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Frontiers in diabetic retinal disease☆

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ABSTRACT

Diabetic retinal disease (DRD) remains a leading cause of vision loss and blindness globally. Although treatments can be effective when given at vision-threatening stages of DRD, there is a lack of knowledge about the earliest mechanisms leading to the development of clinically evident DRD. Recent advances in retinal imaging methods for patients with diabetes allow a more precise and granular characterization of the different stages of DRD than is provided by the classic Diabetic Retinopathy Severity Scale based on fundus photographs. In addition, recent clinical studies have yielded more information on how to adjust blood glucose levels, lipid levels and blood pressure to minimize the risk of DRD. Given the incomplete success of current therapies, there is a critical need for better understanding of the mechanisms underlying DRD and novel treatment targets that address the entire neurovascular retina. Moreover, the causes for interindividual variability in the development of DRD in patients with similar glycemic history and other metabolic factors are not yet clarified either. Finally, greater focus on patients' experience with visual disabilities and treatment effects should be addressed in research in this field.

1. Problem statement

Diabetic retinal disease (DRD) is the most common complication of diabetes mellitus, and one of the leading causes of vision-loss and blindness globally. Over the next few decades it will increase in incidence worldwide with correspondingly greater socioeconomic costs.[1,2] Currently, approximately 103 million persons with diabetes have DRD, of which 19 million have clinically significant diabetic macular edema and 29 million have vision-threatening retinopathy.[3]

Current therapies with anti-vascular endothelial growth factor (anti-VEGF) and corticosteroid injections or laser photocoagulation address late-stage retinal vascular disease and do not target the factors initiating the disruption of normal visual function. Thus, a better understanding of the basic mechanisms and pathophysiology of DRD may provide new clinical practices to fully optimize visual outcomes for patients with diabetes.

The concepts included herein were formulated at the "Frontiers in Diabetes Complications" Conference held at the University of Michigan Caswell Diabetes Institute, May 11 and 12, 2022. The aim of this paper is to address how research should move forward to enable patients to maintain good vision by focusing on three interrelated areas of investigation.

2. What is the pathophysiology of DRD and its relation to vision impairment?

Many aspects of the pathophysiology underlying DRD onset and worsening remain unresolved. The historical perception of DRD as a microvascular disease has been challenged by numerous studies which have shown that the entire neurovascular unit (blood vessels, neurons, macroglia and microglia) is affected in the preclinical phases of the disease.[4–6] The cellular alterations have been examined mostly in rodent

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models, and the clinically evident mild microvascular signs only in patients.[7,8]

Autoregulation of the capillary bed is impaired early in the course of preclinical retinal dysfunction. Under physiologic conditions, autoregulation maintains blood flow to match retinal metabolism[9,10] and is a function of neurovascular coupling controlled by the retinal macroglia (Müller cells and astrocytes) and mediated by lipid small molecules and nitric oxide.[11,12] Thus, impaired autoregulation implies impaired neurovascular coupling, and it can be assessed indirectly as reduced arterial vasodilation in response to flickering light and reduced vasoconstriction in response to hyperoxic breathing.[13,14]

Alterations of the capillary walls by glycemic variability include basement membrane thickening, pericyte loss and eventually endothelial cell loss. The latter compose the inner blood-retinal-barrier together with the Müller cells (astrocytes surround only the innermost vascular plexuses). Thus, wall dysfunction may lead to leakiness or dilations of the capillaries resulting in early clinical signs of DRD (described on page 4).[15]

Retinal macroglia express vascular endothelial growth factor (VEGF) and may therefore play a role in neovascularization.[16,17] One of the most used biomarkers of glial activation in preclinical studies is glial fibrillary acidic protein,[18] a marker also demonstrated to be upregulated in tissues from human donor eyes with DRD.[19] Overall, specific populations of Müller cells have been proposed to play a particularly critical role in central visual function[20] since they are the only non-neuronal cells in the fovea which is free of astrocytes, microglia, and endothelial cells.[21,22]

Inflammation has also been suggested to contribute to DRD. Microglia are the resident immune cells of the retina expressing both pro- and anti-inflammatory molecules and being capable of phagocytosis in response to inflammation. Microglia are normally resident in the inner and outer plexiform layers[23] and the ganglion and nerve fiber layers, but when activated, they have been seen to migrate outwards to the photoreceptor layer[24] or inwards towards microaneurysms and hemorrhages.[25] Additionally, microglia have a function in neurogenesis, mostly via nerve growth factor and tumor necrosis factor, and may serve neuroprotective functions, while if activated uncontrollably may contribute to neurodegeneration.[26]

