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It is time for a moonshot to find "Cures" for diabetic retinal disease

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ABSTRACT

Diabetic retinal disease (DRD), the most common complication of diabetes and a leading cause of blindness in working age individuals, is now understood to be a form of sensory neuropathy or neurovascular degeneration. Current treatments are focused on advanced vision-threatening disease and a single molecular target, vascular endothelial growth factor, has an approved therapy. We trace the evolution of understanding of DRD pathogenesis, identify new approaches to clinical assessment, trials infrastructure and design, and target identification to accelerate selection and evaluation of new therapeutics that will speed development of potentially curative interventions. Critically, the "Restoring Vision Moonshot" framework will address gaps in knowledge to be filled to achieve the goal of restoring sight and preventing vision loss in persons with diabetes.

1. Overview

Diabetic retinopathy [or, preferably, "Diabetic Retinal Disease (DRD)] (Abramoff et al., 2018) remains a major cause of vision loss in working age persons despite improvements in metabolic control and ocular treatments of diabetic macular edema (DME) and proliferative diabetic retinopathy (PDR). The term "DRD" refers to the effects of diabetes on the entire retina whereas "diabetic retinopathy" has been defined by the clinically evident vascular lesions without inclusion of neurosensory retinal changes. At present the cellular and clinical implications of DRD remain poorly understood, but we submit that this term should stimulate studies to resolve many open questions.The subject of diabetic retinopathy (DR) has been covered by multiple authorities (Antonetti et al., 2021; Duh et al., 2017; Simo and Hernandez, 2015), and here we build on the work of colleagues. We are motivated to enhance our understanding and find new approaches to DRD by seven-time Emmy Award winner, Oscar nominated actor, and prominent diabetes research advocate, Mary Tyler Moore's struggles with vision loss despite "successful" panretinal photocoagulation (PRP).

We present this paper not merely to summarize past work, but to understand the long trajectory to gaining a clinically applicable understanding of the pathophysiology of DRD that enables maintenance of good vision, its restoration when lost, and an organizational structure to deploy this information in trials and practice. Thus, in this review we:

1. Trace the evolution of the understanding of DRD, its clinical assessment, and its treatment;
2. Argue that the limited information provided by photographic imaging of vascular lesions has constrained progress in what is now understood to also be a form of sensory neuropathy. Whereas previous work has led to an emphasis on surgical treatment of advanced, vision-threatening disease, new efforts should focus on approaches that address underlying pathophysiology at earlier stages of disease progression;
3. Present a general theory of the current understanding of underlying metabolic and other pathophysiologic mechanisms at the root of DRD;
4. Identify new approaches to clinical assessment, trials infrastructure and design, and target identification to support more rapid selection

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and evaluation of new therapeutics and help speed development of potentially "curative" interventions,

5. Affirm that better understanding of the full spectrum of neurovascular pathophysiology in persons with DRD, as well as pathophysiologic correlates of visual function and patient autonomy are necessary to enable patients to maintain vision and achieve optimal health-related quality of life and function; and
6. Outline a "Restoring Vision Moonshot" initiative – inspired by U.S. President Kennedy's challenge in 1961 (at the height of the Cold War with the Soviet Union and following their successful launch of the first artificial Earth satellite, Sputnick 1) to land a man on the moon within a decade as defense against Soviet domination of space. This Moonshot can serve as an organizing framework to address critical knowledge gaps along the path to achieve goals of restoring reduced vision, protecting the retina, and preventing vision loss in persons with diabetes.

2. An evolving understanding of DRD

2.1. The history of our understanding of diabetic retinal disease, including as a neurovascular disorder

Diabetic retinopathy has been long considered to be a "microvascular disease" despite evidence that the neurosensory retina is also affected dating back over 100 years. Recent publications suggest the term "diabetic retinal disease" (DRD), to include vascular and neural retinal pathophysiology (parallel to diabetic kidney disease (DKD)), to better reflect diabetes' impact on the whole retina (Abramoff et al., 2018; Pan et al., 2021). Excellent histories of DR have been provided by Wolfensberger (Wolfensberger and Hamilton, 2001) and Lynch (Lynch and Abramoff, 2017). The current review seeks to provide greater insight into the times and the people who were involved in this early discovery work (prior to 2010). Many additional investigators have subsequently extended the early findings into important clinical and laboratory-based studies.

In 1875, Leber (1875) and Dickinson (1875) presented arguments for primary involvement of vascular and neural elements in the retina in DRD. Here we summarize the chronology of some key observations (Fig. 1). The first recognized publication to directly state that diabetes affects the neurosensory retina was provided by Dr. Orlando Orlandini in Venice, Italy in 1904 (Orlandini, 1904). He stated, "histological examination of the retina in de-pancreatised animals (rabbits and quail) showed two [types of alterations] "alterations of nervous tissue (especially) (above all) ganglion cells and nerve fibers, and of blood vessels". He also found vacuolization of retinal ganglion cells and degeneration of blood vessels and intraretinal hemorrhages. The next contribution on this topic was by Dr. Donato Lo Russo at the Clinica Oculistica di Roma in 1927. His study of post-mortem human eyes revealed "reduced thickness of the nerve fiber layer, reduction in the number of retinal ganglion cells and the formation of "cavities" in the inner retina". These two papers were published in a primeval era of diabetes investigation; *i.e.*, within five decades of the first description of DRD by von Jaeger (Fischer, 1989; von Jaeger, 1856) and within two decades of von Mering's and Minkowski's discovery (von Mering and Minkowski, 1889) that pancreatectomy in a dog induces diabetes.

The field was quiet for three decades after Lo Russo's paper, then several landmark papers appeared in the 1960's. Dr. Reimer Wolter (Fig. 2) provided the next key report in 1961 (Wolter, 1961). He trained as an ophthalmologist in Hamburg, Germany and later as a neuropathologist at the University of Michigan. In Hamburg a medical school classmate gained recognition as Professor Gerd Meyer-Schwickerath, who developed xenon arc retinal photocoagulation in the 1950's (Henderson, 1986; Meyer-Schwickerath, 1959). At Michigan Dr. Wolter combined clinical practice with pathology and laboratory studies, including use of the xenon photocoagulator. He reported findings from eight patients with diabetes who had died at the Wayne County General Hospital, an institution that cared for psychiatric and indigent persons. The patients had undergone clinical ophthalmoscopic examination during their lifetimes and showed typical signs of DRD. His pathologic examinations revealed hyalinized and sclerotic retinal blood vessels, as well as intervascular connections consistent with what were later termed, "intraretinal microvascular anomalies (IRMA)". He also found degenerated ganglion cell axons, neurites and dendrites, phagocytosis of neurons, and fat accumulation by microglia as noted with lipid staining dyes. Marked gliosis with hyperplastic astrocytes were noted around microaneurysms and areas of neovascularization. In contrast,

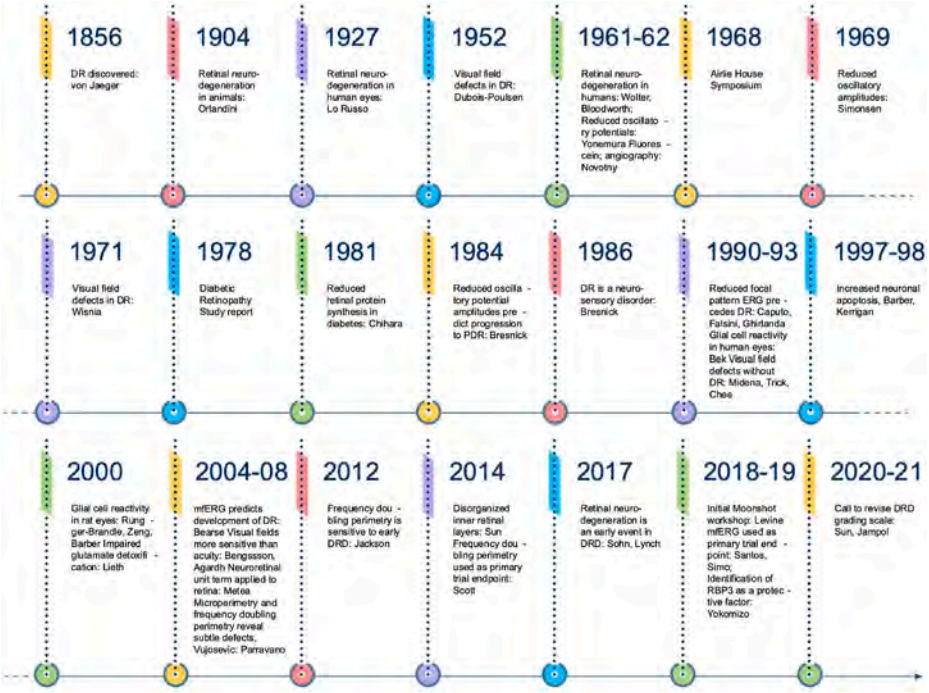

Fig. 1. Chronology of developments in retinal neurodegeneration in diabetes.

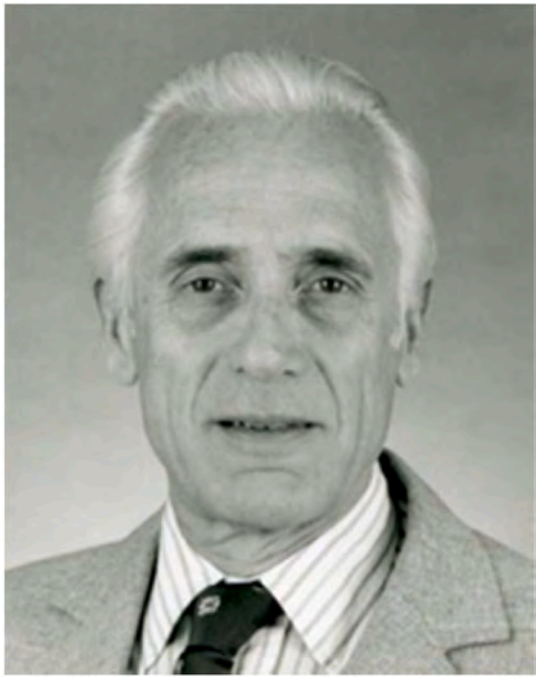

Fig. 2. Reimer Wolter, M.D. Image courtesy of Gale Oren, MILS, AHIP, John W. Henderson Library, Kellogg Eye Center, University of Michigan.

centrifugal nerve fibers that originate in the brain and supply retinal blood vessels were increased in number. Of note, he suggested that retinal "lipid" accumulation represents lipids and proteins from digested neurons rather than a true exudate or transudate that we currently consider as a mark of increased vascular permeability. He opined, "the primary retinal pathology in diabetes mellitus seems to be neuronal."

In 1962, a time when post-mortem examinations in hospitals were common, Dr. James Bloodworth, Jr. (Fig. 3), a pathologist at the Ohio State University, studied 300 eyes from 160 patients with diabetes and

Fig. 3. James Bloodworth, Jr. M.D. Image courtesy of the University of Wisconsin-Madison Archives, #511352.

157 eyes from non-diabetic persons. He observed features of microaneurysms and added that, "degeneration of nervous elements is always present in the surrounding retina." He stated, "The ganglion cells, most of the inner plexiform layer and nerve fiber layer are usually missing in the region of the cotton-wool 'exudate'. He concluded, "There is, as yet, no concrete evidence that the capillary changes initiate the conditions, and … our studies suggest that the degenerative changes in the nervous elements of the retina may precede the vascular changes." He further suggested that the presence of phagocytic cells in areas of ganglion cell and nerve fiber degeneration, independent of exudates, indicates that "gitter" cell nodules are sometimes concerned with the phagocytosis of neuron and nerve fiber debris (Bloodworth, 1962). Dr. Bloodworth subsequently moved to the University of Wisconsin and received the 1963 American Diabetes Association Eli Lilly award.

These two pathologic studies described the vascular and neural cell alterations in persons with established DRD. However, they did not provide direct correlation between the medical history, the *in vivo* ocular status of the subjects and post-mortem pathologic findings. They also did not define macular vs peripheral retina variations, and did not quantify the lesions. Further, the histochemical stains used at the time did not provide the cell-specific data that can now be inferred with mRNA and protein detection technologies. Nonetheless, these two seminal papers were cited in the major textbook of the 1960's, Sir Stewart Duke-Elder's magnum opus, *System of Ophthalmology,* in 1967 (Duke-Elder and Dobree, 1967).

Two other notable papers in the same decade emphasized the vascular nature of DR. First, in 1961, Drs. David Cogan, Daniel Toussaint and Toichiro Kuwabara at the Massachusetts Eye & Ear Infirmary described a trypsin digest method to remove the neurosensory retina from post-mortem retinas and they emphasized the loss of pericytes in eyes of persons with diabetes (Cogan et al., 1961). However, as noted previously (T.W. Gardner, 1995), their study did not include non-diabetic controls, examine areas without microaneurysms, nor present quantitative data or statistical analysis to confirm a "preferential" loss of pericytes. Nevertheless, this paper was cited frequently to support the concept of sorbitol accumulation mediated via aldose reductase and a primary role of pericyte loss (Kinoshita, 1986). Multiple studies have since employed the method to reveal microaneurysms, areas of capillary non-perfusion and neovascularization in relation to regions seen in trypsin digest preparations (De Venecia, Davis and Engerman, 1976; Kohner and Henkind, 1970). A second major development in 1961 was from Novotny and Alvis (Novotny and Alvis, 1961) who demonstrated the technique of fluorescein angiography of the human retina and revealed the extent of microvascular lesions. These two studies corresponded closely with the clinical phenotype and perpetuated the concept that DR is strictly a microvascular disease.

Beyond the information gathered and assumptions generated by pathological studies, early studies investigated retinal function in persons with diabetes. Dr. A. Dubois-Poulsen published evidence of depressed visual sensitivity using rod "scotometry" (measuring visual field defects) in 1952 (Dubois-Poulsen, 1952). In 1969, Dr. J.A. Roth at the Nuffield Laboratory of Ophthalmology at Oxford University, working under the tutelage of Professor Eva Kohner, found scotomas in all of the 31 diabetic patients examined, including those without clinically visible DR, but none in the non-diabetic control subjects (Roth, 1969). The scotomas were relative and detected only with small or dim test objects, but were generally larger than predicted by the fundus appearance. All patients with ophthalmoscopically visible DR had scotomata, as did half of those without visible DR. Two years later, Drs. Wisznia, Lieberman and Leopold (Wisznia et al., 1971) from the Mount Sinai School of Medicine Department of Ophthalmology in New York studied patients with Goldmann perimetry compared to ocular examination, fundus photography and fluorescein angiography. The patients with non-proliferative diabetic DR (NPDR) demonstrated small arcuate field defects. Both Roth and Wisznia et al. ascribed the visual field defects to microvascular lesions and did not mention involvement of the

neurosensory retina or provide details of the medical history of the subjects. Nevertheless, they documented that visual fields reveal functional retinal defects beyond what necould be discerned with standard clinical examination. Chee and Trick (Chee and Flanagan, 1993; Trick et al., 1990) used automated perimetry in the 1990s to confirm increased depth and size of scotomas related to severity of the microvascular features of DR.

Studies that emphasized involvement of the neurosensory retina in diabetes and its role in visual loss were pioneered by investigators on three continents. Daizo Yonemura, M.D. at the Kanazawa, Japan University School of Medicine Ophthalmology Department demonstrated reduced oscillatory potential amplitudes in 15 patients with mild DR and in seven of 13 patients with diabetes but no visible DR (Yonemura et al., 1962). He speculated that neuronal and non-neuronal cell defects may contribute to the electrophysiologic defects but did not publish subsequent papers on the topic.

Svend-Erik Simonsen, M.D (Fig. 4). conducted his work at the Gentofte Hospital and Steno Diabetes Hospital in Copenhagen, Denmark. His initial findings, "ERG in Juvenile Diabetics: a prognostic study," (S.E. Simonsen, 1969) were presented at the landmark 1968 Airlie House Symposium organized by Drs. Stuart Fine and Morton Goldberg in northern Virginia; a follow-up paper was published in 1974 (S. E. Simonsen, 1974). In 1980, he published a remarkable longitudinal study of 137 persons with type 1 diabetes who he examined after 6–8 years and 13–15 years of follow-up (S. E. Simonsen, 1980). His data showed that reduced oscillatory potential amplitudes predicted the onset of proliferative retinopathy and he concluded, "Alterations in the oscillatory potential in diabetics without retinopathy must reflect functional changes in the neurophysiologic properties of the retina before ophthalmoscopical changes are detectable." He published two additional papers that included assessment of dark adaptation (Frost-Larsen, Larsen and Simonsen, 1981a; 1981b). Nonetheless, measures of functional changes of the retina were not included in the Airlie House classification of DR that emerged from the Symposium (Anonymous, 1991; Goldberg and Fine, 1969).

The two preceding authors emphasized changes in the oscillatory potential amplitude whereas George Bresnick, M.D., M.P.A. at the University of Wisconsin (Fig. 5) also investigated their temporal aspects. The "implicit time" reflects the speed of cell-to-cell electrical signal transmission through the retina, arising in the plexiform and nuclear layers. Dr. Bresnick and colleagues showed that reduced oscillatory amplitudes predict progression to high-risk PDR (Bresnick et al., 1984). They also found that delays of the a-wave, b-wave and oscillatory potentials in 72 patients with diabetes but in none of 29 control subjects; the delays increased in correlation with severity of DR (Bresnick and Palta, 1987). They also proposed that the response to flicker stimulation (30 Hz) might be a useful clinical test of retinal function in diabetes. Indeed, Dr. Mitchell Brigell and colleagues (Brigell et al., 2020) recently found that oscillatory potential implicit times and pupillary responses (the RETeval score) improved prediction of NPDR progression better than risk estimates based on Early Treatment Diabetic Retinopathy Study (ETDRS) 7-field photography alone. Patients having scores $\geq$23.5 were 11 times more likely to require a future ocular intervention than patients having scores $<$23.5. In 1986, Dr. Bresnick succinctly proposed that DR should be considered a disorder of the neurosensory retina, not merely a microvascular disease (Bresnick, 1986). That is, diabetes imposes similar impact on retinal neural cells as on other peripheral sensory and autonomic nerves. These changes, however, are not detectable by clinical examination or fundus photographs, in contrast to the evident red "vascular" lesions, and so are not appreciated by clinicians. The implication of his thesis was that like glaucoma, DR disease status evaluation should include tests of visual function, such as visual fields or contrast sensitivity, not merely visual acuity or fundus appearance.

The role of the neurosensory retina in diabetes was also explored in detail by Benedetto Falsini, MD, an ophthalmologist (Fig. 6), and Giovanni Ghirlanda, MD, an endocrinologist, in Rome. They used steady-state focal pattern electroretinography in persons with type 1 diabetes and no or minimal clinically evident retinopathy (Caputo et al., 1990; Falsini et al., 1989; Ghirlanda et al., 1991). In 1989, they reported reduced amplitudes in persons with angiographically normal retinas and greater impairment in persons with mild retinopathy. Dr. Ghirlanda and colleagues stated in 1997, "The pre-retinopathy stage is characterized by the absence of lesions on ophthalmological examination, while clinically overt retinopathy, which may begin long after the onset of diabetes, represents a *late stage* (emphasis added) in a chronic underlying process" (Ghirlanda et al., 1997). This insightful lesson from an endocrinologist has been adopted very slowly by ophthalmologists. Dr. Falsini recalled to the authors:

Fig. 4. Svend-Erik Simonsen, M.D. circa 1975. Image courtesy of Lone Simonsen, Ph.D.

Fig. 5. George Bresnick, M.D., M.P.A. Image courtesy of Dr. Bresnick.

Fig. 6. Benedetto Falsini, M.D. Image courtesy of Dr. Falsini.

"In the late 80's I conducted studies on the pattern ERG in diabetes with the hypothesis that pattern ERG could detect early inner retinal dysfunction in pre-retinopathic diabetes. These studies were prompted by some clinical, preliminary observations suggesting that, unlike the flash ERG (derived from the outer retina), the pattern ERG was significantly reduced in diabetic patients. At that time no imaging techniques studying retinal microanatomy were available, so these functional changes could not be correlated with retinal layer abnormalities. However, several studies published by my group in collaboration with Professor Vittorio Porciatti, my mentor and friend, confirmed that in IDDM patients with normal appearing retina and short disease duration, there was already a dysfunction of inner retina."

Concurrent events in ophthalmology from the 1950's through the 1980's included the treatment of PDR with hypophysectomy, xenon arc photocoagulation, ruby and argon laser panretinal photocoagulation (PRP), the development of the Airlie House and ETDRS grading scales, and the use of focal macular photocoagulation for DME. The risks and benefits of intensive metabolic control were still vigorously debated and home glucose monitors first appeared in the early 1980's. Studies of the impact on the neurosensory retina were considered to be "research" without clinical application; few retina specialists were trained in retinal physiology or in the use and interpretation of functional tests (present authors included). Most publications still focused on microvascular pathology, and a cellular basis for a neurosensory retinopathy did not appear to match the clinical phenotype.

The story returns to Japan where Etsuo Chihara, M.D., (Fig. 7)., an ophthalmologist at the University of Kyoto and his colleagues determined the effects of experimental diabetes in rabbits on retinal protein synthesis beginning in 1981 (Chihara, 1981; Chihara et al., 1982; Tsukada and Chihara, 1986). They found that diabetes markedly reduces retinal protein synthesis, mostly within ganglion cells, including those proteins that accumulate within the optic nerve, within weeks of diabetes onset–well before the onset of clinically evident microvascular lesions. These were the first studies to define a defect in a specific anabolic process in the neurosensory retina. Protein synthesis is a central activity of all cells that results from the incorporation of amino acids into peptides, as mediated by growth factors, notably insulin. Accelerated protein degradation and reduced protein synthesis are cardinal features of diabetes and account for skeletal muscle wasting and

Fig. 7. Etsuo Chihara, M.D. Image courtesy of Dr. Chihara.

elevated plasma amino acid concentrations (Kimball and Jefferson, 1998).

Dr. Chihara recently provided the following recollections about his early studies:

"When I was a resident I was impressed that the hard exudates in diabetic patient were so beautifully lined radially, and I postulated an association between the axonal lining and accumulation of lipoproteins. In later days, I recognized that the orientation is associated with glia and not with axons. But this experience inspired me to start works on the relationship between diabetes and neural functions.

Before start of my study, reduced oscillation patterns of the electroretinogram, abnormal color sensation and impaired contrast sensitivity prior to diabetic vasculopathy had been reported, however pathogenesis was unknown. Also, before my start of investigation, I was interested in neurosecretion of oxytocin from the pituitary gland. This peptide was synthesized in the neural soma, transported in the axon and secreted from nerve terminal. Even though the basic mechanism of this system had not been elucidated at that time, I estimated an association with axoplasmic transport (axonal flow), a hot topic evolving at that time. I thought that the similar mechanism may be found in the eye, and I selected this topic as a subject for my thesis.

Fortunately, the eyeball is a closed system, and most of ^{3}H-leucine injected into the vitreous cavity stays in the eye, a constant amount of ^{3}H-leucine is incorporated into retinal proteins, and a constant amount is transported in the optic nerve as axonal transport, so the optic system was more appropriate for quantitative research of neural tissue than the sciatic nerve which was used by the basic science researchers.

When diabetes was induced in rabbits by alloxan, there was a significant association between blood sugar levels and a decrease in amount of fast axonally transported radioactive proteins and retinal protein synthesis (*J Neurochem* 1981: 27: 247–250). The decrease in slow axonal transport also was parallel to the decrease in protein synthesis in the retina (Brain Res 1982: 250: 363–366). So, I thought

that the decrease in the amount of axonal transport in optic nerve of diabetic rabbit is reflecting decreased protein synthesis in the retina.

We thought that depleted supply of cytoskeleton such as microtubules and neurofilament via axon transport in diabetes is appropriate to explain diabetic nerve abnormalities such as dying back type diabetic neuropathy (a phenomenon where the caliber of peripheral axon selectively thinned, in contrast caliber of axonal hillock or proximal axon does not).

After publishing our first reports my interest was directed to specific proteins or enzymes which may be selectively impaired in diabetic subjects, because there are more than 100 proteins such as neurotrophic factors, mitochondria, synaptic vesicles which are transported by axonal transport. Each should work to maintain nerve functions. We separated axonally transported proteins by SDS electrophoresis and studied if change in component of axonally transported materials occur in diabetic optic nerve.

We thought these kinds of changes in the component of transported proteins may lead to nerve dysfunction. I was interested what kind of proteins or enzymes are affected."

Dr. Chihara complemented his laboratory studies with clinical findings that nerve fiber layer density is reduced in persons with type 2 diabetes in proportion to the severity of retinopathy as defined by red-free fundus photographs in 1993 and by scanning laser polarimetry in 2008 (Chihara et al., 1993; Takahashi and Chihara, 2008). The specific cause of these defects is still not known but loss of ganglion cell protein synthesis could be a contributing factor. Retinal nerve fiber layer dysfunction and thinning parallel reduced visual evoked potential amplitude and velocity found in early stages of DRD (Papakostopoulos et al., 1985; Riva et al., 2005; Varkonyi et al., 2002). These observations are best appreciated in light of our understanding of glaucoma. The average nerve fiber layer thinning in persons with glaucoma is 20 mm and in those with diabetes, 12 mm (Takahashi and Chihara, 2008). Dr. Michael Abràmoff (Abramoff et al., 2018) has emphasized that the degree of visual field depression in persons with mild to moderate NPDR is equivalent to that in persons with glaucoma, yet the field loss due to diabetes is not currently treated.

The cellular consequences of impaired neuroretinal homeostasis and function received additional attention in the 1990's. Kathryn LaNoue, Ph.D (Fig. 8). at the Penn State College of Medicine, was a biochemist who had discovered new pathways of brain glutamate metabolism (Hutson et al., 1998). She, Erich Lieth, Ph.D. and Alistair Barber, Ph.D. applied these principles to the retina in diabetic rats, which revealed that elevated retinal levels of branched chain amino acids (leucine, isoleucine and valine) due to diabetes-induced systemic (mostly skeletal muscle) protein degradation increases retinal glutamate levels in these diabetic rats. Moreover, diabetes impairs detoxification of glutamate to glutamine via glutamine synthase (Gowda et al., 2011; Lieth et al., 2000; Lieth et al., 2001). The net elevated retinal glutamate levels thus contribute to glutamate excitotoxicity. These metabolic changes reflect impaired glial cell function, as described in human tissues by Dr. Toke Bek in 1997 (Bek, 1997) and separately by Drs. Rungger-Brandle, Zeng and Barber in 2000 (Barber et al., 2000; Rungger-Brandle et al., 2000; X. X. Zeng, Ng and Ling, 2000). Microglia, innate immune cells of the retina, are also activated by diabetes (H. Y. Zeng, Green and Tso, 2008; X. X. Zeng et al., 2000). Increased retinal vascular permeability and decreased occludin tight junction protein content occur contemporaneously with the neural cell changes (Antonetti et al., 1998; Barber et al., 2000). Thus, the entire neurovascular unit is altered by diabetes. Goncalves et al. (2021) elegantly showed the intimate relationship between vascular permeability and vision. They created mice with mutations in the occludin tight junction gene such that the protein was either constitutively phosphorylated on the amino acid (Ser490) that mediates VEGF-induced vascular permeability, or genetically blocked this phosphorylation event so the effect of VEGF is inhibited. They found that this phosphorylation not only regulated permeability as predicted, but it also determined whether or not visual acuity and contrast sensitivity were impaired. Of note, neither neural cell death nor electroretinographic responses typical of diabetes were impacted by the vascular cell manipulations. Thus, there are permeability-dependent consequences, and there are also permeability-independent effects in experimental DR. These studies demonstrate the interrelationships within the neurovascular unit and will hopefully spur additional interrogations of cell-cell interactions in the retina to elucidate the mechanisms of vision impairment.

