspective. Curr Neuropharmacol. 2016;14(8):805–9.
21. Wu SM, Maple BR. Amino acid neurotransmitters in the retina: a functional overview. Vis Res. 1998;38(10):1371–84.
22. Choi DW. Glutamate neurotoxicity and diseases of the nervous system. Neuron. 1988;1(8):623–34.
23. Lucas DR, Newhouse JP. The toxic effect of sodium l-glutamate on the inner layers of the retina. AMA Arch Ophthalmol. 1957;58(2):193–201.
24. Vorwerk CK, Lipton SA, Zurakowski D, Hyman BT, Sabel BA, Dreyer EB. Chronic low-dose glutamate is toxic to retinal ganglion cells. Toxicity blocked by memantine. Invest Ophthalmol Vis Sci. 1996;37(8):1618–24.
25. Sucher NJ, Lipton SA, Dreyer EB. Molecular basis of glutamate toxicity in retinal ganglion cells. Vis Res. 1997;37(24):3483–93.
26. Ambati J, Chalam KV, Chawla DK, D'Angio CT, Guillet EG, Rose SJ, et al. Elevated gamma-aminobutyric acid, glutamate, and vascular endothelial growth factor levels in the vitreous of patients with proliferative diabetic retinopathy. Arch Ophthalmol. 1997;115(9):1161–6.
27. Diederen RMH, La Heij EC, Deutz NEP, Kijlstra A, Kessels AGH, van Eijk HMH, et al. Increased glutamate levels in the vitreous of patients with retinal detachment. Exp Eye Res. 2006;83(1):45–50.
28. Santiago AR, Hughes JM, Kamphuis W, Schlingemann RO, Ambrosio AF. Diabetes changes ionotropic glutamate receptor subunit expression level in the human retina. Brain Res. 2008;1198:153–9.
29. Bloodworth JM Jr. Diabetic retinopathy. Diabetes. 1962;11:1–22.
30. Lieth E, Barber AJ, Xu B, Dice C, Ratz MJ, Tanase D, et al. Glial reactivity and impaired glutamate metabolism in short-term experimental diabetic retinopathy. Penn State Retina Research Group. Diabetes. 1998;47(5):815–20.
31. Barber AJ. A new view of diabetic retinopathy: a neurodegenerative disease of the eye. Prog Neuro-Psychopharmacol Biol Psychiatry. 2003;27(2):283–90.
32. Szabó K, Énzsöly A, Dékány B, Szabó A, Hajdú RI, Radovits T, et al. Histological evaluation of diabetic neurodegeneration in the retina of Zucker diabetic fatty (ZDF) rats. Sci Rep. 2017;7:8891.
33. Bogdanov P, Corraliza L, Villena JA, Carvalho AR, Garcia-Arumi J, Ramos D, et al. The db/db mouse: a useful model for the study of diabetic retinal neurodegeneration. PLoS One. 2014;9(5):e97302.
34. Barber AJ, Lieth E, Khin SA, Antonetti DA, Buchanan AG, Gardner TW. Neural apoptosis in the retina during experimental and human diabetes. Early onset and effect of insulin. J Clin Invest. 1998;102(4):783–91.
35. Barber AJ, Antonetti DA, Kern TS, Reiter CE, Soans RS, Krady JK, et al. The Ins2Akita mouse as a model of early retinal complications in diabetes. Invest Ophthalmol Vis Sci. 2005;46(6):2210–8.
36. Aizu Y, Oyanagi K, Hu J, Nakagawa H. Degeneration of retinal neuronal processes and pigment epithelium in the early stage of the streptozotocin-diabetic rats. Neuropathology. 2002;22(3):161–70.
37. Seigel GM, Lupien SB, Campbell LM, Ishii DN. Systemic IGF-I treatment inhibits cell death in diabetic rat retina. J Diabetes Complicat. 2006;20(3):196–204.
38. Podesta F, Romeo G, Liu WH, Krajewski S, Reed JC, Gerhardinger C, et al. Bax is increased in the retina of diabetic subjects and is associated with pericyte apoptosis in vivo and in vitro. Am J Pathol. 2000;156(3):1025–32.
39. Oshitari T, Yamamoto S, Hata N, Roy S. Mitochondria- and caspase-dependent cell death pathway involved in neuronal degeneration in diabetic retinopathy. Br J Ophthalmol. 2008;92(4):552–6.