By extension, a major unresolved question is the nature of the metabolic dysfunction in DRD. Analysis of postmortem eyes has revealed a potential role for photoreceptor-secreted retinol-binding protein 3 as a protective factor in persons with long-standing type 1 diabetes,[27] and suggests a therapeutic potential. In addition, Fort and colleagues[28] identified defective phosphorylation of alphaA-crystallin as a key regulatory step in the loss of intrinsic neuroprotective pathways for retinal neurons. Fort et al.[29] also found that persons with DRD exhibit decreased retinal complex lipid biosynthesis and impaired fatty acid oxidation, and that similar patterns occur in the serum of persons with type 2 diabetes. Thus, studies of human tissues will provide information that is more directly relevant to clinical care than studies only in rodents.

The main diabetes-induced retinal microvascular alterations observed clinically are increased permeability, microaneurysm formation and non-perfusion, which contribute to the transudation of plasma into the neurosensory retina and eventually macular edema, and to retinal ischemia and eventually neovascularization.[15] Balaratnasingam and colleagues have summarized multiple details of human diabetes-induced retinal vascular features of DRD.[30]

Increased vessel permeability occurs early in the course of experimental retinal dysfunction[18,31] and in patients with diabetic macular edema.[32,33] The "hard" exudates seen in diabetic macular edema have been considered to result from exudation of lipoproteins through the vessel wall. However, Wolter[34] proposed that they may result from phagocytosis of neuronal debris, demanding further study of their origin.

Non-perfused (ischemic) areas are defined by lack of fluorescein dye perfusion. These coexist in the region of microaneurysms,[35,36] and retinal thinning colocalizes with capillary non-perfusion.[37,38] The capillary plexuses of the retina can be visualized on fluorescein angiography and optical coherence tomography angiography,[39] respectively, simultaneously or separately. When viewed by ophthalmoscopy or fundus photography, regions of non-perfusion may be indicated by adjacent white-appearing blood vessels but can also appear relatively normal. Over time, the surrounding retinal area can become generally devoid of hemorrhages, to which the term "featureless retina" has been applied.[40]

A central concept in the DRD field has been that the nonperfused areas in the retina lead to hypoxia; i.e., the pO_2 is less than the normal 25 mmHg. However, Einar Stefansson, who has long studied retinal oxygenation, stated, "There is no direct evidence confirming the presence of retinal hypoxia in proliferative retinopathies such as diabetic or branch retinal vein occlusion…The finding of capillary nonperfusion or retinal ischemia suggests that retinal hypoxia may be present. However, the tissue compensates for ischemia through increased blood flow in adjacent blood vessels as well as atrophy of the ischemic tissue to reduce its oxygen consumption, and this may partially or completely relieve the hypoxic state".[41] A new imaging technique, adaptive optics scanning laser ophthalmoscopy, showed that retinal blood flow was increased in eyes of diabetes patients without diabetic retinopathy, but decreased in eyes after the onset of diabetic retinopathy, compared to healthy controls.[42] This finding indicates that there may be a limit to for how long the retina can adjust its blood flow to avoid hypoxia. If diabetes does not initially cause overall retinal tissue hypoxia, then does the increase in VEGF expression result from another mechanism, such as metabolic stress? Or is it vice versa, that metabolic stresses within the retina predispose to capillary involution?

Regional nonperfusion is also considered to result in infarcts in the nerve fiber layer. This process is believed to be the cause of the loss of transparency of the ganglion cell axons seen on fundus photography, termed "cotton wool spots", due to impaired axonal transport. However, David McLeod[43] has cogently argued that cotton-wool spots are not necessarily areas of ischemia, but that their presence in inflammatory conditions and poorly controlled diabetes suggests they may result from impaired retinal cell metabolism. This could also explain why they are seen in the "early-worsening" phenomenon in which a minority of persons having a rapid improvement in diabetes control experiences a worsening of their retinopathy.[44] Could cotton wool spots reflect energy issues, e.g. in the mitochondria, of poor delivery or utilization of substrates of glucose homeostasis? Understanding the formation of cotton wool spots may resolve some basic mechanisms of DRD.