Fig. 8. Kathryn F. LaNoue, Ph.D. Image courtesy of Penn State Department of Cellular & Molecular Physiology.

The consequences of altered glutamate metabolism, which also impairs synaptic transmission, may include neuronal cell death via apoptosis. Wolter (1961) and Bloodworth (1962) both noted condensed ganglion cell nuclei, the histologic hallmark of programmed cell death (apoptosis). Kerrigan et al. (Kerrigan et al., 1997) noted condensed nuclei in ganglion cells of human eyes with primary open angle glaucoma and in eyes without a history of glaucoma but with a history of diabetes. Other work investigating retinal cell death in diabetes did so in rat retinas subjected to trypsin digestion, but removal of the neurosensory retina precluded detection of neuronal death (Mizutani et al., 1996). Dr. Barber (Fig. 9), utilized his experience as a neuroscientist to investigate the question of retinal cell apoptosis in diabetes and provided this perspective:

"We observed occasional tantalizing terminal deoxynucleotidyl transferase-dUTP end labeling (TUNEL)-positive nuclei in histological cross-sections of retinas from diabetic rats, and based on their position and appearance they were likely to be retinal ganglion cells. As a neuroscientist who had most recently studied cell death and neuroprotection in cerebral ischemia, I was unfamiliar with the emphasis on vascular pathology in diabetic retinopathy, so I made the erroneous assumption that diabetes-induced loss of neurons was already well established. It was only after looking at the histology slides with other members of the Penn State Retina Research Group that I learned that we were seeing something important. Those early conversations determined a personal shift in interest that defined the

Fig. 9. Alistair Barber, Ph.D.; Image courtesy of Dr. Barber.

rest of my research career. Since that time I have always maintained that the best conversations take place at the microscope.

Observation of TUNEL-positive cells was not enough evidence to establish that diabetes increased neuronal cell death. We needed a valid method to quantify these transient and rare events. After examining many histological sections we finally developed a TUNEL protocol that would work on whole retinas, based on communication with Annett Bien at her Society for Neuroscience conference poster (Bien, A., M.F. Humphrey, C. Seidenbecher, B.A. Sabel, and M.R. Kreutz. 1996. *Soc. Neurosci.* 22:126.2. Abstract), who was using the technique to quantify retinal ganglion cell apoptosis in whole retinas after optic nerve crush (later published as (Bien et al., 1999). Other important additions to the protocol were positive controls. Luckily our lab technician, Sonny Khin, knew how to dissect and section the ventral prostate from castrated rats; a procedure be had learned when working for John Isaacs, who pioneered studies on mechanisms of apoptosis in the prostate. Without this positive control and that from retinas subjected to ischemia-reperfusion, we would not have been able to confirm that the TUNEL protocol was working. Later we confirmed the increase in apoptosis by immunohistochemistry for activated caspase-3, and then developed biochemical assays for caspase-3 enzyme activity and cytoplasmic nucleosome content, which eliminated the need to spend hours at the microscope counting TUNEL-positive nuclei."

Dr. Barber used the flat mount approach and found consistent, quantitative and rapid onset nuclear cleavage and condensation in diabetic rat and human retinas in contrast to minimal apoptotic cells in non-diabetic controls (Barber et al., 1998). The ability to detect TUNEL-positive cells in flat mounts versus cross sections is analogous to seeing a multitude of stars while viewing the night sky (a "flat mount") but there are many light years between stars so it would be difficult to see a star in a "slice" through space. Thus, cross section studies may lead to sampling error.

The evidence supporting a role for neurosensory retina involvement in human DRD continued to accumulate during the 1990's and 2000's. Dr. Edoardo Midena et al. (1990) (Fig. 10) reported defective nyctometry in persons with diabetes in the absence of visible DR (Midena et al., 1990); Dr. Boel Bengtsson observed in 2005 that white-on-white perimetry better correlates with retinopathy severity than does visual acuity (Bengtsson et al., 2005); Dr. Elisabet Agardh (Fig. 11) also demonstrated that visual fields are more sensitive than visual acuity in detecting retinal dysfunction in eyes with DME (Agardh et al., 2006); Dr. Stela Vujosevic, in 2006, correlated microperimetry and optical coherence tomographic findings in eyes with DME (Vujosevic et al., 2006); and Dr. Mariacristina Parravano (Parravano et al., 2008), in 2008,

Fig. 10. Edoardo Midena, M.D.; Image courtesy of Dr. Midena.

Fig. 11. Elisabet Agardh, M.D. Image courtesy of Dr. Agardh.

reported decreased Humphrey Matrix frequency doubling perimetry sensitivity in 73% of persons with type 1 diabetes. The extensive experience with visual field defects in persons with varying degrees of DRD suggests one or more types of visual field instruments may reveal important pathology in diabetes. The scotomas in persons with glaucoma tend to follow stereotypical patterns (arcuate and nasal step defects) related to nerve fiber layer loss. Diabetes may cause more randomly arranged areas of sensitivity loss due to dysfunction of photoreceptors, bipolar, amacrine and/or ganglion cells and their connections. Hopefully additional studies will define which cell types are responsible for the patterns of visual field sensitivity loss.

These collective data support Dr. Fiona Ewing's 1998 summary of electrophysiological and psychophysical abnormalities in diabetes in

which she stated, "… It becomes clear that the examination of visual function provides a case study in integrative neuroscience." (Ewing et al., 1998). That is, the entire visual system, not just the retina, is impaired by diabetes.

The evidence summarized above provided strong evidence for a neurodegenerative component to DR by the first decade of the 21st century. Additional important contributions continue to accumulate that have provided new diagnostic and therapeutic insights. Dr. Jennifer Sun and colleagues at the Joslin Diabetes Center, Harvard University showed that "disorganized inner retinal layers (DRIL)" as revealed by optical coherence tomography within the central macula are associated with worse visual acuity in eyes with DME than those with intact neuronal layers (J. K. Sun et al., 2014; J. K. Sun et al., 2015). Dr. Katherine Joltikov and colleagues (Joltikov et al., 2018) found that subjects with DRIL had reduced contrast sensitivity and automated perimetry performance than persons without DRIL. Drs. Elliott Sohn and Michael Abràmoff at the University of Iowa combined extensive human and rodent data to strongly argue in 2016 that neurodegeneration may precede vascular changes in diabetes (Lynch and Abramoff, 2017; Sohn et al., 2016), although the sensitivity to detect microvascular lesions in color fundus photographs is limited compared to fluorescein angiography and OCT angiography which reveals microvascular lesions in approximately one third of eyes with normal clinical examinations (de Carlo et al., 2015). Thus, it is possible that undetectable microvascular lesions exist in the human eyes despite normal fundus photographs.

Pinilla et al. (2019) provided important longitudinal data regarding retinal thickness measures in patients with stable type 1 diabetes but no visible retinopathy, nephropathy, or peripheral neuropathy. The mean foveal thickness of their patients remained stable during 8 years of follow up, but patients with diabetes exhibited significant reduction in total retinal thickness due to the thinning of the inner retinal layers (inner nuclear layer, ganglion cell layer, and retinal nerve fiber layer). The controls showed a significant diminution in only the retinal nerve fiber layer and in the ganglion cell layer areas. Collectively, these and other clinical studies (Bao et al., 2019) indicate there is little doubt that a neural component is part of DRD and "microvascular disease" is an incomplete term (Antonetti et al., 2006).

Diabetes affects many aspects of vision but the relationship of various defects remains uncertain. Drs. Midena and Bini at the University of Padova, Italy reviewed the contributions of OCT imaging, fundus autofluorescence and microperimetry in persons with DME (Midena and Bini, 2016). Each of these techniques contributes distinct information, such as hyperreflective intraretinal spots suggestive of microglial cell activation, the extent of intraretinal fluid and regions of reduced retinal sensitivity, respectively. Dr. Riccardo Sacconi and colleagues (Sacconi et al., 2019) in Milan found impaired retinal vascular reactivity using the Dynamic Vessel Analyzer (Imedos, Jena, Germany) and reduced vascular density (Swept-Source OCT-A PLEX® Elite 9000; Carl Zeiss Meditec, Inc., Dublin, CA, USA) but intact macular microperimetry sensitivity (via the MP-1 microperimeter (Nidek Technologies, Padova, Italy) in patients with type 1 diabetes without clinically evident retinopathy after a mean duration of disease of 12 years. They concluded that vascular changes are the earliest detectable features of retinopathy. This conclusion overlooks the fact that "vascular reactivity" reflects neurovascular unit coupling as discussed in Section 4. Lorenzi and colleagues (Tecilazich et al., 2016) found in a small cohort that defective myogenic responses to increased perfusion pressure was associated with an accelerated onset of DR. Joltikov et al. (2017) assessed multiple aspects of retinal structure and function in persons with no to mild NPDR. They found that the quick contrast sensitivity function (qCSF) method, standard automated perimetry, and frequency doubling perimetry all revealed defective retinal sensitivity in concert with ganglion cell layer thinning. Moreover, they found that the percent of subjects with abnormal tests increased with the severity of retinopathy and even moderate NPDR did not universally impact the visual function tests. The basis for these inconsistencies is currently unknown.

The aforementioned studies examined primarily the cone photoreceptor system. During the 2000's, and in opposition to Sacconi's et al., 2019 report of primary vascular dysfunction in DRD (Sacconi et al., 2019), multiple studies from Drs. Anthony Adams, Michael Bearse and colleagues at the University of California, Berkeley School of Optometry showed that delays in mfERG implicit times precede microvascular lesions (as evaluated by color photographs) and predicted the regions where vascular lesions and macular edema would appear within one to two years (Bearse et al., 2004; Harrison, Bearse, Ng, et al., 2011a, 2011b; Harrison, Bearse, Schneck, et al., 2011a, 2011b). These changes also primarily reflect cone photoreceptor transduction. The effect of diabetes on the rod photoreceptor system has received relatively less specific attention but delays in dark-adapted (scotopic) ERG implicit times occur in diabetic rats (Pardue et al., 2014) and humans (Holfort et al., 2011; Motz et al., 2020). By contrast, Longhin et al. (2016) did not detect rod defects in persons with mild NPDR using scotopic microperimetry and dark-adapted ERGs. Dr. Greg Jackson and colleagues (Jackson et al., 2012) found reduced frequency doubling sensitivity in persons with mild to moderate NPDR compared to controls. In addition, 25% of the patients exhibited reduced dark adaptation and scotopic visual field sensitivity was reduced by 2.9 dB in spite of having normal plasma retinol and retinal binding protein levels. Thus, a minority of patients have subtle defects in rod photoreceptor function that are apparently independent of systemic vitamin A deficiency. Further investigations of rod function will help to better understand the impact of diabetes on the dominant cellular pathways of the retina.

Based on the evidence in the literature presented over decades, the authors believe it is substantively established that: 1) DRD includes dysfunction of both the inner and outer retina in a manner that may vary between patients and across the spectrum of severity; 2) the peripheral retina is affected early in the course of disease; and 3) tests of visual function are more sensitive indices of DRD than visual acuity. To date, investigators have applied available test methods to DRD but further investigation is required to determine the optimal indices to predict the stage and course of the disease in patients. Further details of the cellular anatomy of human DRD may help to guide development of these test strategies.

Summary point: Diabetic retinal disease involves the entire neurovascular unit–including blood vessels and neural cells–so the assessment and staging of people with diabetes at risk for and with DRD needs to account for this observation. Through its DRD Staging Update project (described in Section 3, below), the Restoring Vision Moonshot will address: 1) the implications that DRD is present in the absence of clinical evidence of retinopathy on ophthalmologic exams or fundus photographs; and 2) the evidence that neural component of retinopathy must be accounted for in staging and therapeutic approaches.

2.2. Retinal physiology and the effects of diabetes

The evidence that diabetes disrupts the entire retina in humans is now overwhelming, as summarized by Simo et al. (Simo et al., 2018), but important questions remain. The retina as the specialized visual organ, operates under its own distinctive physiologic principles, and understanding normal visual processes can provide a framework to learn how diabetes impacts its homeostasis. The human retina is the product of eons of evolution that collects and transfers light energy captured by opsin in cones and rhodopsin in rods into electrical signals that confer visual and non-visual sensory information. That is, photoreceptive cells are paired to horizontally and vertically oriented cells that form integrated signals that are transmitted to the occipital lobe via the optic nerve and optic radiations. The visual information includes spatial and temporal resolution that is translated into perceptions of shapes, depth, color, contrast and motion. This system adapts across a wide range of illumination from starlight to bright sunlight (Hildebrand and Fielder, 2011). In addition, non-image forming "vision" operates under ambient light captured by melanopsin in intrinsically photosensitive ganglion

cells, and controls pupil responses, sets the circadian cycle, sleep/alertness and mood (Mure, 2021).

The general requirements for normal vision include:

1. Phototransduction and daily photoreceptor outer segment renewal;
2. Electrical signal transmission and integration between interneurons;
3. Biosynthesis of macromolecules (lipids, nucleic acids and proteins);
4. Transport of ribosomes and macromolecules along the length of ganglion cell axons to the lateral geniculate body; and
5. Maintenance of high sodium and potassium gradients across unmyelinated neuronal cell membranes via sodium-potassium ATPase and sodium channels, which in turn, enables generation of action potentials.

These functions require both glycolytic and oxidative metabolism, with the highest degree of oxidative activity in photoreceptors. Ganglion cells, by contrast, reside in a lower oxygen environment (pO_2 approximately 30 mm Hg in macaque retina) (Linsenmeier and Zhang, 2017), yet have a rate of anabolic activity, as measured by protein synthesis, that is the highest of all cellular layers (Losiewicz et al., 2019).

The term, "neurovascular unit," as originally applied to the brain (Hawkins and Davis, 2005; Zlokovic, 2008) and now to the retina (Metea et al., 2007; Newman, 2013) encompasses the physiologic and anatomic interactions between neurons, glial cells, blood vessels and microglia. Angiogenesis and neurogenesis are closely linked during retinal embryogenesis and are essential for normal retinal development (Joyal et al., 2018). This relationship is illustrated by the lack of formation of the superficial retinal vascular plexus in the absence of ganglion cells following genetic ablation (Edwards et al., 2012; Sapieha et al., 2008) and in humans with the outer retinal vascular plexus appearing (Dreher and Robinson, 1988; Hughes et al., 2000) with the first visually functional neurons (Dreher and Robinson, 1988).

During adult life normal functional integration of these cells enables adaptation to metabolic fluctuations, such as variable nutrient and oxygen/carbon dioxide levels and blood pressure, known as autoregulation (Pournaras et al., 2008). Notably, 30 Hz flickering light stimulation increases retinal activity, so vessels dilate to provide increased blood flow; in contrast, breathing 100% oxygen and systemic arterial hypertension each induce vasoconstriction to maintain blood flow within a physiologic range. Usui et al. (2015) elegantly demonstrated that amacrine and horizontal cells and their associated capillaries are interdependent for normal function and survival. In addition, Chou and Porciatti (Chou and Porciatti, 2020) illustrated that retinal ganglion cell activity adapts to visual stimuli and energy demands, a concept that is likely relevant to DRD.

Pathologic conditions also illustrate neurovascular unit interactions. For example, patients with presumably primary optic nerve disorders—autosomal dominant optic atrophy (Cesareo et al., 2021) and glaucoma (Rao et al., 2020)—exhibit reduced peripapillary vascular density, likely consequent to reduced ganglion cell function. In the streptozotocin-induced mouse model of type 1 diabetes, ganglion cells upregulate embryonic factors such as semaphorin 3A that induce vascular permeability at early stages of disease and prior to induction of VEGF (Cerani et al., 2013). Semaphorin3A can prevent physiological vascular regeneration (Joyal et al., 2012) and Kwon et al. found that plasma levels of Semaphorin3A correlate with severity of DR phenotypes (Kwon et al., 2016). Moran and colleagues (Moran et al., 2016) recently summarized how neural and vascular cells interact in the context of diabetes.

Moreover, inherited photoreceptor degeneration also leads to attenuation of vascular caliber and increased blood-retinal barrier permeability (Ivanova et al., 2019). Thus, it is understandable that diabetes impairs the physiologic adaptations to flickering light and hyperoxia prior to the onset of visible vascular lesions (Lott et al., 2012, 2015; Pournaras et al., 2008; Sousa et al., 2020). These early onset alterations reveal how the metabolically active retina responds to physiologic variations and dysmetabolic conditions. Newman and colleagues (Metea et al., 2007; Newman, 2013, 2015; Nippert and Newman, 2020) have demonstrated the critical role of Müller cells and astrocytes in mediating neurovascular interactions via prostaglandin E2, epoxyeicosatrienoic acids and nitric oxide. Glial dysfunction in diabetes impairs these interactions, which lead to vasodilation ("reactive hyperemia"), a common feature of DR (Grunwald et al., 1996) and reflects impaired autoregulatory mechanisms. Reactive hyperemia is reduced after panretinal and focal macular photocoagulation and anti-VEGF therapy (Blindbaek et al., 2020; Gottfredsdottir et al., 1993; Grunwald et al., 1989). Thus, the critical communications between the neurosensory retina and its vascular supply are progressively impaired by diabetes and improved by its treatments.

***Summary point*:** Normal retinal neurovascular physiology and primary disorders of ganglion cells and photoreceptors provide insights into how diabetes impairs cell—cell interactions within the retina.

2.3. Retinal neuroplasticity

Homeostatic neuroplasticity refers to the ability of neural tissue to adapt to physiologic and pathologic alterations during development, and particularly involves dendrites and synapses (Turrigiano, 2012); (Leinonen et al., 2020; Shen et al., 2020). The retina exhibits remarkable neuroplasticity to slowly recover function after injury, such as after treatment of retinal detachment and advanced photoreceptor degenerations (Levi et al., 2021; Nuzbrokh et al., 2021). That is, visual acuity can progressively recover up to a year after repair of macula-off rhegmatogenous retinal detachments in the absence of persistent submacular fluid, due to recovery of photoreceptor outer segments (Mitry et al., 2013; Shimoda et al., 2010). Moreover, the Zacks lab (Chinskey et al., 2014; Pawar et al., 2017; Ross et al., 2020; Xiao et al., 2021) has shown that modulation of autophagy and apoptosis prolongs photoreceptor survival in mice with experimental retinal detachment, and their work enabled a phase I clinical trial of a Fas receptor inhibitor (NCT03780972). Leinonen (Leinonen et al., 2020) showed that homeostatic plasticity accompanies genetically determined photoreceptor cell death. Neural development and growth-related pathways, ion channel activity and post-synaptic elements are the most highly upregulated transcriptomic pathways. Thus, if photoreceptors can adapt to stress, what about inner retinal neurons?

Glaucoma affects primarily ganglion cells and their axons. Fry et al. (2018) reviewed the literature and suggested that ganglion cells are dysfunctional before they die, and can recover function (visual field sensitivity) after reduction of intraocular pressure (Caprioli et al., 2016; Musch et al., 2014). The mouse optic nerve crush model is a severe injury which has been used to test ganglion cell axon recovery, and Peterson et al. (2021) showed that complement C3 and myeloid cells mediate recovery. Ganglion cells also adapt to rod photoreceptor damage with preservation of cone receptor pathways (Care et al., 2020). Thus, ganglion cell function can improve, and understanding of the underlying injury processes is important. The role of dendrites and synapses in homeostatic plasticity is particularly relevant to early stage DRD (Gastinger et al., 2001; Gastinger et al., 2008; vanGuilder, Brucklacher, Conboy, Bronson, & Barber, 2008) (Castilho et al., 2015; Cui et al., 2019). Hence, there may be specific therapeutic opportunities to enhance visual function in DRD. By comparison, a six year prospective followup study suggests that corneal nerve sensitivity improves in association with enhanced metabolic control (Misra et al., 2022). Peripheral sensory neuropathy in patients with type 1 diabetes can improve with intensive treatment, though those with type 2 diabetes are less responsive to treatment (Callaghan et al., 2012), but the potential plasticity of the retina in persons with type 2 diabetes has not been directed tested.

The vascular features of DRD can also resolve after control of hyperglycemia and/or hypertension as illustrated in Fig. 12. Pancreas transplantation in persons with type 1 diabetes can also stabilize DRD

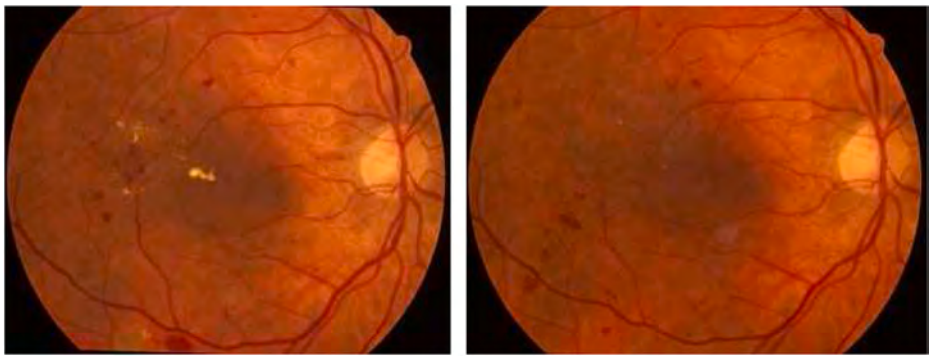

Fig. 12. Improvement in diabetic retinopathy. Fundus photographs from a patient with clinically significant diabetic macular edema before (left) and 9 months after (right) control of type 2 diabetes, hypertension and hyperlipidemia but without any ophthalmic therapy.

(Giannarelli et al., 2005; Jenssen et al., 2017; D. M. Thompson et al., 2008). The metabolic and cellular basis for this recovery, and after anti-vascular endothelial growth factor therapy is poorly understood but offers opportunities to guide future therapeutic development efforts.

***Summary point*:** Retinal functional and pathophysiologic studies provide important clues that are not evident in fundus photographs to guide clinical trials and interventions. Thus, it is essential to view the retina from the perspective of its normal functions and how it responds to stresses.

2.4. Diabetes alters retinal electrical signals

Sensation of stimuli such as touch and pain that yield electrical signals transmitted to brain centers via axons has been a fundamental mechanism of the nervous system since the rise of multicellular organisms. Retinal action potentials are generated by ganglion cells, conveyed to the lateral geniculate nucleus of the thalamus, and relayed to the visual cortex. Thus, ganglion cell electrical properties are the integrated signals from the entire retina.

Reduced amplitude and velocity of these signals in the earliest phases of DRD have been long recognized in the visual evoked potential responses (Papakostopoulos et al., 1985; Parisi et al., 1994; Varkonyi et al., 2002). These retinal responses are analogous to and correlated with reduced signals in peripheral nerves of persons with diabetes (Shahidi et al., 2012). Recent studies in diabetic mice have revealed new details of ganglion cell electrical dysfunction. Yu et al. (J. Yu et al., 2013) showed an increased spontaneous ON-cell spiking rate due to reduced inhibitory firing. Cui et al. (2019) also found increased firing rates, increased resting membrane potentials and decreased membrane capacitance in transient ON-RGA2 cells, but not in transient OFF-RGA2 cells. (RGA cells have large somas and dendritic fields; (W. Sun, Li and He, 2002). Flood et al. (Flood et al., 2020) also found that ON sustained ganglion cells receive excessive excitation under dark- and light-adapted conditions. Chen and colleagues (W.-Y. Chen et al., 2021) found impaired pupillary responses in diabetic mice associated with enlarged cell bodies and increased dendritic branching complexity of M2/M3 subtypes of intrinsically photosensitive ganglion cells. Moore-Dotson and Eggers (Moore-Dotson and Eggers, 2019) determined that reduced and shortened calcium signals in presynaptic GABAergic amacrine cells reduces light-evoked inhibitory input to rod bipolar cells, which in turn, creates an imbalance in ganglion cell function. Together, decreased gamma aminobutyric acid (GABA) release, increased glutamate release, and increased excitation of retinal ganglion cells contribute to this essential common pathway of vision (Eggers and Carreon, 2020). Catalani and Cervia (Catalani and Cervia, 2020) succinctly summarized these findings as shown in Fig. 13. Collectively, these data provide a new dimension for understanding previously unappreciated aspects of how diabetes alters retinal neuronal structure and function and impairs vision. Hopefully, studies in rodents can be translated into clinical studies of ganglion cell function for diagnostic or therapeutic purposes.

***Summary point*:** Preserving and restoring retinal ganglion cell integrity should be a goal of future therapies to preserve and restore vision in DRD.

2.5. Human data is essential

One hundred sixty-five years have passed since the initial observations of DR to the present understanding of a whole retina diabetic retinal disease. As evidence of progress in the advances in diabetes and DRD care over the past five decades, at least half to two-thirds of persons with decades of type 1 diabetes and stable metabolic control do *not* develop vision-threatening eye disease (Antonetti et al., 2012; Diabetes et al., 2015; Sabanayagam et al., 2019)–an approximately 80% reduction in significant vision loss compared to prior decades. However, vast expansion of the global population with diabetes (Teo et al., 2021) continues to impair the quality of life and productivity of tens of millions of persons due to diabetes-related visual loss, combined with vast financial burden to society, making it imperative to move beyond the microvascular-centric view of DR, and find therapeutic approaches that can be deployed early in disease progression (including before there is photographic evidence of DRD) and address the function of the entire neurovascular unit (Antonetti et al., 2006). This expansion includes adolescents with both type 1 and type 2 diabetes who already have mild DRD and other complications (Dabelea et al., 2017; Mayer-Davis et al., 2017).

