Photoreceptors in diabetic retinopathy

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Keywords
Diabetes, Diabetic retinopathy, Photoreceptors

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J Diabetes Invest 2015; 6: 371–380
doi: 10.1111/jdi.12312

INTRODUCTION
Diabetic retinopathy (DR), a leading cause of visual impairment and blindness, is clinically defined as a microvascular disease, but the unique susceptibility of the retina (compared to other tissues) to this disease has never been explained4. Here, we review the accumulating data that suggests a new hypothesis that photoreceptors in the outer retina might play an important role in the development of the retinopathy. Photoreceptors are light-sensing cells unique to the retina. The present review will summarize current data linking retinal photoreceptors in the pathogenesis of early stages of diabetic retinopathy. The discussion will focus primarily on rods, as most of the animal-based work on this topic has been carried out in rodents (which have a rod-rich retina)2. Less is known about how cone function is affected with diabetes, although new animal models (such as nrl−/− mice3,4) might be useful in addressing this in the future.

EVIDENCE SUGGESTING THAT PHOTORECEPTORS CONTRIBUTE TO VASCULAR DISEASE IN DIABETIC RETINOPATHY
Photoreceptors of the outer retina have not usually been regarded as important in the pathogenesis of early diabetic retinopathy, likely due in part to the substantial distance between the photoreceptors and the retinal microvasculature that is affected by diabetes (Figure 1). Nevertheless, available evidence raises a possibility that the unique susceptibility of the retina to injury in diabetes could in fact be as a result of the presence of photoreceptors. In support of the photoreceptor hypothesis, Arden et al.5 sent a survey sent to a group of diabetic patients who also had retinitis pigmentosa. The results of these responses suggested that DR was less severe in patients who also had retinitis pigmentosa (and therefore, photoreceptor degeneration). Stitt et al.6 subsequently reported that diabetes did not cause the expected decrease in density of the retinal microvasculature in mice lacking rhodopsin (Rho−/−), and thus lacking most photoreceptors. These data suggest that loss of photoreceptors in the outer retina reduced the severity of vascular degeneration in that model of diabetic retinopathy. There are at least two hypotheses to explain how photoreceptors might influence the development of DR, and they are not mutually exclusive:

Hypoxia
It is well known that photoreceptors account for much of the oxygen consumed by the retina, and that this metabolism is increased in the dark7,8, when the rod dark current becomes maximal9–11. Studies in cat and macaque retinae, in which oxygen microelectrodes were inserted into the retina, found a 30–40% PO2 difference between the inner retina and the vicinity of rods12,13. There was no detectable oxygen next to dark-adapted rods. In the dark, oxygen consumption is greater than any other cell in the retina14.

Arden6,15,16 incorporated available data showing the high metabolic activity of photoreceptors at night (dark current), and postulated that in the presence of a compromised retinal vasculature (such as in diabetes), photoreceptor activity in the dark would make the retina even more hypoxic than usual.
The extent to which this hypothesis applies also to the development of early diabetic retinopathy (before the vasculature is compromised) requires additional study.

Oxidative Stress
Diabetes results in increased generation of superoxide and other reactive oxygen species in the retina. This oxidative stress is important in the pathogenesis of at least the vascular lesions of diabetic retinopathy, because inhibition of oxidative stress has been shown to inhibit development of inflammation and subsequent vascular lesions of early DR. It has commonly been assumed that the diabetes-induced increases in oxidative stress arises in retinal cells known to be affected by diabetes (including endothelial cells and pericytes), but recent data shows that photoreceptors are the major site of superoxide generation in diabetes. Consistent with a role of photoreceptors in oxidative stress, the presence or absence of light affects retinal oxidative stress (oxidative stress caused by diabetes is worsened in the dark), and oxidative stress contributes to the induction of pro-inflammatory proteins (which participate in the development of retinal microvascular pathology in diabetes). Photoreceptors express nicotinamide adenine dinucleotide phosphate oxidase, and contain most of the mitochondria found in the retina, and both of these sources of reactive oxygen species seem to contribute to the observed retinal superoxide generation in diabetes.

Based on these considerations, our working model of how photoreceptors play a critical role in the pathogenesis of diabetic retinopathy is summarized in Figure 2. Work is ongoing to elucidate how oxidative stress, inflammation and microvascular disease in diabetes are linked, and these are not the central topic of the present review. Here, we will largely focus on the impact of diabetes on rod morphology, cell biology and function as they related to oxidative stress generation in early stages of diabetic retinopathy.

MORPHOLOGICAL CHANGES TO PHOTORECEPTORS, RETINAL PIGMENT EPITHELIUM AND CHOROID IN DIABETES
A number of animal studies (summarized here) have reported that at least some photoreceptors degenerate in diabetes.
Nevertheless, it is important to recognize that diabetes has not been reported to cause widespread degeneration of photoreceptors, unlike in several other important retinal diseases.

**Animal Studies**

Some studies in diabetic rodents have reported photoreceptor degeneration early in the course of diabetes. Retinas from diabetic rats have been found to have increased caspase-3, as well as photoreceptor atrophy. A reduction in the thickness of the outer nuclear layer was seen 24 weeks after the onset of diabetes, resulting in only half of the normal cellular layers in the outer nuclear layer remaining at 24 weeks of diabetes. A few photoreceptors showed evidence of apoptosis at 4 weeks of diabetes, and the number of apoptotic photoreceptors increased thereafter. Diabetes has also been reported to cause a reduction in the length of the rod outer segments in male Sprague–Dawley rats over a study duration of 24 weeks. Morphological signs of degeneration in the outer segments of rods, most M-cones and some S-cones has been reported in Male Wistar and Sprague–Dawley rats killed 12 weeks after the induction of diabetes.