Indeed, it is unresolved whether retinal neurons or capillaries are affected first in diabetes and how they interplay in non-perfusion: What is the status of astrocytes, Müller cells and retinal neurons adjacent to non-perfused areas? Is nonperfusion the consequence of intravascular events; e.g. platelet or leucocyte thrombi, migration of Müller cells into vascular lumens,[49,50] or intraretinal events whereby loss of metabolic activity and trophic support from neurons and glia leads to secondary closure of capillaries because they have no stimulus to provide blood to inactive cells? Could semaphorins secreted by metabolically starved neurons diminish vascular viability,[51] or could downregulation of key gene expression in ganglion cells or astrocyte-derived trophic factors influence on retinal vessels? That is, does ischemia represent a cause or a consequence? Answering these questions may have important implications for understanding how diabetes impairs vision.

Hyperglycemia has been considered to be the underlying cause of the above retinal microvascular alterations, while the significance of hypoglycemia is unknown. Remarkably, mild microvascular lesions develop in about 7 % of persons who do not have overt diabetes[45] and even advanced DRD can occur in individuals with normal glucose tolerance.[46–48] These findings suggest that metabolic factors other than glucose may also contribute to the development of DRD.

As described, the role of blood vessels in DRD remains central from a clinical standpoint but therapies that have primarily targeted blood

vessels, aside from VEGF inhibition (i.e., advanced glycation end-product inhibitors, aldose reductase inhibitors, protein kinase C inhibitors, histamine receptor inhibitors, Tie2 activators, kallikrein inhibitors) have not demonstrated clinically significant utility. Primary neuroprotective therapies have not been tested yet but may provide future opportunities, particularly if combined with vasoprotective strategies. Future studies of the mechanisms by which diabetes disturbs the neurovascular unit may yield indices that could serve as physiologic endpoints for clinical studies with short-term durations by which to test potential therapies.[52]

Together, the above findings reveal clues about mechanisms by which diabetes impairs vision, and that some long-held assumptions about the pathophysiology of DRD require additional investigation. We submit that multimodal structure-function studies in patients and in post-mortem human eyes provide the best potential opportunity to resolve these issues.

3. How can visual function and quality of life best be determined?

Fear of vision loss is a common concern among persons with diabetes.[53] Standard clinical assessments of DRD severity include visual acuity and image-based tests—fundus photographs, optical coherence tomography, and fluorescein angiograms—while the impact on quality of life is seldom systematically determined. Whereas functional assessments are central to the evaluation of cardiac, neurologic and renal components of diabetes by guiding diagnostic testing and therapeutic decisions, the field of DRD research has to date not incorporated aspects of visual function other than central visual acuity into routine assessment of patients. This historical clinical convention is in contrast to the standard practice of including visual fields and other aspects of visual function for glaucoma, optic neuropathies and inherited retinal degenerations.[54]

Retinal nerve functions have been evaluated in diabetes patients with or without DRD via electrophysiology, visual fields, contrast sensitivity, microperimetry and color vision testing, revealing e.g. defects in cone and rod photoreceptor pathways, diminished amplitudes of retinal interneurons, and impaired contrast and peripheral vision.[54–57] The methods have, however, not been incorporated into clinical practice due to lack of standardization, time required, and lack of understanding how they can be used to assess clinical phenotypes and patient symptoms, provide prognostic information and guide therapy. In addition, there has not been sufficient long-term prospective data in clinical trials to result in their regulatory approval. A National Eye Institute/Food and Drug Administration Diabetic Retinopathy Clinical Trial Design and Endpoints Workshop in 2016 led to the first statement that validated functional endpoints could be acceptable.[58] Functional tests of visual fields and contrast sensitivity are now secondary endpoints in the Diabetic Retinopathy Clinical Research Network Study AF (NCT04661358) and the Hoffman-LaRoche-sponsored CANBERRA study of a cannabinoid receptor agonist (NCT04265261).

The latter studies have also utilized psychophysical tests of vision, but do they reflect the subjective difficulties experienced by patients? Chen et al.[59] evaluated persons who had previously undergone panretinal photocoagulation for proliferative diabetic retinopathy. The subjects had excellent visual acuity but marked impairment of macular and peripheral vision revealed by frequency doubling perimetry, Humphrey 60-4 visual field sensitivity and a low luminance questionnaire. The patients also had poor quality of life as revealed by the low luminance- and the National Eye Institute VFQ-25 questionnaires, both broadly used in ophthalmology. A recent DRD-specific instrument (RetCAT™)[60,61] incorporates several quality of life tests and correlates the test result with DRD and/or visual impairment severity, but has been tested in Asian populations only.