40. Abu-El-Asrar AM, Dralands L, Missotten L, Al-Jadaan IA, Geboes K. Expression of apoptosis markers in the retinas of human subjects with diabetes. Invest Ophthalmol Vis Sci. 2004;45(8):2760–6.
41. Valverde AM, Miranda S, García-Ramírez M, González-Rodriguez Á, Hernández C, Simó R. Proapoptotic and survival signaling in the neuroretina at early stages of diabetic retinopathy. Mol Vis. 2013;19:47–53.
42. Fu S, Dong S, Zhu M, Sherry DM, Wang C, You Z, et al. Müller glia are a major cellular source of survival signals for retinal neurons in diabetes. Diabetes. 2015;64(10):3554–63.
43. Gastinger MJ, Singh RSJ, Barber AJ. Loss of cholinergic and dopaminergic amacrine cells in streptozotocin-diabetic rat and

Ins2Akita-diabetic mouse retinas. Invest Ophthalmol Vis Sci. 2006;47(7):3143–50.
44. Park SH, Park JW, Park SJ, Kim KY, Chung JW, Chun MH, et al. Apoptotic death of photoreceptors in the streptozotocin-induced diabetic rat retina. Diabetologia. 2003;46(9):1260–8.
45. Kowluru RA, Mishra M. Oxidative stress, mitochondrial damage and diabetic retinopathy. Biochim Biophys Acta. 2015;1852(11): 2474–83.
46. Du Y, Veenstra A, Palczewski K, Kern TS. Photoreceptor cells are major contributors to diabetes-induced oxidative stress and local inflammation in the retina. Proc Natl Acad Sci U S A. 2013;110(41):16586–91.
47. Tonade D, Liu H, Palczewski K, Kern TS. Photoreceptor cells produce inflammatory products that contribute to retinal vascular permeability in a mouse model of diabetes. Diabetologia. 2017;60(10):2111–20.
48. Tonade D, Liu H, Kern TS. Photoreceptor cells produce inflammatory mediators that contribute to endothelial cell death in diabetes. Invest Ophthalmol Vis Sci. 2016;57(10):4264–71.
49. de Gooyer TE, Stevenson KA, Humphries P, Simpson DA, Gardiner TA, Stitt AW. Retinopathy is reduced during experimental diabetes in a mouse model of outer retinal degeneration. Invest Ophthalmol Vis Sci. 2006;47(12):5561–8.
50. Carrasco E, Hernandez C, Miralles A, Huguet P, Farres J, Simo R. Lower somatostatin expression is an early event in diabetic retinopathy and is associated with retinal neurodegeneration. Diabetes Care. 2007;30(11):2902–8.
51.•• Sohn EH, van Dijk HW, Jiao C, Kok PH, Jeong W, Demirkaya N, et al. Retinal neurodegeneration may precede microvascular changes characteristic of diabetic retinopathy in diabetes mellitus. Proc Natl Acad Sci U S A. 2016;113(19):E2655–64. **This 4-year longitudinal study in people with DM with no or minimal DR shows that DRN precedes signs of microvasculopathy and is progressive and independent of glycated hemoglobin, age, and sex.**
52. Masser DR, Otalora L, Clark NW, Kinter MT, Elliott MH, Freeman WM. Functional changes in the neural retina occur in the absence of mitochondrial dysfunction in a rodent model of diabetic retinopathy. J Neurochem. 2017;143(5):595–608.
53. Cogan DG, Toussaint D, Kuwabara T. Retinal vascular patterns. IV. Diabetic retinopathy. Arch Ophthalmol. 1961;66:366–78.
54. Papachristodoulou D, Heath H, Kang SS. The development of retinopathy in sucrose-fed and streptozotocin-diabetic rats. Diabetologia. 1976;12(4):367–74.
55. Kanamori A, Nakamura M, Mukuno H, Maeda H, Negi A. Diabetes has an additive effect on neural apoptosis in rat retina with chronically elevated intraocular pressure. Curr Eye Res. 2004;28(1):47–54.
56. LoDuca AL, Zhang C, Zelkha R, Shahidi M. Thickness mapping of retinal layers by spectral domain optical coherence tomography. Am J Ophthalmol. 2010;150(6):849–55.
57. Costa RA, Skaf M, Melo LA Jr, Calucci D, Cardillo JA, Castro JC, et al. Retinal assessment using optical coherence tomography. Prog Retin Eye Res. 2006;25(3):325–53.
