HHS Public Access

Author manuscript

Prog Retin Eye Res. Author manuscript; available in PMC 2024 July 22.

Published in final edited form as:

Prog Retin Eye Res. 2024 July ; 101: 101271. doi:10.1016/j.preteyeres.2024.101271.

"Resilience to diabetic retinopathy"

Anara Serikbaeva[1,*], **Yanliang Li**[2,*], **Simon Ma**[3], **Darvin Yi**[2,3], **Andrius Kazlauskas**[1,2,*]

[1]Department of Physiology and Biophysics, University of Illinois at Chicago, 1905 West Taylor St, Chicago, IL 60612

[2]Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago, 1905 West Taylor St, Chicago, IL 60612

[3]Department of Biomedical Engineering, University of Illinois at Chicago, 1905 West Taylor St, Chicago, IL 60612

Abstract

Chronic elevation of blood glucose at first causes relatively minor changes to the neural and vascular components of the retina. As the duration of hyperglycemia persists, the nature and extent of damage increases and becomes readily detectable. While this second, overt manifestation of diabetic retinopathy (DR) has been studied extensively, what prevents maximal damage from the very start of hyperglycemia remains largely unexplored. Recent studies indicate that diabetes (DM) engages mitochondria-based defense during the retinopathy-resistant phase, and thereby enables the retina to remain healthy in the face of hyperglycemia. Such resilience is transient, and its deterioration results in progressive accumulation of retinal damage. The concepts that co-emerge with these discoveries set the stage for novel intellectual and therapeutic opportunities within the DR field. Identification of biomarkers and mediators of protection from DM-mediated damage will enable development of resilience-based therapies that will indefinitely delay the onset of DR.

1. DR is one of the complications of DM

Types of diabetes mellitus (DM)

Diabetes mellitus (DM) is a prevalent and chronic metabolic disorder characterized by elevated blood glucose caused by aberrations in the production and/or response to insulin. The World Health Organization (WHO) ranked DM as the ninth leading cause of global mortality in 2019. As of 2021, approximately 529 million individuals worldwide were living with DM, with projections estimating a staggering increase to about 1.31 billion by 2050 (Collaborators, 2023). According to the American Diabetes Association (ADA) "Standards of Care in Diabetes" (American Diabetes Association Professional Practice, 2024), DM is classified into 4 categories: type 2 (T2D), type 1 (T1D), DM that is related to gestation, and DM that results from other causes such as monogenic diabetes syndromes, diseases of the

**corresponding author: Andrius Kazlauskas, [1]Department of Physiology and Biophysics, [2]Department of Ophthalmology and Visual Sciences, [3]Department of Bioengineering, University of Illinois at Chicago, 1905 W Taylor St, Chicago, IL 60612, ak20@uic.edu.
*Equal contribution

exocrine pancreas, and exposure to drugs or chemicals. Of the four categories of DM, T2D and T1D are the most common (American Diabetes, 2014).

Type 2 diabetes mellitus (T2D), also known as adult-onset diabetes, constitutes about 90–95% of DM. This type of DM is characterized by two main insulin-related anomalies: insulin resistance and β-cell dysfunction (Roden and Shulman, 2019). Insulin resistance results from decreased insulin responsiveness of cells in the peripheral tissues, in particular the muscle, liver, and adipose. In the early stage of T2D, insulin-resistance triggers β-cells hyperfunction (hyperinsulinemia) to achieve normal glucose homeostasis. As T2D progresses, β-cells fail to produce enough insulin, resulting in hyperglycemia.

High body mass index (BMI) is the predominant risk factor for T2D, contributing to over 50% of global disability-adjusted life-years in 2021 (Collaborators, 2023). Factors such as increased availability of shelf-stable and high-calorie products, limited access to healthy foods, increased consumption of ultra-processed foods (Delpino et al., 2022), and sedentary lifestyles (Popkin et al., 2012) have fueled the rise in obesity rates, thereby exacerbating the prevalence of T2D. Despite the potential of obesity management as a readily available and effect approach to mitigate the progression of DM, current trends indicate a persistent increase in obesity rates (Popkin et al., 2012). Effective interventions for T2D, which could be sustained over two years, encompass weight reduction through stringent caloric control, enhanced physical activity, and bariatric surgery (Schauer et al., 2017; Taheri et al., 2020). Early detection, patient education, regular healthcare consultations, lifestyle modifications, and early pharmacological intervention constitute pivotal strategies in preventing or delaying the onset of T2D (Gong et al., 2019; Nauck et al., 2021). However, the implementation of proactive healthcare systems and infrastructural support for early interventions remains limited in many countries.