It is also useful to obtain insights directly from patients. Three persons with 20-plus years of type 1 diabetes who had undergone successful panretinal photocoagulation volunteered the following observations[1]:

- Driving at night is tough and I avoid it when possible.
- My peripheral vision does not cover objects near my feet.
- I cannot find my friends in dimly lit rooms.
- Going from light to dark - it takes me a long time for my eyes to adjust.
- I cannot take hikes as well without a walking stick due to depth perception issues.
- I avoid passing a vehicle on my affected side.
- I avoid brick sidewalks, or uneven sidewalks, especially at night.
- I can't see my pump readings on the Omnipod™ when it is too light because they accommodate for the lighting being too dark, but not too bright.
- I need to use a high wattage bulb in all of my lamps.
- I often can't see the edge of steps and need to feel the edge.

Thus, the time has come for a concerted effort to define which functional tests and patient-reported outcome metrics that are sensitive, predictive and useful in clinical trials as well as in clinical practice. Therefore, JDRF and the Mary Tyler Moore Vision Initiative™[2] held a workshop in October 2022 with key patient, industry, clinical and regulatory stakeholders to launch this effort. The measures of visual function and quality of life identified by this work will be incorporated into an updated grading scale for DRD severity, as proposed recently by two groups,[62,63] along with measures of basic mechanisms of disease and retinal neurodegeneration. Also, the scale will include advanced imaging technologies to better assess the periphery of the retina and the whole neurovascular unit.

4. How do we develop more effective therapeutics?

Results from Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications study (DCCT/EDIC) clearly showed that DRD and other complications of type 1 diabetes can be reduced with intensive metabolic control. However, there are other factors, many not well characterized, that contribute to the development and progression of DRD, and the degree of glucose control required for optimal glucose management benefit is difficult for many persons to achieve due to risks of hypoglycemia and the expenses of medications and devices. Thus, the goal is to determine means to benefit persons whose control is suboptimal as well as account for the other contributors to the development and progression of DRD. We submit that a multifaceted approach offers a potential way forward to eliminate visual loss and blindness from diabetes.

New understanding of DRD pathophysiology may arise from studies of human eyes using targeted and untargeted -omic analyses, similar to the path used to develop therapeutic targets for diabetic kidney disease.[64] Structure-function analyses of various grades of DRD severity (no detectable DRD, mild nonproliferative retinopathy, moderate-severe nonproliferative retinopathy, diabetic macular edema and proliferative diabetic retinopathy before and after standard treatment) can define the cellular defects that can be modified. Patients with diabetic macular edema and proliferative diabetic retinopathy can continue to have impaired visual acuity despite anti-VEGF therapy.[65–67] These conditions are prime candidates for restorative approaches that benefit from the

[1] Heather Stuckey-Peyrot, Kendra Durdock and Amy Heffelfinger provided comments to TWG with permission April 2022.

[2] The Mary Tyler Moore Vision Initiative™ is a joint effort of the Mary Tyler Moore and S. Robert Levine, MD Charitable Foundation, the Caswell Diabetes Institute, and the JDRF. Its mission is to accelerate development of methods to preserve, restore, and protect visual function in people with diabetes, including those with significant visual loss.

lessons derived from successful gene therapy for photoreceptor dystrophies.

Having the patient in focus is crucial for optimized treatment. How do the patients perceive a difference in vision after treatment? Is an improvement in visual acuity always related to a subjective improvement of vision? How can we better study how patients with diabetes live with visual disabilities? It is important to find ways to address psychosocial impacts of DRD on patients' lives. For this purpose, we suggest development of up-to-date, cloud-based, computer-assisted questionnaires for patient-reported outcomes. Also, we need to consider how we can best explain to newly-diagnosed diabetes patients the risk of late complications, taking into consideration the benefits of newer continuous blood glucose measurement devices, to combat fears of vision loss and blindness. We also believe that bringing patient voices into the research lab will help scientists think about disease within the context of patients' lives.

An organizational structure should be established to provide long-term progress with the key stakeholders, as exemplified by the Kidney Precision Medicine Project (https://www.kpmp.org/). Existing organizations such as the DRCR Retina Network (formerly the Diabetic Retinopathy Clinical Research Network) and the European Association for the Study of Diabetes Eye Complications (EASDEC.org) together with research and expert collaborations such as the Mary Tyler Moore Vision Initiative™ will be key partners.