These photoreceptor abnormalities seem not to be secondary to chemical induction of diabetes, because they have also been detected in spontaneously diabetic animal models. A spontaneous model of type 1 diabetes in Ins2Akita diabetic mice has been reported as showing cone, but not rod, photoreceptor loss after just 3 months of diabetes, and severe impairment of synaptic connectivity at the outer plexiform layer was detected in 9-month-old animals, suggesting cone photoreceptor degeneration. A model of type 2 diabetes, the db/db mouse, showed thinning of the inner and outer nuclear (photoreceptor) layers, with defects in the integrity of the retinal pigment epithelium (RPE) over 8–24 weeks of diabetes. Photoreceptors in less-studied animal models have also been reported to be affected by diabetes or experimental hyperglycemia. In Otsuka Long-Evans Tokushima Fatty (OLETF) rats (duration of diabetes not reported), the number of photoreceptor cell nuclei decreased, RPE decreased in height and basal infoldings were poorly developed. Retinas from (mRen2)27 rats (a transgenic model showing greater than normal plasma prorenin levels) who were diabetic for just 3 weeks showed increased apoptotic cell death of both inner retinal neurons and photoreceptors. Diabetes narrowed the layers of rods and cones after 6 weeks in rabbits, and these changes were exacerbated after 3–6 months of diabetes (including atrophy of the RPE and damage to photoreceptor discs). Adult zebrafish, in which the zebrafish were subjected to oscillating hyperglycemia for 30 days, showed degeneration of cone photoreceptor neurons and dysfunction of cone-mediated electroretinograms. Diabetes-induced defects or degeneration of photoreceptors in animals have been reported to be inhibited therapeutically. These defects have been reported to be inhibited by administration of hesperetin, wolfberry, aliskiren, or exendin-4a, an agonist of glucagon-like factor-1.

Not all studies show photoreceptor death in diabetes. Studies of male Wistar and Sprague–Dawley rats diabetic for 12 weeks reported that retinal thickness, the number of apoptotic cells,
and the density of cones expressing middle (M)- and shortwave (S)-sensitive opsins were similar in diabetic and control retinas. In male C57BL/6J mice diabetic for 2 months, no significant difference in the number of layers in the outer nuclear layer was detected. Other morphological studies at substantially longer durations of diabetes and in multiple species have not found evidence of photoreceptor loss, or have not commented on (or noticed) it. The lack of consistent conclusions among investigators about whether or not photoreceptor loss occurs in diabetes raises possibilities that some reports of photoreceptor loss might be due less to diabetes than to other differences (including strain differences), or that duration of diabetes plays an important role in the process.

Patient Studies
Evidence showing photoreceptor death is even less abundant in diabetic patients. Occasional case reports suggest photoreceptor loss in diabetes or diabetic macular edema (DME), but there has been no systematic demonstration that photoreceptors are lost in diabetic patients, with the exception of autopsy evidence showing that the S-cones selectively are lost in DR.

Less severe changes to photoreceptor morphology have been associated with changes in visual acuity in diabetes. Also, the photoreceptor inner and outer segment junction, and external limiting membrane have been identified as useful parameters for optical coherence tomography evaluation of foveal photoreceptor layer integrity in DME. In DME, photoreceptor outer segment length of the central subfield was less than the mean cone OS length in the fovea of healthy subjects, suggesting shortening of the photoreceptor outer segment length in diabetes or macular edema.

Summarizing
Anatomical changes in the photoreceptors elicited by diabetes appear modest, but this needs to be studied more, especially in patients.

MOLECULAR CHANGES IN PHOTORECEPTORS IN DIABETES
Animal Studies
Molecular techniques provide evidence that proteins important for photoreceptor function become altered before the appearance of microangiopathy in diabetes. For example, the content of rhodopsin, transducin, recoverin and optical density of photopigment have been reported to become subnormal in diabetes. Reduced levels of genes involved in the phototransduction pathway [photoreceptor-specific opsin (Opm1mw), arrestin (Arr3) and increased transducin (Gnb3)] also suggests altered photoreceptor function, and whole transcriptome rino-nucleic acid (RNA) sequencing (RNA-seq) has identified changes in transcripts including cyclic nucleotide gated channel (Cngb3), arrestin (Arr), guanine nucleotide binding protein (Gnb3) and phosphodiesterase (Pde6 h). Marginal decreases were also noticed in messenger RNA (mRNA) for RPE65, c transducin (Gnat2) and Crxos1. A significant decrease in RPE65 protein immunoreactivity was apparent in Wistar rats diabetic for 12 weeks, but was less evident in diabetic Sprague–Dawley rats. Rhodopsin kinase (Grk1) mRNA was subnormal in diabetic Brown Norway and Sprague–Dawley rats (but not in diabetic Long Evans rats), but expression of rhodopsin kinase protein was reported to be increased in the retinas of Sprague–Dawley rats diabetic for 6 weeks. Despite the changes in rhodopsin kinase and arrestin identified above, diabetes of 12 weeks’ duration in rats did not alter the rate of deactivation of the photoreponse.

Notably, insulin (independently of glucose uptake) has direct effects on photoreceptors. Insulin directly binds to photoreceptors, and initiates signaling within those cells. Photoactivation of rhodopsin causes tyrosine phosphorylation of the insulin receptor and subsequent activation of phosphoinositide 3-kinase, a neuron survival factor. This activation has been speculated to protect the photoreceptors from light damage. The retinal insulin receptor shows a high level of basal autophosphorylation, and this autophosphorylation is reduced in diabetic mouse retinas. Thus, the absence or relative absence of insulin in diabetes might have effects on photoreceptors that have not been fully characterized yet.

Na’/K’-adenosine 5’-triphosphatase activity, which is concentrated in outer segments of rods, plays a major role in a-wave maintenance, and is responsible for sustaining the dark current. Na’/K’-adenosine 5’-triphosphatase activity has been found to be impaired in diabetes. It is possible that this diminished activity contributes to the diabetes-induced reduction in photoreceptor amplitude.

Not all defects affecting photoreceptor function in diabetes directly involve the photoreceptors. Some investigators have shown that the availability of vitamin A (retinol; the parent compound for retinoids) is subnormal in diabetes.