The 60 year old studies of Wolter (1961) and Bloodworth (1962)

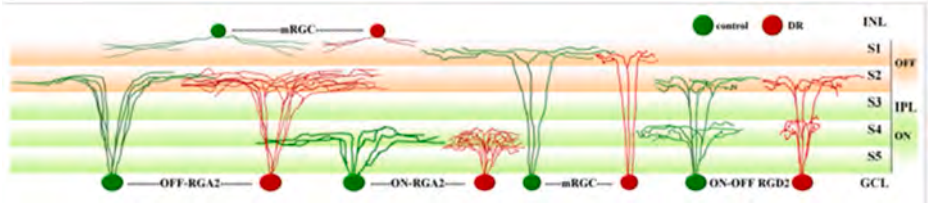

Fig. 13. A summary of major changes induced by DRD affecting the dendritic field of different retinal ganglion cells (RGCs). RGC branching pattern and branching level in the five strata (S1–S2: OFF sublaminae; S3–S5: ON sublaminae) of the inner plexiform layer (IPL) was depicted. The dendritic arborization of melanopsin-expressing RGCs (mRGCs) located in the inner nuclear layer (INL) was showed as well. DR: Diabetic retinopathy; GCL: ganglion cell layer; RGA: RGC type A; RGC: retinal ganglion cell; RGD: RGC type D (Catalani and Cervia, 2020). Note the reduced dendritic length and arborization induced by diabetes.

were the last large scale examinations of the cellular features of DRD, and the majority of studies over the past several decades have been based on rodents. A recent paper by Obara et al. (Obara et al., 2017) showed that melanopsin-expressing retinal ganglion cells are reduced in post-mortem eyes of persons with DR. Nonetheless, the cellular and molecular features that underlie human retinal vascular nonperfusion, macular edema, disorganized retinal inner layers (DRIL), neuroinflammation and the consequences of chronic anti-VEGF therapy and panretinal photocoagulation remain unknown. A similar state of ignorance existed about the pancreas in normal and diabetic humans until creation of the Network for Pancreatic Organ Donors with Diabetes (jdrfnpod.org) in 2007 to collect post-mortem tissues and analyze their morphology and cellular features. Key findings from nPOD to date include: 1) normal pancreases vary widely in size and number of islets; 2) insulitis is a feature of type 1 but not type 2 diabetes; and 3) insulin-positive beta cells persist in persons with longstanding type 1 diabetes (Atkinson et al., 2020). The success of nPOD and similar organizations, notably the European Innovative Medicines Initiative for Diabetes and the NIH-funded Human Pancreas Analysis Program (Kaestner et al., 2019; Marchetti et al., 2019) portends substantial potential gains from a human eye biosample recovery, preparation, and sharing program for DRD.

The inability to biopsy the human retina or to routinely collect vitreous as a proximal biofluid has substantially limited the ability to determine the effects of diabetes and its treatment and the importance of this approach was recently emphasized by Midena (Midena et al., 2021). The value of vitreous fluid analysis was clearly shown by the use of vitreous samples to confirm VEGF as a major angiogenic factor and therapeutic target in patients with PDR (Adamis et al., 1994; Aiello et al., 1994). A similar approach identified upregulation of the plasma kallikrein-kinin system in vitreous (Gao et al., 2007; Kita et al., 2015), giving rise to a therapeutic trial in persons with DME (NCT03466099). Proteomic analyses of human retina and vitreous from the Joslin 50-Year Medalists, who have survived 50 years or more with insulin-dependent diabetes, have also identified the photoreceptor-secreted protein, retinol binding protein 3 (RBP3) as a potential protective factor against advanced DRD (Yokomizo et al., 2019). Weber et al. (Weber et al., 2021a, 201b) recently reviewed the published data regarding vitreous proteomics and proposed a standardized methodology for sample collection, analysis and publication of data that is in line with standards of the Human Proteome Organization (hupo.org). The most frequently identified upregulated pathways in vitreous of persons with PDR include inflammation, complement and angiogenesis, and downregulated pathways are related to neuronal survival. They recommended vitreous proteomic studies follow rigorous methods of quantification, data normalization, and deposition of full datasets in public repositories. Weber et al. (Weber et al., 2021a, 201b) also found that metabolic pathways such glycolysis, glycogenolysis, protein kinase A signaling, NRF2-mediated oxidative stress, and the SPINK1 pancreatic cancer pathway are upregulated in PDR vitreous, whereas semaphorin neuronal repulsive signaling pathway", "IL-15 production", "LXR/RXR activation", and "synaptogenesis signaling pathway" are inhibited compared to controls. This study depleted high abundance vitreous proteins such as antibodies, alpha1-antitrypsin, alpha2-macroglobulin, albumin, apolipoprotein A1 and A2, fibrinogen, haptoglobin, and transferrin, prior to liquid chromatography and mass spectrometry. This step enabled detection of retina-derived proteins such as metabolic enzymes and axonal guidance proteins in the vitreous. This reduction in semaphorin content and signaling as measured by liquid chromatography/mass spectrometry contrasts with increased semaphorin 3A content in persons with PDR as measured by ELISA and immunoblotting (Dejda et al., 2014) but the basis of the different results is uncertain at this time. Multiple vitreous cytokines have been found to be changed in eyes with DRD (Daruich et al., 2018; Gariano and Gardner, 2005; Ghodasra et al., 2016) but this information has yet to yield therapies other than anti-VEGF antibodies. The ability to investigate the status of the retina via vitreous fluid biopsy may offer new opportunities for better understanding of the metabolic attributes of DRD.

Post-mortem human retinas also offer the opportunity to gain direct insight into disease mechanisms. Unbiased proteomic analysis of human retinas revealed previously unrecognized neurodegenerative pathways, such as dopamine degradation, Parkinson's signaling, synaptic long-term potentiation and amyloid processing (Sundstrom et al., 2018). The eyes studied by Sundstrom et al. were obtained from patients who had undergone fundus examination within two years prior to death to exclude the presence of clinically evident DRD and the average death to harvest time was 3.8 h. Eyes were classified as controls, and diabetic with or without increased glial fibrillary acidic protein immunoreactivity. The altered metabolic pathways detected were not predicted on the basis of prior DRD studies but clearly show the impact of diabetes on retinal homeostasis prior to the onset of visible microvascular lesions. Notably, neuroprotective role of THOP1 in Alzheimer's disease and unfolded protein response' pathways were uniquely enriched in control retinas in contrast to those from donors with diabetes. By contrast, dopamine degradation and Parkinson's Signaling were enriched only in diabetic retinas with glial activation. Neuregulin signaling, synaptic long term potentiation, and amyloid processing pathways were enriched in diabetic retinas with no glial cell activation. Of interest, the dopamine degradation pathway activation is consistent with the finding of reduced retinal dopamine signaling in diabetic rats and mice (Aung et al., 2014) and the preliminary results that two weeks of carbidopa/levodopa therapy improved dark-adapted ERG oscillatory potential implicit times in patients with diabetes (Motz et al., 2020).

Becker et al. (2021) performed transcriptomic analysis of a large cohort of post-mortem human retinas. In summary, they noted: a) marked differences between the macula and peripheral retina of normal and diabetic donors; b) upregulation of ADAMTS4 (ADAM Metallopeptidase with Thrombospondin Type 1 Motif 4), CCND1 (Cyclin D1), FZD7 (Frizzled Class Receptor 7), and RGS5 (Regulator Of G Protein Signaling 5); c) pathway analysis revealed marked differences in sphingolipid, carbon, tricyclic acid cycle, synaptic signaling, and other central aspects of metabolism; and d) multiple gene expression changes consistent with retinal ganglion cell loss. Interestingly, neither VEGF-A nor VEGF-B mRNAs were upregulated in eyes with advanced DR. They did not report associated proteomic or phosphor-proteomic data which might reveal a separate level of adaptive responses.

Eisma et al. (Eisma et al., 2015) reviewed prior studies using human eyes that revealed retinal vascular cell death, increased retinal VEGF expression, altered vascular integrin expression, gliosis, and neuroinflammation. They also pointed out the limitations due to post-mortem tissue degradation and small sample sizes derived from multiple donors. Nevertheless, analysis of post-mortem human eyes has advanced the understanding of age-related macular degeneration, particularly when combined with clinical data of the patients while alive (Curcio et al., 2020; M. Li et al., 2021; Williams et al., 2021).

We argue strongly for increased use of human data, but add that integrated human and animal studies can confirm molecular targets of preclinical disease (T. W. Gardner, Abcouwer, Barber and Jackson, 2011). For example, Dr. Xuwen Liu et al. (2016) demonstrated that phosphorylation of occludin protein on Serine 490 has a causal role in VEGF-induced endothelial cell permeability and tube formation in vitro, which was confirmed by the expression of Serine 490 immunoreactivity in neovascular complexes from patients with PDR. Likewise, Dr. Donald Puro et al. (Puro et al., 2016) found that neovascular complexes from rats with oxygen-induced retinopathy and humans with PDR share similar very high hyperpolarization which may further promote hypoxia-driven neovascularization, perhaps independent of VEGF. Furthermore, the Kim, D'Amore and Arboleda-Velasquez labs identified Runt-related transcription factor 1 (RUNX1) in $CD31^{+}$ vascular endothelial cells in human PDR fibrovascular membranes, and inhibiton of RUNX1 reduced neovascularization in oxygen-induced retinopathy

(Lam et al., 2017). Clearly, integrated analysis of human and pre-clinical data can provide important insights into disease pathogenesis. This point demonstrates the benefits of collaborations between laboratory-based scientists and clinicians.

Summary point: Human data from blood, vitreous fluid and retinas are essential to integrate with structural data from imaging and visual function data to advance the development of better diagnostic and therapeutic approaches, including the selection of subjects for clinical trials.

2.6. Key issues in clinical study design for DRD

The clinical evaluation of neural structure and its relationship to visual function in persons with DRD has been frequently been limited by the modest numbers of patients being studied and cross-sectional rather than longitudinal approaches. Past studies have also provided an incomplete understanding of the relative timing of neural versus vascular pathophysiologic changes over the course of evolution from no to severe DRD and the implications for prognosis and treatment.

In addition, DRD may be heterogeneous in that some persons may have predominantly vascular or neural features. Indeed, the European Consortium for the Early Treatment of Diabetic Retinopathy (EUROCONDOR) found: 1) neurodysfunction occurred in 61% of patients with ETDRS grade <20 and in 68% of patients with ETDRS grade 20–35; and 2) a third of patients with ETDRS grade 20–35 had no abnormalities on mfERG or spectral domain optical coherence tomography retinal thickness measurements (Santos et al., 2017). These data reflect primarily cone photoreceptor pathway function and the study did not evaluate rod photoreceptor function in the mid-peripheral retina where the earliest stages of vascular non-perfusion typically occur (Shimizu et al., 1981). Since comprehensive determination of DRD severity cannot be made by a single neural or vascular-based index, a single test is likely to miss responses to therapy in patients with heterogenous phenotypes.

It is also important to realize that differences in group means from clinical trials may or may not provide sufficient information to identify subsets of patients who might respond, predict their prognosis and their potential responses to interventions. Additional challenges in studies that have attempted to elucidate neural biomarkers or endpoints in DRD include:

1. Lack of definition of test-retest variability and effect sizes;
2. Potential neural retinal differences between individuals of varying ages, gender and types of diabetes;
3. Controlling for varying diabetes duration, systemic status and degree of metabolic control of study participants;
4. Lack of target confirmation and biological response parameters in subjects enrolled in studies; and
5. Lack of data from a diversity of populations so that results may not reflect real world situations

The true duration of diabetes and degree of control of subjects prior to entering clinical trials is often difficult to discern, particularly in persons with type 2 diabetes, so their position on a curve of systemic progression is unknowable. Segregating data from persons with type 1 versus type 2 diabetes might help to reveal underlying differences in DRD between these forms of diabetes since the metabolic bases are distinct, and partly overlapping. It is possible that DRD represents a common response to variable metabolic and inflammatory insults, similar to the manner in which radiation-induced injury exhibits a phenotype similar to DRD (Simo et al., 2018). Moreover, Ahlqvist and colleagues (Ahlqvist et al., 2018) categorized persons with type 2 diabetes into five subtypes based on six clinical parameters: glutamic acid decarboxylase (GAD) autoantibodies, age at diabetes onset, HbA1c, body mass index, and measures of insulin resistance and insulin secretion. They found that the highest risk of DRD and peripheral neuropathy is associated with insulin deficiency, whereas the highest risk of diabetic nephropathy and fatty liver disease was found in those who have severe insulin resistance (Ahlqvist et al., 2020). These findings may have substantial impact on the design of future clinical trials for the major complications, including DRD.

Clinical trials of retinal diseases have the advantage of sensitive imaging endpoints but for non-genetically defined diseases we currently lack the ability to confirm the presence of drug targets in the eye or to follow a biological parameter to monitor responses to therapies. For example, recent Phase 3 trials of the complement factor D antibody, lampalizumab (Roche; NCT02745119) and Fovista® (anti-platelet derived growth factor (PDGF) BB; and Ophthotech; NCT01940887 and NCT01944839) for geographic atrophy were terminated for lack of efficacy. It was not considered possible to assay for intraocular complement factor D or anti-PDGF BB in the subjects who were recruited to the trials. Similar limitations have been overcome in oncology where the expression of an oncogene, such as HER-2/neu defines the prognosis and response to treatment with monoclonal antibodies for a subset of women with breast cancer (Pegram et al., 1998) and the bcr-abl gene defines the defect in chronic myelogenous leukemia that paved the way for successful therapy with imatinib (Bartram et al., 1983). However, in ophthalmology, patients are frequently given therapy for which there is no confirmation that the target is manifest in individuals. There is yet no analogous molecular diagnostic test for any stage of DRD; even vitreous VEGF concentrations are not elevated in all patients with PDR (Aiello et al., 1994) and they are not tested in practice to predict or monitor anti-VEGF therapy. We have argued that target identification of retinal diseases would allow for greater specificity for clinical trial design and clinical care (T. W. Gardner and Sundstrom, 2017).

The effects of systemic medications targeting DRD can be monitored to confirm that patients are taking their medications and that the medications are having the intended pharmacologic response. The DRCR Retina Network Protocol AF (NCT04661358) that examines the effects of fenofibrate on DR worsening will test serum cholesterol as a potential index of drug effectiveness and to understand whether fenofibrate's effects on DR are mediated through its actions on lipid pathways.

Summary point: Future clinical studies in DRD should have rigorously defined metrics for standardized and thorough ocular and systemic evaluations, including functional assessment of the neural retina and patient-specific molecular diagnostic tests.

Alternative metrics of visual function beyond best corrected visual acuity and reliable, reproducible quality of life measures are sorely needed for future studies to understand the full impact of DRD. Several pilot clinical trials have employed neurosensory retina function tests as primary endpoints. Scott et al. used frequency doubling perimetry in two separate randomized 24 month studies of low-dose treatment with the antibiotic, doxycycline, which has anti-inflammatory properties. Doxycycline administration had no discernible effect on retinal sensitivity in 33 subjects with mild-to-moderate NPDR (Scott et al., 2014a, 2014b). However, in a separate study of 30 patients with severe NPDR to non-high risk PDR (Scott et al., 2014a, 2014b) the subjects had a baseline reduction in foveal sensitivity of −3 dB. Mean FDP foveal sensitivity further decreased in the placebo group (−1.9 dB) and increased in the doxycycline group (+1.8 dB) ($P = 0.02$) with no change in retinopathy severity. Further studies of doxycycline were not pursued, due in part to concern about induction of antibiotic resistance.

The EUROCONDOR study group employed mfERG implicit times as an index of neuroretinal function in persons with type 2 diabetes in response to placebo, or topically applied brimonidine or somatostatin (Simo et al., 2019). The primary outcome was the change in implicit time between baseline and after 96 weeks of follow-up. Patients randomly received brimonidine (n = 152), somatostatin (n = 145), or placebo (n = 152) eyedrops. Neither brimonidine or somatostatin treatment caused a neuroprotective effect in the entire cohort. However, a minority of patients (34.7%) with baseline prolonged implicit times

worsened if they received a placebo ($P < 0.001$), but the brimonidine and somatostatin treated groups remained stable, suggesting that the medications may have been protective. However, whether or not the topically applied medications engaged the target receptors in human retina remains an open question. Nonetheless, this study remarkably collected mfERG data from 11 clinical sites across Europe and maintained 76% patient follow up for 96 months in subjects with no visual symptoms.

The DRCR Retina Network (formerly the Diabetic Retinopathy Clinical Research Network or DRCR.net) (DRCR.net) used automated Humphrey perimetry as a secondary endpoint in the Protocol S comparison of ranibizumab versus PRP in persons with PDR. The patients had baseline visual field loss of approximately −6 dB (equivalent to moderately severe glaucoma), and from years three to five of follow-up both groups had a similar rate of visual field loss (Maguire et al., 2020). The cause of the unpredicted field loss in the ranibizumab treated patients is not known, but this study shows the ability to employ visual fields across multiple clinical sites and the importance of monitoring patients for visual function deficits that may occur in the natural history of disease or as adverse consequences of therapies.

Collectively, these studies address Dr. Bresnick's 35 year old admonition to include measures of visual function in the evaluation of persons with DRD (Bresnick, 1986). Two ongoing therapeutic trials (NCT04265261 and NCT04661358) now include visual fields and contrast sensitivity testing as secondary endpoints. Such tests have the ability to assess the separate functions of the macula and mid-peripheral retina, and rod versus cone pathways, depending on the stage of disease and hypothesis being tested.

***Summary point*:** Visual function data can be successfully acquired in longitudinal trials, which can be a useful way to determine the benefits and risks of new therapeutics.

2.7. Lessons from inherited retinal diseases

The success of other fields can provide important lessons. Dr. George Bresnick challenged the DRD field to take a comprehensive view of vision and disease state in 1986 (Bresnick, 1986), and the unpredicted and marked progressive loss of visual field sensitivity after 5 years of anti-VEGF therapy reported in 2020 (Maguire et al., 2020) forcefully confirmed the wisdom of his words. The field of inherited retinal photoreceptor and pigment epithelial cell disorders has seen remarkable advances over the past 20 years. The discovery of gene mutations in humans, rodents and dogs led to huge advances in understanding of the physiology of the outer retina, and enabled the development of successful gene therapy for persons with late-stage Leber congenital amaurosis (Bainbridge et al., 2015; Testa et al., 2013). Progress in this field has been accelerated by the biannual International Retinal Degeneration Symposium, now in its 19th meeting, organized by Drs. Robert (Gene) Anderson, Matthew LaVail and Joe Hollyfield. In addition, private donors have supported two Montaciano Symposia in Italy to address key challenges in opportunities and needs, such as novel outcome measures and endpoints in small patient populations, study design and ethical considerations. The Symposium's goal is to stimulate discussions among researchers, funding agencies, industry, and policy makers to further the design, conduct, and analysis of clinical trials (D. A. Thompsonet al., 2015; D. A. Thompsonet al., 2020). The European Association for the Study of Diabetes Eye Complications (http://easdec.org) has met annually since 1991 and brings ophthalmologists and endocrinologists together. The DRD field, which affects several orders of magnitude more persons, would benefit greatly from more frequent goal-oriented meetings.

***Summary point*:** Regularly scheduled international meetings are needed to accelerate the field.

2.8. A general theory of DRD pathogenesis

Decades of fundamental research using reductionist investigations of specific signaling pathways in rodent models have provided important insights into the pathophysiology of preclinical phases of DRD, notably mechanisms of neuroinflammation, vascular permeability and neovascularization, gliosis and cell death. These findings have helped to confirm the role of the neurovascular unit in retinal physiology and disease (T. W. Gardner and Davila, 2017; Moran et al., 2016; Newman, 2013; Y. Zeng et al., 2019). However, most of these mechanisms that have been proposed to lead to vascular lesions (polyol pathway activation, nonenzymatic glycation, oxidative stress) have not been validated in human tissue or causally linked to vision loss, so a comprehensive, testable concept of DRD remains elusive. Several factors contribute to this challenge.

First, diabetes is a complex metabolic disorder and unlike genetically defined disorders, cannot be defined by a linear pathway that can be replicated in animal models and cells, thus limiting the ability to perform effective drug screens. Type 1 diabetes results from autoimmune driven beta cell destruction and insulin deficiency, which leads to hyperglycemia, dyslipidemia and altered amino acid metabolism. Peripheral insulin resistance often accompanies Type 1 diabetes in both lean and overweight individuals (Cree-Green et al., 2018; Greenbaum, 2002; Nadeau et al., 2010; Wurtz et al., 2012). That is, type 1 diabetes is a catabolic disorder that leads to accelerated tissue breakdown, notably fat and protein, in which excess levels of glucose, amino acids—particularly branched chain amino acids—and triglycerides and fatty acids, accumulate in the blood, and ATP generation is reduced (Hebert and Nair, 2010; Nair et al., 1983). The lack of appropriate levels of insulin in tissues (not just in plasma) or appropriate quantities of macromolecules being synthesized and turned over in a steady state fashion leads, in the short term, to signs and symptoms of hyperglycemia and ketosis, and in the long term to stunted growth and wasting of insulin-sensitive tissues, such as skeletal muscle and adipose. Thus, tissues essentially starve in the presence of abundant substrates that cannot be metabolized efficiently (Okar, 2002). For example, brief (9 h) of systemic insulin withdrawal in persons with controlled type 1 diabetes reduces muscle ATP generation and the content of muscle mitochondrial enzymes involved in oxidative phosphorylation, with secondary increases in VEGF signaling, inflammation, cytoskeleton signaling, and integrin signaling pathways (Karakelides et al., 2007). If these effects define the consequences of insulin deficiency (with secondary hyperglucagonemia and elevated corticoid levels) in peripheral tissues, is the retina affected in a similar manner? We submit that the retina is likely affected by multiple aspects of systemic diabetes dysmetabolism, and perhaps by the accompanying autoimmune processes of type 1 diabetes, and that prevention and treatment of DRD depends on controlling these multiple processes. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) established a strong correlation between levels of glycemia and complications but has not examined other metabolites to date (Nathan and Group, 2014).

Second, our current incomplete knowledge of normal retinal homeostatic processes limits the understanding of how diabetes impairs retinal viability. One can think of the retina as a complex highly active metabolic factory that has to meet specific requirements in specific cells to achieve normal visual functions. In a simplistic example, an efficient factory receives raw materials of various types at its loading dock and the amounts and qualities of these raw materials are carefully validated and controlled (Fig. 14). At appropriate times raw materials enter the factory where they undergo inspection and quality control, are fed into an assembly line, and where the output is defined by needs of customers on the outside. In a similar fashion, cells take in raw materials for cell function—glucose, amino acids, fatty acids, ions, vitamin cofactors—and have well-regulated pathways to incorporate raw materials into macromolecules, and the breakdown of some nutrients to yield

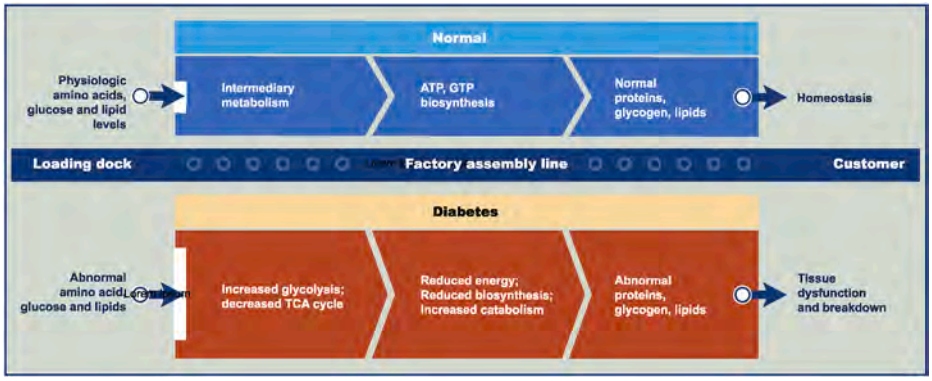

Fig. 14. A cellular "factory" showing substrates at the entry port on the left, their normal processing along an "assembly line" and output to the tissue on the right (top), compared to changes from diabetes (bottom), in which metabolic pathways are impaired with reducing biosynthesis and increased catabolism, leading eventually to tissue dysfunction and breakdown.

energy in the form of ATP and GTP. When the raw materials are of poor quality or in wrong quantities, or the assembly line malfunctions, the products are defective. Understanding how a factory functions under normal conditions is a prerequisite to restoring it to efficient production.

Some of these processes have been investigated in the retina. For example, insulin deficient rats have 30–40% less of the high normal basal retinal insulin receptor activity and downstream phosphatidyl inositol 3-kinase and Akt kinase activity (Rajala et al., 2009; Reiter et al., 2006). Subconjunctival insulin delivery and systemic administration of the competitive inhibitor of sodium/glucose cotransporter 1 & 2, phloridzin, restores the depressed signaling pathways and retinal protein synthesis and reduces apoptosis and expression of pro-inflammatory genes (Fort et al., 2011). Insulin deficient rats also exhibit reduced retinal lipid synthesis in (Tikhonenko et al., 2010) and diabetic *db/db* mice have increased retinal glycolytic intermediates and ß-oxidation metabolites after systemic administration of radiolabeled glucose, but reduced citrate metabolism. Palmitate uptake increases in *db/db* mice but its flux through the tricitric acid (Krebs) cycle was blocked at succinate; *i.e.*, it is not fully utilized for energy production (Sas et al., 2016). Indeed, multiple lipid classes are reduced in retinas of *db/db* mice (Sas et al., 2017) indicating reduced tissue uptake and/or increased oxidation. Moreover, succinate buildup can trigger production of growth factors that regulate vascular permeability and angiogenesis such as VEGF and Angiopoietin-2 and further exacerbate DRD (Sapieha et al., 2008). Collectively, these studies confirm that retinal anabolic functions are impaired within several months of the onset of insulin deficient and insulin resistant models of diabetes. Patrice Fort and colleagues (Fort et al., 2021) using postmortem human eyes and plasma from persons with type 2 diabetes, revealed a graded decrease from no diabetes to diabetes with DRD in long chain acylcarnitines, longer chain fatty acid esters of hydroxyl fatty acids, diacylglycerols, triacylglycerols, phosphatidylcholines, and ceramides in central retina biopsies. Moreover, sera from Pima Indians with type 2 diabetes also exhibited a similar graded decrease in circulating long-chain acyl-carnitines and a graded increase in the intermediate length saturated and monounsaturated triacylglycerols from no DRD to moderate NPDR. These findings indicate diminished synthesis of complex lipids and impaired mitochondrial beta-oxidation of fatty acids in retinal DRD with parallel changes in circulating lipids. In short, the metabolic "factory" in the retina is damaged by diabetes before the onset of clinically evident lesions but in parallel with reduced electroretinographic responses and visual acuity (Aung et al., 2013; Q. Li, Zemel, Miller and Perlman, 2002).