5. Final recommendations based on the provided solutions

To summarize, more research in pathophysiology and early detection of DRD is needed to understand the gap between its subclinical stages and vision-threatening abnormalities, and to find new therapeutic targets for preventing DRD development. Keeping the patient voice in focus is important to have accurate evaluations of treatment effects. The latter should not be restricted to visual acuity or structural measures, but also parameters of subjective visual function. For this purpose, computer-assisted, patient-reported outcome questionnaires could be developed that can be widely applied to any context of care, region, or community. Additionally, not only better characterization of disease development, but also of its relation with metabolic and inflammatory factors, such as blood glucose levels, serum lipid levels, blood pressure, oxidative stress and mitochondrial function, is key to understanding DRD and developing more effective therapeutics.

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References

1. Lee R, Wong TY, Sabanayagam C. Epidemiology of diabetic retinopathy, diabetic macular edema and related vision loss. *Eye Vis*. 2015;2:17. https://doi.org/10.1186/s40662-015-0026-2.
2. Duh EJ, Sun JK, Stitt AW. Diabetic retinopathy: current understanding, mechanisms, and treatment strategies. *JCI Insight*. 2017;2. https://doi.org/10.1172/jci.insight.93751.
3. Teo ZL, Tham YC, Yu M, et al. Global prevalence of diabetic retinopathy and projection of burden through 2045: systematic review and meta-analysis. *Ophthalmology*. 2021;128:1580–1591. https://doi.org/10.1016/J.OPHTHA.2021.04.027.
4. Gardner TW, Antonetti DA, Barber AJ, LaNoue KF, Levison SW. Diabetic retinopathy: more than meets the eye. *Surv Ophthalmol*. 2002;47. https://doi.org/10.1016/S0039-6257(02)00387-9 (SUPPL. 2).
5. Sohn EH, Van Dijk HW, Jiao C, et al. Retinal neurodegeneration may precede microvascular changes characteristic of diabetic retinopathy in diabetes mellitus. *Proc Natl Acad Sci U S A*. 2016;113:E2655–E2664. https://doi.org/10.1073/PNAS.1522014113.
6. Simó R, Stitt AW, Gardner TW. Neurodegeneration in diabetic retinopathy: does it really matter? *Diabetologia*. 2018;61:1902–1912. https://doi.org/10.1007/S00125-018-4692-1.
7. Simó R, Stitt AW, Gardner TW. Neurodegeneration in diabetic retinopathy: does it really matter? *Diabetologia*. 2018;61:1902–1912. https://doi.org/10.1007/s00125-018-4692-1.
8. Antonetti DA, Barber AJ, Bronson SK, et al. Diabetic retinopathy: seeing beyond glucose-induced microvascular disease. *Diabetes*. 2006;55:2401–2411. https://doi.org/10.2337/db05-1635.
9. Bek T, Hajari J, Jeppesen P. Interaction between flicker-induced vasodilatation and pressure autoregulation in early retinopathy of type 2 diabetes. *Graefes Arch Clin Exp Ophthalmol*. 2008;246:763–769. https://doi.org/10.1007/s00417-008-0766-y.
10. Chou TH, Porciatti V. Adaptable retinal ganglion cell function: assessing autoregulation of inner retina pathways. *Neural Regen Res*. 2020;15:2237–2238. https://doi.org/10.4103/1673-5374.284984.
11. Newman EA. Functional hyperemia and mechanisms of neurovascular coupling in the retinal vasculature. *J Cereb Blood Flow Metab*. 2013;33:1685–1695. https://doi.org/10.1038/jcbfm.2013.145.
12. Nippert AR, Newman EA. Regulation of blood flow in diabetic retinopathy. *Vis Neurosci*. 2020. https://doi.org/10.1017/S0952523820000036. Published online.