The other common type of DM is type 1 diabetes mellitus (T1D), also known juvenile-onset diabetes. It is an autoimmune disorder characterized by T-cell-mediated destruction of pancreatic β-cells, which results in insulin deficiency and hyperglycemia (Kahaly and Hansen, 2016; Knip and Siljander, 2008).

While both genetic and environmental factors contribute to the risk of developing T1D, the precise mechanism remains incompletely elucidated (Banday et al., 2020). Genetic predisposition, infectious agents, nutrition, toxins, psychosocial and socioeconomic determinants, prenatal conditions, and environmental exposures have been implicated in T1DM development (Stene et al., 2023). Although relatives of individuals with T1D exhibit a significantly elevated risk, almost 90% of newly diagnosed patients have no family history of the disease (Turtinen et al., 2019). Despite extensive research, few non-genetic factors have been consistently associated with the risk of islet autoimmunity or T1D onset (Herold et al., 2024). Ongoing large-scale observational studies, such as ENDIA, GPPAD, TEDDY, and DAISY, are investigating potential environmental triggers, including viral exposures, dietary patterns, and the microbiome, to better understand their role in the pathogenesis of T1D (Herold et al., 2024).

Types of complications

DM damages both large and small blood vessels throughout the body, and thereby compromises a patients' health outcomes and quality of life. Complications of the macrovasculature, including the coronary and cerebrovascular arteries, are the leading cause of death in individuals with DM (Morrish et al., 2001). Complications of the microvascular network in the kidneys, eyes and peripheral nerves also develop in patients with DM, and are more common the macrovascular complications (Deshpande et al., 2008). Among the microvascular complications, diabetic retinopathy (DR) is the most common; its overall prevalence in individuals with DM is 34.6% (Yau et al., 2012). Even though the prevalence of DR is higher in people with T1D (77.3 vs. 25.2%) (Yau et al., 2012), both T1D and T2D patients are equally susceptible to DR (Skyler et al., 2017).

Despite extensive research efforts, there are currently no definitive cures for DM-related complications. Consequently, prevention is paramount, and such strategies include controlling blood glucose levels, blood pressure, and lipid profiles. The effectiveness of these options varies among individuals, necessitating a personalized approach. Additionally, the burden of complications significantly impairs patients' quality of life, affecting an individual’s physical and psychological well-being, daily functioning, and socioeconomic status. Addressing complications remains a critical aspect of the management of patients who develop DM.

Types of DR

DR is the leading cause of visual loss amongst working-age adults within the western population (Teo et al., 2021). DR is classified according to a severity scale, which is based on the morphology and functionality of the retinal vasculature. There are two main types of DR: non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR) (Wong et al., 2016). NPDR typically precedes PDR and is characterized by vascular aberrations such as microaneurysms and hemorrhages, as well as exudates. NPDR is staged across a spectrum of severity as mild, moderate, or severe. PDR, on the other hand, represents an advanced stage of DR that is characterized by the presence of retinal neovascularization resulting from DM-induced ischemia. These vessels are fragile and prone to bleeding, leading to severe vision loss and even blindness if left untreated. Diabetic macular edema (DME), or swelling of the central retina, can develop in patients with any stage of DR, independent of DR progression. It results from increased vascular permeability and can lead to significant vision loss. The definition and classification of DME in the current guidelines are updated with information from OCT (optical coherence tomography) (Table 1). The impact of DR on a patient's vision and quality of life depends on various factors, including the stage of the disease, the effectiveness of treatment, and individual health factors. Early-stage DR may not cause noticeable symptoms, but as it progresses, it can impair vision and even blindness if left untreated.

Diagnosing DR

Diagnosis of DR in patients is based on the appearance of the retinal vasculature and thickness of the retina (Table 1). While DM also affects the neural compartment of the retina, such changes are not included in determining a patient’s diagnosis. DM can cause

loss of neural cells, neural dysfunction and a decline in vision (Simo et al., 2014; Simo et al., 2018). In some instances, neural dysfunction can be detected prior to the vascular abnormalities that are diagnostic of DR (Sohn et al., 2016; Srinivasan et al., 2022).