In addition to better-known changes in carbohydrate and lipid metabolism, insulin deficient diabetes also alters retinal amino acid concentrations and their metabolism (Frayser and Buse, 1978; Ola et al., 2011; Ward et al., 2005). Notably, retinal leucine, isoleucine and valine concentrations are increased by 50–96% after 4 months of insulin deficiency in rats. Ola et al. (2002) concluded that elevated branched chain amino acid concentrations inhibit glutamate transamination to glutamine and subsequent conversion to malate. The altered retinal amino acid composition may also contribute to reduced retinal protein synthesis that occurs in diabetic rats (Fort et al., 2014). Elevated vitreous concentrations of proline and pentose phosphate pathway components, along with reduced ascorbate levels, occur in vitreous of patients with PDR versus controls (Haines, Manoharan, Olson, D'Alessandro and Reisz, 2018). Also, Zuo et al. (2021) employed multidimensional network biomarker analysis of serum in a case-control study of patients with type 2 diabetes. They found that multiple polyunsaturated fatty acids were decreased in the vitreous of persons with diabetes and DR, and that a panel of linoleic acid, nicotinuric acid, ornithine and phenylacetylglutamine was highly specific and sensitive to distinguish retinopathy from diabetes. They did not include information about the status of other complications in their cohort. Separately, Hou and colleagues (Hou et al., 2021) reviewed 9 public databases and concluded that altered amino acid and energy pathways reflected in plasma, vitreous and aqueous fluids are associated with the presence of retinopathy, as illustrated in Fig. 15. Collectively, these studies strongly suggest that multiple aspects of diabetes dysmetabolism contribute to DRD, yet

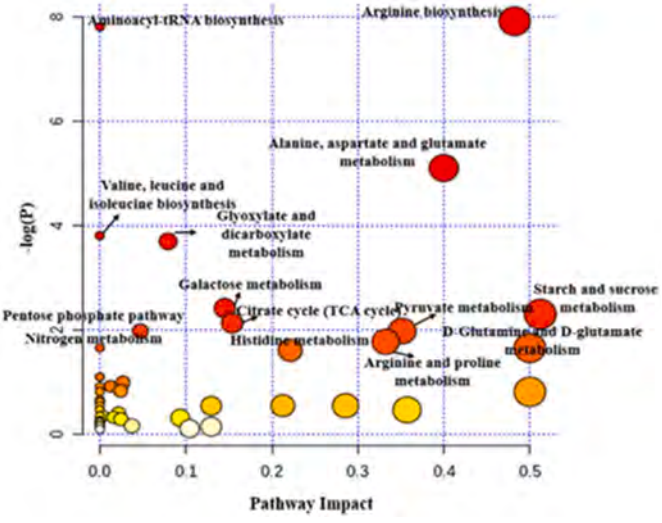

Fig. 15. Results of pathway analysis of metabolic biomarkers of DR. The color of the circle indicates the significance level in the enrichment analysis; darker color (more red) indicates greater significance. The size of the circle reflects the pathway impact value in the topology analysis, such that the larger the circle, the larger the impact value. The x-axis is the pathway impact value calculated based on topology analysis from (Hou et al., 2021).

how these central metabolic abnormalities lead to retinal neurovascular and inflammatory consequences remains an important open question.

The DCCT showed 50–70% reduction in retinopathy, nephropathy and peripheral neuropathy in patients who received intensive therapy versus those who received conventional therapy (Anonymous, 1993). Both groups of patients received the same total dose of insulin, while the intensively treated group received their insulin in more frequent intervals so the periods of insulin deficiency were shorter. How did this relatively minor adjustment lead to major improvement in outcomes? The benefits of intensive control are strongly associated with HbA1c levels but to date the roles of other metabolites have only recently been studied. Mathew et al. (2019) found that cardiac autonomic neuropathy in persons with type 1 diabetes is associated with higher plasma fumarate and lower asparagine and glutamine concentrations, independent of baseline glucose, hemoglobin A1c, body mass index and duration of diabetes. Moreover, Afshinnia et al. (2021) have now shown that elevated serum levels of unsaturated free fatty acid and phosphotidyethanolamine and reduced levels of saturated free fatty acids and lipid species predict rapid progression of estimated glomerular filtration rates in persons with type 1 diabetes. Similar studies regarding DRD are now in progress.

These studies identify risk factors for developing DRD but another perspective is to define the factors that are associated with *lack* of progression to vision-threatening DRD. Analysis of DCCT/EDIC subjects 15–18 years after DCCT closeout (with a mean diabetes duration of 29.7 years) showed that 67.9% of those in the intensive therapy group and 49.1% of those in the conventional therapy group had only mild NPDR (grade 35/<35) or less (Diabetes et al., 2015). Further, the Joslin 50-Year Medalist Study revealed that patients who were protected from PDR (42.6%) had lower plasma carboxyethyl-lysine and pentosidine concentrations than those with PDR (J. K. Sun et al., 2011), and the pancreases of all Medalists studied retained ß-cell insulin positivity (M. G. Yu et al., 2019). The presence of DRD as assessed by OCT angiography was associated with cognitive impairment (Fickweiler et al., 2021). Thus, a significant minority of persons with long-standing type 1 diabetes remain relatively free of complications. What additional metabolic, socioeconomic or genetic factors conferred this protection and might be applied to those at greater risk to minimize disease progression?

We propose that complications-prone tissues have distinct baseline energy and biosynthetic requirements that make them vulnerable to long-term quantitative and or qualitative impairment of macromolecule synthesis and/or degradation. The high metabolic requirements of the retina for normal function may be an “Achilles heel” that compromises its viability over the course of years of diabetes (Antonetti et al., 2006). Chronic metabolic stresses may lead initially to adapative responses to preserve tissue function and viability (reduced electrical responses in the retina, and hyperfiltration and hypertrophy in the kidney), followed by failure of adaptive changes (cell death), then maladaptive pathology (fibrosis, neovascularization and loss of function (vision) as outlined previously (Abcouwer and Gardner, 2014; T. W. Gardner and Davila, 2017; Gray and Gardner, 2015).

A corollary to this approach is that the signaling pathways that determine normal fetal growth of the retina with neurons, glial cells and vessels migrating peripherally from the optic nerve to form the neurovascular unit may provide clues to disease states. For example, proteins typically associated with embryonic development such as semaphorins (Cerani et al., 2013; Dejda et al., 2014; Kwon et al., 2016) and a proteolytically cleaved fragment of Netrin-1 are found in the vitreous of patients with various forms of DRD (Miloudi et al., 2016). Joyal and colleagues (Joyal et al., 2018) summarized how energy demands drive retinal vascularization during development and how impaired energy availability and biosynthesis compromises adult retinal cell viability. Ahmad et al. (Ahmad et al., 2020) illustrated how developmental mechanisms may be used to promote retinal regeneration. Properly timed and localized expression of vascular endothelial growth factor, Wnt and biosynthetic signaling pathways such as the insulin receptor and mechanistic target of rapamycin are also likely to be central to maintenance of the adult retina over many years. It may be instructive to analyze which of these pathways are reduced at various stages of human diabetes and in which regions of the retina, such that replacement strategies could be developed to restore retinal function and regenerate or reactivate cellular neuronal and vascular cell activity. Indeed, augmentation of the mTOR pathway can sustain photoreceptor function in mice with photoreceptor mutations (Punzo et al., 2009; Zhang et al., 2016), and ganglion cells following optic nerve injury (Teotia et al., 2019), as does ocular and systemic augmentation of the Akt/mTOR pathway in diabetic rats (Fort et al., 2011, 2014; Zolov et al., 2021). These findings may stimulate clinical trials in persons with DRD under the aegis of the Restoring Vision Moonshot (see below).

Additional pathways that are altered by diabetes may also provide therapeutic opportunities. For example, the mineralocorticoid receptor (Behar-Cohen and Zhao, 2021) is upregulated in photoreceptor, inner nuclear layer, ganglion cells of Goto-Kakizaki diabetic rats and human diabetic retinas, and the mineralocorticoid receptor inhibitor, spironolactone, reduces retinal inflammation and restores the blood-retinal barrier (Zhao et al., 2021). Also, the sulfonylurea receptor 1 (SUR1) is expressed in human retina axons colocalizes with the Kir6.2 channel, and the SUR1 inhibitor, glyburide, a common anti-diabetic drug, mitigates retinal gliosis in Goto-Kakizaki diabetic rats (Berdugo et al., 2021). Several investigators have suggested that choroidal vascular lesions are correlated with photoreceptor defects in persons with no visible DR and non-proliferative retinopathy (Choi et al., 2017; Parravano et al., 2021). The understanding of choroidal processes in DRD remains an area of continued investigation.

***Summary point*:** DRD is likely the consequence of the tissue responses to chronic multiple metabolic alterations in persons with type 1 and type 2 diabetes, and identifying and inhibiting aggravating and/or enhancing protective pathways may lead to opportunities to prevent DRD and/or to restore vision.

3. It is time for a moonshot to find “cures” for DRD

3.1. The worldwide threat of DRD

The very real threat of visual loss is a chief concern among people with diabetes and their families, with tens of millions of individuals, worldwide, who suffer from blindness and vision threatening retinal disease as a complication of their diabetes. In fact, DR increasingly affects children with type 2 diabetes (Lin et al., 2021). DR is the most common cause of blindness in working age adults, worldwide, with global prevalence of DR in people with type 1 diabetes being 77% and in type 2, 25% (Teo et al., 2021):

1. 463 million people with diabetes mellitus in the world, estimated to increase to 700 million people in 2045
2. In 2020, 103 million people worldwide with DR, projected to increase to 160.5 million by 2045
3. In 2020, 28.4 million people with VTDR, projected to increase to 44.82 million by 2045
4. In 2020, 18,83 million people with CSME, projected to increase to 28.61 by 2045

Our broadening understanding of DRD as a disease of the neurovascular unit (Antonetti et al., 2012; T. W. Gardner and Davila, 2017) parallels major advances in analytic techniques at the cellular and molecular (“omic”) level (Becker et al., 2021) progress in tissue nanotransfection (Gallego-Perez et al., 2017; Roy et al., 2020; Xuan et al., 2021); regenerative medicine and bioprinting (Lahne et al., 2020; Ruiz-Alonso et al., 2021; Wang et al., 2018), availability of advanced imaging technologies (Channa, Wolf, & Abramoff, 2021a, 2021b), greater access to well characterized cohorts of people with diabetes (Levi et al.,

2021; Chua et al., 2019; Hietala et al., 2008; Sobrin et al., 2021; J. K. Sun et al., 2011), and potential utility of artificial intelligence (Grzybowski et al., 2020). Thus, the authors argue the time is right for a science-with-a-mission "moonshot" to find "cures" for low vision and blindness due to DRD, to better identify, early, and arrest progression of DRD, and to protect the retina from the deleterious effects of diabetes. The model for such a moonshot is the highly successful United States National Aeronautics and Space Administration's (NASA's) lunar mission of the 1960's.

3.2. *The "Restoring Vision Moonshot" inspired by Mary Tyler Moore and President John F. Kennedy (JFK)'s "Moonshot"*

In January 2018 JDRF (www.jdrf.org) partnered with the Mary Tyler Moore and S. Robert Levine, MD Charitable Foundation to launch the "Restoring Vision Moonshot" as a way to honor Ms.Moore's singular efforts in support of finding cures for type 1 diabetes and its complications. In their 2017 "Profile in Progress" for *Diabetes Care* (Atkinson and Nierras, 2017), following her death, Drs. Mark Atkinson and Concepcion Nierras wrote of Ms. Moore's extraordinary impact on type 1 diabetes research funding (Atkinson and Nierras, 2017):"Beginning in 1995, [Ms. Moore] led a group of government relations volunteers, individuals with diabetes and their family members to petition the U.S. Congress for increased funding for the National Institutes of Health (NIH) and, in particular, for special funding for type 1 diabetes research (Fig. 16).

In response to these persistent charm offenses and advocacy initiatives, from fiscal year 1998 through 2017, Congress appropriated a cumulative total of $2.46 billion in special funding for type 1 research, with an additional equal amount to support diabetes care and education programs for Native Americans, who are disproportionately affected by type 2 diabetes. The so-called Special Diabetes Program (SDP) funding augments regularly appropriated funds that the U.S. Department of Health and Human Services receives for diabetes research. The SDP has fostered unique collaborations among the NIH, the Centers for Disease Control and Prevention, and the broader research community to accelerate the pace of progress in type 1 diabetes research. Thanks largely to Ms. Moore's leadership, the diabetes research community has benefitted greatly from opportunities made possible by the SDP as illustrated in a few examples below".

Drs. Atkinson and Nierras further describe related progress in the areas of Genetic Discovery; Epidemiology, Disease Prediction, and Pathogenesis; Beta Cell Replacement; and Complications Prevention and conclude by emphasizing that:

> "The progress enabled by this long-term investment in diabetes research underscores the power of partnership among government, foundations, scientists, and people with diabetes – an alliance that counted Ms. Moore among its most effective leaders".

From 1991 to 2017, Ms. Moore also helped the JDRF raise over $2 billion for direct type 1 diabetes research funding (making JDRF the world's largest non-profit funder of diabetes research) along with leading advocacy for an initiative which doubled the NIH's funding (1998–2003, approximately $14 billion increased to approximately $28 billion) as well as taking the lead (along with Michael J. Fox, Christopher Reeve and others) in advocating for federal support for embryonic stem cell research. In the years Ms. Moore worked with JDRF to help find cures for diabetes and its complications, her diabetes had a devastating impact on her life including causing significant visual loss from diabetic retinal disease.

It is a fair assumption that most may know Ms. Moore by her work as an actress and diabetes research advocate. But there is something readers may not know about her – in her heart she was a dancer. She worshipped Fred Astaire, Gene Kelly, Ginger Rogers, and Cyd Charisse and wished she could dance like them (Fig. 17). She had a dancer's discipline, work ethic, drive to perfection, and willingness to take risk (literal leaps of faith), with the clear understanding that in order to innovate, to create something beautiful, your toes were going to get bloody, but most of all, it was in dance that she found her true joy. Diabetes stole this joy from her. Remarkably, Ms. Moore was able to manage through the numbness and loss of position sense due to her neuropathy, and was even able to train herself to overcome the exertional pain of her peripheral vascular disease, and ultimately benefitted from revascularization surgery.

But she was never able to overcome the vision-stealing impact of her retinopathy and the severe narrowing of her visual fields and diminished night vision that accompanied her pan-retinal photocoagulation laser therapy. Indeed, over time, it became a great challenge for her to simply walk across a room and avoid obstacles, or judge changes in grades, or walk down stairs, or be physically active in low light, making this once fiercely independent woman, this joyful dancer, unable to get around on her own, unable to sustain her autonomy.

Fig. 16. Mary Tyler Moore helping to launch the new journal, *NIH MedlinePlus*, Fall 2006 (Atkinson and Nierras, 2017).

Fig. 17. Mary Tyler Moore dances with Gene Kelly on the "Mary Tyler Moore Hour" an MTM Enterprises Production, aired on CBS, April 1, 1979.

3.3. The Restoring Vision Moonshot workshop

The Restoring Vision Moonshot launch workshop in January 2018 benefitted from participation by a diverse group of leaders in basic and clinical research; tissue regeneration and bioengineering; therapeutics development, pharma, biotech; bioinformatics and artificial intelligence; funding and regulatory agencies; as well as people personally affected by diabetes and its complications (see Table 1).

Table 1
Participants in the restoring vision moonshot initiative program workshop.

January 2018
• Wei Liu, Assistant Professor, Albert Einstein College of Medicine
• Tom Bollenbach, Chief Technology Officer, Advanced Regenerative Manufacturing Institute (ARMI)
• Katrina Norfleet, Director of Communications, Association for Research in Vision and Ophthalmology (ARVO)
• Timothy Kern, Professor, Case Western Reserve University
• Stephen Rose, Chief Research Officer, Foundation Fighting Blindness
• Peter Loskill, Doctor, Fraunhofer Institute for Interfacial Engineering and Biotechnology
• Jason Erlich, Global Head, Clinical Ophthalmology Produce Development, Genentech/Roche
• Lily Peng, Product Manager, Google Research
• Joseph Boneventre, Chief, Renal Division, Harvard Institute of Medicine
• Lloyd Paul Aiello, Professor of Ophthalmology, Harvard Medical School
• Jennifer Sun, Assistant Professor, Harvard Medical School and the Joslin Diabetes Center
• Bhavna Antony, Research Scientist, IBM Research
• Eileen Koski, Program Director, Health Data & Insights, IBM Research
• Ashwani Malhotra, Research Business Alliances Executive, IBM Research
• Roy Beck, Executive Director, Jaeb Center for Health Research
• Adam Glassman, Coordinating Center Director, Jaeb Center for Health Research
• Elia Duh, Professor of Ophthalmology, Wilmer Eye Institute, Johns Hopkins School of Medicine
• Leonard Levin, Professor and Chair, McGill University
• Steven Becker, Audacious Goals Initiative Program Coordinator, National Eye Institute (NEI)
• Kapil Bharti, Tenure-Track Investigator, National Eye Institute (NEI)
• Emily Chew, Director, Division of Epidemiology and Clinical Applications, National Eye Institute (NEI)
• Paul Sieving, Director, National Eye Institute (NEI)
• Bjarki Johannesson, Investigator, New York Stem Cell Foundation
• Elizabeth Schwarzbach, Vice President, Business Development, New York Stem Cell Foundation
• Dhanuraj Shetty, Senior Director, Strategic Alliances and Partnership, New York Stem Cell Foundation
• Susan Solomon, CEO, New York Stem Cell Foundation
• Lisa Strovink, Chief Strategy Officer, New York Stem Cell Foundation
• Richard Kirk, Chief Executive, PolyPhotonix
• Patrick McCrossen, PolyPhotonix
• Brian Hofland, President, Research to Prevent Blindness
• Chandan Sen, Professor and Vice Chairman (Research), Department of Surgery, The Ohio State University Wexner Medical Center
• Brent Toto, Program Director, Center for Regenerative Medicine and Cell Based Therapies, The Ohio State University College of Medicine
• Robin Ali, Professor, Kings College London
• Pete Coffey, Professor, University College London, University of California, Santa Barbara
• Mark Atkinson, American Diabetes Association Eminent Scholar, Director, University of Florida Diabetes Institute
• Michael Abramoff, Professor of Ophthalmology and Visual Sciences, University of Iowa
• Erin Lavik, Professor, University of Maryland, Baltimore County
• Daniel Pelaez, Assistant Research Professor of Ophthalmology and Biomedical Engineering, University of Miami Miller School of Medicine
• David Antonetti, Professor, Ophthalmology and Visual Sciences, University of Michigan
• Jim Weiland, Professor, Biomedical Engineering, University of Michigan
• Thomas Gardner, Professor of Ophthalmology & Visual Sciences, University of Michigan Medical School
• William Murphy, Professor, Biomedical Engineering, University of Wisconsin
• David Gamm, Associate Professor, University of Wisconsin, School of Medicine and Public Health
• Dimitri Azar, Director, Verily and University of Illinois College of Medicine
• Pete DiStefano, Chief Scientific Officer, Zebra Biologics Inc.
• Ron Lindsay, CEO, Zebra Biologics Inc.

The launch workshop featured a day of moderated panel presentations and well engaged discussions. Topics addressed included: an overview of Type 1 diabetes and the state of DR; key factors leading to visual loss in T1D; solving blindness in other retinal disorders; understanding DR/DME; current treatments for DME; landscape of care for PDR; approaches for DR in development; lessons learned from other diseases; next generation approaches; tissue nanotransfection; regenerative therapies; optogenetic modulation; photoreceptor replacement by cell transplantation; reconstructing the macula; bioprinting and bioengineering; technological approaches to regain vision, including retinal prostheses; hydrogel scaffolds for regeneration; and perspectives from funders. A summary of some of the proposed opportunities coming out of the launch workshop is included in Table 2.

3.4. President Kennedy's moonshot challenge

The Restoring Vision Moonshot finds its inspiration in JFK's May 1961 challenge to the US to "commit itself to achieving the goal, before this decade is out, of landing a man on the Moon and returning him safely to the Earth" (Kennedy, 1961). In his September 1962 speech at Rice University, JFK amplified the challenge, noting that, "We choose to go to the Moon in this decade and do the other things, not because they are easy, but because they are hard; because that goal will serve to organize and measure the best of our energies and skills, because that

Table 2
Summary of Opportunities generated by Restoring Vision Moonshot Launch Workshop, January 2018.

• Use human bio-samples, patient registries and very large datasets to better identify new pathways and medical therapies; perform detailed phenotyping of patients to further generate well characterized populations to develop metrics to quantify what parameters of disease occurrence, progression, and reversal can be identified.
• Biomarkers of anatomic and functional state are critically important. The key is to find biomarkers that can be readily measured over time and that will correlate well with clinical/functional status. One caveat is that while biomarkers can be used to describe disease situations, they may not have predictive effect on a particular individual patient. There will need to be a clinical end-target before these can be used as a registerable endpoint with the FDA.
• Technological and engineering approaches can be electrical chemical, biologic, or cellular-based to address cell dysfunction and cell death.
• Stem cell research in the stroke field has attempted to be neuroprotective and is now moving toward the vasculature, whereas in the retina, it's been the opposite. Both fields can learn from each other about what does and does not work in the human body. Using endothelial progenitors derived from stem cells may be able to "re-plumb" an ischemic retina, considered an "easier ask" than developing a complete neural network to replace the retina, recognizing that neither is "easy." A key factor will be how the new cells implanted ill react to the underlying diabetes effects, and will it be in a similar fashion to the native cells that died as a result of the disease.
• Specific polymers in the form of hydrogels may be able to repair the blood-brain barrier and, by extension, possibly the blood-retinal barrier as well as support regeneration with nerve resprouting. These polymers may be used as a means of drug delivery or cell-based therapy delivery.
• Making modifications to blood vessels or to retinal ganglion cells may have a beneficial effect beyond what is currently thought would be likely to happen.
• Optogenetics or photoreceptor transplantation may also need improved vascularity for optimal results.
• Transcription factors take a differentiated cell and transform it into another cell type, to generate cells of a desired phenotype/function. One potential question is whether this can be achieved in situ in the living human eyes and how to assess the long-term effects.
• Delivery of drugs and cells to the inner retina is challenging in terms of maintenance, accuracy and function over extended periods of time. How well they penetrate into the retina remains unanswered and needs to be addressed along with addressing other therapeutics (be they drugs, trophic factors, cells, etc.) delivery challenges.
• Encapsulated technologies for delivery of therapeutics have been used in other ophthalmic disease sates in clinical trials, and may be applicable in retinopathy. Key question remains: how many targets have been validated in the eye for retinal disease?
• Replacing the whole retina through bioprinting is critical for advanced disease. At this point, bioprinting to replace the whole retina may represent the most feasible biologic solution.

challenge is one that we are willing to accept, one we are unwilling to postpone, and one we intend to win." In kicking off the Restoring Vision Moonshot Workshop, Dr. Levine, repeated JFK's framing of the nature of the challenge he had put forth in the context of the challenges ahead to achieve the goals of this new moonshot and added: "We further understand what every dancer, like my wife intrinsically knew, that to achieve something no one has done before requires passion, clarity of vision, discipline, a willingness to take risk, collaboration, and the clear understanding that in order to succeed, your toes are likely to get bloodied." Like the imperative driving JFK's moonshot was fear of Soviet dominance of space, and the threat this posed to the US's liberty and sovereignty, the Restoring Vision Moonshot's imperative is the fear of the millions of people with diabetes about visual loss and blindness, and the threat this poses to their personal liberty and sovereignty.

3.5. The NASA moonshot program as a model for "science-with-a-mission"

In their response to JFK's challenge, NASA leadership organized the US space program around the achievement of critical milestones required to complete their manned mission to the moon (Fig. 18).

In order to achieve its goals NASA established "partnerships with industry, not as vendors but as research and development partners" (Webb, 1982), as well as with academia and the military services, while sharing decision-making with the various collaborating centers "to the fullest extent possible" (Webb, 1982). NASA strove to sustain a balanced approach to administration, fostering decentralization of program innovation and management while sustaining centralized goal-setting, oversight, and contracting/financing support. In his foreword to Dr. Arnold Levine's *Managing NASA in the Apollo Era*, James Webb, further notes:

> "Dr. Dryden [Deputy NASA Administrator], Dr. Seamans [Associate Administrator], and I, in making the substantive and administrative decisions, constantly and deliberately sought to spread our most difficult problems over the largest possible number of able minds and to develop means to evaluate, from the broadest national and international viewpoints, the concepts and proposals that resulted. We could not know what some of this large number could invent, but we strongly felt many innovative ideas would emerge from a widespread invitation to work on the problems related to an understanding of the solar system and the universe beyond."

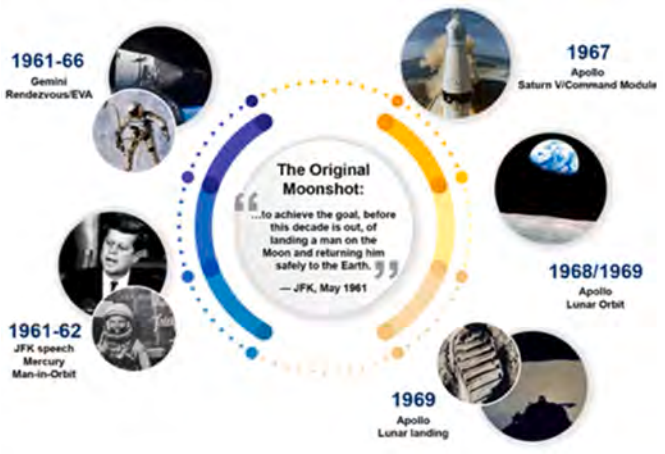

Fig. 18. Major Moon Mission Milestones. JFK speech to joint session of Congress, May 1961; First Manned Orbital Flight by John Glenn in "Friendship 7" in February 1962; Gemini completing rendezvous and dock and space walks,1965; First un-crewed test flight of the Saturn V launch vehicle, November 1967; first manned orbit of the moon, Christmas, 1968; first lunar landing July 1969.

This openness to a broad range of ideas, decentralization of decision-making, and program innovation and management in partnership with industry, academia, and the military services, along with clarity and focus on the overarching goal of "landing a man on the moon and returning him safely to earth" (Kennedy, 1961) and its timeline of achieving this goal "before the decade is out" (Kennedy, 1961) were keys to NASA's many successes in the decade of the 1960's, capped by JFK's challenge being achieved on July 20, 1969 with Neil Armstrong's famous "one small step for [a] man …" on the lunar surface and the Apollo 11 spacecraft he was commanding splashing down safely in the Pacific on July 24, 1969.

3.6. The Restoring Vision Moonshot

NASA's approach to achieving its complex, large scale, extraordinary mission provides guidance for other such undertakings, such as the Restoring Vision Moonshot, starting with recognition of the nature of the problem, the depth of the basic science and translational challenges that must be overcome to succeed, stating an overall goal clearly at the outset with a timeline to achieve that goal, and establishing intermediate milestones against which program managers can organize efforts.

Like NASA did in service of the moon mission, the Restoring Vision Moonshot has assembled participation that is inclusive, bringing together individuals from across the globe of diverse expertise (e.g., physician-scientists, engineers, cell biologists, epidemiologists, regulators), people affected by DRD, and multiple funding entities. The Restoring Vision Moonshot provides a supportive context for all to share their unique insights while contemplating novel approaches to vision preservation and restoration in people with diabetes. Its goals are to organize its efforts (Fig. 19) to respond to patient-oriented concerns and help develop the means and methods to protect retinas from the deleterious effects of diabetes, preserve retinal neuronal and vascular integrity and function, and restore patients to normal visual function.

The Restoring Vision Moonshot takes further organizational development and programmatic cues from the Brehm Coalition (www.brehmcoalition.org) and the Kidney Health Initiative (https://khi.asn-online.org). Founded by Bill and Dee Brehm in 2007, the Brehm Coalition is a self-organizing scientific effort involving 130 experts in the fields of immunology and beta cell biology from 12 institutions whose purpose is, "to accelerate the search for a cure for type 1 diabetes through establishing a new paradigm for medical research based on unparalleled collaboration." It is founded on principles of scientific engagement and interaction which establish a unique "phenotype" (Table 3) supporting sustainability, satisfaction, and success. Of note, Mr. Brehm, a systems engineer and Department of Defense staff member in the Johnson and Nixon Administrations, had first hand exposure to the NASA moon mission efforts, and Mrs. Brehm has had type 1 diabetes for over 70 years. Thus, the organizational structure and purpose of the Brehm Coalition is of great personal relevance to them.