Summarizing
Diabetes causes a number of molecular alterations within photoreceptors, but there is not yet a clear understanding of how these changes occur, or their significance with regard to photoreceptor (and retinal) function. Whether these abnormalities are a cause or result of the oxidative stress that develops in photoreceptors in diabetes is not known.

CHANGES IN PHOTORECEPTOR/RPE UNIT FUNCTION IN DIABETES
Photoreceptors are the most metabolically active neuron in the central nervous system. One common method for evaluating the function of photoreceptors non-invasively is by electroretinogram (ERG), and specifically by analysis of the ERG a-wave and b-wave.

Animal Studies
Diabetes-induced defects in both amplitude and latency of the a-wave have been detected in some studies of diabetic rats. This
Defect has been reported to develop as rapidly as 2 days after the onset of diabetes, but whether this rapid development of a functional defect was a result of diabetes or the rapidly changing metabolic milieu immediately after the initiation of hyperglycemia and insulin deficiency is not yet clear. Defects have also been reported at 4 and 12 weeks of diabetes, and the defects in photoreceptor function detected at 12 weeks of diabetes in rats encompassed several different parameters, including abnormal response amplitudes in the presence of normal sensitivity. Diabetes did not affect deactivation of the photoreceptor response, and dark adaptation occurred faster than normal in those diabetic animals. The authors interpreted these data as likely showing a decrease in the amount of rhodopsin present in the rod outer segments associated with a proportional decrease in outer segment lengths.

Likewise, some studies involving diabetic mice showed defects in the a-wave. Diabetic db/db mice showed significant a-wave amplitude and implicit time defect in the interval of 8–24 weeks of diabetes. Spontaneously diabetic Ins2akita mice showed subnormal a-wave amplitude and implicit time at 9 months-of-age, but not at 3 or 6 months-of-age.

Diabetes has been reported to result in subnormal rhodopsin generation. Rhodopsin regeneration was also reported to be impaired by decreased pH in rod photoreceptors based on studies in the excised mouse eye. These data appear consistent with recent data from Linsenmeier et al., who reported a significant acidosis in rod nuclei of rats diabetic for 1 month.

Not all investigators detected diabetes-induced alterations in a-wave. Responses of the a-wave were not significantly reduced by experimental diabetes of 3 months’ duration in male Sprague–Dawley rats or in male Long Evans rats. Likewise, the a-wave at the brightest luminous energy was unaffected by 12 weeks of diabetes in male SD rats, and amplitudes in such rats were significantly reduced only at 10 and 15 weeks of diabetes, but not at 2, 6, 20 or 25 weeks. No significant differences were observed in the sensitivity or amplitude of the a- or b-wave components of the ERG between female diabetic and control rats, but this might be due to the less severe diabetes that developed in the female rats (compared with male rats). A-wave amplitudes were not subnormal in C57Bl/6j mice tested at 22 weeks of diabetes. Thus, there seems to be no consensus on a-wave involvement in diabetic rodents at present.

**Patient Studies**

Clinical data provide evidence for rod and cone receptor defects in patients with diabetic retinopathy. Studies of diabetic patients by Holopigian et al. detected both rod-isolated and cone-isolated changes in a-wave that were primarily in the log S (sensitivity) parameter. Based on the mathematical model that they used to interpret the results, changes in the sensitivity parameter show that the receptors could have transduction abnormalities, although this was not confirmed experimentally. Losses of selective S-cone pathway sensitivity have been identified in diabetic patients. Alterations in rod and cone signaling have been detected in newly onset type 2 diabetes patients with normal fundus appearance. Patients with diabetes show retinal regions with early neuroretinal dysfunction that are predictive of the eventual locations that develop microvascular histopathology, but the contributions of photoreceptors to the multifocal ERG signal remains unclear. More light than usual is required to bleach an equivalent amount of photopigment in some diabetic patients, suggesting that the photopigment is not bleaching normally.

Elevations of glucose in diabetes seems itself to play an important role in the development of photoreceptor defects, as rod adaptation (but not cone adaptation) was enhanced by transiently increased blood glucose.

Photoreceptors and the RPE have multiple close interactions related to many important functions of the outer retina including recovery of photoreceptor sensitivity after a bleach.

Rod sensitivity was subnormal in patients with early diabetic retinopathy, and mean thresholds were abnormal at all eccentricities and in all four quadrants of retina. Abnormalities in dark adaptation and absolute threshold have also been reported in human subjects with diabetes. Electrooculogram amplitudes (thought to reflect ionic fluxes across the RPE) have been shown to fluctuate with elevation of blood glucose in healthy human subjects. In addition, the RPE response was found to be abnormal in diabetic mice with prolonged diabetes.

**Summarizing**

The electrophysiology data suggest that photoreceptors and/or RPE show variable impairments in diabetes. Whether or not these changes can serve as biomarkers for impending development of aspects of diabetic retinopathy is still unclear.

**DIABETES-INDUCED ALTERATIONS IN ION FLUX IN PHOTORECEPTORS**

As discussed here, electrophysiological and biochemical (adenosine 5’-triphosphatase) evidence suggests that diabetes alters photoreceptor ion homeostasis. However, these data focus on the entire retina and movement of monovalent ions, such as sodium. L-type calcium channels (LTCCs) are the major entry route of calcium into photoreceptors, and play a major role in photoreceptor function. For example, sustained influx of calcium into photoreceptors through open LTCCs is essential for the regulated release of the neurotransmitter glutamate (among many other critical functions). Photoreceptors also have a relatively weak calcium buffering capacity and contain at least 75% of total retinal mitochondria. Together, these calcium handling features greatly facilitate rapid signaling in photoreceptors, but also substantially promote susceptibility to increased reactive oxygen species production relative to other cell types in the retina.