58. Ctori I, Huntjens B. Repeatability of foveal measurements using spectralis optical coherence tomography segmentation software. PLoS One. 2015;10(6):e0129005.
59. Massin P, Vicaut E, Haouchine B, Erginay A, Paques M, Gaudric A. Reproducibility of retinal mapping using optical coherence tomography. Arch Ophthalmol. 2001;119(8):1135–42.
60. Scarinci F, Picconi F, Virgili G, Giorno P, Di Renzo A, Varano M, et al. Single retinal layer evaluation in patients with type 1 diabetes with no or early signs of diabetic retinopathy: the first hint of neurovascular crosstalk damage between neurons and capillaries? Ophthalmologica. 2017;237(4):223–31.
61. Ng DS, Chiang PP, Tan G, Cheung CG, Cheng CY, Cheung CY, et al. Retinal ganglion cell neuronal damage in diabetes and diabetic retinopathy. Clin Exp Ophthalmol. 2016;44(4):243–50.
62. Gundogan FC, Akay F, Uzun S, Yolcu U, Cagiltay E, Toyran S. Early neurodegeneration of the inner retinal layers in type 1 diabetes mellitus. Ophthalmologica. 2016;235(3):125–32.
63. El-Fayoumi D, Badr Eldine NM, Esmael AF, Ghalwash D, Soliman HM. Retinal nerve fiber layer and ganglion cell complex thicknesses are reduced in children with type 1 diabetes with no evidence of vascular retinopathy. Invest Ophthalmol Vis Sci. 2016;57(13):5355–60.
64. Dhasmana R, Sah S, Gupta N. Study of retinal nerve fibre layer thickness in patients with diabetes mellitus using Fourier domain optical coherence tomography. J Clin Diagn Res. 2016;10(7): Nc05–9.
65. Carpineto P, Toto L, Aloia R, Ciciarelli V, Borrelli E, Vitacolonna E, et al. Neuroretinal alterations in the early stages of diabetic retinopathy in patients with type 2 diabetes mellitus. Eye. 2016;30(5):673–9.
66. Rodrigues EB, Urias MG, Penha FM, Badaro E, Novais E, Meirelles R, et al. Diabetes induces changes in neuroretina before retinal vessels: a spectral-domain optical coherence tomography study. Int J Retina Vitreous. 2015;1:4.
67. Demir M, Oba E, Sensoz H, Ozdal E. Retinal nerve fiber layer and ganglion cell complex thickness in patients with type 2 diabetes mellitus. Indian J Ophthalmol. 2014;62(6):719–20.
68. van Dijk HW, Verbraak FD, Kok PH, Stehouwer M, Garvin MK, Sonka M, et al. Early neurodegeneration in the retina of type 2 diabetic patients. Invest Ophthalmol Vis Sci. 2012;53(6):2715–9.
69. van Dijk HW, Verbraak FD, Kok PHB, Garvin MK, Sonka M, Lee K, et al. Decreased retinal ganglion cell layer thickness in patients with type 1 diabetes. Invest Ophthalmol Vis Sci. 2010;51(7): 3660–5.
70. Chen Y, Li J, Yan Y, Shen X. Diabetic macular morphology changes may occur in the early stage of diabetes. BMC Ophthalmol. 2016;16:12.
71. Cabrera DeBuc D, Somfai GM. Early detection of retinal thickness changes in diabetes using optical coherence tomography. Med Sci Monit. 2010;16(3):Mt15–21.
72. Verma A, Raman R, Vaitheeswaran K, Pal SS, Laxmi G, Gupta M, et al. Does neuronal damage precede vascular damage in subjects with type 2 diabetes mellitus and having no clinical diabetic retinopathy? Ophthalmic Res. 2012;47(4):202–7.
73. Pierro L, Iuliano L, Cicinelli MV, Casalino G, Bandello F. Retinal neurovascular changes appear earlier in type 2 diabetic patients. Eur J Ophthalmol. 2017;27(3):346–51.
74. van Dijk HW, Kok PHB, Garvin M, Sonka M, DeVries JH, Michels RPJ, et al. Selective loss of inner retinal layer thickness in type 1 diabetic patients with minimal diabetic retinopathy. Invest Ophthalmol Vis Sci. 2009;50(7):3404–9.