Drs.Matthias von Herrath and Peter Arvan, long-standing Brehm Coalition members, make the following observations about its benefits:

- "The Brehm Coalition is my best professional experience–a forum where honesty, trust and collaboration reign (versus competition, secrecy and mistrust), the latter being major impediments in productive science, especially for complex translational research/medicine questions.
- direct real-time sharing of negative data to accelerate scientific development
- integration of a wide range of expertise from academia, large academic consortia, clinical consortia and industry as well as smaller biotech
- the coalition is much bigger than the single individual; $1 + 1 = 10$ or more
- catalyzation funds have resulted in multiplicity of original investment

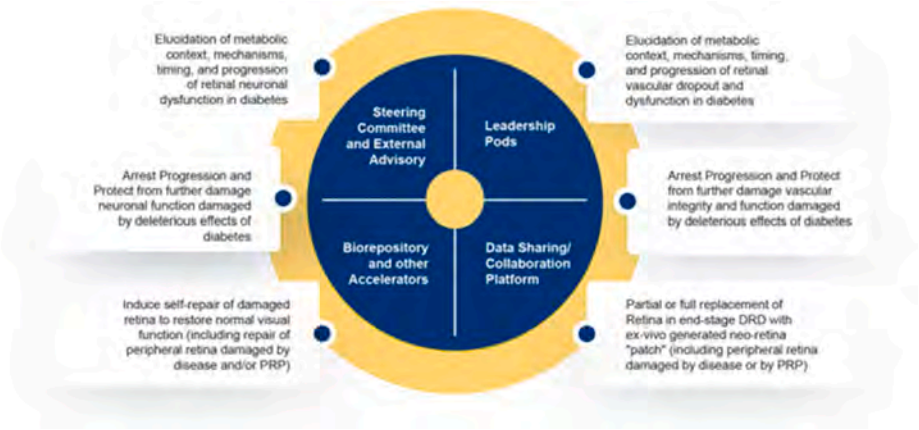

Fig. 19. The Restoring Vision Moonshot organizational structure. The Moonshot positions mission-oriented program areas around a hub/core of administrative and scientific leadership, a confidential communications and data sharing platform, and essential shared resources such as: a biorepository for human eyes, tissue and fluids; a retinal image data bank; biomarker-evaluation variable development/validation; and well characterized patient cohorts and clinical trial networks.

Table 3
Phenotype of the Brehm coalition.

1. Fun to work with
2. Self-organizing style not compromised by rigid management approach imposed by others.
3. Intimate collaboration facilitated and convenient videoconferences involving all Coalition members
4. Emphasis on the competence of the group rather than solely on the competence of individuals.
5. Respect for and trust in each other with real-time sharing of data.
6. Dependence on each other for results
7. Cooperation on, and performance of one another's experiments.
8. Sharing of institutional cores and other resources.

- o ability to openly discuss complex political and scientific enterprise issues, position papers
- o exposure of younger trainees, post-docs, and junior faculty to senior scientists in an open, supportive mentoring environment
- o bringing together investigators from two distinct scientific fields has brought cross-collaborative efforts that would not have been possible otherwise.
- o There are unfortunately too few examples where this collegiality is fostered. Industry employs a similar basis though to establish functional teams, academia often unfortunately not."

The Kidney Heath Initiative (KHI) was founded in 2012 as a public-private partnership between the American Society of Nephrology and the Food and Drug Administration. Its mission is to "catalyze innovation and the development of safe and effective patient-centered therapies for people living with kidney diseases." Its efforts provide proof points for the importance of the Restoring Vision Moonshot's emphasis on bringing the patient voice and perspective into its process as a key driver of how DRD's scientific challenges and clinical issues are framed and how the patient point of view can be incorporated into programmatic decision-making and approach, including through the development of patient reported outcomes measures (PROMs) (Conway and Knight, 2021; Sheldon, 2021; Tarver and Neuland, 2021). The KHI's efforts further substantiate the value the Restoring Vision Moonshot assigns to efforts to develop updated taxonomies of disease and the use of science and technology road-mapping as stimulus for innovation.

The question raised by the successes of cross-field, cross-institutional, cross-sector collaboratives, like the Brehm Coalition, Kidney Health Initiative, nPOD, Artificial Pancreas Program of the JDRF (Kowalski, 2015) and others is simply – why not for Diabetic Retinal Disease? The Restoring Vision Moonshot is a response, and the time is now.

The Restoring Vision Moonshot is centering its initial efforts on updating the staging system and severity scale for DRD (Abramoff et al., 2018; Jampol et al., 2021; J. K. Sun et al., 2021) as well as on development of essential shared resources – "accelerators" (Fig. 20). Accelerators in the pre-launch planning, fund-raising, and early implementation stages include:

1. A biobank for human eyes and vitreous, with access to advanced -omic analytic capacity and including an associated data-sharing platform and data repository;
2. Support for validation of biomarkers identified as promising by the DRD staging system update project, including through collaboration with existing clinical trial networks;
3. A global digital image repository linked to clinical records and against which artificial intelligence (AI) tools can be used to generate new insights regarding risk prediction, prognosis, potential response to therapy (Gilbert and Sun, 2020).
4. Organize large cohorts of well characterized individuals with diabetes for global research access;
5. Develop validated patient reported outcome measures that can be used to facilitate new therapeutics development and clinical decision-making; and
6. Establish a confidential communications platform for scientific exchange, mentoring, and real-time data-sharing.

Restoring Vision Moonshot facilitating activities in plan also include creating incentives for young investigators to enter the field, and convening regular meetings targeting cross-field expert participation to encourage further shared learning and collaboration.

3.7. An international initiative to update the staging of diabetic retinal disease

In their scientific statement (Insel et al., 2015) on the staging of type 1 diabetes, the JDRF, American Diabetes Association and Endocrine Society offered that adoption of their newly proposed staging classification of pre-symptomatic T1D would:

1. Provide a new standardized taxonomy for human type 1 diabetes;
2. Accelerate the clinical development of therapies to prevent symptomatic disease;
3. Aid the design of clinical trials through the use of risk profiles, subject stratification, and stage-specific clinical trial end-points;
4. Promote precision medicine involving the tailoring of optimal therapies to specific individuals at specific stages of the disease; and

Fig. 20. Restoring Vision Moonshot (RVM) Accelerators, their need and responses.

5. Provide a framework and approach for an optimized benefit/risk ratio that should impact regulatory approval, reimbursement, and adoption of interventions in the early stages of type 1 diabetes to prevent symptomatic disease

Adopting this construct of the importance of advanced staging systems for the acceleration of disease research and enhancement of patient care, the first Restoring Vision Moonshot project launched was tasked with developing an updated, multidimensional DRD staging system and severity scale (J. K. Sun et al., 2021) that can better define DRD, stage individual risk for disease worsening, predict and measure response to therapy, and support clinical trials evaluating novel therapies while having a readily useable interface for both researchers and clinicians. We argue an updated DRD staging system serves a vital role in advancing research and care for persons with DRD by providing a framework for a common language of DRD risk, progression, severity and interventions that can help accelerate our path to "cures" for along the full course of disease.

The Restoring Vision Moonshot's DRD Staging Project benefits from the leadership and participation of global experts organized in six working groups (Table 4) comprised of 50 persons from 12 countries charged with examining variables relevant to:

1. Retinal Vascular Disease
2. Retinal Neuronal Disease
3. Basic and Cellular Mechanisms of DRD
4. Systemic factors in DRD
5. Visual Function in DRD
6. Quality of Life related to DRD

Each of the DRD Staging Project working groups undertook structured literature reviews to map the landscape of variables that are useful, promising, or have potential for inclusion in an updated staging system and severity scale (Fig. 21). The DRD Staging Project working groups will deliver their recommendations for an updated system and scale, along with outlining needs for additional research, in a series of reviews that are currently in preparation. Promising and potential variables identified as needing additional validation by this project will inform the Restoring Vision Moonshot's support for such gap-filling research as financial resources become available. To accelerate inclusion of promising and potential variables in clinical trial protocols, the Moonshot will leverage its relationships with established clinical research networks to develop validated endpoints for clinical research and practice.

3.8. The Restoring Vision Moonshot ocular biorepository

Better understanding of human disease and acceleration of target identification and development of new therapeutics for DRD will be enhanced by the ability to study human tissue (Eisma et al., 2015). Further, the research opportunities that can be generated by a human tissue resource can become a catalyst for building a community of shared interest among a diverse group of investigators and a focal point for novel collaborations (Quinn et al., 2021). Recognizing this critical role of global access to human tissue with real-time data-sharing among collaborating researchers, the first of the Restoring Vision Moonshot's accelerators to be launched is an Ocular Biorepository. This biorepository is being established at the Elizabeth Caswell Diabetes Institute (CDI) of the University of Michigan under the scientific direction of Patrice Fort and in association with the JDRF Center of Excellence there. Dr. Fort has developed a dual approach to human sample analysis using: a) vitreous obtained from patients in the clinic with accompanying medical and ocular histories; and b) post-mortem retinal and vitreous tissues recovered in a standardized fashion. This approach enables a high-quality assessment of the disease state through molecular and cellular analyses extending beyond traditional histopathology (Fort et al., 2021).

We contend that access to human tissues can benefit our understanding of the pathogenesis of a human disease at the cellular and molecular level, facilitate biomarker identification, inform us on the formation of disease associated complications, and support the design of innovative therapies capable of providing patient benefit. In type 1 diabetes, this approach has been substantiated through data emanating from the many biorepositories and sample distribution efforts that have, over the last two decades, been created by the community to address such issues. Indeed, efforts like the NIH sponsored TrialNet (www.trialnet.org/our-research) (serum, plasma, and peripheral blood mononuclear cells), Integrated Islet Distribution Program (iidp.coh.org) (data from islets), and the Human Islet Research Network (hirnetwork.org) (data from islets and immune cell studies) have led to dramatic improvements in our understanding of type 1 diabetes associated autoimmunity and beta cell biology. In terms of the pathogenesis of type 1 diabetes, perhaps the most pronounced knowledge gains (Atkinson et al., 2020; Battaglia et al., 2017; Hart and Powers, 2019; Kaestner et al., 2021) have occurred through studies resulting from the Network for Pancreatic Organ donors with Diabetes (nPOD) program, upon which the Restoring Vision Moonshot's ocular biorepository is modeled. The nPOD was founded in 2007 with support from the JDRF and The Leona M. and Harry B. Helmsley Charitable Trust. nPOD organ donors with type 1

Table 4
Working group areas and members.

Vascular Retina

Lead:
Tien Wong, M.D., Ph.D., Singapore Eye Research Institute, Singapore, Singapore National Eye Centre, Singapore and Duke-National University of Singapore Medical School, Singapore
Members:
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Lee Jampol, M.D., Department of Ophthalmology, Feinberg School of Medicine, Northwestern University, Chicago, IL
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Ramin Tadayoni, M.D., Ophthalmology Department, AP-HP, Hôpital Lariboisière, Université de Paris, Paris, France
Tien-En Tan, MBBS (Hons), MMed (Ophth), FRCOphth, Singapore Eye Research Institute, Singapore, Singapore National Eye Centre, Singapore and Duke-National University of Singapore Medical School, Singapore

Neural Retina
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Members:
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Christine Curcio, Ph.D., FARVO, White-McKee Endowed Professor, Department of Ophthalmology and Visual Sciences, University of Alabama at Birmingham School of Medicine
Roomasa Channa, M.D., Assistant Professor, Department of Ophthalmology and Visual Sciences, University of Wisconsin-Madison, WI
Patrice Fort, Ph.D., Associate Professor of the departments of Ophthalmology and Visual Sciences, and Molecular and Integrative Physiology; Director of the Vision Research Training Program (NEI-T32) of the University of Michigan Kellogg Eye Center, Ann Arbor, MI
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Rafael Simó, M.D., Ph.D., Professor of Medicine & Endocrinology, Head of Endocrinology & Nutrition Department, Vall d'Hebron University Hospital; Head, Diabetes Research and Metabolism Unit, Institut de Recerca Hospital Universitari Vall d'Hebron, Barcelona, Spain
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Risa Wolf, M.D., Assistant Professor, Division of Pediatric Endocrinology, Johns Hopkins Hospital, Baltimore, MD

Basic and cellular mechanisms
Lead:
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David Antonetti, Ph.D., Department of Ophthalmology and Visual Sciences, Kellogg Eye Center, University of Michigan Medical School, Ann Arbor, MI;

Table 4 (*continued*)

Vascular Retina

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Mary Elizabeth Hartnett, M.D., FACS, FARVO, Distinguished Professor Calvin S and JeNeal N. Hatch Presidential Endowed Chair in Ophthalmology and Visual Sciences, Vitreoretinal Medical and Surgical Service, John A. Moran Eye Center, Salt Lake City, UT
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Anita Jeyam, M.D., Institute of Genetics and Cancer, University of Edinburgh, Edinburgh, Scotland

Visual function
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(*continued on next page*)

Table 4 (*continued*)

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Members:
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Ecosse Lamoureux, Ph.D., FARVO, Duke-NUS Medical School, Singapore, Singapore Eye Research Institute, Singapore
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diabetes, those at increased risk for the disease (autoantibody positive individuals), and about 600 persons without the disease have been investigated using a variety of techniques designed to address a number of fundamental questions. Indeed, nPOD currently supports approximately 300 projects by investigators in over 20 countries. Importantly, the results of investigating nPOD tissues have been quite dramatic in terms of addressing both knowledge voids and overturning common "dogmas" associated with the disease (Atkinson et al., 2020). Specifically, studies of human tissues afforded by nPOD have identified a series of key immunological and endocrine differences (Y. G. Chen, Mathews and Driver, 2018; Peters et al., 2019) between type 1 diabetes as it appears in humans versus the prototype animal model, the NOD mouse. These include, but are not limited to, quantitative and qualitative differences in insulitis (Campbell-Thompson et al., 2016; In't Veld, 2014) and the ability for cellular replication of insulin producing beta cells (Saunders and Powers, 2016; Sharma et al., 2021). Such disparities, over time, have contributed to our ability to prevent/reverse diabetes in animal models while efforts to do so in humans remain unsuccessful. Beyond these observations is new appreciation of the extent of disease heterogeneity. Investigations of nPOD tissues have uncovered a pronounced degree of variation in terms of their pathologies, providing support for the emerging concept of disease "endotypes" (Battaglia et al., 2020); i.e, not all type 1 diabetes is the same. Perhaps most importantly, nPOD has provided a scientific basis for argument that type 1 diabetes may not result solely from autoimmunity against beta cells but that the endocrine cells may, themselves, contribute to their own demise (Atkinson et al., 2011). Prior to the development of this important tissue bank, such thoughts were not widely considered but now are considered essential to understanding the pathogenesis of this disorder.

In standing up its Ocular Biorepository the Restoring Vision Moonshot has adopted the nPOD's three main strategic goals, paraphrased as follows (Pugliese et al., 2014):

1. Obtain specimens from organ donors with and without diabetes and with and without DRD and establish a research resource;
2. Distribute specimens to Restoring Vision Moonshot approved investigators, anywhere in the world, for comprehensive and diversified investigations of human DRD
3. Promote collaborations by using tissue and real-time data-sharing; and developing and managing synergistic project interactions as well as focused working groups, all to facilitate a more complete understanding of human DRD.

The Restoring Vision Moonshot leadership believes that intellectual advances, similar in importance to those of the nPOD can be made through its development of a tissue bank dedicated to studies of diabetic retinal disease which serves as a core for global collaborations sharing data in real-time.

3.9. Science with a mission needs a "roadmap"

A great challenge of mission-driven science, such as that proposed by the Restoring Vision Moonshot, is to clearly state goals consistent with achieving the mission without being prescriptive from the top down in how the related problems will be solved, including in areas for which there remain significant gaps in fundamental knowledge. As with NASA's approach to the moon-mission, the Restoring Vision Moonshot will encourage bottom-up exploration of basic science and translational

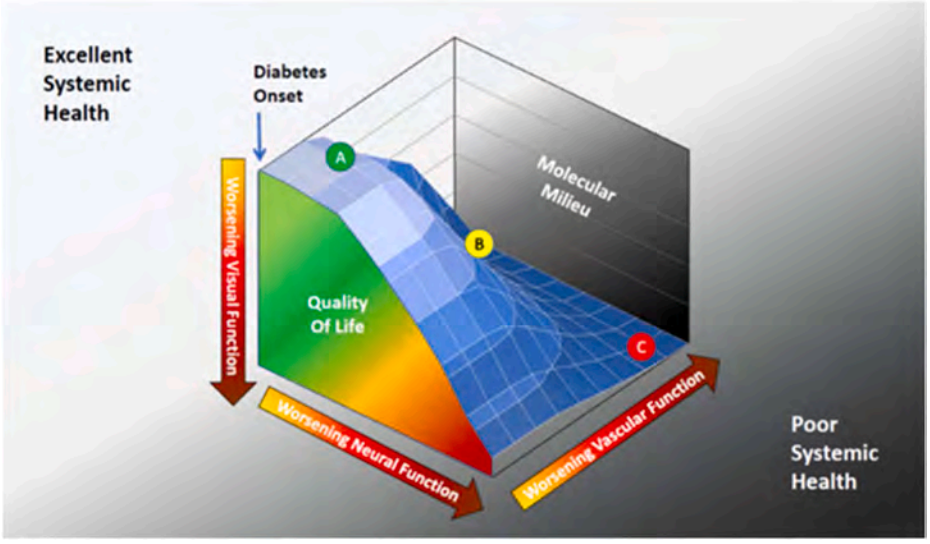

Fig. 21. An updated staging system should incorporate variables reflecting on the state of the neural as well as the vascular retina; the molecular milieu; visual function; and patient reported quality of life and function; and acknowledge relationship to systemic health and access to screening, general, and specialist health care services (J. K. Sun et al., 2021).

gaps aided by Moonshot accelerators. It is through collaborative engagement and the initiative and ingenuity of individual researchers and research teams that the means of mission success will emerge. To facilitate collaboration and establish a framework for programmatic activity, the Restoring Vision Moonshot will utilize a process of science road-mapping, reflecting approaches that have been deployed by NASA, in industry, and elsewhere.

Former Motorola CEO Robert Galvin described "Science Roadmaps" in an editorial in *Science* Magazine in 1998 (Galvin, 1998), as follows:

> "A 'roadmap' is an extended look at the future of a chosen field of inquiry composed from the collective knowledge and imagination of the brightest drivers of change in that field. Roadmaps can comprise statements of theories and trends, the formulation of models, identification of linkages among and within sciences, identification of discontinuities and knowledge voids, and interpretation of investigations and experiments. Roadmaps can also include the identification of instruments needed to solve problems, as well as graphs, charts, and showstoppers.
>
> The optimal process for gathering and selecting the content of roadmaps is to include as many practicing professionals as possible in workshops periodically in order to allow all suggestions to be considered and to objectively evaluate the consensuses that will more often than not emerge. Equal treatment should be given to minority views and individual advocacies.
>
> Roadmaps communicate visions, attract resources from business and government, stimulate investigations, and monitor progress. They become the inventory of possibilities for a particular field, thus stimulating earlier, more targeted investigations. They facilitate more interdisciplinary networking and teamed pursuit. Even "white spaces" can conjure promising investigations. NASA has used roadmaps built on basic themes for years and encourages others to do the same".

The purpose of a science mapping process is not to deliver a two-dimensional representation of "a map" that might be made attractive with interesting graphics or narrative, but be obsolete the day it's published. It is about harnessing the dynamic that's created through the interaction of diverse expertise and voices, the exchange of ideas, the cacophony of conflicting opinion and points of view–all key elements of innovation and progress.

Further, science mapping is about recognizing the need to develop new models to capture the promise of scientific advancement and charting a path to translating advances into often multifactorial solutions to complex problems. It is also about managing the added complexity of bringing together diverse expertise from multiple disciplines and multiple institutions, and focusing their creativity on achieving shared ultimate goals. The Restoring Vision Moonshot will establish mission-oriented research networks to utilize the shared resources provided through its accelerator programs, enhance collaborative communications and data sharing, map research opportunities and needs against goals, and, as financial resources become available, support ground breaking research directed to key basic and translational milestones associated with preservation, restoration, and protection of visual function in people with diabetes, including:

1. Elucidate metabolic context, mechanisms, timing, and progression of retinal neuronal dysfunction in diabetes.
2. Elucidate metabolic context, mechanisms, timing, and progression of retinal vascular drop-out and dysfunction in diabetes.
3. Arrest pathologic progression and protect from further damage neuronal function damaged by deleterious effects of diabetes including retinal neuronal damage from DME.
4. Arrest pathologic progression and protect from further damage vascular integrity and function damaged by deleterious effects of diabetes and revascularize diabetic retinas with healthy vessels, where therapeutically indicated.
5. Induce self-repair of damaged retina to restore normal visual function, including repair of peripheral retina damaged by disease and/or PRP.
6. Partial or full replacement of retina in end-stage DRD with ex vivo generated neo-retina "patch" (including peripheral retina damaged by disease and/or by PRP)(Bharti, 2018).

In the context of the Moonshot efforts to develop methods to preserve and restore visual function in people with DRD, it is important to acknowledge that current "gold standards" of treatment—PRP and intraocular anti-VEGF therapy—are not without their limitations. Panretinal photocoagulation reduces peripheral visual field sensitivity, visual acuity, night vision and contrast sensitivity (Fong et al., 2007). Bavinger et al. (2016) using high resolution OCT imaging found modest thinning of the RGC and inner plexiform layers, and pigment epithelium after PRP. Anti-VEGF therapy has as much as a 40% rate of suboptimal responses (less than 5 letters gained) for DME {Gonzalez, 2016 #19466; Walsh and Gallemore (2021). Balancing the clinical imperatives of using available, though imperfect, therapeutic modalities with the need to identify approaches which might be more accessible, less costly, applicable at earlier stages of disease, and more efficacious in preserving and restoring visual function will likely direct the Restoring Vision Moonshot to focus its first translational efforts on improving visual function and quality of life in people with reduced vision following treatment for DME and PDR, including persons whose retinas have been damaged by PRP and who have failed treatment with intra-ocular anti-VEGF treatment (Wallsh & Gallemore, 2021).

4. Future directions and conclusions

Prevention and treatment of diseases continually evolves with better understanding of the underlying pathophysiology. Notable examples include the recognition of peptic ulcer disease as consequence of *Heliobacter pylori* infection, not stress or spicy foods; somatic mutations as a cause for leukemias, lymphomas and cancers of the breast, colon, and melanomas; identification of the role of VEGF in ocular angiogenesis; and, recognizing the importance of dental hygiene to prevent tooth loss and reduce risk of cardiovascular disease. Diabetic retinal disease is more complex than simply glucose-induced microvascular disease {Antonetti et al., 2006 #2390} given the host of systemic metabolic and other factors that impact the retina with its multiple cell types, along with the difficulty in evaluating its pathophysiology in humans. We propose that a combination of structural and functional assessments and the development of safe methods for routine human eye "biopsies" via the vitreous fluid will enable clinicians to better assess severity of disease and risk for progression and design more personalized therapeutic plans, thereby better enabling patients to maintain good vision and vision-related quality of life. We further audaciously propose it is important to *restore* vision in persons who have late stage DRD, as is now possible for inherited photoreceptor degenerations. The quality of vision and quality of life of the patient must be considered as the primary determinant of success. The role of ophthalmologists will likely evolve from treating advanced vision-threatening PDR and DME ("retinal failure") (Gray and Gardner, 2015) with surgical approaches, to a future in which we can focus on early detection and intervention to arrest progression of DRD for most of the millions of affected and at risk patients, as nephrologists now are able to do to prevent/forestall end-stage renal failure (Limonte et al., 2020; Pan et al., 2021). In this future world, ophthalmologists will also likely perform patient-specific treatments to repair, rejuvenate, or replace damaged retinas, for those patients with low vision or blindness (thereby curing DRD). This progress will result from recognizing the patient and science needs along with the research and development opportunities.

This is also a call to action to coalesce all stakeholders – patients,

funders, drug and device makers, regulators and payers – globally, to address a major healthcare gap that we are confident is surmountable with a unified "vision". The coalition is necessary not only to expand resources and reach, but also to galvanize a multidisciplinary approach that requires the seamless yet imperative collaborations between ophthalmologists and other disciplines due to the systemic influences causing and exacerbating DRD. Another critical element will be the development and implementation of education campaigns to create awareness, seek out-of-the-box solutions, stay abreast of current and emerging standards of care, and aspire for the holy grail of eliminating DRD. Like U.S. President John F. Kennedy's original Moonshot challenge, the Restoring Vision Moonshot will bring together the talent and resources to achieve the goal of eliminating blindness and low vision from diabetes.

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References

Abcouwer, S.F., Gardner, T.W., 2014. Diabetic retinopathy: loss of neuroretinal adaptation to the diabetic metabolic environment. Ann. N. Y. Acad. Sci. 1311, 174–190. https://doi.org/10.1111/nyas.12412.

Abramoff, M.D., Fort, P.E., Han, I.C., Jayasundera, K.T., Sohn, E.H., Gardner, T.W., 2018. Approach for a clinically useful comprehensive classification of vascular and neural aspects of diabetic retinal disease. Invest. Ophthalmol. Vis. Sci. 59 (1), 519–527. https://doi.org/10.1167/iovs.17-21873.

Adamis, A.P., Miller, J.W., Bernal, M.T., D'Amico, D.J., Folkman, J., Yeo, T.K., Yeo, K.T., 1994. Increased vascular endothelial growth factor levels in the vitreous of eyes with proliferative diabetic retinopathy. Am. J. Ophthalmol. 118 (4), 445–450. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=7943121.

Afshinnia, F., Rajendiran, T.M., He, C., Byun, J., Montemayor, D., Darshi, M., et al., 2021. Circulating free fatty acid and phospholipid signature predicts early rapid kidney function decline in patients with type 1 diabetes. Diabetes Care 44 (9), 2098–2106. https://doi.org/10.2337/dc21-0737.

Agardh, E., Stjernquist, H., Heijl, A., Bengtsson, B., 2006. Visual acuity and perimetry as measures of visual function in diabetic macular oedema. Diabetologia 49 (1), 200–206. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16341838.

Ahlqvist, E., Storm, P., Karajamaki, A., Martinell, M., Dorkhan, M., Carlsson, A., et al., 2018. Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. Lancet Diabetes Endocrinol. 6 (5), 361–369. https://doi.org/10.1016/S2213-8587(18)30051-2.

Ahlqvist, E., Prasad, R.B., Groop, L., 2020. Subtypes of type 2 diabetes determined from clinical parameters. Diabetes 69 (10), 2086–2093. https://doi.org/10.2337/dbi20-0001.

Ahmad, I., Teotia, P., Erickson, H., Xia, X., 2020. Recapitulating developmental mechanisms for retinal regeneration. Prog. Retin. Eye Res. 76, 100824. https://doi.org/10.1016/j.preteyeres.2019.100824.

Aiello, L.P., Avery, R.L., Arrigg, P.G., Keyt, B.A., Jampel, H.D., Shah, S.T., et al., 1994. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. N. Engl. J. Med. 331 (22), 1480–1487. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=7526212.