Manganese-enhanced magnetic resonance imaging (MEMRI) is a new method that measures aspects of photoreceptor function not evaluated using electrophysiology, such as the influx of divalent ions like calcium into central retinal photoreceptors of
awake and freely moving animals. Manganese ($\text{Mn}^{2+}$, a strong magnetic resonance imaging contrast agent) is a calcium ion surrogate that is taken into excitable cells through LTCCs$^{20,107–116}$. After systemic injection of a non-toxic dose of $\text{MnCl}_2$, manganese uptake into photoreceptors and other retinal cells can be non-invasively and quantitatively measured using MEMRI. This technique is being used to investigate diabetes-induced changes in calcium channels in photoreceptors.

Early in the course of diabetes, MEMRI studies have shown that photoreceptor uptake of manganese is significantly reduced in dark-adapted mice and rats, suggesting that diabetes causes a paradoxical closure of LTCCs in the dark (as if the photoreceptors were light adapted)$^{20,111,117}$. Because these ion channels are essential for regulated release of neurotransmitter at the photoreceptor synapse, paradoxically closed photoreceptor LTCCs in the dark (together with the normally closed LTCCs in the light) likely have significant consequences on function of photoreceptors and the whole retina.

Several possibilities exist as to how diabetes might inhibit opening of ion channels in dark:

1. The diabetes-induced defect in photoreceptor ion channel regulation is apparently secondary to oxidative stress. Preventing oxidative stress in diabetic mice or rats, using either genetic overexpression of Cu,Zn superoxide dismutase or systemic administration of $\alpha$-lipoic acid, respectively, corrected the diabetes-induced reduction in ion flux into photoreceptors in the dark$^{20,111}$. Interestingly, both of these treatments have also been shown to inhibit the diabetes-induced degeneration of retinal capillaries$^{20,118}$. Ongoing experiments are testing the possibility that closed LTCCs might also contribute to the oxidative stress.

2. Diabetes alters electron chain efficiency, resulting in excessive generation of superoxide. Thus, the reduction in mitochondrial function might reduce the energy available for keeping the cyclic guanosine monophosphate channels open in the dark$^{111,119}$. Available data does not provide support for this hypothesis, however, as retinal adenosine triphosphate levels (measured during daylight hours) have not been found to be abnormal in diabetes$^{69,120}$. Furthermore, 11-cis-retinal supplementation partly restored manganese uptake, suggesting that enough energy was available to maintain open channels, at least to some degree$^{117}$.

3. Activated protein kinase C suppresses LTCC activity (at least in cardiac tissue)$^{121}$. Protein kinase C activity is known to be increased in the retina in diabetes patients, and has been implicated in diabetes-induced reductions in visual function$^{122,123}$.

Summarizing

Accumulating evidence shows that diabetes alters ion flux in photoreceptors, and that these abnormalities are linked to oxidative stress. The contribution of photoreceptor calcium channels and ion flux to the oxidative stress and to the development of the lesions clinically accepted as diabetic retinopathy is vastly unexplored, and is actively being investigated.

CONCLUSION

Photoreceptors are unique to the retina, and thus might account for the unique susceptibility of the retina to damage in diabetes. Although photoreceptors account for most of the mass and metabolic activity of the retina, and they clearly influence the function of all other cell types in the retina, their role in DR has not been clearly delineated. The present review provides a rationale for further study of a role of photoreceptors in the pathogenesis of diabetic retinopathy. The contributions of surrounding cells, such as RPE and choriocapillaris, to the photoreceptor alterations in diabetes remain to be investigated.

ACKNOWLEDGMENTS

This work was supported by grants from the National Eye Institute (R01EY00300 and R01EY022938 to TSK, and R21 EY021619 to BAB), the Medical Research Service of the Department of Veteran Affairs (to TSK), NIH Animal Models of Diabetic Complications Consortium and Mouse Metabolic Phenotyping Centers Pilot and Feasibility Programs (to BAB), and an unrestricted grant from Research to Prevent Blindness (Kresge Eye Institute). The authors declare no conflict of interest.

REFERENCES

1. Kern TS, Engerman RL. Capillary lesions develop in retina rather than cerebral cortex in diabetes and experimental galactosemia. Arch Ophthalmol 1996; 114: 306–310.
2. Carter-Dawson LD, LaVail MM. Rods and cones in the mouse retina. I. Structural analysis using light and electron microscopy. J Comp Neurol 1979; 188: 245–262.
3. Nikonov SS, Daniele LL, Zhu X, et al. Photoreceptors of Nrl−/− mice coexpress functional S- and M-cone opsins having distinct inactivation mechanisms. J Gen Physiol 2005; 125: 287–304.
4. Mears AJ, Kondo M, Swain PK, et al. Nrl is required for rod photoreceptor development. Nat Genet 2001; 29: 447–452.
5. Arden GB. The absence of diabetic retinopathy in patients with retinitis pigmentosa: implications for pathophysiology and possible treatment. Br J Ophthalmol 2001; 85: 366–370.
6. de Gooyer TE, Stevenson KA, Humphries P, et al. Retinopathy is reduced during experimental diabetes in a mouse model of outer retinal degeneration. Invest Ophthalmol Vis Sci 2006; 47: 5561–5568.
7. Arden GB, Wolf JE, Tsang Y. Does dark adaptation exacerbate diabetic retinopathy? Evidence and a linking hypothesis. Vision Res 1998; 38: 1723–1729.
8. Arden GB, Sidman RL, Arap W, et al. Spare the rod and spoil the eye. Br J Ophthalmol 2005; 89: 764–769.
9. Linsenmeier RA. Effects of light and darkness on oxygen distribution and consumption in the cat retina. J Gen Physiol 1986; 88: 521–542.
10. Haugh LM, Linsenmeier RA, Goldstick TK. Mathematical models of the spatial distribution of retinal oxygen tension and consumption, including changes upon illumination. *Ann Biomed Eng* 1990; 18: 19–36.

11. Yu DY, Cringle SJ. Oxygen distribution and consumption within the retina in vascularised and avascular retinas and in animal models of retinal disease. *Prog Retin Eye Res* 2001; 20: 175–208.