The growing appreciation that DM affects both vascular and neural components of the retina is the rationale to develop diagnostic approaches that include both of these retinal "compartments". However, such efforts must overcome substantial hurdles. Processing visual information by the retina is complex; while it is known that many neural cell types participate, their relative contribution is still emerging (Joesch and Meister, 2016; Kim et al., 2008; Nath and Schwartz, 2017; Vlasits et al., 2019). The advent of this type of information is a prerequisite for assessing the impact of DM on this neural-based process. Furthermore, the intricate physical and functional relationship between the neural and vascular compartments of the retina present a challenge to determining the direct effect of DM on either. Finally, analysis of neural function in patients can be time consuming and require specialized equipment and staff, which is not as widely available as the infrastructure for analyzing the retinal vasculature. Some of these challenges may be circumvented by artificial intelligence-based diagnosis, which is described in the section below.

Artificial intelligence to diagnose DR

Individuals who have developed DM, and are therefore at risk of developing DR, are encouraged to undergo retinal screening, so that timely treatment can be implemented to prevent vision loss (American Diabetes, 2020). Such screening/diagnosis has historically been done by ophthalmologists who view retinal fundus images. Both the time-consuming nature of this process, and the advent of artificial intelligence (AI) have contributed to the development of an AI-based approach for diagnosing DR.

The initial strategy in the development of automated detection of DR from fundus images was machine learning models. These models used image-processing techniques to extract features of early DR that are listed in Table 1 such as microaneurysms, hemorrhages, exudates, and cotton wool spots (Abramoff et al., 2008; Abramoff et al., 2010; Gardner et al., 1996; Quellec et al., 2011). Additional feature extraction could be geometric or morphological features (such as Scale Invariant Feature Transform (Lowe, 1999) or Histogram of Gradient features (Dalal and Triggs, 2005)) that were not directly tied with the clinical definition of DR, but more general image-based features that could hopefully be correlated with the signal of DR.

Recently, deep learning has advanced many areas of computer vision, such as image classification (He, 2016; Huang, 2017; Krizhevsky, 2012; Simonyan and Zisserman, 2014; Szegedy, 2016), object detection (He, 2017; Lin et al., 2020; Redmon, 2017; Ren et al., 2017), and semantic segmentation (Badrinarayanan et al., 2017; Chen et al., 2017a; He et al., 2015; Ronneberger et al., 2015; Shelhamer et al., 2017). In contrast to the feature-based models discussed before, deep learning is usually an end-to-end approach. This means that the model sees the full image and the target label (e.g. DR stages), and learns to extract features that would help it differentiate images of the different target labels most efficiently in the training dataset. As with in several other medical image classification tasks (Dunnmon et al., 2019; Esteva et al., 2017; Park et al., 2019), this proved to be a more efficient means

of learning to detect DR stages from fundus photographs. (Abramoff et al., 2018; Abramoff et al., 2016; Dai et al., 2021; Gulshan et al., 2016; Gulshan et al., 2019; Ruamviboonsuk et al., 2022; Ting et al., 2017). Deep learning may have the potential to detect signals in biomedical image analysis, which humans haven't been looking for, such as gender prediction using fundus images. Currently, clinicians are not aware of specific retinal feature variations related to gender, highlighting the importance of model explainability. Therefore, deep learning may enable clinician-driven discovery of novel biomarkers for early detection of disease (Babenko et al., 2022; Korot et al., 2021). Similarly, AI may uncover biomarkers of resilience to DR.

AI performs well on feature extraction, classification on specific eye disease given large amount of labeled retinal images from human experts; but not as good as doctors in terms of explainability of image details. We provide a comparison of the pros and cons of AI in healthcare, including (1) accuracy performance, (2) time and cost efficiency, (3) model robustness and generalization (Table 2).

(1) Accuracy performance. AI system tends to have higher sensitivity but lower specificity than general ophthalmologists or retina specialists. Before applying deep learning, the diagnostic accuracy of computer detection programs has been reported to be comparable to that of specialists and expert readers. The model sensitivity/specificity was 97%/59% compared to 71%-91%/95%-100% from three retinal specialists (Abramoff et al., 2013). Later on, as deep learning, where all transformations are determined from training data, instead of being designed by experts, has been highly successful and popular in computer vision, the AI system exceeded all superiority endpoints (from FDA) at sensitivity of 87% (>85%), specificity of 91% (>82.5%), demonstrating AI's ability to bring specialty-level diagnostics to primary care settings (Abramoff et al., 2018). For consistency of predicting results, as AI is usually a static algorithm, its behavior does not differ based on workload, day of the week, or other human factors. On the other hand, AI is vulnerable to adversarial attacks from crafted input data (Shah, 2018).