13. Lott MEJ, Slocomb JE, Shivkumar V, et al. Comparison of retinal vasodilator and constrictor responses in type 2 diabetes. *Acta Ophthalmol*. 2012;90. https://doi.org/10.1111/j.1755-3768.2012.02445.x.
14. Pournaras CJ, Rungger-Brändle E, Riva CE, Hardarson SH, Stefansson E. Regulation of retinal blood flow in health and disease. *Prog Retin Eye Res*. 2008;27:284–330. https://doi.org/10.1016/J.PRETEYERES.2008.02.002.
15. Joussen AM, et al. *Retinal vascular disease*. Springer; 2007.
16. Biswas S, Bachay G, Chu J, Hunter DD, Brunken WJ. Laminin-dependent interaction between astrocytes and microglia: a role in retinal angiogenesis. *Am J Pathol*. 2017;187:2112–2127. https://doi.org/10.1016/J.AJPATH.2017.05.016.
17. Stone J, Itin A, Alon T, et al. Development of retinal vasculature is mediated by hypoxia-induced vascular endothelial growth factor (VEGF) expression by neuroglia. *J Neurosci*. 1995;15:4738–4747. https://doi.org/10.1523/JNEUROSCI.15-07-04738.1995.
18. Barber AJ, Antonetti DA, Gardner TW. Altered expression of retinal occludin and glial fibrillary acidic protein in experimental diabetes. *Investig Ophthalmol Vis Sci*. 2000;41:3561–3568.
19. Carrasco E, Hernández C, de Torres I, Farrés J, Simó R. Lowered cortistatin expression is an early event in the human diabetic retina and is associated with apoptosis and glial activation. *Mol Vis*. 2008;14:1496–1502.
20. Bringmann A, Unterlauft JD, Wiedemann R, Barth T, Rehak M, Wiedemann P. Two different populations of Müller cells stabilize the structure of the fovea: an optical coherence tomography study. *Int Ophthalmol*. 2020;40:2931–2948. https://doi.org/10.1007/S10792-020-01477-3.
21. Delaunay K, Khamsy L, Kowalczuk L, et al. Glial cells of the human fovea. *Mol Vis*. 2020;26:235–245.
22. Kugler EC, Greenwood J, MacDonald RB. The "Neuro-glial-vascular" unit: the role of glia in neurovascular unit formation and dysfunction. *Front Cell Dev Biol*. 2021;9, 732820. https://doi.org/10.3389/fcell.2021.732820.
23. Zhang Y, Zhao L, Wang X, et al. Repopulating retinal microglia restore endogenous organization and function under CX3CL1-CX3CR1 regulation. *Sci Adv*. 2018;4, eaap8492. https://doi.org/10.1126/sciadv.aap8492.
24. Omri S, Behar-Cohen F, de Kozak Y, et al. Microglia/macrophages migrate through retinal epithelium barrier by a transcellular route in diabetic retinopathy: role of PKCzeta in the goto kakizaki rat model. *Am J Pathol*. 2011;179:942–953. https://doi.org/10.1016/j.ajpath.2011.04.018.
25. Zeng HY, Green WR, Tso MOM. Microglial activation in human diabetic retinopathy. *Arch Ophthalmol (Chicago, Ill 1960)*. 2008;126:227–232. https://doi.org/10.1001/ARCHOPHTHALMOL.2007.65.
26. Altmann C, Schmidt MHH. The role of microglia in diabetic retinopathy: inflammation, microvasculature defects and neurodegeneration. *Int J Mol Sci*. 2018;19. https://doi.org/10.3390/IJMS19010110.
27. Yokomizo H, Maeda Y, Park K, et al. Retinol binding protein 3 is increased in the retina of patients with diabetes resistant to diabetic retinopathy. *Sci Transl Med*. 2019;11. https://doi.org/10.1126/scitranslmed.aau6627.
28. Ruebsam A, Dulle JE, Myers AM, et al. A specific phosphorylation regulates the protective role of αA-crystallin in diabetes. *JCI Insight*. 2018;3. https://doi.org/10.1172/JCI.INSIGHT.97919.
29. Fort PE, Rajendiran TM, Soni T. Diminished retinal complex lipid synthesis and impaired fatty acid β-oxidation associated with human diabetic retinopathy. *JCI Insight*. 2021;6. https://doi.org/10.1172/jci.insight.152109.