12. Linsenmeier RA, Braun RD. Oxygen distribution and consumption in the cat retina during normoxia and hypoxemia. *J Gen Physiol* 1992; 99: 177–197.

13. Ahmed J, Braun RD, Dunn R Jr, et al. Oxygen distribution in the macaque retina. *Invest Ophthalmol Vis Sci* 1993; 34: 516–521.

14. Wang S, Birol G, Budzynski E, et al. Metabolic responses to light in monkey photoreceptors. *Curr Eye Res* 2010; 35: 510–518.

15. Arden GB, Sivaprasad S. Hypoxia and oxidative stress in the causation of diabetic retinopathy. *Curr Diabetes Rev* 2011; 7: 291–304.

16. Arden GB, Sivaprasad S. The pathogenesis of early retinal changes of diabetic retinopathy. *Doc Ophthalmol* 2012; 124: 15–26.

17. Du Y, Veenstra A, Palczewski K, et al. Photoreceptor cells are major contributors to diabetes-induced oxidative stress and local inflammation in the retina. *Proc Natl Acad Sci USA* 2013; 110: 16586–16591.

18. Kowluru RA, Tang J, Kern TS. Abnormalities of retinal metabolism in diabetes and experimental galactosemia. VII. Effect of long-term administration of antioxidants on the development of retinopathy. *Diabetes* 2001; 50: 1938–1942.

19. Kanwar M, Chan PS, Kern TS, et al. Oxidative damage in the retinal mitochondria of diabetic mice: possible protection by superoxide dismutase. *Invest Ophthalmol Vis Sci* 2007; 48: 3805–3811.

20. Berkowitz BA, Gradianu M, Bissig D, et al. Retinal ion regulation in a mouse model of diabetic retinopathy: natural history and the effect of Cu/Zn superoxide dismutase overexpression. *Invest Ophthalmol Vis Sci* 2009; 50: 2351–2358.

21. Zheng L, Kern T. Role of nitric oxide, superoxide, peroxynitrite and poly(ADP-ribose) polymerase in diabetic retinopathy. *Front Biosci* 2009; 14: 3974–3987.

22. Lee SG, Lee CG, Yun IH, et al. Effect of lipoic acid on expression of angiogenic factors in diabetic rat retina. *Clin Experiment Ophthalmol* 2012; 40: e47–e57.

23. Hoang QV, Linsenmeier RA, Chung CK, et al. Photoreceptor inner segments in monkey and human retina: mitochondrial density, optics, and regional variation. *Vis Neurosci* 2002; 19: 395–407.

24. Kowluru RA, Kowluru A, Veluthakal R, et al. TIAM1-RAC1 signalling axis-mediated activation of NADPH oxidase-2 initiates mitochondrial damage in the development of diabetic retinopathy. *Diabetologia* 2014; 57: 1047–1056.

25. Santos JM, Tewari S, Lin JY, et al. Interrelationship between activation of matrix metalloproteinases and mitochondrial dysfunction in the development of diabetic retinopathy. *Biochem Biophys Res Commun* 2013; 438: 760–764.

26. Kumar B, Gupta SK, Srinivasan BP, et al. Hesperetin rescues retinal oxidative stress, neuroinflammation and apoptosis in diabetic rats. *Microvasc Res* 2013; 87: 65–74.

27. Park SH, Park JW, Park SJ, et al. Apoptotic death of photoreceptors in the streptozotocin-induced diabetic rat retina. *Diabetologia* 2003; 46: 1260–1268.

28. Enzsolı A, Szabo A, Kantor O, et al. Pathologic alterations of the outer retina in streptozotocin-induced diabetes. *Invest Ophthalmol Vis Sci* 2014; 55: 3686–3699.

29. Zhang Y, Wang Q, Zhang J, et al. Protection of exendin-4 analogue in early experimental diabetic retinopathy. *Graefes Arch Clin Exp Ophthalmol* 2009; 247: 699–706.

30. Hombrebueno JR, Chen M, Penalva RG, et al. Loss of synaptic connectivity, particularly in second order neurons is a key feature of diabetic retinal neuropathy in the Ins2Akita mouse. *PLoS ONE* 2014; 9: e97970.

31. Tang L, Zhang Y, Jiang Y, et al. Dietary wolfberry ameliorates retinal structure abnormalities in db/db mice at the early stage of diabetes. *Exp Biol Med* 2011; 236: 1051–1063.

32. Bogdanov P, Corraliza L, Villena JA, et al. The db/db mouse: a useful model for the study of diabetic retinal neurodegeneration. *PLoS ONE* 2014; 9: e97302.

33. Lu ZY, Bhatta IA, Amemiyi T. Retinal changes in Otsuka long-evans Tokushima Fatty rats (spontaneously diabetic rat)–possibility of a new experimental model for diabetic retinopathy. *Jpn J Ophthalmol* 2003; 47: 28–35.

34. Batenburg WW, Verma A, Wang Y, et al. Combined renin inhibition/(Pro)Renin receptor blockade in diabetic retinopathy- a study in transgenic (mREN2)27 rats. *PLoS ONE* 2014; 9: e100954.

35. Zarebska A, Czerny K, Bakiera K, et al. Histological changes in the retina in experimental alloxan-induced diabetes in rabbits. *Ann Univ Mariae Curie Sklodowska Med* 2001; 56: 81–84.

36. Zarebska A, Lancut M, Bakiera K, et al. Ultrastructural changes in the receptor parts of retinal rods in experimental alloxan-induced diabetes in rabbits. *Ann Univ Mariae Curie Sklodowska Med* 2001; 56: 77–80.

37. Alvarez Y, Chen K, Reynolds AL, et al. Predominant cone photoreceptor dysfunction in a hyperglycaemic model of non-proliferative diabetic retinopathy. *Dis Model Mech* 2010; 3: 236–245.

38. Barber AJ, Lieth E, Khin SA, et al. Neural apoptosis in the retina during experimental and human diabetes. Early onset and effect of insulin. *J Clin Invest* 1998; 102: 783–791.