(2) Time and cost efficiency. For AI development, the cost is relatively low compared to training experts in healthcare systems. For AI application, the model inference time is in less than 25 seconds, provided at the point of care. For most people, the model will eliminate the need for a separate visit to an ophthalmologist (Abramoff et al., 2013).

(3) Model robustness and generalization. AI is susceptible to Out-of-Distribution robustness problems. A Model trained at one institution might not perform highly when deployed at another institution, often due to different imaging protocols, imaging vendors, and patient populations (Rashidisabet et al., 2023).

The FDA authorized IDx-DR "for use by health care providers to automatically detect more than mild DR (mtmDR), including moderate, severe nonproliferative DR, proliferative DR, and/or clinically significant macular edema in patients (22 years of age or older) diagnosed with DM who have not been previously diagnosed with DR", as the first autonomous diagnostic AI system in 2018 (Administration, 2018). It is a clinically-inspired algorithm, and therefore has independent, validated detectors for pathological lesions (microaneurysms,

hemorrhages, etc.), the output of which are then fused into a disease level output, using a separately trained and validated machine learning algorithm. Detectors of diagnostic features (e.g., hemorrhages, exudates) were developed with deep learning, except the microaneurysm detector which was from the featurebank (Abramoff et al., 2018). Many patients with mtmDR need to be referred for eye specialist care (hence, mtmDR is typically considered referable), but vision threatening diabetic retinopathy (vtDR) may need more urgent intervention. In 2021, another fully autonomous AI system for detecting DR without human oversight was The EyeArt system (Eyenuk, Inc) with 96% sensitivity and 88% specificity. It was used in detecting both mtmDR and, for the first time, vtDR, including severe nonproliferative DR, proliferative DR, and/or clinically significant macular edema) (Ipp et al., 2021). Further study evaluated and compared the performances of the EyeArt system and dilated ophthalmoscopy on a diverse cohort of subjects enrolled at multiple centers with geographic diversity, which indicated that the AI system had a higher sensitivity for detecting mtmDR than either general ophthalmologists or retina specialists (Lim et al., 2023).

Current approaches to manage DR

Current approaches to manage DR encompass both preventive strategies and mitigation of symptoms. Although lifestyle factors such as maintaining a healthy diet, regular physical activity, and strict control of blood glucose levels are associated with a reduced risk of developing DR, no approach works for all individuals. Furthermore, aggressively reducing blood glucose using either insulin- or non-insulin-based approaches can worsen DR in some patients (Bain et al., 2019). For patients who have already developed DR, treatment options aim to halt disease progression and preserve vision. Anti-vascular endothelial growth factor (anti-VEGF) therapy, corticosteroids, and laser photocoagulation are among the mainstay treatments for DR (Wykoff, 2017; Wykoff et al., 2016). Agents that neutralize VEGF (anti-VEGFs), such as bevacizumab, ranibizumab and aflibercept, work by inhibiting abnormal blood vessel growth and reducing vascular leakage, thereby improving visual outcomes. Corticosteroids typically reduce inflammation and edema in the retina. Laser photocoagulation, including focal/grid and pan-retinal photocoagulation, aim to seal leaking blood vessels and prevent the growth of abnormal ones. These treatment modalities, often used in combination or sequentially, have demonstrated efficacy in slowing the progression of DR and preserving vision in affected patients (Wong et al., 2018).

DM induces changes within the retina prior to the onset of DR

Technological advances that improve our ability to observe the retina reveal DM-driven changes that precede the earliest signs of clinical retinopathy (Table 1) (Cheung et al., 2015). These include changes in the perfused capillary density (Chen et al., 2017b; Dimitrova et al., 2017; Rosen et al., 2019; Scarinci et al., 2018), leakage (Tichauer et al., 2015), expansion of the foveal avascular zone (Ashraf et al., 2018) and loss of vascular mural cells (Huang et al., 2024). Loss of neural cells and function also occurs prior to the onset of clinically-recognized DR (Channa et al., 2021; Motz et al., 2020). As compared with the nature and extent of the damage the accumulates following prolonged DM, the early changes are minor.