Anonymous, 1991. Grading diabetic retinopathy from stereoscopic color fundus photographs–an extension of the modified Airlie House classification. ETDRS report number 10. Early Treatment Diabetic Retinopathy Study Research Group. Ophthalmology 98 (5 Suppl), 786–806.

Anonymous, 1993. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. N. Engl. J. Med. 329 (14), 977–986. https://doi.org/10.1056/NEJM199309303291401.

Antonetti, D.A., Barber, A.J., Khin, S., Lieth, E., Tarbell, J.M., Gardner, T.W., Group, P.S. R.R., 1998. Vascular permeability in experimental diabetes is associated with reduced endothelial occludin content: vascular endothelial growth factor decreases occludin in retinal endothelial cells. Diabetes 47 (12), 1953–1959.

Antonetti, D.A., Barber, A.J., Bronson, S.K., Freeman, W.M., Gardner, T.W., Jefferson, L. S., et al., 2006. Diabetic retinopathy: seeing beyond glucose-induced microvascular disease. Diabetes 55 (9), 2401–2411. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16936187.

Antonetti, D.A., Klein, R., Gardner, T.W., 2012. Diabetic retinopathy. N. Engl. J. Med. 366 (13), 1227–1239. https://doi.org/10.1056/NEJMra1005073.

Antonetti, D.A., Silva, P.S., Stitt, A.W., 2021. Current understanding of the molecular and cellular pathology of diabetic retinopathy. Nat. Rev. Endocrinol. https://doi.org/10.1038/s41574-020-00451-4.

Atkinson, M.A., Nierras, C.R., 2017. Mary Tyler Moore (1936-2017): diabetes educator and advocate. Diabetes Care 40 (6), 732–735. https://doi.org/10.2337/dci17-0015.

Atkinson, M.A., Bluestone, J.A., Eisenbarth, G.S., Hebrok, M., Herold, K.C., Accili, D., et al., 2011. How does type 1 diabetes develop?: the notion of homicide or beta-cell suicide revisited. Diabetes 60 (5), 1370–1379. https://doi.org/10.2337/db10-1797.

Atkinson, M.A., Campbell-Thompson, M., Kusmartseva, I., Kaestner, K.H., 2020. Organisation of the human pancreas in health and in diabetes. Diabetologia 63 (10), 1966–1973. https://doi.org/10.1007/s00125-020-05203-7.

Aung, M.H., Kim, M.K., Olson, D.E., Thule, P.M., Pardue, M.T., 2013. Early visual deficits in streptozotocin-induced diabetic long evans rats. Invest. Ophthalmol. Vis. Sci. 54 (2), 1370–1377. https://doi.org/10.1167/iovs.12-10927.

Aung, M.H., Park, H.N., Han, M.K., Obertone, T.S., Abey, J., Aseem, F., et al., 2014. Dopamine deficiency contributes to early visual dysfunction in a rodent model of type 1 diabetes. J. Neurosci. 34 (3), 726–736. https://doi.org/10.1523/JNEUROSCI.3483-13.2014.

Bainbridge, J.W., Mehat, M.S., Sundaram, V., Robbie, S.J., Barker, S.E., Ripamonti, C., et al., 2015. Long-term effect of gene therapy on Leber's congenital amaurosis. N. Engl. J. Med. 372 (20), 1887–1897. https://doi.org/10.1056/NEJMoa1414221.

Bao, Y.K., Yan, Y., Gordon, M., McGill, J.B., Kass, M., Rajagopal, R., 2019. Visual field loss in patients with diabetes in the absence of clinically-detectable vascular retinopathy in a nationally representative survey. Invest. Ophthalmol. Vis. Sci. 60 (14), 4711–4716. https://doi.org/10.1167/iovs.19-28063.

Barber, A.J., Lieth, E., Khin, S.A., Antonetti, D.A., Buchanan, A.G., Gardner, T.W., 1998. Neural apoptosis in the retina during experimental and human diabetes. Early onset and effect of insulin. J. Clin. Invest. 102 (4), 783–791.

Barber, A.J., Antonetti, D.A., Gardner, T.W., 2000. Altered expression of retinal occludin and glial fibrillary acidic protein in experimental diabetes. The Penn State Retina Research Group. Investigat. Ophthalmol. Vis. Sci. 41 (11), 3561–3568.

Bartram, C.R., de Klein, A., Hagemeijer, A., van Agthoven, T., Geurts van Kessel, A., Bootsma, D., et al., 1983. Translocation of c-ab1 oncogene correlates with the presence of a Philadelphia chromosome in chronic myelocytic leukaemia. Nature 306 (5940), 277–280. https://doi.org/10.1038/306277a0.

Battaglia, M., Anderson, M.S., Buckner, J.H., Geyer, S.M., Gottlieb, P.A., Kay, T.W.H., et al., 2017. Understanding and preventing type 1 diabetes through the unique working model of TrialNet. Diabetologia 60 (11), 2139–2147. https://doi.org/10.1007/s00125-017-4384-2.

Battaglia, M., Ahmed, S., Anderson, M.S., Atkinson, M.A., Becker, D., Bingley, P.J., et al., 2020. Introducing the endotype concept to address the challenge of disease heterogeneity in type 1 diabetes. Diabetes Care 43 (1), 5–12. https://doi.org/10.2337/dc19-0880.

Bavinger, J.C., Dunbar, G.E., Stem, M.S., Blachley, T.S., Kwark, L., Farsiu, S., et al., 2016. The effects of diabetic retinopathy and pan-retinal photocoagulation on photoreceptor cell function as assessed by dark adaptometry. Invest. Ophthalmol. Vis. Sci. 57 (1), 208–217. https://doi.org/10.1167/iovs.15-17281.

Bearse Jr., M.A., Han, Y., Schneck, M.E., Barez, S., Jacobsen, C., Adams, A.J., 2004. Local multifocal oscillatory potential abnormalities in diabetes and early diabetic retinopathy. Invest. Ophthalmol. Vis. Sci. 45 (9), 3259–3265. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15326149.

Becker, K., Klein, H., Simon, E., Viollet, C., Haslinger, C., Leparc, G., et al., 2021. In-depth transcriptomic analysis of human retina reveals molecular mechanisms underlying diabetic retinopathy. Sci. Rep. 11 (1), 10494. https://doi.org/10.1038/s41598-021-88698-3.

Behar-Cohen, F., Zhao, M., 2021. Mineralocorticoid pathway in retinal health and diseases. Br. J. Pharmacol. https://doi.org/10.1111/bph.15770.

Bek, T., 1997. Immunohistochemical characterization of retinal glial cell changes in areas of vascular occlusion secondary to diabetic retinopathy. Acta Ophthalmol. Scand. 75 (4), 388–392.

Bengtsson, B., Heijl, A., Agardh, E., 2005. Visual fields correlate better than visual acuity to severity of diabetic retinopathy. Diabetologia 48 (12), 2494–2500. https://doi.org/10.1007/s00125-005-0001-x.

Berdugo, M., Delaunay, K., Naud, M.C., Guegan, J., Moulin, A., Savoldelli, M., et al., 2021. The antidiabetic drug glibenclamide exerts direct retinal neuroprotection. Transl. Res. 229, 83–99. https://doi.org/10.1016/j.trsl.2020.10.003.

Bharti, K., 2018. Patching the retina with stem cells. Nat. Biotechnol. 36 (4), 311–313. https://doi.org/10.1038/nbt.4118.

Bien, A., Seidenbecher, C.I., Bockers, T.M., Sabel, B.A., Kreutz, M.R., 1999. Apoptotic versus necrotic characteristics of retinal ganglion cell death after partial optic nerve injury. J. Neurotrauma 16 (2), 153–163. https://doi.org/10.1089/neu.1999.16.153.

Blindbaek, S.L., Peto, T., Grauslund, J., 2020. Alterations in retinal arteriolar microvascular structure associate with higher treatment burden in patients with diabetic macular oedema: results from a 12-month prospective clinical trial. Acta Ophthalmol. 98 (4), 353–359. https://doi.org/10.1111/aos.14278.

Bloodworth, J.M.B., 1962. Diabetic retinopathy. Diabetes 2, 1–22.

Bresnick, G.H., 1986. Diabetic retinopathy viewed as a neurosensory disorder. Arch. Ophthalmol. 104 (7), 989–990.

Bresnick, G.H., Palta, M., 1987. Temporal aspects of the electroretinogram in diabetic retinopathy. Arch. Ophthalmol. 105 (5), 660–664. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=3619742.

Bresnick, G.H., Korth, K., Groo, A., Palta, M., 1984. Electroretinographic oscillatory potentials predict progression of diabetic retinopathy. Preliminary report. Arch. Ophthalmol. 102 (9), 1307–1311. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=6383303.

Brigell, M.G., Chiang, B., Maa, A.Y., Davis, C.Q., 2020. Enhancing risk assessment in patients with diabetic retinopathy by combining measures of retinal function and structure. Transl. Vis. Sci. Technol. 9 (9), 40. https://doi.org/10.1167/tvst.9.9.40.

Callaghan, B.C., Little, A.A., Feldman, E.L., Hughes, R.A., 2012. Enhanced glucose control for preventing and treating diabetic neuropathy. Cochrane Database Syst. Rev. 6, CD007543. https://doi.org/10.1002/14651858.CD007543.pub2.

Campbell-Thompson, M., Fu, A., Kaddis, J.S., Wasserfall, C., Schatz, D.A., Pugliese, A., Atkinson, M.A., 2016. Insulitis and beta-cell mass in the natural history of type 1 diabetes. Diabetes 65 (3), 719–731. https://doi.org/10.2337/db15-0779.

Caprioli, J., de Leon, J.M., Azarbod, P., Chen, A., Morales, E., Nouri-Mahdavi, K., et al., 2016. Trabeculectomy can improve long-term visual function in glaucoma. Ophthalmology 123 (1), 117–128. https://doi.org/10.1016/j.ophtha.2015.09.027.

Caputo, S., Di Leo, M.A., Falsini, B., Ghirlanda, G., Porciatti, V., Minella, A., Greco, A.V., 1990. Evidence for early impairment of macular function with pattern ERG in type I diabetic patients. Diabetes Care 13 (4), 412–418. https://care.diabetesjournals.org/content/diacare/25/9/1661.full.pdf.

Care, R.A., Anastassov, I.A., Kastner, D.B., Kuo, Y.M., Della Santina, L., Dunn, F.A., 2020. Mature retina compensates functionally for partial loss of rod photoreceptors. Cell Rep. 31 (10), 107730. https://doi.org/10.1016/j.celrep.2020.107730.

Castilho, A., Ambrosio, A.F., Hartveit, E., Veruki, M.L., 2015. Disruption of a neural microcircuit in the rod pathway of the mammalian retina by diabetes mellitus. J. Neurosci. 35 (13), 5422–5433. https://doi.org/10.1523/JNEUROSCI.5285-14.2015.

Catalani, E., Cervia, D., 2020. Diabetic retinopathy: a matter of retinal ganglion cell homeostasis. Neural Regen. Res. 15 (7), 1253–1254. https://doi.org/10.4103/1673-5374.272577.

Cerani, A., Tetreault, N., Menard, C., Lapalme, E., Patel, C., Sitaras, N., et al., 2013. Neuron-derived semaphorin 3A is an early inducer of vascular permeability in diabetic retinopathy via neuropilin-1. Cell Metabol. 18 (4), 505–518. https://doi.org/10.1016/j.cmet.2013.09.003.

Cesareo, M., Giannini, C., Di Marino, M., Aloe, G., Martucci, A., Aiello, F., et al., 2021. Optical coherence tomography angiography in the multimodal assessment of the retinal posterior pole in autosomal dominant optic atrophy. Acta Ophthalmol. https://doi.org/10.1111/aos.14972.

Channa, R., Lee, K., Staggers, K.A., Mehta, N., Zafar, S., Gao, J., et al., 2021a. Detecting retinal neurodegeneration in people with diabetes: findings from the UK Biobank. PLoS One 16 (9), e0257836. https://doi.org/10.1371/journal.pone.0257836.

Channa, R., Wolf, R., Abramoff, M.D., 2021b. Autonomous artificial intelligence in diabetic retinopathy: from algorithm to clinical application. J. Diabetes Sci. Technol. 15 (3), 695–698. https://doi.org/10.1177/1932296820909900.

Chee, C.K., Flanagan, D.W., 1993. Visual field loss with capillary non-perfusion in preproliferative and early proliferative diabetic retinopathy. Br. J. Ophthalmol. 77 (11), 726–730. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=8280688.

Chen, Y.G., Mathews, C.E., Driver, J.P., 2018. The role of NOD mice in type 1 diabetes research: lessons from the past and recommendations for the future. Front. Endocrinol. 9, 51. https://doi.org/10.3389/fendo.2018.00051.

Chen, W.-Y., Han, X., Cui, L.-J., Yu, C.-X., Sheng, W.-L., Yu, J., et al., 2021. Cell-subtype-specific remodeling of intrinsically photosensitive retinal ganglion cells in streptozotocin-induced diabetic mice. Diabetes.

Chihara, E., 1981. Impairment of protein synthesis in the retinal tissue in diabetic rabbits: secondary reduction of fast axonal transport. J. Neurochem. 37 (1), 247–250.

Chihara, E., Sakugawa, M., Entani, S., 1982. Reduced protein synthesis in diabetic retina and secondary reduction of slow axonal transport. Brain Res. 250 (2), 363–366.

Chihara, E., Matsuoka, T., Ogura, Y., Matsumura, M., 1993. Retinal nerve fiber layer defect as an early manifestation of diabetic retinopathy. Ophthalmology 100 (8), 1147–1151.

Chinskey, N.D., Zheng, Q.D., Zacks, D.N., 2014. Control of photoreceptor autophagy after retinal detachment: the switch from survival to death. Invest. Ophthalmol. Vis. Sci. 55 (2), 688–695. https://doi.org/10.1167/iovs.13-12951.

Choi, W., Waheed, N.K., Moult, E.M., Adhi, M., Lee, B., De Carlo, T., et al., 2017. Ultrahigh speed swept source optical coherence tomography angiography of retinal and choriocapillaris alterations in diabetic patients with and without retinopathy. Retina 37 (1), 11–21. https://doi.org/10.1097/IAE.0000000000001250.

Chou, T.H., Porciatti, V., 2020. Adaptable retinal ganglion cell function: assessing autoregulation of inner retina pathways. Neural Regen. Res. 15 (12), 2237–2238. https://doi.org/10.4103/1673-5374.284984.

Chua, S.Y.L., Thomas, D., Allen, N., Lotery, A., Desai, P., Patel, P., et al., 2019. Cohort profile: design and methods in the eye and vision consortium of UK Biobank. BMJ Open 9 (2), e025077. https://doi.org/10.1136/bmjopen-2018-025077.

Cogan, D.G., Toussaint, D., Kuwabara, T., 1961. Retinal vascular patterns. IV. Diabetic retinopathy. Arch. Ophthalmol. 66, 366–378.

Conway, P.T., Knight, R., 2021. Legitimization and incorporation of patient preferences: the arrow that hit the achilles heel of status quo kidney care. Clin. J. Am. Soc. Nephrol. 16 (4), 645–647. https://doi.org/10.2215/CJN.11780720.

Cree-Green, M., Stuppy, J.J., Thurston, J., Bergman, B.C., Coe, G.V., Baumgartner, A.D., et al., 2018. Youth with type 1 diabetes have adipose, hepatic, and peripheral insulin resistance. J. Clin. Endocrinol. Metab. 103 (10), 3647–3657. https://doi.org/10.1210/jc.2018-00433.

Cui, R.Z., Wang, L., Qiao, S.N., Wang, Y.C., Wang, X., Yuan, F., et al., 2019. ON-type retinal ganglion cells are preferentially affected in STZ-induced diabetic mice. Invest. Ophthalmol. Vis. Sci. 60 (5), 1644–1656. https://doi.org/10.1167/iovs.18-26359.

Curcio, C.A., McGwin Jr., G., Sadda, S.R., Hu, Z., Clark, M.E., Sloan, K.R., et al., 2020. Functionally validated imaging endpoints in the Alabama study on early age-related macular degeneration 2 (ALSTAR2): design and methods. BMC Ophthalmol. 20 (1), 196. https://doi.org/10.1186/s12886-020-01467-0.

Dabelea, D., Stafford, J.M., Mayer-Davis, E.J., D'Agostino Jr., R., Dolan, L., Imperatore, G., et al., 2017. Association of type 1 diabetes vs type 2 diabetes diagnosed during childhood and adolescence with complications during teenage years and young adulthood. JAMA 317 (8), 825–835. https://doi.org/10.1001/jama.2017.0686.

Daruich, A., Matet, A., Moulin, A., Kowalczuk, L., Nicolas, M., Sellam, A., et al., 2018. Mechanisms of macular edema: beyond the surface. Prog. Retin. Eye Res. 63, 20–68. https://doi.org/10.1016/j.preteyeres.2017.10.006.

de Carlo, T.E., Chin, A.T., Bonini Filho, M.A., Adhi, M., Branchini, L., Salz, D.A., et al., 2015. Detection of microvascular changes in eyes of patients with diabetes but not clinical diabetic retinopathy using optical coherence tomography angiography. Retina 35 (11), 2364–2370. https://doi.org/10.1097/IAE.0000000000000882.

De Venecia, G., Davis, M., Engerman, R., 1976. Clinicopathologic correlations in diabetic retinopathy. I. Histology and fluorescein angiography of microaneurysms. Arch. Ophthalmol. 94 (10), 1766–1773. Retrieved from. http://www.ncbi.nlm.nih.gov/pubmed/973822.

Dejda, A., Mawambo, G., Cerani, A., Miloudi, K., Shao, Z., Daudelin, J.F., et al., 2014. Neuropilin-1 mediates myeloid cell chemoattraction and influences retinal neuroimmune crosstalk. J. Clin. Invest. 124 (11), 4807–4822. https://doi.org/10.1172/JCI76492.

Diabetes, C., , Complications Trial/Epidemiology of Diabetes, I, Complications Research, G, Lachin, J.M., White, N.H., Hainsworth, D.P., et al., 2015. Effect of intensive diabetes therapy on the progression of diabetic retinopathy in patients with type 1 diabetes: 18 years of follow-up in the DCCT/EDIC. Diabetes 64 (2), 631–642. https://doi.org/10.2337/db14-0930.

Dickinson, W.H., 1875. Chapter II pathology. In: Diseases of the Kidney and Urinary Derangements [part 1. Diabetes]. Longmans, Green & Co, London, pp. 30–66.

Dreher, B., Robinson, S.R., 1988. Development of the retinofugal pathway in birds and mammals: evidence for a common 'timetable. Brain Behav. Evol. 31 (6), 369–390. https://doi.org/10.1159/000116602.

Dubois-Poulsen, W., 1952. Le Champ Visuel. Verlag, Masson, Paris, p. 654.

Duh, E.J., Sun, J.K., Stitt, A.W., 2017. Diabetic retinopathy: current understanding, mechanisms, and treatment strategies. JCI Insight 2 (14). https://doi.org/10.1172/jci.insight.93751.

Duke-Elder, S., Dobree, J.H., 1967. Diseases of the retina. In: Duke-Elder, S. (Ed.), System of Ophthalmology, X. C.V. Mosby, St. Louis, MO, pp. 408–448.

Edwards, M.M., McLeod, D.S., Li, R., Grebe, R., Bhutto, I., Mu, X., Lutty, G.A., 2012. The deletion of Math5 disrupts retinal blood vessel and glial development in mice. Exp. Eye Res. 96 (1), 147–156. https://doi.org/10.1016/j.exer.2011.12.005.

Eggers, E.D., Carreon, T.A., 2020. The effects of early diabetes on inner retinal neurons. Vis. Neurosci. 37, E006. https://doi.org/10.1017/S095252382000005X.

Eisma, J.H., Dulle, J.E., Fort, P.E., 2015. Current knowledge on diabetic retinopathy from human donor tissues. World J. Diabetes 6 (2), 312–320. https://doi.org/10.4239/wjd.v6.i2.312.

Ewing, F.M., Deary, I.J., Strachan, M.W., Frier, B.M., 1998. Seeing beyond retinopathy in diabetes: electrophysiological and psychophysical abnormalities and alterations in vision. Endocr. Rev. 19 (4), 462–476. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9715375.

Falsini, B., Porciatti, V., Scalia, G., Caputo, S., Minnella, A., Di Leo, M.A., Ghirlanda, G., 1989. Steady-state pattern electroretinogram in insulin-dependent diabetics with no or minimal retinopathy. Doc. Ophthalmol. 73 (2), 193–200. https://doi.org/10.1007/BF00155037.

Fickweiler, W., Wolfson, E.A., Paniagua, S.M., Yu, M.G., Adam, A., Bahnam, V., et al., 2021. Association of cognitive function and retinal neural and vascular structure in type 1 diabetes. J. Clin. Endocrinol. Metab. 106 (4), 1139–1149. https://doi.org/10.1210/clinem/dgaa921.

Fischer, F., 1989. The first case of diabetic retinopathy. In: von Englehardt, D. (Ed.), Diabetes: its Medical and Cultural History. Springer-Verlag, Berlin, pp. 363–369.

Flood, M.D., Wellington, A.J., Cruz, L.A., Eggers, E.D., 2020. Early diabetes impairs ON sustained ganglion cell light responses and adaptation without cell death or dopamine insensitivity. Exp. Eye Res. 200, 108223. https://doi.org/10.1016/j.exer.2020.108223.

Fong, D.S., Girach, A., Boney, A., 2007. Visual side effects of successful scatter laser photocoagulation surgery for proliferative diabetic retinopathy: a literature review. Retina 27 (7), 816–824. https://doi.org/10.1097/IAE.0b013e318042d32c.00006982-200709000-00003 [pii]).

Fort, P.E., Losiewicz, M.K., Reiter, C.E., Singh, R.S., Nakamura, M., Abcouwer, S.F., et al., 2011. Differential roles of hyperglycemia and hypoinsulinemia in diabetes induced retinal cell death: evidence for retinal insulin resistance. PLoS One 6 (10), e26498. https://doi.org/10.1371/journal.pone.0026498.

Fort, P.E., Losiewicz, M.K., Pennathur, S., Jefferson, L.S., Kimball, S.R., Abcouwer, S.F., Gardner, T.W., 2014. mTORC1-independent reduction of retinal protein synthesis in type 1 diabetes. Diabetes 63 (9), 3077–3090. https://doi.org/10.2337/db14-0235.

Fort, P.E., Rajendiran, T.M., Soni, T., Byun, J., Shan, Y., Looker, H.C., et al., 2021. Diminished retinal complex lipid synthesis and impaired fatty acid beta-oxidation associated with human diabetic retinopathy. JCI Insight. https://doi.org/10.1172/jci.insight.152109.

Frayser, R., Buse, M.G., 1978. Branched chain amino acid metabolism in the retina of diabetic rats. Diabetologia 14 (3), 171–176. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=658633.

Frost-Larsen, K., Larsen, H.W., Simonsen, S.E., 1981a. The value of dark-adaptation as a prognostic tool in diabetic retinopathy. Metab. Pediatr. Ophthalmol. 5 (1), 39–44. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=7253765.

Frost-Larsen, K., Larsen, H.W., Simonsen, S.E., 1981b. Value of electroretinography and dark adaptation as prognostic tools in diabetic retinopathy. Dev. Ophthalmol. 2, 222–234. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=7262405.

Fry, L.E., Fahy, E., Chrysostomou, V., Hui, F., Tang, J., van Wijngaarden, P., et al., 2018. The coma in glaucoma: retinal ganglion cell dysfunction and recovery. Prog. Retin. Eye Res. 65, 77–92. https://doi.org/10.1016/j.preteyeres.2018.04.001.

Gallego-Perez, D., Pal, D., Ghatak, S., Malkoc, V., Higuita-Castro, N., Gnyawali, S., et al., 2017. Topical tissue nano-transfection mediates non-viral stroma reprogramming and rescue. Nat. Nanotechnol. 12 (10), 974–979. https://doi.org/10.1038/nnano.2017.134.

Galvin, R., 1998. Science roadmaps. Science 280. Retrieved from. https://science-sciencemag-org.proxy.lib.umich.edu/content/280/5365/803.1.full.

Gao, B.B., Clermont, A., Rook, S., Fonda, S.J., Srinivasan, V.J., Wojtkowski, M., et al., 2007. Extracellular carbonic anhydrase mediates hemorrhagic retinal and cerebral vascular permeability through prekallikrein activation. Nat. Med. 13 (2), 181–188. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17259996.

Gardner, T.W., 1995. Histamine, ZO-1 and blood-retinal barrier permeability in diabetic retinopathy. Trans. Am. Ophthalmol. Soc. 93, 583–621.

Gardner, T.W., Davila, J.R., 2017. The neurovascular unit and the pathophysiologic basis of diabetic retinopathy. Graefes Arch. Clin. Exp. Ophthalmol. 255 (1), 1–6. https://doi.org/10.1007/s00417-016-3548-y.

Gardner, T.W., Sundstrom, J.M., 2017. A proposal for early and personalized treatment of diabetic retinopathy based on clinical pathophysiology and molecular phenotyping. Vision Res. 139, 153–160. https://doi.org/10.1016/j.visres.2017.03.006.

Gardner, T.W., Abcouwer, S.F., Barber, A.J., Jackson, G.R., 2011. An integrated approach to diabetic retinopathy research. Arch. Ophthalmol. 129 (2), 230–235. https://doi.org/10.1001/archophthalmol.2010.362.

Gariano, R.F., Gardner, T.W., 2005. Retinal angiogenesis in development and disease. Nature 438 (7070), 960–966. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16355161.

Gastinger, M.J., Barber, A.J., Khin, S.A., McRill, C.S., Gardner, T.W., Marshak, D.W., 2001. Abnormal centrifugal axons in streptozotocin-diabetic rat retinas. Investigat. Ophthalmol. Vis. Sci. 42 (11), 2679–2685.

Gastinger, M.J., Kunselman, A.R., Conboy, E.E., Bronson, S.K., Barber, A.J., 2008. Dendrite remodeling and other abnormalities in the retinal ganglion cells of Ins2 Akita diabetic mice. Invest. Ophthalmol. Vis. Sci. 49 (6), 2635–2642, 49/6/2635 [pii]10.1167/iovs.07-0683.

Ghirlanda, G., Di Leo, M.A., Caputo, S., Falsini, B., Porciatti, V., Marietti, G., Greco, A.V., 1991. Detection of inner retina dysfunction by steady-state focal electroretinogram pattern and flicker in early IDDM. Diabetes 40 (9), 1122–1127. https://doi.org/10.2337/diab.40.9.1122.

Ghirlanda, G., Di Leo, M.A., Caputo, S., Cercone, S., Greco, A.V., 1997. From functional to microvascular abnormalities in early diabetic retinopathy. Diabetes Metab. Rev. 13 (1), 15–35. Retrieved from. https://onlinelibrary.wiley.com/doi/abs/10.1002/%28SICI%291099-0895%28199703%2913%3A1%3C15%3A%3AAID-DMR176%3E3.0.CO%3B2-M?sid=nlm%3Apubmed.