75. Tavares Ferreira J, Alves M, Dias-Santos A, Costa L, Santos BO, Cunha JP, et al. Retinal neurodegeneration in diabetic patients without diabetic retinopathy. Invest Ophthalmol Vis Sci. 2016;57(14):6455–60.
76. Zhu T, Ma J, Li Y, Zhang Z. Association between retinal neuronal degeneration and visual function impairment in type 2 diabetic patients without diabetic retinopathy. Sci China Life Sci. 2015;58(6):550–5.
77. Vujosevic S, Midena E. Retinal layers changes in human preclinical and early clinical diabetic retinopathy support early retinal neuronal and Müller cells alterations. J Diabetes Res. 2013;2013:8.
78. Araszkiewicz A, Zozulinska-Ziolkiewicz D, Meller M, Bernardczyk-Meller J, Pilacinski S, Rogowicz-Frontczak A, et al. Neurodegeneration of the retina in type 1 diabetic patients. Pol Arch Med Wewn. 2012;122(10):464–70.

79. van Dijk HW, Verbraak FD, Stehouwer M, Kok PH, Garvin MK, Sonka M, et al. Association of visual function and ganglion cell layer thickness in patients with diabetes mellitus type 1 and no or minimal diabetic retinopathy. Vis Res. 2011;51(2):224–8.
80. Verma A, Rani PK, Raman R, Pal SS, Laxmi G, Gupta M, et al. Is neuronal dysfunction an early sign of diabetic retinopathy? Microperimetry and spectral domain optical coherence tomography (SD-OCT) study in individuals with diabetes, but no diabetic retinopathy. Eye (Lond). 2009;23(9):1824–30.
81. Oshitari T, Hanawa K, Adachi-Usami E. Changes of macular and RNFL thicknesses measured by stratus OCT in patients with early stage diabetes. Eye (Lond). 2009;23(4):884–9.
82. Asefzadeh B, Fisch BM, Parenteau CE, Cavallerano AA. Macular thickness and systemic markers for diabetes in individuals with no or mild diabetic retinopathy. Clin Exp Ophthalmol. 2008;36(5):455–63.
83. Demirkaya N, van Dijk HW, van Schuppen SM, Abramoff MD, Garvin MK, Sonka M, et al. Effect of age on individual retinal layer thickness in normal eyes as measured with spectral-domain optical coherence tomography. Invest Ophthalmol Vis Sci. 2013;54(7):4934–40.
84. Do carmo A, Ramos P, Reis A, Proenca R, Cunha-vaz JG. Breakdown of the inner and outer blood retinal barrier in streptozotocin-induced diabetes. Exp Eye Res. 1998;67(5):569–75.
85. Bowd C, Weinreb RN, Williams JM, Zangwill LM. The retinal nerve fiber layer thickness in ocular hypertensive, normal, and glaucomatous eyes with optical coherence tomography. Arch Ophthalmol. 2000;118(1):22–6.
86. Rauscher FM, Sekhon N, Feuer WJ, Budenz DL. Myopia affects retinal nerve fiber layer measurements as determined by optical coherence tomography. J Glaucoma. 2009;18(7):501–5.
87. Chhablani J, Sharma A, Goud A, Peguda HK, Rao HL, Begum VU, et al. Neurodegeneration in type 2 diabetes: evidence from spectral-domain optical coherence tomography. Invest Ophthalmol Vis Sci. 2015;56(11):6333–8.
88. Falsini B, Porciatti V, Scalia G, Caputo S, Minnella A, Di Leo MA, et al. Steady-state pattern electroretinogram in insulin-dependent diabetics with no or minimal retinopathy. Doc Ophthalmol. 1989;73(2):193–200.
89. Di Leo MA, Falsini B, Caputo S, Ghirlanda G, Porciatti V, Greco AV. Spatial frequency-selective losses with pattern electroretinogram in type 1 (insulin-dependent) diabetic patients without retinopathy. Diabetologia. 1990;33(12):726–30.
90. Caputo S, Di Leo MA, Falsini B, Ghirlanda G, Porciatti V, Minella A, et al. Evidence for early impairment of macular function with pattern ERG in type I diabetic patients. Diabetes Care. 1990;13(4):412–8.
91. Bearse MA Jr, Han Y, Schneck ME, Adams AJ. Retinal function in normal and diabetic eyes mapped with the slow flash multifocal electroretinogram. Invest Ophthalmol Vis Sci. 2004;45(1):296–304.