39. Kern TS, Kowluru R, Engerman RL. Dog and rat models of diabetic retinopathy. In: Shafrir E (ed). Lessons from Animal Diabetes. Smith-Gordon, London, 1996; 395–408.
40. Joussen AM, Poulaki V, Le ML, et al. A central role for inflammation in the pathogenesis of diabetic retinopathy. *Faseb J* 2004; 18: 1450–1452.

41. Barber AJ, Antonetti DA, Kern TS, et al. The Ins2Akita mouse as a model of early retinal complications in diabetes. *Invest Ophthalmol Vis Sci* 2005; 46: 2210–2218.

42. Kern TS. In vivo models of diabetic retinopathy. In: Duh EJ (ed.). Diabetic Retinopathy. Humana Press, Totowa, New Jersey, 2008; 137–156.

43. Frank RN. The Optic UK Lecture: bench-to-bedside adventures of a diabetes researcher: results past, results present. *Eye (Lond)* 2011; 25: 331–341.

44. Cho NC, Poulsen GL, Ver Hoeve JN, et al. Selective loss of S-cones in diabetic retinopathy. *Arch Ophthalmol* 2000; 118: 1393–1400.

45. Uji A, Murakami T, Unoki N, et al. Parallelism as a novel marker for structural integrity of retinal layers in optical coherence tomographic images in eyes with epiretinal membrane. *Am J Ophthalmol* 2014; 157: 227–236.

46. Murakami T, Yoshimura N. Structural changes in individual retinal layers in diabetic macular edema. *J Diabetes Res* 2013; 2013: 920713.

47. Shin HJ, Lee SH, Chung H, et al. Association between photoreceptor integrity and visual outcome in diabetic macular edema. *Graefes Arch Clin Exp Ophthalmol* 2012; 250: 61–70.

48. Foroozian F, Stetson PF, Meyer SA, et al. Relationship between photoreceptor outer segment length and visual acuity in diabetic macular edema. *Retina* 2010; 30: 63–70.

49. Ito S, Miyamoto N, Ishida K, et al. Association between external limiting membrane status and visual acuity in diabetic macular edema. *Br J Ophthalmol* 2013; 97: 228–232.

50. Srinivasan VJ, Adler DC, Chen Y, et al. Ultrahigh-speed optical coherence tomography for three-dimensional and en face imaging of the retina and optic nerve head. *Invest Ophthalmol Vis Sci* 2008; 49: 5103–5110.

51. Ostrow SE, Frede SM, Wagner EF, et al. Decreased rhodopsin regeneration in diabetic mouse eyes. *Invest Ophthalmol Vis Sci* 1994; 35: 3905–3909.

52. Kowluru A, Kowluru RA, Yamazaki A. Functional alterations of G-proteins in diabetic rat retina: a possible explanation for the early visual abnormalities in diabetes mellitus. *Diabetologia* 1992; 35: 624–631.

53. Kim YH, Kim YS, Noh HS, et al. Changes in rhodopsin kinase and transducin in the rat retina in early-stage diabetes. *Exp Eye Res* 2005; 80: 753–760.

54. Kirwin SJ, Kanaly ST, Linke NA, et al. Strain-dependent increases in retinal inflammatory proteins and photoreceptor FGF-2 expression in streptozotocin-induced diabetic rats. *Invest Ophthalmol Vis Sci* 2009; 50: 5396–5404.

55. Kandpal RP, Rajasimha HK, Brooks MJ, et al. Transcriptome analysis using next generation sequencing reveals molecular signatures of diabetic retinopathy and efficacy of candidate drugs. *Mol Vis* 2012; 18: 1123–1146.

56. Phipps JA, Yee P, Fletcher EL, et al. Rod photoreceptor dysfunction in diabetes: activation, deactivation, and dark adaptation. *Invest Ophthalmol Vis Sci* 2006; 47: 3187–3194.

57. Stella SL Jr, Bryson EJ, Thoresen WB. Insulin inhibits voltage-dependent calcium influx into rod photoreceptors. *NeuroReport* 2001; 12: 947–951.

58. Rajala A, Dighe R, Agbagba MP, et al. Insulin receptor signaling in cones. *J Biol Chem* 2013; 288: 19503–19515.

59. Gupta VK, Rajala A, Rajala RV. Insulin receptor regulates photoreceptor CNG channel activity. *Am J Physiol Endocrinol Metab* 2012; 303: E1363–E1372.

60. Rajala A, Tanito M, Le YZ, et al. Loss of neuroprotective survival signal in mice lacking insulin receptor gene in rod photoreceptor cells. *J Biol Chem* 2008; 283: 19781–19792.

61. Yu X, Rajala RV, McGinnis JF, et al. Involvement of insulin/phosphoinositide 3-kinase/Akt signal pathway in 17 beta-estradiol-mediated neuroprotection. *J Biol Chem* 2004; 279: 13086–13094.

62. Rajala RV, McClellan ME, Chan MD, et al. Interaction of the retinal insulin receptor beta-subunit with the P85 subunit of phosphoinositide 3-kinase. *Biochemistry* 2004; 43: 5637–5650.

63. Rajala A, Gupta VK, Anderson RE, et al. Light activation of the insulin receptor regulates mitochondrial hexokinase. A possible mechanism of retinal neuroprotection. *Mitochondrion* 2013; 13: 566–576.

64. Rajala RV, Wiskur B, Tanito M, et al. Diabetes reduces autophosphorylation of retinal insulin receptor and increases protein-tyrosine phosphatase-1B activity. *Invest Ophthalmol Vis Sci* 2009; 50: 1033–1040.

65. Wetzel RK, Arystarkhova E, Svedaner KJ. Cellular and subcellular specification of Na, K-ATPase alpha and beta isoforms in the postnatal development of mouse retina. *J Neurosci* 1999; 19: 9878–9889.