Ghodasra, D.H., Fante, R., Gardner, T.W., Langue, M., Niziol, L.M., Besirli, C., et al., 2016. Safety and feasibility of quantitative multiplexed cytokine analysis from office-based vitreous aspiration. Invest. Ophthalmol. Vis. Sci. 57 (7), 3017–3023. https://doi.org/10.1167/iovs.15-18721.

Giannarelli, R., Coppelli, A., Sartini, M., Aragona, M., Boggi, U., Vistoli, F., et al., 2005. Effects of pancreas-kidney transplantation on diabetic retinopathy. Transpl. Int. 18 (5), 619–622. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15819813.

Gilbert, M.J., Sun, J.K., 2020. Artificial Intelligence in the assessment of diabetic retinopathy from fundus photographs. Semin. Ophthalmol. 35 (7–8), 325–332. https://doi.org/10.1080/08820538.2020.1855358.

Goldberg, M.F., Fine, S.L., 1969. Symposium on the Treatment of Diabetic Retinopathy. US Govt Printing Office, Washington, DC.

Goncalves, A., Dreffs, A., Lin, C.M., Sheskey, S., Hudson, N., Keil, J., et al., 2021. Vascular expression of permeability-resistant occludin mutant preserves visual function in diabetes. Diabetes 70 (7), 1549–1560. https://doi.org/10.2337/db20-1220.

Gottfredsdottir, M.S., Stefansson, E., Jonasson, F., Gislason, I., 1993. Retinal vasoconstriction after laser treatment for diabetic macular edema. Am. J. Ophthalmol. 115 (1), 64–67.

Gowda, K., Zinnanti, W.J., Lanoue, K.F., 2011. The influence of diabetes on glutamate metabolism in retinas. J. Neurochem. https://doi.org/10.1111/j.1471-4159.2011.07206.x.

Gray, E.J., Gardner, T.W., 2015. Retinal failure in diabetes: a feature of retinal sensory neuropathy. Curr. Diabetes Rep. 15 (12), 107. https://doi.org/10.1007/s11892-015-0683-5.

Greenbaum, C.J., 2002. Insulin resistance in type 1 diabetes. Diabetes/Metabol. Res. Rev. 18 (3), 192–200.

Grunwald, J.E., Brucker, A.J., Petrig, B.L., Riva, C.E., 1989. Retinal blood flow regulation and the clinical response to panretinal photocoagulation in proliferative diabetic retinopathy. Ophthalmology 96 (10), 1518–1522. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=2587047.

Grunwald, J.E., DuPont, J., Riva, C.E., 1996. Retinal haemodynamics in patients with early diabetes mellitus. Br. J. Ophthalmol. 80 (4), 327–331. https://doi.org/10.1136/bjo.80.4.327.

Grzybowski, A., Brona, P., Lim, G., Ruamviboonsuk, P., Tan, G.S.W., Abramoff, M., Ting, D.S.W., 2020. Artificial intelligence for diabetic retinopathy screening: a review. Eye 34 (3), 451–460. https://doi.org/10.1038/s41433-019-0566-0.

Haines, N.R., Manoharan, N., Olson, J.L., D'Alessandro, A., Reisz, J.A., 2018. Metabolomics analysis of human vitreous in diabetic retinopathy and rhegmatogenous retinal detachment. J. Proteome Res. 17 (7), 2421–2427. https://doi.org/10.1021/acs.jproteome.8b00169.

Harrison, W.W., Bearse Jr., M.A., Ng, J.S., Jewell, N.P., Barez, S., Burger, D., et al., 2011a. Multifocal electroretinograms predict onset of diabetic retinopathy in adult patients with diabetes. Investigat. Ophthalmol. Vis. Sci. 52 (2), 772–777. https://doi.org/10.1167/iovs.10-5931.

Harrison, W.W., Bearse Jr., M.A., Schneck, M.E., Wolff, B.E., Jewell, N.P., Barez, S., et al., 2011b. Prediction, by retinal location, of the onset of diabetic edema in patients with nonproliferative diabetic retinopathy. Investigat. Ophthalmol. Vis. Sci. 52 (9), 6825–6831. https://doi.org/10.1167/iovs.11-7533.

Hart, N.J., Powers, A.C., 2019. Use of human islets to understand islet biology and diabetes: progress, challenges and suggestions. Diabetologia 62 (2), 212–222. https://doi.org/10.1007/s00125-018-4772-2.

Hawkins, B.T., Davis, T.P., 2005. The blood-brain barrier/neurovascular unit in health and disease. Pharmacol. Rev. 57 (2), 173–185. https://doi.org/10.1124/pr.57.2.4.

Hebert, S.L., Nair, K.S., 2010. Protein and energy metabolism in type 1 diabetes. Clin. Nutr. 29 (1), 13–17. S0261-5614(09)00181-2 [pii]10.1016/j.clnu.2009.09.001.

Henderson, J.W., 1986. The University of Michigan Department of Ophthalmology; A Proud Heritage, p. 168.

Hietala, K., Forsblom, C., Summanen, P., Groop, P.H., FinnDiane Study, G., 2008. Heritability of proliferative diabetic retinopathy. Diabetes 57 (8), 2176–2180. https://doi.org/10.2337/db07-1495.

Hildebrand, G.D., Fielder, A.R., 2011. Anatomy and physiology of the retina. In: Reynolds, J., Olitsky, S. (Eds.), Pediatric Retina. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 39–65.

Holfort, S.K., Norgaard, K., Jackson, G.R., Hommel, E., Madsbad, S., Munch, I.C., et al., 2011. Retinal function in relation to improved glycaemic control in type 1 diabetes. Diabetologia 54 (7), 1853–1861. https://doi.org/10.1007/s00125-011-2149-x.

Hou, X.W., Wang, Y., Pan, C.W., 2021. Metabolomics in diabetic retinopathy: a systematic review. Invest. Ophthalmol. Vis. Sci. 62 (10), 4. https://doi.org/10.1167/iovs.62.10.4.

Hughes, S., Yang, H., Chan-Ling, T., 2000. Vascularization of the human fetal retina: roles of vasculogenesis and angiogenesis. Invest. Ophthalmol. Vis. Sci. 41 (5), 1217–1228. Retrieved from. https://www.ncbi.nlm.nih.gov/pubmed/10752963.

Hutson, S.M., Berkich, D., Drown, P., Xu, B., Aschner, M., LaNoue, K.F., 1998. Role of branched-chain aminotransferase isoenzymes and gabapentin in neurotransmitter metabolism. J. Neurochem. 71 (2), 863–874. https://doi.org/10.1046/j.1471-4159.1998.71020863.x.

In't Veld, P., 2014. Insulitis in human type 1 diabetes: a comparison between patients and animal models. Semin. Immunopathol. 36 (5), 569–579. https://doi.org/10.1007/s00281-014-0438-4.

Insel, R.A., Dunne, J.L., Atkinson, M.A., Chiang, J.L., Dabelea, D., Gottlieb, P.A., et al., 2015. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. Diabetes Care 38 (10), 1964–1974. https://doi.org/10.2337/dc15-1419.

Ivanova, E., Alam, N.M., Prusky, G.T., Sagdullaev, B.T., 2019. Blood-retina barrier failure and vision loss in neuron-specific degeneration. JCI Insight 5. https://doi.org/10.1172/jci.insight.126747.

Jackson, G.R., Scott, I.U., Quillen, D.A., Walter, L.E., Gardner, T.W., 2012. Inner retinal visual dysfunction is a sensitive marker of non-proliferative diabetic retinopathy. Br. J. Ophthalmol. 96 (5), 699–703. https://doi.org/10.1136/bjophthalmol-2011-300467.

Jampol, L.M., Tadayoni, R., Ip, M., 2021. Need for a new classification of diabetic retinopathy. Retina 41 (3), 459–460. https://doi.org/10.1097/IAE.0000000000003070.

Jenssen, T., Hartmann, A., Birkeland, K.I., 2017. Long-term diabetes complications after pancreas transplantation. Curr. Opin. Organ Transplant. 22 (4), 382–388. https://doi.org/10.1097/MOT.0000000000000436.

Joltikov, K.A., de Castro, V.M., Davila, J.R., Anand, R., Khan, S.M., Farbman, N., et al., 2017. Multidimensional functional and structural evaluation reveals neuroretinal impairment in early diabetic retinopathy. Invest. Ophthalmol. Vis. Sci. 58 (6), BIO277–BIO290. https://doi.org/10.1167/iovs.17-21863.

Joltikov, K.A., Sesi, C.A., de Castro, V.M., Davila, J.R., Anand, R., Khan, S.M., et al., 2018. Disorganization of retinal inner layers (DRIL) and neuroretinal dysfunction in early diabetic retinopathy. Invest. Ophthalmol. Vis. Sci. 59 (13), 5481–5486. https://doi.org/10.1167/iovs.18-24955.

Joyal, J.S., Omri, S., Sitaras, N., Rivera, J.C., Sapieha, P., Chemtob, S., 2012. Neovascularization in retinopathy of prematurity: opposing actions of neuronal factors GPR91 and semaphorins 3A. Acta Paediatr. 101 (8), 819–826. https://doi.org/10.1111/j.1651-2227.2012.02692.x.

Joyal, J.S., Gantner, M.L., Smith, L.E.H., 2018. Retinal energy demands control vascular supply of the retina in development and disease: the role of neuronal lipid and glucose metabolism. Prog. Retin. Eye Res. 64, 131–156. https://doi.org/10.1016/j.preteyeres.2017.11.002.

Kaestner, K.H., Powers, A.C., Naji, A., Consortium, H., Atkinson, M.A., 2019. NIH initiative to improve understanding of the pancreas, islet, and autoimmunity in type 1 diabetes: the human pancreas analysis program (HPAP). Diabetes 68 (7), 1394–1402. https://doi.org/10.2337/db19-0058.

Kaestner, K.H., Campbell-Thompson, M., Dor, Y., Gill, R.G., Glaser, B., Kim, S.K., et al., 2021. What is a beta cell? - chapter I in the Human Islet Research Network (HIRN) review series. Mol. Metabol. 53, 101323. https://doi.org/10.1016/j.molmet.2021.101323.

Karakelides, H., Asmann, Y.W., Bigelow, M.L., Short, K.R., Dhatariya, K., Coenen-Schimke, J., et al., 2007. Effect of insulin deprivation on muscle mitochondrial ATP production and gene transcript levels in type 1 diabetic subjects. Diabetes 56 (11), 2683–2689. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17660267.

Kennedy, J.F., 1961, May 25, 1961. The decision to go to the moon. Retrieved from. https://history.nasa.gov/moondec.html#:~:text=On%20May%2025%2C%201961%2C%20President,the%20end%20of%20the%20decade.

Kerrigan, L.A., Zack, D.J., Quigley, H.A., Smith, S.D., Pease, M.E., 1997. TUNEL-positive ganglion cells in human primary open-angle glaucoma. Arch. Ophthalmol. 115 (8), 1031–1035.

Kimball, S.R., Jefferson, L.S., 1998. Cellular mechanisms involved in the action of insulin on protein synthesis. Diabetes Metab. Rev. 4, 773–787.

Kinoshita, J.H., 1986. Aldose reductase in the diabetic eye. XLIII Edward Jackson memorial lecture. Am. J. Ophthalmol. 102 (6), 685–692. https://doi.org/10.1016/0002-9394(86)90394-6.

Kita, T., Clermont, A.C., Murugesan, N., Zhou, Q., Fujisawa, K., Ishibashi, T., et al., 2015. Plasma kallikrein-kinin system as a VEGF-independent mediator of diabetic macular edema. Diabetes 64 (10), 3588–3599. https://doi.org/10.2337/db15-0317.

Kohner, E.M., Henkind, P., 1970. Correlation of fluorescein angiogram and retinal digest in diabetic retinopathy. Am. J. Ophthalmol. 69 (3), 403–414. Retrieved from. http://www.ncbi.nlm.nih.gov/pubmed/4907465.

Kowalski, A., 2015. Pathway to artificial pancreas systems revisited: moving downstream. Diabetes Care 38 (6), 1036–1043. https://doi.org/10.2337/dc15-0364.

Kwon, S.H., Shin, J.P., Kim, I.T., Park, D.H., 2016. Association of plasma semaphorin 3A with phenotypes of diabetic retinopathy and nephropathy. Invest. Ophthalmol. Vis. Sci. 57 (7), 2983–2989. https://doi.org/10.1167/iovs.16-19468.

Lahne, M., Nagashima, M., Hyde, D.R., Hitchcock, P.F., 2020. Reprogramming muller glia to regenerate retinal neurons. Ann. Rev. Vis. Sci. 6, 171–193. https://doi.org/10.1146/annurev-vision-121219-081808.

Lam, J.D., Oh, D.J., Wong, L.L., Amarnani, D., Park-Windhol, C., Sanchez, A.V., et al., 2017. Identification of RUNX1 as a mediator of aberrant retinal angiogenesis. Diabetes 66 (7), 1950–1956. https://doi.org/10.2337/db16-1035.

Leber, T.K., 1875. Uber die Erkrankenungen des Auges bei Diabetes mellitus. Albrecht Von Graefes Arch. Klin. Exp. Ophthalmol. 21, 206–237.

Leinonen, H., Pham, N.C., Boyd, T., Santoso, J., Palczewski, K., Vinberg, F., 2020. Homeostatic plasticity in the retina is associated with maintenance of night vision during retinal degenerative disease. Elife 9. https://doi.org/10.7554/eLife.59422.

Levi, S.R., Ryu, J., Liu, P.K., Tsang, S.H., 2021. Precision medicine trials in retinal degenerations. Ann. Rev. Vis. Sci. 7, 851–865. https://doi.org/10.1146/annurev-vision-100419-111701.

Li, Q., Zemel, E., Miller, B., Perlman, I., 2002. Early retinal damage in experimental diabetes: electroretinographical and morphological observations. Exp. Eye Res. 74 (5), 615–625.

Li, M., Dolz-Marco, R., Messinger, J.D., Ferrara, D., Freund, K.B., Curcio, C.A., 2021. Neurodegeneration, gliosis, and resolution of haemorrhage in neovascular age-related macular degeneration, a clinicopathologic correlation. Eye 35 (2), 548–558. https://doi.org/10.1038/s41433-020-0896-y.

Lieth, E., LaNoue, K.F., Antonetti, D.A., Ratz, M., Group, T. P. S. R. R, 2000. Diabetes reduces glutamate oxidation and glutamine synthesis in the retina. Exp. Eye Res. 70 (6), 723–730.

Lieth, E., LaNoue, K.F., Berkich, D.A., Xu, B.Y., Ratz, M., Taylor, C., Hutson, S.M., 2001. Nitrogen shuttling between neurons and glial cells during glutamate synthesis. J. Neurochem. 76 (6), 1712–1723.

Limonte, C.P., Valo, E., Montemayor, D., Afshinnia, F., Ahluwalia, T.S., Costacou, T., et al., 2020. A targeted multiomics approach to identify biomarkers associated with rapid eGFR decline in type 1 diabetes. Am. J. Nephrol. 1–10. https://doi.org/10.1159/000510830.

Lin, T., Gubitosi-Klug, R.A., Channa, R., Wolf, R.M., 2021. Pediatric diabetic retinopathy: updates in prevalence, risk factors, screening, and management. Curr. Diabetes Rep. 21 (12), 56. https://doi.org/10.1007/s11892-021-01436-x.

Linsenmeier, R.A., Zhang, H.F., 2017. Retinal oxygen: from animals to humans. Prog. Retin. Eye Res. 58, 115–151. https://doi.org/10.1016/j.preteyeres.2017.01.003.

Liu, X., Dreffs, A., Diaz-Coranguez, M., Runkle, E.A., Gardner, T.W., Chiodo, V.A., et al., 2016. Occludin S490 phosphorylation regulates vascular endothelial growth factor-induced retinal neovascularization. Am. J. Pathol. 186 (9), 2486–2499. https://doi.org/10.1016/j.ajpath.2016.04.018.

Longhin, E., Tormene, A.P., Olivato, E., Convento, E., Vujosevic, S., Pilotto, E., et al., 2016. Rod function in diabetic patients without and with early diabetic retinopathy. Eur. J. Ophthalmol. 26 (5), 418–424. https://doi.org/10.5301/ejo.5000800.

Losiewicz, M.K., Elghazi, L., Kong, D., Finger, D., Rajala, R.V.S., Fort, P.E., et al., 2019. Retinal Ganglion Cell Protein Synthesis Is Regulated by Glycolysis, mTORC1 Signaling and Diabetes. Paper presented at the ARVO, Seattle, WA.

Lott, M.E., Slocomb, J.E., Shivkumar, V., Smith, B., Gabbay, R.A., Quillen, D., et al., 2012. Comparison of retinal vasodilator and constrictor responses in type 2 diabetes. Acta Ophthalmol. 90 (6) https://doi.org/10.1111/j.1755-3768.2012.02445.x e434-441.

Lott, M.E., Slocomb, J.E., Gao, Z., Gabbay, R.A., Quillen, D., Gardner, T.W., Bettermann, K., 2015. Impaired coronary and retinal vasomotor function to hyperoxia in Individuals with Type 2 diabetes. Microvasc. Res. 101, 1–7. https://doi.org/10.1016/j.mvr.2015.05.002.

Lynch, S.K., Abramoff, M.D., 2017. Diabetic retinopathy is a neurodegenerative disorder. Vision Res. 139, 101–107. https://doi.org/10.1016/j.visres.2017.03.003.

Maguire, M.G., Liu, D., Glassman, A.R., Jampol, L.M., Johnson, C.A., Baker, C.W., et al., 2020. Visual field changes over 5 Years in patients treated with panretinal photocoagulation or ranibizumab for proliferative diabetic retinopathy. JAMA Ophthalmol 138 (3), 285–293. https://doi.org/10.1001/jamaophthalmol.2019.5939.

Marchetti, P., Schulte, A.M., Marselli, L., Schoniger, E., Bugliani, M., Kramer, W., et al., 2019. Fostering improved human islet research: a European perspective. Diabetologia 62 (8), 1514–1516. https://doi.org/10.1007/s00125-019-4911-4.

Mathew, A.V., Jaiswal, M., Ang, L., Michailidis, G., Pennathur, S., Pop-Busui, R., 2019. Impaired amino acid and TCA metabolism and cardiovascular autonomic neuropathy progression in type 1 diabetes. Diabetes 68 (10), 2035–2044. https://doi.org/10.2337/db19-0145.

Mayer-Davis, E.J., Lawrence, J.M., Dabelea, D., Divers, J., Isom, S., Dolan, L., et al., 2017. Incidence trends of type 1 and type 2 diabetes among youths, 2002-2012. N. Engl. J. Med. 376 (15), 1419–1429. https://doi.org/10.1056/NEJMoa1610187.

Metea, M.R., Kofuji, P., Newman, E.A., 2007. Neurovascular coupling is not mediated by potassium siphoning from glial cells. J. Neurosci. 27 (10), 2468–2471. Retrieved from. http://www.ncbi.nlm.nih.gov/pubmed/17344384.

Meyer-Schwickerath, G., 1959. Licktkoagulation. Buech Augenarzt 33, 1–96.

Midena, E., Bini, S., 2016. Multimodal retinal imaging of diabetic macular edema: toward new paradigms of pathophysiology. Graefes Arch. Clin. Exp. Ophthalmol. 254 (9), 1661–1668. https://doi.org/10.1007/s00417-016-3361-7.

Midena, E., Segato, T., Giuliano, M., Zucchetto, M., 1990. Macular recovery function (nyctometry) in diabetics without and with early retinopathy. Br. J. Ophthalmol. 74 (2), 106–108. https://doi.org/10.1136/bjo.74.2.106.

Midena, E., Frizziero, L., Midena, G., Pilotto, E., 2021. Intraocular fluid biomarkers (liquid biopsy) in human diabetic retinopathy. Graefes Arch. Clin. Exp. Ophthalmol. https://doi.org/10.1007/s00417-021-05285-y.

Miloudi, K., Binet, F., Wilson, A., Cerani, A., Oubaha, M., Menard, C., et al., 2016. Truncated netrin-1 contributes to pathological vascular permeability in diabetic retinopathy. J. Clin. Invest. 126 (8), 3006–3022. https://doi.org/10.1172/JCI84767.

Misra, S.L., Slater, J.A., McGhee, C.N.J., Pradhan, M., Braatvedt, G.D., 2022. Corneal confocal microscopy in type 1 diabetes mellitus: a six-year longitudinal study. Transl. Vis. Sci. Technol. 11 (1), 17. https://doi.org/10.1167/tvst.11.1.17.

Mitry, D., Awan, M.A., Borooah, S., Syrogiannis, A., Lim-Fat, C., Campbell, H., et al., 2013. Long-term visual acuity and the duration of macular detachment: findings from a prospective population-based study. Br. J. Ophthalmol. 97 (2), 149–152. https://doi.org/10.1136/bjophthalmol-2012-302330.

Mizutani, M., Kern, T.S., Lorenzi, M., 1996. Accelerated death of retinal microvascular cells in human and experimental diabetic retinopathy. J. Clin. Invest. 97 (12), 2883–2890.

Moore-Dotson, J.M., Eggers, E.D., 2019. Reductions in calcium signaling limit inhibition to diabetic retinal rod bipolar cells. Invest. Ophthalmol. Vis. Sci. 60 (12), 4063–4073. https://doi.org/10.1167/iovs.19-27137.

Moran, E.P., Wang, Z., Chen, J., Sapieha, P., Smith, L.E., Ma, J.X., 2016. Neurovascular cross talk in diabetic retinopathy: pathophysiological roles and therapeutic implications. Am. J. Physiol. Heart Circ. Physiol. 311 (3), H738–749. https://doi.org/10.1152/ajpheart.00005.2016.