92. Shimada Y, Li Y, Bearse MA Jr, Sutter EE, Fung W. Assessment of early retinal changes in diabetes using a new multifocal ERG protocol. Br J Ophthalmol. 2001;85(4):414–9.
93. Han Y, Bearse MA Jr, Schneck ME, Barez S, Jacobsen CH, Adams AJ. Multifocal electroretinogram delays predict sites of subsequent diabetic retinopathy. Invest Ophthalmol Vis Sci. 2004;45(3):948–54.
94. Han Y, Adams AJ, Bearse MA Jr, Schneck ME. Multifocal electroretinogram and short-wavelength automated perimetry measures in diabetic eyes with little or no retinopathy. Arch Ophthalmol. 2004;122(12):1809–15.
95. Lakhani E, Wright T, Abdolell M, Westall C. Multifocal ERG defects associated with insufficient long-term glycemic control in adolescents with type 1 diabetes. Invest Ophthalmol Vis Sci. 2010;51(10):5297–303.
96. Bronson-Castain KW, Bearse MA Jr, Neuville J, Jonasdottir S, King-Hooper B, Barez S, et al. Adolescents with type 2 diabetes: early indications of focal retinal neuropathy, retinal thinning, and venular dilation. Retina. 2009;29(5):618–26.
97. Han Y, Schneck ME, Bearse JMA, Barez S, Jacobsen CH, Jewell NP, et al. Formulation and evaluation of a predictive model to identify the sites of future diabetic retinopathy. Invest Ophthalmol Vis Sci. 2004;45(11):4106–12.
98. Ng JS, Bearse JMA, Schneck ME, Barez S, Adams AJ. Local diabetic retinopathy prediction by multifocal ERG delays over 3 years. Invest Ophthalmol Vis Sci. 2008;49(4):1622–8.
99.• Santos AR, Ribeiro L, Bandello F, Lattanzio R, Egan C, Frydkjaer-Olsen U, et al. Functional and structural findings of neurodegeneration in early stages of diabetic retinopathy: cross-sectional analyses of baseline data of the EUROCONDOR project. Diabetes. 2017;66(9):2503–10. **Findings from this study suggest that neurodegeneration plays a role in the pathogenesis of early stages of DR in a large proportion but not in all patients with T2DM.**
100. Nilsson M. The rarebit fovea test: a new measure of visual function: Institutionen för klinisk neurovetenskap/Department of Clinical Neuroscience; 2008.
101. Balta O, Sungur G, Yakin M, Unlu N, Balta OB, Ornek F. Pattern visual evoked potential changes in diabetic patients without retinopathy. J Ophthalmol. 2017;2017:6.
102. Heravian J, Ehyaei A, Shoeibi N, Azimi A, Ostadi-Moghaddam H, Yekta A-A, et al. Pattern visual evoked potentials in patients with type II diabetes mellitus. J Ophthalmic Vis Res. 2012;7(3):225–30.
103. Mariani E, Moreo G, Colucci GB. Study of visual evoked potentials in diabetics without retinopathy: correlations with clinical findings and polyneuropathy. Acta Neurol Scand. 1990;81(4):337–40.
104. Ponte F, Giuffre G, Anastasi M, Lauricella M. Involvment of the visual evoked potentials in type I insulin-dependent diabetes. Metab Pediatr Syst Ophthalmol (1985). 1986;9(2–4):77–80.
105. Raman P, Sodani A, George B. A study of visual evoked potential changes in diabetes mellitus. Int J Diab Dev Countries. 1997;17:69–73.
106. Simonsen SE. The value of the oscillatory potential in selecting juvenile diabetics at risk of developing proliferative retinopathy. Acta Ophthalmol. 1980;58(6):865–78.
107. van der Torren K, van Lith G. Oscillatory potentials in early diabetic retinopathy. Doc Ophthalmol. 1989;71(4):375–9.
108. Yonemura D, Kawasaki K. New approaches to ophthalmic electrodiagnosis by retinal oscillatory potential, drug-induced responses from retinal pigment epithelium and cone potential. Doc Ophthalmol. 1979;48(1):163–222.
109. Bresnick GH, Korth K, Groo A, Palta M. Electroretinographic oscillatory potentials predict progression of diabetic retinopathy: preliminary report. Arch Ophthalmol. 1984;102(9):1307–11.
110. Bresnick GH, Palta M. Oscillatory potential amplitudes: relation to severity of diabetic retinopathy. Arch Ophthalmol. 1987;105(7):929–33.