66. Ottelez A, Garcia CA, Eichberg J, et al. Alterations in retinal Na+, K+-ATPase in diabetes: streptozotocin-induced and Zucker diabetic fatty rats. *Curr Eye Res* 1993; 12: 1111–1121.

67. Bensaoula T, Ottelez A. Decreased activity of retinal ATPases and Na+, K+-ATPase isozymes in the microvascular and neural compartments of the experimentally diabetic retina. *ARVO abstracts*. *Invest Ophthalmol Vis Sci* 1995; 36: S897.

68. Ottelez A, Bensaoula T. Captopril ameliorates the decreased Na+, K+-ATPase activity in the retina of streptozotocin-induced diabetic rats. *Invest Ophthalmol Vis Sci* 1996; 37: 1633–1641.

69. Kern TS, Kowluru R, Engerman RL. Abnormalities of retinal metabolism in diabetes or galactosemia. ATPases and glutathione. *Invest Ophthalmol Vis Sci* 1994; 35: 2962–2967.

70. Tuitoek PJ, Lakey JR, Rajotte RV, et al. Strain variation in vitamin A (retinol) status of streptozotocin-induced diabetic rats. *Int J Vitam Nutr Res* 1996; 66: 101–105.
71. Tuittoek PJ, Ziarí S, Tsin AT, et al. Streptozotocin-induced diabetes in rats is associated with impaired metabolic availability of vitamin A (retinol). Br J Nutr 1996; 75: 615–622.

72. Ames A 3rd, Li YY, Heher EC, et al. Energy metabolism of rabbit retina as related to function: high cost of Na+ transport. J Neurosci 1992; 12: 840–853.

73. Hood DC, Birch DG. A quantitative measure of the electrical activity of human rod photoreceptors using electroretinography. Vis Neurosci 1990; 5: 379–387.

74. Hood DC, Birch DG. Rod phototransduction in retinitis pigmentosa: estimation and interpretation of parameters derived from the rod a-wave. Invest Ophthalmol Vis Sci 1994; 35: 2948–2961.

75. Breton ME, Schueller AW, Lamb TD, et al. Analysis of ERG a-wave amplification and kinetics in terms of the G-protein cascade of phototransduction. Invest Ophthalmol Vis Sci 1994; 35: 295–309.

76. Phipps JA, Fletcher EL, Vingrys AJ. Paired-flash identification of rod and cone dysfunction in the diabetic rat. Invest Ophthalmol Vis Sci 2004; 45: 4592–4600.

77. Kusari J, Zhou S, Padillo E, et al. Effect of memantine on neuroretinal function and retinal vascular changes of streptozotocin-induced diabetic rats. Invest Ophthalmol Vis Sci 2007; 48: 5152–5159.

78. Ostroy SE. Altered rhodopsin regeneration in diabetic mice caused by acid conditions within the rod photoreceptors. Curr Eye Res 1998; 17: 979–985.

79. Dmitriev AV, Henderson D, Lau JC, et al. Retinal acidosis at an early stage of diabetes in the rat. Invest Ophthalmol Vis Sci 2014; 55: 1049.

80. Kohzaki K, Vingrys AJ, Bui BV. Early inner retinal dysfunction in streptozotocin-induced diabetic rats. Invest Ophthalmol Vis Sci 2008; 49: 3595–3604.

81. Aung MH, Kim MK, Olson DE, et al. Early visual deficits in streptozotocin-induced diabetic long evans rats. Invest Ophthalmol Vis Sci 2013; 54: 1370–1377.

82. Bui BV, Loeliger M, Thomas M, et al. Investigating structural and biochemical correlates of ganglion cell dysfunction in streptozotocin-induced diabetic rats. Exp Eye Res 2009; 88: 1076–1083.

83. Li Q, Zemel E, Miller B, et al. Early retinal damage in experimental diabetes: electoretinographical and morphological observations. Exp Eye Res 2002; 74: 615–625.

84. Ramsey DJ, Rippis H, Qian H. An electrophysiological study of retinal function in the diabetic female rat. Invest Ophthalmol Vis Sci 2006; 47: 5116–5124.

85. Samuels IS, Lee CA, Petrah JM, et al. Exclusion of Aldose Reductase as a mediator of ERG deficits in a mouse model of diabetic eye disease. Vis Neurosci 2012; 29: 267–274.

86. Holopigian K, Greenstein VC, Seiple W, et al. Evidence for photoreceptor changes in patients with diabetic retinopathy. Invest Ophthalmol Vis Sci 1997; 38: 2355–2365.

87. Greenstein V, Sarber B, Hood D, et al. Hue discrimination and S cone pathway sensitivity in early diabetic retinopathy. Invest Ophthalmol Vis Sci 1990; 31: 1008–1014.

88. Tyberg M, Lindblad U, Melander A, et al. Electrophysiological studies in newly onset type 2 diabetes without visible vascular retinopathy. Doc Ophthalmol 2011; 123: 193–198.

89. Harrison WW, Bease MA Jr, Ng JS, et al. Multifocal electrotetnograms predict onset of diabetic retinopathy in adult patients with diabetes. Invest Ophthalmol Vis Sci 2011; 52: 772–777.

90. Harrison WW, Bease MA Jr, Schneck ME, et al. Prediction, by retinal location, of the onset of diabetic edema in patients with nonproliferative diabetic retinopathy. Invest Ophthalmol Vis Sci 2011; 52: 6825–6831.

91. Elsner AE, Burns SA, Lobes LA Jr, et al. Cone photopigment bleaching abnormalities in diabetes. Invest Ophthalmol Vis Sci 1987; 28: 718–724.

92. Holfort SK, Jackson GR, Larsen M. Dark adaptation during transient hyperglycemia in type 2 diabetes. Exp Eye Res 2010; 91: 710–714.

93. Strauss O. The retinal pigment epithelium in visual function. Physiol Rev 2005; 85: 845–881.

94. Greenstein VC, Thomas SR, Blaustein H, et al. Effects of early diabetic retinopathy on rod system sensitivity. Optom Vis Sci 1993; 70: 18–23.