30. Balaratnasingam C, An D, Hein M, Yu P, Yu DY. Studies of the retinal microcirculation using human donor eyes and high-resolution clinical imaging: insights gained to guide future research in diabetic retinopathy. *Prog Retin Eye Res.* 2022 (in press).
31. Antonetti DA, Barber AJ, Khin S, Lieth E, Tarbell JM, Gardner TW. Vascular permeability in experimental diabetes is associated with reduced endothelial occludin content: vascular endothelial growth factor decreases occludin in retinal endothelial cells. Penn State Retina Research Group. *Diabetes*. 1998;47:1953–1959. https://doi.org/10.2337/DIABETES.47.12.1953.
32. Sander B, Larsen M, Engler C, Lund-Andersen H, Parving H-H. Early changes in diabetic retinopathy: capillary loss and blood-retina barrier permeability in relation to metabolic control. *Acta Ophthalmol.* 1994;72:553–559. https://doi.org/10.1111/j.1755-3768.1994.tb07179.x.
33. Sander B, Larsen M, Moldow B, Lund-Andersen H. Diabetic macular edema: passive and active transport of fluorescein through the blood-retina barrier. *Investig Ophthalmol Vis Sci.* 2001;42:433–438.
34. Wolter JR. Diabetic retinopathy. *Am J Ophthalmol.* 1961;51:1123–1141. https://doi.org/10.1016/0002-9394(61)91802-5.
35. Kohner EM, Henkind P. Correlation of fluorescein angiogram and retinal digest in diabetic retinopathy. *Am J Ophthalmol.* 1970;69:403–414. https://doi.org/10.1016/0002-9394(70)92273-7.
36. Bresnick GH, Davis MD, Myers FL, Venecia G. Clinicopathologic correlations in diabetic retinopathy: II. Clinical and histologic appearances of retinal capillary microaneurysms. *Arch Ophthalmol.* 1977;95:1215–1220. https://doi.org/10.1001/archopht.1977.04450070113010.
37. Bek T. Transretinal histopathological changes in capillary-free areas of diabetic retinopathy. *Acta Ophthalmol.* 1994;72:409–415. https://doi.org/10.1111/J.1755-3768.1994.TB02787.X.
38. Dodo Y, Murakami T, Uji A, Yoshitake S, Yoshimura N. Disorganized retinal lamellar structures in nonperfused areas of diabetic retinopathy. *Invest Ophthalmol Vis Sci.* 2015;56:2012–2020. https://doi.org/10.1167/iovs.14-15924.
39. Spaide RF, Fujimoto JG, Waheed NK, Sadda SR, Staurenghi G. Optical coherence tomography angiography. *Prog Retin Eye Res.* 2018;64:1–55. https://doi.org/10.1016/j.preteyeres.2017.11.003.
40. Shukla D, Dhawan A, Kalliath J. Featureless retina in diabetic retinopathy: clinical and fluorescein angiographic profile. *Indian J Ophthalmol.* 2021;69:3194–3198. https://doi.org/10.4103/IJO.IJO_1352_21.
41. Stefansson E. Oxygen and diabetic eye disease. *Graefes Arch Clin Exp Ophthalmol.* 1990;228:120–123. https://doi.org/10.1007/BF00935719.
42. Palochak CMA, Lee HE, Song J, et al. Retinal blood velocity and flow in early diabetes and diabetic retinopathy using adaptive optics scanning laser ophthalmoscopy. *J Clin Med.* 2019;8. https://doi.org/10.3390/JCM8081165.
43. McLeod D. Why cotton wool spots should not be regarded as retinal nerve fibre layer infarcts. *Br J Ophthalmol.* 2005;89:229–237. https://doi.org/10.1136/bjo.2004.058347.
44. Early worsening of diabetic retinopathy in the diabetes control and complications trial. *Arch Ophthalmol.* 1998;116:874–886. https://doi.org/10.1001/archopht.116.7.874 (in press).
45. White NH, Pan Q, Knowler WC, et al. Risk factors for the development of retinopathy in prediabetes and type 2 diabetes: the diabetes prevention program experience. *Diabetes Care.* 2022;45:2653–2661. https://doi.org/10.2337/DC22-0860.
46. Chan JY, Cole E, Hanna AK. Diabetic nephropathy and proliferative retinopathy with normal glucose tolerance. *Diabetes Care.* 1985;8:385–390. https://doi.org/10.2337/DIACARE.8.4.385.
47. Harrower ADB, Clarke BF. Diabetic retinopathy with normal glucose tolerance. *Br J Ophthalmol.* 1976;60:459–463. https://doi.org/10.1136/BJO.60.6.459.