111. Juen S, Kieselbach GF. Electrophysiological changes in juvenile diabetics without retinopathy. Arch Ophthalmol. 1990;108(3):372–5.
112. Andrade LCO, Souza GS, Lacerda EMCB, Nazima MTST, Rodrigues AR, Otero LM, et al. Influence of retinopathy on the achromatic and chromatic vision of patients with type 2 diabetes. BMC Ophthalmol. 2014;14:104.
113. Feitosa-Santana C, Paramei GV, Nishi M, Gualtieri M, Costa MF, Ventura DF. Color vision impairment in type 2 diabetes assessed by the D-15d test and the Cambridge Colour Test. Ophthalmic Physiol Opt. 2010;30(5):717–23.
114. Sokol S, Moskowitz A, Skarf B, Evans R, Molitch M, Senior B. Contrast sensitivity in diabetics with and without background retinopathy. Arch Ophthalmol. 1985;103(1):51–4.

115. Dosso AA, Bonvin ER, Morel Y, Golay A, Assal JP, Leuenberger PM. Risk factors associated with contrast sensitivity loss in diabetic patients. Graefes Arch Clin Exp Ophthalmol. 1996;234(5):300–5.
116. Greenstein VC, Thomas SR, Blaustein H, Koenig K, Carr RE. Effects of early diabetic retinopathy on rod system sensitivity. Optom Vis Sci. 1993;70(1):18–23.
117. Amemiya T. Dark adaptation in diabetics. Ophthalmologica. 1977;174(6):322–6.
118. Di Leo MA, Caputo S, Falsini B, Porciatti V, Minnella A, Greco AV, et al. Nonselective loss of contrast sensitivity in visual system testing in early type I diabetes. Diabetes Care. 1992;15(5):620–5.
119. Wachtmeister L. Oscillatory potentials in the retina: what do they reveal. Prog Retin Eye Res. 1998;17(4):485–521.
120. Bresnick GH, Palta M. Predicting progression to severe proliferative diabetic retinopathy. Arch Ophthalmol. 1987;105(6):810–4.
121. Green FD, Ghafour IM, Allan D, Barrie T, McClure E, Foulds WS. Colour vision of diabetics. Br J Ophthalmol. 1985;69(7):533–6.
122. Trick GL, Burde RM, Gordon MO, Santiago JV, Kilo C. The relationship between hue discrimination and contrast sensitivity deficits in patients with diabetes mellitus. Ophthalmology. 1988;95(5):693–8.
123. Shoji T, Sakurai Y, Sato H, Chihara E, Takeuchi M. Do type 2 diabetes patients without diabetic retinopathy or subjects with impaired fasting glucose have impaired colour vision? The Okubo Color Study Report. Diabet Med. 2011;28(7):865–71.
124. Gella L, Raman R, Kulothungan V, Pal SS, Ganesan S, Sharma T. Impairment of colour vision in diabetes with no retinopathy: Sankara Nethralaya diabetic retinopathy epidemiology and molecular genetics study (SNDREAMS- II, report 3). PLoS One. 2015;10(6):e0129391.
125. Fong DS, Barton FB, Bresnick GH. Impaired color vision associated with diabetic retinopathy: early treatment diabetic retinopathy study report no. 15. Am J Ophthalmol. 1999;128(5):612–7.
126. Nguyen TT, Kawasaki R, Wang JJ, Kreis AJ, Shaw J, Vilser W, et al. Flicker light–induced retinal vasodilation in diabetes and diabetic retinopathy. Diabetes Care. 2009;32(11):2075–80.
127. Barnstable CJ, Tombran-Tink J. Neuroprotective and antiangiogenic actions of PEDF in the eye: molecular targets and therapeutic potential. Prog Retin Eye Res. 2004;23(5):561–77.
128. Zheng B, Li T, Chen H, Xu X, Zheng Z. Correlation between ficolin-3 and vascular endothelial growth factor-to-pigment epithelium-derived factor ratio in the vitreous of eyes with proliferative diabetic retinopathy. Am J Ophthalmol. 2011;152(6):1039–43.
129. Shen X, Zhong Y, Xie B, Cheng Y, Jiao Q. Pigment epithelium derived factor as an anti-inflammatory factor against decrease of glutamine synthetase expression in retinal Müller cells under high glucose conditions. Graefes Arch Clin Exp Ophthalmol. 2010;248(8):1127–36.