95. Henson DB, North RV. Dark adaptation in diabetes mellitus. Br J Ophthalmol 1979; 63: 539–541.

96. Amemiya T. Dark adaptation in diabetics. Eur J Neurosci 1997; 174: 322–326.

97. Schneck ME, Fortune B, Adams AJ. The fast oscillation of the electrooculogram reveals sensitivity of the human outer retina/retinal pigment epithelium to glucose level. Vision Res 2000; 40: 3447–3453.

98. Schmitz Y, Witkovsky P. Dependence of photoreceptor glutamate release on a dihydropyridine-sensitive calcium channel. Neuroscience 1997; 78: 1209–1216.

99. Mann M, Haq W, Zabel T, et al. Age-dependent changes in the regulation mechanisms for intracellular calcium ions in ganglion cells of the mouse retina. Eur J Neurosci 2005; 22: 2735–2743.

100. Nikonov S, Engheta N, Pugh EN Jr. Kinetics of recovery of the dark-adapted salamander rod photoresponse. J Gen Physiol 1998; 111: 7–37.

101. Chiquet C, Dkhissi-Benyahya O, Cooper HM. Calcium-binding protein distribution in the retina of strepsirhine and haplorhine primates. Brain Res Bull 2005; 68: 185–194.

102. Demontis GC, Longoni B, Marchiafava PL. Molecular steps involved in light-induced oxidative damage to retinal rods. Invest Ophthalmol Vis Sci 2002; 43: 2421–2427.

103. Yang JH, Basinger SF, Gross RL, et al. Blue light-induced generation of reactive oxygen species in photoreceptor ellipsoids requires mitochondrial electron transport. Invest Ophthalmol Vis Sci 2003; 44: 1312–1319.
104. Krizaj D, Copenhagen DR. Calcium regulation in photoreceptors. Front Biosci 2002; 7: d2023–d2044.
105. Morgans CW, El Far O, Bernston A, et al. Calcium extrusion from mammalian photoreceptor terminals. J Neurosci 1998; 18: 2467–2474.
106. Johnson JE Jr, Perkins GA, Giddabasappa A, et al. Spatiotemporal regulation of ATP and Ca2+ dynamics in vertebrate rod and cone ribbon synapses. Mol Vis 2007; 13: 887–919.
107. Drapeau P, Nachshen DA. Manganese fluxes and manganese-dependent neurotransmitter release in presynaptic nerve endings isolated from rat brain. J Physiol 1984; 348: 493–510.
108. Lin YJ, Koretsky AP. Manganese ion enhances T1-weighted MRI during brain activation: an approach to direct imaging of brain function. Magn Reson Med 1997; 38: 378–388.
109. Berkowitz BA, Roberts R, Goebel DJ, et al. Noninvasive and simultaneous imaging of layer-specific retinal functional adaptation by manganese-enhanced MRL. Invest Ophthal Vis Sci 2006; 47: 2668–2674.
110. Berkowitz BA, Roberts R, Luan H, et al. Manganese-enhanced MRL studies of alterations of intraretinal ion demand in models of ocular injury. Invest Ophthal Vis Sci 2007; 48: 3796–3804.
111. Berkowitz BA, Roberts R, Stemmler A, et al. Impaired ion demand in experimental diabetic retinopathy: correction by lipoic Acid. Invest Ophthal Vis Sci 2007; 48: 4753–4758.
112. Berkowitz BA, Roberts R, Oleske DA, et al. Quantitative mapping of ion channel regulation by visual cycle activity in rodent photoreceptors in vivo. Invest Ophthal Vis Sci 2009; 50: 1880–1885.
113. Ivanova E, Roberts R, Bissig D, et al. Retinal channelrhodopsin-2-mediated activity in vivo evaluated with manganese-enhanced magnetic resonance imaging. Mol Vis 2010; 16: 1059–1067.
114. Berkowitz BA, Roberts R, Bissig D. Light-dependant intraretinal ion regulation by melanopsin in young awake and free moving mice evaluated with manganese-enhanced MRL. Mol Vis 2010; 16: 1776–1780.
115. Bissig D, Goebel D, Berkowitz BA. Diminished vision in healthy aging is associated with increased retinal L-type voltage gated calcium channel ion influx. PLoS ONE 2013; 8: e56340.
116. Berkowitz BA, Grady EM, Roberts R. Confirming a prediction of the calcium hypothesis of photoreceptor aging in mice. Neurobiol Aging 2014; 35: 1883–1891.
117. Berkowitz BA, Bissig D, Patel P, et al. Acute systemic 11-cis-retinal intervention improves abnormal outer retinal ion channel closure in diabetic mice. Mol Vis 2012; 18: 372–376.
118. Kowluru RA, Odenbach S. Effect of long-term administration of alpha-lipoic acid on retinal capillary cell death and the development of retinopathy in diabetic rats. Diabetes 2004; 53: 3233–3238.
119. Braun RD, Gradianu M, Vistisen KS, et al. Manganese-enhanced MRI of human choroidal melanoma xenografts. Invest Ophthal Vis Sci 2007; 48: 963–967.
120. Diederen RM, Stormes CA, Berkowitz BA, et al. Reexamining the hyperglycemic pseudohypoxia hypothesis of diabetic oculopathy. Invest Ophthal Vis Sci 2006; 47: 2726–2731.
121. Kamp TJ, Hell JW. Regulation of cardiac L-type calcium channels by protein kinase A and protein kinase C. Circ Res 2000; 87: 1095–1102.
122. Aiello LP, Davis MD, Girach A, et al. Effect of ruboxistaurin on visual loss in patients with diabetic retinopathy. Ophthalmology 2006; 113: 2221–2230.
123. Davis MD, Sheetz MJ, Aiello LP, et al. Effect of ruboxistaurin on the visual acuity decline associated with long-standing diabetic macular edema. Invest Ophthal Vis Sci 2009; 50: 1–4.