Motz, C.T., Chesler, K.C., Allen, R.S., Bales, K.L., Mees, L.M., Feola, A.J., et al., 2020. Novel detection and restorative levodopa treatment for preclinical diabetic retinopathy. Diabetes 69 (7), 1518–1527. https://doi.org/10.2337/db19-0869.
Mure, L.S., 2021. Intrinsically photosensitive retinal ganglion cells of the human retina. Front. Neurol. 12, 636330. https://doi.org/10.3389/fneur.2021.636330.
Musch, D.C., Gillespie, B.W., Palmberg, P.F., Spaeth, G., Niziol, L.M., Lichter, P.R., 2014. Visual field improvement in the collaborative initial glaucoma treatment study. Am. J. Ophthalmol. 158 (1) https://doi.org/10.1016/j.ajo.2014.04.003, 96-104 e102.
Nadeau, K.J., Regensteiner, J.G., Bauer, T.A., Brown, M.S., Dorosz, J.L., Hull, A., et al., 2010. Insulin resistance in adolescents with type 1 diabetes and its relationship to cardiovascular function. J. Clin. Endocrinol. Metab. 95 (2), 513–521. https://doi.org/10.1210/jc.2009-1756.
Nair, K.S., Garrow, J.S., Ford, C., Mahler, R.F., Halliday, D., 1983. Effect of poor diabetic control and obesity on whole body protein metabolism in man. Diabetologia 25 (5), 400–403.
Nathan, D.M., Group, D.E.R., 2014. The diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: overview. Diabetes Care 37 (1), 9–16. https://doi.org/10.2337/dc13-2112.
Newman, E.A., 2013. Functional hyperemia and mechanisms of neurovascular coupling in the retinal vasculature. J. Cerebr. Blood Flow Metabol. 33 (11), 1685–1695. https://doi.org/10.1038/jcbfm.2013.145.
Newman, E.A., 2015. Glial cell regulation of neuronal activity and blood flow in the retina by release of gliotransmitters. Philos. Trans. R. Soc. Lond. B Biol. Sci. 370 (1672) https://doi.org/10.1098/rstb.2014.0195.
Nippert, A.R., Newman, E.A., 2020. Regulation of blood flow in diabetic retinopathy. Vis. Neurosci. 37, E004. https://doi.org/10.1017/S0952523820000036.
Novotny, H.R., Alvis, D.L., 1961. A method of photographing fluorescence in circulating blood of the human retina. Circulation 24, 82–86.
Nuzbrokh, Y., Ragi, S.D., Tsang, S.H., 2021. Gene therapy for inherited retinal diseases. Ann. Transl. Med. 9 (15), 1278. https://doi.org/10.21037/atm-20-4726.
Obara, E.A., Hannibal, J., Heegaard, S., Fahrenkrug, J., 2017. Loss of melanopsin-expressing retinal ganglion cells in patients with diabetic retinopathy. Invest. Ophthalmol. Vis. Sci. 58 (4), 2187–2192. https://doi.org/10.1167/iovs.16-21168.
Okar, D.A., 2002. Starvation amidst plenty: PDKs and diabetes mellitus. Trends Biochem. Sci. 27 (5), 227–228. Retrieved from. http://www.ncbi.nlm.nih.gov/pubmed/12076530.
Ola, M.S., Moore, P., Maddox, D., El-Sherbeny, A., Huang, W., Roon, P., et al., 2002. Analysis of sigma receptor (sigmaR1) expression in retinal ganglion cells cultured under hyperglycemic conditions and in diabetic mice. Brain Res. Mol. Brain Res. 107 (2), 97–107. S0169328X02004448 [pii].
Ola, M.S., Hosoya, K., LaNoue, K.F., 2011. Influence of insulin on glutamine synthetase in the Muller glial cells of retina. Metab. Brain Dis. 26 (3), 195–202. https://doi.org/10.1007/s11011-011-9245-y.
Orlandini, O., 1904. Alterazioni della retina nella glicosuria sperimentale. Revistia veneta di scienze mediche 40 (Tomo XXXX), 97–107.
Pan, W.W., Gardner, T.W., Harder, J.L., 2021. Integrative biology of diabetic retinal disease: lessons from diabetic kidney disease. J. Clin. Med. 10 (6) https://doi.org/10.3390/jcm10061254.
Papakostopoulos, D., Dean Hart, J.C., Harney, B., Corrall, R.J., 1985. Visual evoked potentials in diabetics without retinopathy. Br. Med. J. 291, 603. Retrieved from, 6495. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=3929890.
Pardue, M.T., Barnes, C.S., Kim, M.K., Aung, M.H., Amarnath, R., Olson, D.E., Thule, P. M., 2014. Rodent hyperglycemia-induced inner retinal deficits are mirrored in human diabetes. Transl. Vis. Sci. Technol. 3 (3), 6. https://doi.org/10.1167/tvst.3.3.6.
Parisi, V., Uccioli, L., Monticone, G., Parisi, L., Menzinger, G., Bucci, M.G., 1994. Visual evoked potentials after photostress in insulin-dependent diabetic patients with or without retinopathy. Graefes Arch. Clin. Exp. Ophthalmol. 232 (4), 193–198. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=8034206.
Parravano, M., Oddone, F., Mineo, D., Centofanti, M., Borboni, P., Lauro, R., et al., 2008. The role of Humphrey Matrix testing in the early diagnosis of retinopathy in type 1 diabetes. Br. J. Ophthalmol. 92 (12), 1656–1660. https://doi.org/10.1136/bjo.2008.143057.
Parravano, M., Ziccardi, L., Borrelli, E., Costanzo, E., Frontoni, S., Picconi, F., et al., 2021. Outer retina dysfunction and choriocapillaris impairment in type 1 diabetes. Sci. Rep. 11 (1), 15183. https://doi.org/10.1038/s41598-021-94580-z.
Pawar, M., Busov, B., Chandrasekhar, A., Yao, J., Zacks, D.N., Besirli, C.G., 2017. FAS apoptotic inhibitory molecule 2 is a stress-induced intrinsic neuroprotective factor in the retina. Cell Death Differ. 24 (10), 1799–1810. https://doi.org/10.1038/cdd.2017.109.
Pegram, M.D., Pauletti, G., Slamon, D.J., 1998. HER-2/neu as a predictive marker of response to breast cancer therapy. Breast Cancer Res. Treat. 52 (1–3), 65–77. https://doi.org/10.1023/a:1006111117877.
Peters, L., Posgai, A., Brusko, T.M., 2019. Islet-immune interactions in type 1 diabetes: the nexus of beta cell destruction. Clin. Exp. Immunol. 198 (3), 326–340. https://doi.org/10.1111/cei.13349.
Peterson, S.L., Li, Y., Sun, C.J., Wong, K.A., Leung, K.S., de Lima, S., et al., 2021. Retinal ganglion cell axon regeneration requires complement and myeloid cell activity within the optic nerve. J. Neurosci. 41 (41), 8508–8531. https://doi.org/10.1523/JNEUROSCI.0555-21.2021.
Pinilla, I., Idoipe, M., Perdices, L., Sanchez-Cano, A., Acha, J., Lopez-Galvez, M.I., et al., 2019. Changes in total and inner retinal thicknesses in type 1 diabetes with No retinopathy after 8 Years of follow-up. Retina. https://doi.org/10.1097/IAE.0000000000002576.
Pournaras, C.J., Rungger-Brandle, E., Riva, C.E., Hardarson, S.H., Stefansson, E., 2008. Regulation of retinal blood flow in health and disease. Prog. Retin. Eye Res. 27 (3), 284–330. https://doi.org/10.1016/j.preteyeres.2008.02.002.
Pugliese, A., Yang, M., Kusmarteva, I., Heiple, T., Vendrame, F., Wasserfall, C., et al., 2014. The juvenile diabetes research foundation network for pancreatic organ donors with diabetes (nPOD) program: goals, operational model and emerging findings. Pediatr. Diabetes 15 (1), 1–9. https://doi.org/10.1111/pedi.12097.
Punzo, C., Kornacker, K., Cepko, C.L., 2009. Stimulation of the insulin/mTOR pathway delays cone death in a mouse model of retinitis pigmentosa. Nat. Neurosci. 12 (1), 44–52 nn.2234 [pii], 10.1038/nn.2234.
Puro, D.G., Kohmoto, R., Fujita, Y., Gardner, T.W., Padovani-Claudio, D.A., 2016. Bioelectric impact of pathological angiogenesis on vascular function. Proc. Natl. Acad. Sci. U. S. A. 113 (35), 9934–9939. https://doi.org/10.1073/pnas.1604757113.
Quinn, C.M., Porwal, M., Meagher, N.S., Hettiarachi, A., Power, C., Jonnaggadala, J., et al., 2021. Moving with the times: the health science alliance (HSA) biobank, pathway to sustainability. Biomark. Insights 16, 1–10.
Rajala, R.V., Wiskur, B., Tanito, M., Callegan, M., Rajala, A., 2009. Diabetes reduces autophosphorylation of retinal insulin receptor and increases protein-tyrosine phosphatase-1B activity. Invest. Ophthalmol. Vis. Sci. 50 (3), 1033–1040 iovs.08-2851 [pii], 10.1167/iovs.08-2851.
Rao, H.L., Pradhan, Z.S., Suh, M.H., Moghimi, S., Mansouri, K., Weinreb, R.N., 2020. Optical coherence tomography angiography in glaucoma. J. Glaucoma 29 (4), 312–321. https://doi.org/10.1097/IJG.0000000000001463.
Reiter, C.E.N., Wu, X., Sandirasegarane, L., Nakamura, M., Gilbert, K.A., Singh, R.S.J., et al., 2006. Diabetes reduces basal retinal insulin receptor signaling: reversal with systemic and local insulin. Diabetes 55, 1148–1156. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16567541.
Riva, C.E., Logean, E., Falsini, B., 2005. Visually evoked hemodynamical response and assessment of neurovascular coupling in the optic nerve and retina. Prog. Retin. Eye Res. 24 (2), 183–215. https://doi.org/10.1016/j.preteyeres.2004.07.002.
Ross, B.X., Choi, J., Yao, J., Hager, H.M., Abcouwer, S.F., Zacks, D.N., 2020. Loss of high-mobility group box 1 (HMGB1) protein in rods accelerates rod photoreceptor degeneration after retinal detachment. Invest. Ophthalmol. Vis. Sci. 61 (5), 50. https://doi.org/10.1167/iovs.61.5.50.
Roth, J.A., 1969. Central visual field in diabetes. Br. J. Ophthalmol. 53 (1), 16–25. Retrieved from. http://www.ncbi.nlm.nih.gov/pubmed/5775566.
Roy, S., Sen, C.K., Ghatak, S., Higuita-Castro, N., Palakurti, R., Nalluri, N., et al., 2020. Neurogenic tissue nanotransfection in the management of cutaneous diabetic polyneuropathy. Nanomedicine 28, 102220. https://doi.org/10.1016/j.nano.2020.102220.
Ruiz-Alonso, S., Villate-Beitia, I., Gallego, I., Lafuente-Merchan, M., Puras, G., Saenz-Del-Burgo, L., Pedraz, J.L., 2021. Current insights into 3D bioprinting: an advanced approach for eye tissue regeneration. Pharmaceutics 13 (3). https://doi.org/10.3390/pharmaceutics13030308.
Rungger-Brandle, E., Dosso, A.A., Leuenberger, P.M., 2000. Glial reactivity, an early feature of diabetic retinopathy. Invest. Ophthalmol. Vis. Sci. 41 (7), 1971–1980. Retrieved from. http://www.iovs.org/cgi/content/full/41/7/1971.
Sabanayagam, C., Banu, R., Chee, M.L., Lee, R., Wang, Y.X., Tan, G., et al., 2019. Incidence and progression of diabetic retinopathy: a systematic review. Lancet Diabetes Endocrinol. 7 (2), 140–149. https://doi.org/10.1016/S2213-8587(18)30128-1.
Sacconi, R., Casaluci, M., Borrelli, E., Mulinacci, G., Lamanna, F., Gelormini, F., et al., 2019. Multimodal imaging assessment of vascular and neurodegenerative retinal alterations in type 1 diabetic patients without fundoscopic signs of diabetic retinopathy. J. Clin. Med. 8 (9) https://doi.org/10.3390/jcm8091409.
Santos, A.R., Ribeiro, L., Bandello, F., Lattanzio, R., Egan, C., Frydkjaer-Olsen, U., et al., 2017. Functional and structural findings of neurodegeneration in early stages of diabetic retinopathy. Cross-sectional analyses of baseline data of the EUROCONDOR project. Diabetes. https://doi.org/10.2337/db16-1453.
Sapieha, P., Sirinyan, M., Hamel, D., Zaniolo, K., Joyal, J.S., Cho, J.H., et al., 2008. The succinate receptor GPR91 in neurons has a major role in retinal angiogenesis. Nat. Med. 14 (10), 1067–1076. https://doi.org/10.1038/nm.1873.
Sas, K.M., Kayampilly, P., Byun, J., Nair, V., Hinder, L.M., Hur, J., et al., 2016. Tissue-specific metabolic reprogramming drives nutrient flux in diabetic complications. JCI Insight 1 (15), e86976. https://doi.org/10.1172/jci.insight.86976.
Sas, K.M., Lin, J., Rajendiran, T.M., Soni, T., Nair, V., Hinder, L.M., et al., 2017. Shared and distinct lipid-lipid interactions in plasma and affected tissues in a diabetic mouse model. J. Lipid Res. https://doi.org/10.1194/jlr.M077222.
Saunders, D., Powers, A.C., 2016. Replicative capacity of beta-cells and type 1 diabetes. J. Autoimmun. 71, 59–68. https://doi.org/10.1016/j.jaut.2016.03.014.
Scott, I.U., Jackson, G.R., Quillen, D.A., Klein, R., Liao, J., Gardner, T.W., 2014a. Effect of doxycycline vs placebo on retinal function and diabetic retinopathy progression in mild to moderate nonproliferative diabetic retinopathy: a randomized proof-of-concept clinical trial. JAMA Ophthalmol 132 (9), 1137–1142. https://doi.org/10.1001/jamaophthalmol.2014.1422.
Scott, I.U., Jackson, G.R., Quillen, D.A., Larsen, M., Klein, R., Liao, J., et al., 2014b. Effect of doxycycline vs placebo on retinal function and diabetic retinopathy progression in patients with severe nonproliferative or non-high-risk proliferative diabetic retinopathy: a randomized clinical trial. JAMA Ophthalmol 132 (5), 535–543. https://doi.org/10.1001/jamaophthalmol.2014.93.
Shahidi, A.M., Sampson, G.P., Pritchard, N., Edwards, K., Vagenas, D., Russell, A.W., et al., 2012. Retinal nerve fibre layer thinning associated with diabetic peripheral neuropathy. Diabet. Med. 29 (7), e106–111. https://doi.org/10.1111/j.1464-5491.2012.03588.x.

Sharma, R.B., Landa-Galvan, H.V., Alonso, L.C., 2021. Living dangerously: protective and harmful ER stress responses in pancreatic beta-cells. Diabetes 70 (11), 2431–2443. https://doi.org/10.2337/dbi20-0033.
Sheldon, M., 2021. Overview of various components of the science of patient input: advancing the use of patient-reported, real-world evidence for medical device evaluation of innovative products for the treatment of kidney failure using strategically coordinated registry networks. Clin. J. Am. Soc. Nephrol. 16 (4), 634–635. https://doi.org/10.2215/CJN.11760720.
Shen, N., Wang, B., Soto, F., Kerschensteiner, D., 2020. Homeostatic plasticity shapes the retinal response to photoreceptor degeneration. Curr. Biol. 30 (10), 1916–1926 e1913. https://doi.org/10.1016/j.cub.2020.03.033.
Shimizu, K., Kobayashi, Y., Muraoka, K., 1981. Midperipheral fundus involvement in diabetic retinopathy. Ophthalmology 88 (7), 601–612. Retrieved from. http://www.ncbi.nlm.nih.gov/pubmed/6167923.
Shimoda, Y., Sano, M., Hashimoto, H., Yokota, Y., Kishi, S., 2010. Restoration of photoreceptor outer segment after vitrectomy for retinal detachment. Am. J. Ophthalmol. 149 (2), 284–290. https://doi.org/10.1016/j.ajo.2009.08.025.
Simo, R., Hernandez, C., 2015. Novel approaches for treating diabetic retinopathy based on recent pathogenic evidence. Prog. Retin. Eye Res. 48, 160–180. https://doi.org/10.1016/j.preteyeres.2015.04.003.
Simo, R., Stitt, A.W., Gardner, T.W., 2018. Neurodegeneration in diabetic retinopathy: does it really matter? Diabetologia 61 (9), 1902–1912. https://doi.org/10.1007/s00125-018-4692-1.
Simo, R., Hernandez, C., Porta, M., Bandello, F., Grauslund, J., Harding, S.P., et al., 2019. Effects of topically administered neuroprotective drugs in early stages of diabetic retinopathy: results of the EUROCONDOR clinical trial. Diabetes 68 (2), 457–463. https://doi.org/10.2337/db18-0682.
Simonsen, S.E., 1969. ERG in Juvenile Diabetics: a prognostic study. In: Goldberg, M.F., Fine, S.L. (Eds.), Symposium on the Treatment of Diabetic Retinopathy. US Department of Health, Education and Welfare, Arlington, pp. 681–689.
Simonsen, S.E., 1974. Prognostic value of ERG (oscillatory potential) in juvenile diabetics. Acta Ophthalmol. Suppl. 123, 223–224. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=4369070.
Simonsen, S.E., 1980. The value of the oscillatory potential in selecting juvenile diabetics at risk of developing proliferative retinopathy. Acta Ophthalmol. 58 (6), 865–878. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=7331773.
Sobrin, L., Susarla, G., Stanwyck, L., Rouhana, J.M., Li, A., Pollack, S., et al., 2021. Gene set enrichment analsyes identiify pathways involved in genetic risk for diabetic retinopathy. Am. J. Ophthalmol. 233, 111–123. https://doi.org/10.1016/j.ajo.2021.06.014.
Sohn, E.H., van Dijk, H.W., Jiao, C., Kok, P.H., Jeong, W., Demirkaya, N., et al., 2016. Retinal neurodegeneration may precede microvascular changes characteristic of diabetic retinopathy in diabetes mellitus. Proc. Natl. Acad. Sci. U. S. A. 113 (19), E2655–2664. https://doi.org/10.1073/pnas.1522014113.
Sousa, D.C., Leal, I., Moreira, S., do Vale, S., Silva-Herdade, A.S., Aguiar, P., et al., 2020. Retinal vascular reactivity in type 1 diabetes patients without retinopathy using optical coherence tomography angiography. Invest. Ophthalmol. Vis. Sci. 61 (6), 49. https://doi.org/10.1167/iovs.61.6.49.
Sun, W., Li, N., He, S., 2002. Large-scale morphological survey of mouse retinal ganglion cells. J. Comp. Neurol. 451 (2), 115–126. https://doi.org/10.1002/cne.10323.
Sun, J.K., Keenan, H.A., Cavallerano, J.D., Asztalos, B.F., Schaefer, E.J., Sell, D.R., et al., 2011. Protection from retinopathy and other complications in patients with type 1 diabetes of extreme duration: the joslin 50-year medalist study. Diabetes Care 34 (4), 968–974. https://doi.org/10.2337/dc10-1675.
Sun, J.K., Lin, M.M., Lammer, J., Prager, S., Sarangi, R., Silva, P.S., Aiello, L.P., 2014. Disorganization of the retinal inner layers as a predictor of visual acuity in eyes with center-involved diabetic macular edema. JAMA Ophthalmol 132 (11), 1309–1316. https://doi.org/10.1001/jamaophthalmol.2014.2350.
Sun, J.K., Radwan, S.H., Soliman, A.Z., Lammer, J., Lin, M.M., Prager, S.G., et al., 2015. Neural retinal disorganization as a robust marker of visual acuity in current and resolved diabetic macular edema. Diabetes 64 (7), 2560–2570. https://doi.org/10.2337/db14-0782.
Sun, J.K., Aiello, L.P., Abramoff, M.D., Antonetti, D.A., Dutta, S., Pragnell, M., et al., 2021. Updating the staging system for diabetic retinal disease. Ophthalmology 128 (4), 490–493. https://doi.org/10.1016/j.ophtha.2020.10.008.
Sundstrom, J.M., Hernandez, C., Weber, S.R., Zhao, Y., Dunklebarger, M., Tiberti, N., et al., 2018. Proteomic analysis of early diabetic retinopathy reveals mediators of neurodegenerative brain diseases. Invest. Ophthalmol. Vis. Sci. 59 (6), 2264–2274. https://doi.org/10.1167/iovs.17-23678.
Takahashi, H., Chihara, E., 2008. Impact of diabetic retinopathy on quantitative retinal nerve fiber layer measurement and glaucoma screening. Invest. Ophthalmol. Vis. Sci. 49 (2), 687–692. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18235015.
Tarver, M.E., Neuland, C., 2021. Integrating patient perspectives into medical device regulatory decision making to advance innovation in kidney disease. Clin. J. Am. Soc. Nephrol. 16 (4), 636–638. https://doi.org/10.2215/CJN.11510720.
Tecilazich, F., Feke, G.T., Mazzantini, S., Sobrin, L., Lorenzi, M., 2016. Defective myogenic response of retinal vessels is associated with accelerated onset of retinopathy in type 1 diabetic individuals. Invest. Ophthalmol. Vis. Sci. 57 (4), 1523–1529. https://doi.org/10.1167/iovs.15-18356.
Teo, Z.L., Tham, Y.C., Yu, M., Chee, M.L., Rim, T.H., Cheung, N., et al., 2021. Global prevalence of diabetic retinopathy and projection of burden through 2045: systematic review and meta-analysis. Ophthalmology. https://doi.org/10.1016/j.ophtha.2021.04.027.
Teotia, P., Van Hook, M.J., Fischer, D., Ahmad, I., 2019. Human retinal ganglion cell axon regeneration by recapitulating developmental mechanisms: effects of recruitment of the mTOR pathway. Development 146 (13). https://doi.org/10.1242/dev.178012.
Testa, F., Maguire, A.M., Rossi, S., Pierce, E.A., Melillo, P., Marshall, K., et al., 2013. Three-year follow-up after unilateral subretinal delivery of adeno-associated virus in patients with Leber congenital Amaurosis type 2. Ophthalmology 120 (6), 1283–1291. https://doi.org/10.1016/j.ophtha.2012.11.048.
Thompson, D.M., Begg, I.S., Harris, C., Ao, Z., Fung, M.A., Meloche, R.M., et al., 2008. Reduced progression of diabetic retinopathy after islet cell transplantation compared with intensive medical therapy. Transplantation 85 (10), 1400–1405. https://doi.org/10.1097/TP.0b013e318172ca07.
Thompson, D.A., Ali, R.R., Banin, E., Branham, K.E., Flannery, J.G., Gamm, D.M., et al., 2015. Advancing therapeutic strategies for inherited retinal degeneration: recommendations from the Monaciano Symposium. Invest. Ophthalmol. Vis. Sci. 56 (2), 918–931. https://doi.org/10.1167/iovs.14-16049.
Thompson, D.A., Iannaccone, A., Ali, R.R., Arshavsky, V.Y., Audo, I., Bainbridge, J.W.B., et al., 2020. Advancing clinical trials for inherited retinal diseases: recommendations from the second monaciano Symposium. Transl. Vis. Sci. Technol. 9 (7), 2. https://doi.org/10.1167/tvst.9.7.2.
Tikhonenko, M., Lydic, T.A., Wang, Y., Chen, W., Opreanu, M., Sochacki, A., et al., 2010. Remodeling of retinal Fatty acids in an animal model of diabetes: a decrease in long-chain polyunsaturated fatty acids is associated with a decrease in fatty acid elongases Elovl2 and Elovl4. Diabetes 59 (1), 219–227. https://doi.org/10.2337/db09-0728.
Trick, G.L., Trick, L.R., Kilo, C., 1990. Visual field defects in patients with insulin-dependent and noninsulin-dependent diabetes. Ophthalmology 97 (4), 475–482. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=2326027.
Tsukada, T., Chihara, E., 1986. Changes in components of fast axonally transported proteins in the optic nerves of diabetic rabbits. Investigat. Ophthalmol. Vis. Sci. 27 (7), 1115–1122.
Turrigiano, G., 2012. Homeostatic synaptic plasticity: local and global mechanisms for stabilizing neuronal function. Cold Spring Harb Perspect Biol 4 (1), a005736. https://doi.org/10.1101/cshperspect.a005736.
Usui, Y., Westenskow, P.D., Kurihara, T., Aguilar, E., Sakimoto, S., Paris, L.P., et al., 2015. Neurovascular crosstalk between interneurons and capillaries is required for vision. J. Clin. Invest. 125 (6), 2335–2346. https://doi.org/10.1172/JCI80297.
vanGuilder, H.D., Brucklacher, A.R., Conboy, E.E., Bronson, S.K., Barber, A.J., 2008. Diabetes downregulates presynaptic proteins and reduces basal synapsin 1 phosphorylation in rat retina. Eur. J. Neurosci. 28, 1–11.
Varkonyi, T.T., Peto, T., Degi, R., Keresztes, K., Lengyel, C., Janaky, M., et al., 2002. Impairment of visual evoked potentials: an early central manifestation of diabetic neuropathy? Diabetes Care 25 (9), 1661–1662. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12196448.
von Jaeger, E., 1856. Beitrage zur Pathologie des Auges. 2, S33 (Tafel XII). Wien: K.K Hof- und Staatsdruckerei).
von Mering, J., Minkowski, O., 1889. Diabetes mellitus nach Pankreasexstirpation. Zbl Kin Med 10, 393.
Vujosevic, S., Midena, E., Pilotto, E., Radin, P.P., Chiesa, L., Cavarzeran, F., 2006. Diabetic macular edema: correlation between microperimetry and optical coherence tomography findings. Invest. Ophthalmol. Vis. Sci. 47 (7), 3044–3051. https://doi.org/10.1167/iovs.05-1141.
Wallsh, Josh, Gallemore, Ron, 2021. Anti-VEGF-Resistant Retinal Diseases: A Review of the Latest Treatment Options. Cells 10, 1049. https://doi.org/10.3390/ cells10051049.
Wang, P., Li, X., Zhu, W., Zhong, Z., Moran, A., Wang, W., et al., 2018. 3D bioprinting of hydrogels for retina cell culturing. Bioprinting 11. https://doi.org/10.1016/j.bprint.2018.e00029.
Ward, M.M., Jobling, A.I., Kalloniatis, M., Fletcher, E.L., 2005. Glutamate uptake in retinal glial cells during diabetes. Diabetologia 48 (2), 351–360. https://doi.org/10.1007/s00125-004-1639-5.
Webb, J., 1982. Managing NASA in the Apollo Era. Scientific and Technical Information Branch National Aeronautics and Space Administration, Washington, DC.
Weber, S.R., Zhao, Y., Gates, C., Ma, J., da Veiga Leprevost, F., Basrur, V., et al., 2021a. Proteomic analyses of vitreous in proliferative diabetic retinopathy: prior studies and future outlook. J. Clin. Med. 10 (11) https://doi.org/10.3390/jcm10112309.
Weber, S.R., Zhao, Y., Ma, J., Gates, C., Leprevost, F.D., Basrur, V., et al., 2021b. A validated analysis pipeline for mass spectrometry-based vitreous proteomics: new insights into proliferative diabetic retinopathy. Clin. Proteonomics (in press).
Williams, B.L., Seager, N.A., Gardiner, J.D., Pappas, C.M., Cronin, M.C., Amat di San Filippo, C., et al., 2021. Chromosome 10q26-driven age-related macular degeneration is associated with reduced levels of HTRA1 in human retinal pigment epithelium. Proc. Natl. Acad. Sci. U. S. A. 118 (30) https://doi.org/10.1073/pnas.2103617118.
Wisznia, K.I., Lieberman, T.W., Leopold, I.H., 1971. Visual fields in diabetic retinopathy. Br. J. Ophthalmol. 55 (3), 183–188. Retrieved from. https://www.ncbi.nlm.nih.gov/pubmed/5575220.
Wolfensberger, T.J., Hamilton, A.M., 2001. Diabetic retinopathy–an historical review. Semin. Ophthalmol. 16 (1), 2–7. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15487691.
Wolter, J.R., 1961. Diabetic retinopathy. Am. J. Ophthalmol. 51, 1123–1139.
Wurtz, P., Makinen, V.P., Soininen, P., Kangas, A.J., Tukiainen, T., Kettunen, J., et al., 2012. Metabolic signatures of insulin resistance in 7,098 young adults. Diabetes 61 (6), 1372–1380. https://doi.org/10.2337/db11-1355.

Xiao, J., Yao, J., Jia, L., Ferguson, T.A., Weber, S., Sundstrom, J.M., et al., 2021. Autophagy activation and photoreceptor survival in retinal detachment. Exp. Eye Res. 205, 108492. https://doi.org/10.1016/j.exer.2021.108492.

Xuan, Y., Ghatak, S., Clark, A., Li, Z., Khanna, S., Pak, D., et al., 2021. Fabrication and use of silicon hollow-needle arrays to achieve tissue nanotransfection in mouse tissue in vivo. Nat. Protoc. 16 (12), 5707–5738. https://doi.org/10.1038/s41596-021-00631-0.

Yokomizo, H., Maeda, Y., Park, K., Clermont, A.C., Hernandez, S.L., Fickweiler, W., et al., 2019. Retinol binding protein 3 is increased in the retina of patients with diabetes resistant to diabetic retinopathy. Sci. Transl. Med. 11 (499) https://doi.org/10.1126/scitranslmed.aau6627.

Yonemura, D., Aoki, T., Tsuzuki, K., 1962. Electroretinogram in diabetic retinopathy. Arch. Ophthalmol. 68, 19–24. https://doi.org/10.1001/archopht.1962.00960030023005.

Yu, J., Wang, L., Weng, S.J., Yang, X.L., Zhang, D.Q., Zhong, Y.M., 2013. Hyperactivity of ON-type retinal ganglion cells in streptozotocin-induced diabetic mice. PLoS One 8 (9), e76049. https://doi.org/10.1371/journal.pone.0076049.

Yu, M.G., Keenan, H.A., Shah, H.S., Frodsham, S.G., Pober, D., He, Z., et al., 2019. Residual beta cell function and monogenic variants in long-duration type 1 diabetes patients. J. Clin. Invest. 129 (8), 3252–3263. https://doi.org/10.1172/JCI127397.

Zeng, X.X., Ng, Y.K., Ling, E.A., 2000. Neuronal and microglial response in the retina of streptozotocin-induced diabetic rats. Vis. Neurosci. 17 (3), 463–471.

Zeng, H.Y., Green, W.R., Tso, M.O., 2008. Microglial activation in human diabetic retinopathy. Arch. Ophthalmol. 126 (2), 227–232. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18268214.

Zeng, Y., Cao, D., Yu, H., Yang, D., Zhuang, X., Hu, Y., et al., 2019. Early retinal neurovascular impairment in patients with diabetes without clinically detectable retinopathy. Br. J. Ophthalmol. 103 (12), 1747–1752. https://doi.org/10.1136/bjophthalmol-2018-313582.

Zhang, L., Justus, S., Xu, Y., Pluchenik, T., Hsu, C.W., Yang, J., et al., 2016. Reprogramming towards anabolism impedes degeneration in a preclinical model of retinitis pigmentosa. Hum. Mol. Genet. 25 (19), 4244–4255. https://doi.org/10.1093/hmg/ddw256.

Zhao, M., Gelize, E., Levy, R., Moulin, A., Azan, F., Berdugo, M., et al., 2021. Mineralocorticoid receptor pathway and its antagonism in a model of diabetic retinopathy. Diabetes 70 (11), 2668–2682. https://doi.org/10.2337/db21-0099.

Zlokovic, B.V., 2008. The blood-brain barrier in health and chronic neurodegenerative disorders. Neuron 57 (2), 178–201. https://doi.org/10.1016/j.neuron.2008.01.003.

Zolov, S.N., Imai, H., Losiewicz, M.K., Singh, R.S.J., Fort, P.E., Gardner, T.W., 2021. Insulin-like growth factor-2 regulates basal retinal insulin receptor activity. J. Biol. Chem. 100712. https://doi.org/10.1016/j.jbc.2021.100712.

Zuo, J., Lan, Y., Hu, H., Hou, X., Li, J., Wang, T., et al., 2021. Metabolomics-based multidimensional network biomarkers for diabetic retinopathy identification in patients with type 2 diabetes mellitus. BMJ Open Diabetes Res. Care 9 (1). https://doi.org/10.1136/bmjdrc-2020-001443.