130. Yoshida Y, Yamagishi S-I, Matsui T, Jinnouchi Y, Fukami K, Imaizumi T, et al. Protective role of pigment epithelium-derived factor (PEDF) in early phase of experimental diabetic retinopathy. Diabetes Metab Res Rev. 2009;25(7):678–86.
131. Barber AJ, Nakamura M, Wolpert EB, Reiter CE, Seigel GM, Antonetti DA, et al. Insulin rescues retinal neurons from apoptosis by a phosphatidylinositol 3-kinase/Akt-mediated mechanism that reduces the activation of caspase-3. J Biol Chem. 2001;276(35):32814–21.
132. Reiter CE, Wu X, Sandirasegarane L, Nakamura M, Gilbert KA, Singh RS, et al. Diabetes reduces basal retinal insulin receptor signaling: reversal with systemic and local insulin. Diabetes. 2006;55(4):1148–56.
133. Seki M, Tanaka T, Nawa H, Usui T, Fukuchi T, Ikeda K, et al. Involvement of brain-derived neurotrophic factor in early retinal neuropathy of streptozotocin-induced diabetes in rats. Diabetes. 2004;53(9):2412–9.
134. Hu Y, Cho S, Goldberg JL. Neurotrophic effect of a novel TrkB agonist on retinal ganglion cells. Invest Ophthalmol Vis Sci. 2010;51(3):1747–54.
135. Bai Y, Xu J, Brahimi F, Zhuo Y, Sarunic MV, Saragovi HU. An agonistic TrkB mAb causes sustained TrkB activation, delays RGC death, and protects the retinal structure in optic nerve axotomy and in glaucoma. Invest Ophthalmol Vis Sci. 2010;51(9):4722–31.
136. Dostalova I, Haluzikova D, Haluzik M. Fibroblast growth factor 21: a novel metabolic regulator with potential therapeutic properties in obesity/type 2 diabetes mellitus. Physiol Res. 2009;58(1):1–7.
137. Jiang X, Zhang C, Xin Y, Huang Z, Tan Y, Huang Y, et al. Protective effect of FGF21 on type 1 diabetes-induced testicular apoptotic cell death probably via both mitochondrial- and endoplasmic reticulum stress-dependent pathways in the mouse model. Toxicol Lett. 2013;219(1):65–76.
138. Zhang C, Shao M, Yang H, Chen L, Yu L, Cong W, et al. Attenuation of hyperlipidemia- and diabetes-induced early-stage apoptosis and late-stage renal dysfunction via administration of fibroblast growth factor-21 is associated with suppression of renal inflammation. PLoS One. 2013;8(12):e82275.
139. Cheng Y, Zhang J, Guo W, Li F, Sun W, Chen J, et al. Up-regulation of Nrf2 is involved in FGF21-mediated fenofibrate protection against type 1 diabetic nephropathy. Free Radic Biol Med. 2016;93:94–109.
140. Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, et al. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun. 1997;236(2):313–22.
141. Fu Z, Wang Z, Liu CH, Gong Y, Cakir B, Liegl R, et al. Fibroblast growth factor 21 protects photoreceptor function in type 1 diabetic mice. Diabetes. 2018;67(5):974–85.
142. Simó R, Lecube A, Sararols L, García-Arumí J, Segura RM, Casamitjana R, et al. Deficit of somatostatin-like immunoreactivity in the vitreous fluid of diabetic patients. Diabetes Care. 2002;25(12):2282–6.
143. Hernandez C, Bogdanov P, Corraliza L, Garcia-Ramirez M, Sola-Adell C, Arranz JA, et al. Topical administration of GLP-1 receptor agonists prevents retinal neurodegeneration in experimental diabetes. Diabetes. 2016;65(1):172–87.
144. Hernandez C, Bogdanov P, Sola-Adell C, Sampedro J, Valeri M, Genis X, et al. Topical administration of DPP-IV inhibitors prevents retinal neurodegeneration in experimental diabetes. Diabetologia. 2017;60(11):2285–98.
145. Colafrancesco V, Coassin M, Rossi S, Aloe L. Effect of eye NGF administration on two animal models of retinal ganglion cells degeneration. Ann Ist Super Sanita. 2011;47(3):284–9.