48. Barnes AJ, Kohner EM, Johnston DG, Alberti KGMM. Severe retinopathy and mild carbohydrate intolerance: possible role of insulin deficiency and elevated circulating growth hormone. *Lancet (London, England).* 1985;1:1465–1468. https://doi.org/10.1016/S0140-6736(85)92250-0.
49. Bek T. Glial cell involvement in vascular occlusion of diabetic retinopathy. *Acta Ophthalmol Scand.* 1997;75:239–243. https://doi.org/10.1111/j.1600-0420.1997.tb00764.x.
50. Bek T. Inner retinal ischaemia: current understanding and needs for further investigations. *Acta Ophthalmol.* 2009;87:362–367. https://doi.org/10.1111/j.1755-3768.2008.01429.x.
51. Joyal JS, Sitaras N, Binet F, et al. Ischemic neurons prevent vascular regeneration of neural tissue by secreting semaphorin 3A. *Blood.* 2011;117:6024–6035. https://doi.org/10.1182/blood-2010-10-311589.
52. Bek T. Translational research in retinal vascular disease. An approach. *Acta Ophthalmol.* 2019;97:441–450. https://doi.org/10.1111/AOS.14045.
53. Peterson TJ. https://www.healthyplace.com/diabetes/complications/vision-loss-from-diabetes-and-dealing-with-related-anxiety; Jan. 12, 2022.
54. Bresnick GH. Diabetic retinopathy viewed as a neurosensory disorder. *Arch Ophthalmol (Chicago, Ill 1960).* 1986;104:989–990. https://doi.org/10.1001/ARCHOPHT.1986.01050190047037.
55. Simonsen SE. The value of the oscillatory potential in selecting juvenile diabetics at risk of developing proliferative retinopathy. *Acta Ophthalmol.* 1980;58:865–878.
56. Uccioli L, et al. Electrophysiological assessment of visual function in newly-diagnosed IDDM patients. 1995;38:804–808.
57. McAnany JJ, Park JC, Liu K, et al. Contrast sensitivity is associated with outer-retina thickness in early-stage diabetic retinopathy. *Acta Ophthalmol.* 2020;98:e224–e231. https://doi.org/10.1111/AOS.14241.
58. Nair P, Aiello LP, Gardner TW, Jampol LM, Ferris FL. Report from the NEI/FDA diabetic retinopathy clinical trial design and endpoints workshop. *Investig Ophthalmol Vis Sci.* 2016;57:5127–5142. https://doi.org/10.1167/iovs.16-20356.
59. Chen XD, Gardner TW. Patient-reported outcomes reveal impairments not explained by psychophysical testing in patients with regressed PDR. *Transl Vis Sci Technol.* 2019;8:11. https://doi.org/10.1167/tvst.8.4.11.
60. Fenwick EK, Barnard J, Gan A. Computerized adaptive tests: efficient and precise assessment of the patient-centered impact of diabetic retinopathy. *Transl Vis Sci Technol.* 2020;9. https://doi.org/10.1167/TVST.9.7.3.
61. Fenwick EK, Man REK, Gan ATL, et al. Validation of a new diabetic retinopathy knowledge and attitudes questionnaire in people with diabetic retinopathy and diabetic macular edema. *Transl Vis Sci Technol.* 2020;9:32. https://doi.org/10.1167/tvst.9.10.32.
62. Sun JK, Aiello LP, Abràmoff MD, et al. Updating the staging system for diabetic retinal disease. *Ophthalmology.* 2021;128:490–493. https://doi.org/10.1016/J.OPHTHA.2020.10.008.
63. Jampol LM, Tadayoni R, Ip M. Need for a new classification of diabetic retinopathy. *Retina.* 2021;41:459–460. https://doi.org/10.1097/IAE.0000000000003070.
64. Hansen J, Sealfon R, Menon R, et al. A reference tissue atlas for the human kidney. *Sci Adv.* 2022;8. https://doi.org/10.1126/SCIADV.ABN4965.
65. Ciulla TA, Bracha P, Pollack J, Williams DF. Real-world outcomes of anti-vascular endothelial growth factor therapy in diabetic macular edema in the United States. *Ophthalmol Retin.* 2018;2:1179–1187. https://doi.org/10.1016/j.oret.2018.06.004.
66. Ciulla TA, Hussain RM, Pollack JS, Williams DF. Visual acuity outcomes and anti-vascular endothelial growth factor therapy intensity in neovascular age-related macular degeneration patients: a real-world analysis of 49 485 eyes. *Ophthalmol Retin.* 2020;4:19–30. https://doi.org/10.1016/J.ORET.2019.05.017.
67. Gross JG, Glassman AR, Liu D, et al. Five-year outcomes of panretinal photocoagulation vs intravitreous ranibizumab for proliferative diabetic retinopathy: a randomized clinical trial. *JAMA Ophthalmol.* 2018;136:1138–1148. https://doi.org/10.1001/jamaophthalmol.2018.3255.