146. Wilkinson-Berka JL. Angiotensin and diabetic retinopathy. Int J Biochem Cell Biol. 2006;38(5–6):752–65.
147. Ola MS, Ahmed MM, Abuohashish HM, Al-Rejaie SS, Alhomida AS. Telmisartan ameliorates neurotrophic support and oxidative stress in the retina of streptozotocin-induced diabetic rats. Neurochem Res. 2013;38(8):1572–9.
148. Kurihara T, Ozawa Y, Nagai N, Shinoda K, Noda K, Imamura Y, et al. Angiotensin II type 1 receptor signaling contributes to synaptophysin degradation and neuronal dysfunction in the diabetic retina. Diabetes. 2008;57(8):2191–8.
149. Kumar B, Gupta SK, Srinivasan BP, Nag TC, Srivastava S, Saxena R, et al. Hesperetin rescues retinal oxidative stress, neuroinflammation and apoptosis in diabetic rats. Microvasc Res. 2013;87:65–74.
150. Kumar B, Gupta SK, Nag TC, Srivastava S, Saxena R, Jha KA, et al. Retinal neuroprotective effects of quercetin in streptozotocin-induced diabetic rats. Exp Eye Res. 2014;125:193–202.

151. Ibrahim AS, El-Shishtawy MM, Peña A Jr, Liou GI. Genistein attenuates retinal inflammation associated with diabetes by targeting of microglial activation. Mol Vis. 2010;16:2033.
152. Mrudula T, Suryanarayana P, Srinivas PNBS, Reddy GB. Effect of curcumin on hyperglycemia-induced vascular endothelial growth factor expression in streptozotocin-induced diabetic rat retina. Biochem Biophys Res Commun. 2007;361(2):528–32.
153. Quaranta L, Bettelli S, Uva MG, Semeraro F, Turano R, Gandolfo E. Effect of Ginkgo biloba extract on preexisting visual field damage in normal tension glaucoma. Ophthalmology. 2003;110(2): 359–62 discussion 362-354.
154. Guo X, Kong X, Huang R, Jin L, Ding X, He M, et al. Effect of Ginkgo biloba on visual field and contrast sensitivity in Chinese patients with normal tension glaucoma: a randomized, crossover clinical trial. Invest Ophthalmol Vis Sci. 2014;55(1):110–6.
155. Kusari J, Zhou S, Padillo E, Clarke KG, Gil DW. Effect of memantine on neuroretinal function and retinal vascular changes of streptozotocin-induced diabetic rats. Invest Ophthalmol Vis Sci. 2007;48(11):5152–9.
156. Almasieh M, Levin LA. Neuroprotection in glaucoma: animal models and clinical trials. Ann Rev Vis Sci. 2017;3:91–120.
157. Krupin T, Liebmann JM, Greenfield DS, Ritch R, Gardiner S. A randomized trial of brimonidine versus timolol in preserving visual function: results from the low-pressure glaucoma treatment study. Am J Ophthalmol. 2011;151(4):671–81.
158. Sena DF, Lindsley K. Neuroprotection for treatment of glaucoma in adults. Cochrane Database Syst Rev. 2013;2(2):CD006539.
159. Sena DF, Lindsley K. Neuroprotection for treatment of glaucoma in adults. Cochrane Database Syst Rev. 2017;1:Cd006539.
160. Birch DG, Weleber RG, Duncan JL, Jaffe GJ, Tao W, Ciliary Neurotrophic Factor Retinitis Pigmentosa Study G. Randomized trial of ciliary neurotrophic factor delivered by encapsulated cell intraocular implants for retinitis pigmentosa. Am J Ophthalmol. 2013;156(2):283–292.e281.
161. Miao I, Bhakta AS, Sredar N, Ivers KM, Patel NB, Queener HM, et al. In vivo examination of cone photoreceptors in patients with retinitis pigmentosa implanted over five years ago with encapsulated ciliary neurotrophic factor. Invest Ophthalmol Vis Sci. 2014;55(13):2619.
162. Talcott KE, Ratnam K, Sundquist SM, Lucero AS, Lujan BJ, Tao W, et al. Longitudinal study of cone photoreceptors during retinal degeneration and in response to ciliary neurotrophic factor treatment. Invest Ophthalmol Vis Sci. 2011;52(5):2219–26.
163. Chew EY, Clemons TE, Jaffe GJ, Johnson CA, Farsiu S, Lad EM, et al. Effect of ciliary neurotrophic factor on retinal neurodegeneration in patients with macular telangiectasia type 2: a randomized clinical trial. Ophthalmology. 2018.