2. Risk factors for developing DR

HG is the major risk factor for DR

The magnitude and duration of hyperglycemia greatly increases the risk of complications such as DR (Cheung et al., 2010; Chew et al., 2010). This concept emerged from several clinical studies demonstrating that controlling hyperglycemia reduced the incidence of DR and slowed its progression (1991; 1993; 1999; Metascreen Writing et al., 2006; Zoungas et al., 2017). Thus insulin-related dysfunction (the cause of DM) results in hyperglycemia, which substantially elevates an individual's risk of eventually developing DR. While insulin's effect on blood sugar has been the focus of these studies, insulin has many additional functions (such as governing mitochondrial homeostasis (Amorim et al., 2022; Brys et al., 2010; Zarse et al., 2012)), which may contribute to the beneficial effects of insulin-based therapies.

Dyslipidemia, hypertension, may also influence DR. However, the associations between plasma lipids, lipoproteins, and DR are not consistently strong at the individual patient level (Bryl et al., 2022). Similarly, while hypertension has been associated with an elevated risk of DR (Yau et al., 2012), the ACCORD study (Chew et al., 2010) showed that more intensive blood pressure control may not confer additional benefits in slowing retinopathy progression compared to standard control measures. Optimization of systemic risk factors such as HbA1C, blood pressure and serum levels of total cholesterol explain DR progression and PDR development in only 9% and 10% of affected patients, respectively (Antonetti et al., 2021). Other risk factors for DR include genetic predisposition, high BMI, puberty, pregnancy, and cataract surgery (Yau et al., 2012). However, clinical studies on patients living with DM have shown substantial variation in the onset and severity of DR that is not fully explained by known risk factors (Vujosevic et al., 2020). These findings underscore the complex interplay of various factors in the initiation and progression of DR, suggesting that additional unidentified factors may also play critical roles.

Recent studies indicate that sleep protects from many diseases, including DR, and hence poor sleep may be an additional risk factor for DR. There is a growing appreciation that sleep enforces health by preventing a plethora of pathologies (Buysse, 2014; Lopez-Otin and Kroemer, 2021; Sang et al., 2023). Sleep disturbances such as short or long sleep duration, poor sleep quality or mistimed sleep compromise glycemic control and increases an individual's risk of developing DM (Anothaisintawee et al., 2016). Similarly, poor sleep quality is associated with both activation of process that drive DR, and the presence of DR (Besedovsky et al., 2012; Joussen et al., 2004; Mullington et al., 2010). For instance, obstructive sleep apnea contributes to insulin resistance, elevated blood pressure, endothelial dysfunction, increased systemic inflammation and oxidative stress (Anothaisintawee et al., 2016; Garcia-Sanchez et al., 2022; Han et al., 2020; Lee et al., 2017; Reutrakul and Mokhlesi, 2017). Determining if such associations are causative will substantially advance this field of research.

In summary, while elevated and prolonged blood sugar is an established risk factor for DR, there is a growing appreciation that it is not the only one.

DM damages both the neural and vascular compartments of the retina and causes retinal dysfunction

Once resilience (the innate ability of the retina to remain healthy in the face of DM) is lost, the vascular, neural and immune components of the retina progressively accumulate damage. Because such damage is associated with biochemical and molecular changes that accompany DM, they are widely considered to be the drivers of such damage. For instance, hyperglycemia-driven elevation of oxidative stress correlates with the death of both vascular, and neural cell types (Barber et al., 2011). As the endothelium is damaged, it triggers leukostasis, which is binding of leukocytes to endothelial cells within retinal capillaries (Barouch et al., 2000; Lessieur et al., 2022; Miyamoto et al., 1998; Schroder et al., 1991; Serra et al., 2012). Leukostasis is required for experimental DR; preventing leukostasis attenuates injury and death of endothelial cells within the retina (Joussen et al., 2001; Joussen et al., 2004; Joussen et al., 2003). Similarly, genetically or pharmacologically suppressing proteases that are secreted by neutrophils protects diabetic animals from DR (Lessieur et al., 2021). The importance of immune homeostasis in DR is further illustrated by the discovery that perturbing immune homeostasis is sufficient to cause retinopathy in non-DM mice, i.e., in the absence of all other DM-driven perturbations. Eliminating the somatostatinergic neurons within the hypothalamus of non-DM mice skewed the profile of circulating immune cells and caused retinal damage that was typical of DR (Huang et al., 2021). Notably, the number of somatostatinergic neurons are decreased in animal models of DM (Bhatwadekar et al., 2017). The functional interdependence of the various compartments of the retina makes it difficult to define the relative contribution of DM-driven damage on any one of the retinal compartments to resultant retinal dysfunction.

3. Oxidative stress is a central driver of DR

HG increases oxidative stress, which is a central driver of damage to the retina

There are a number of ways that the retina responds to persistent hyperglycemia that accompanies DM. These include changes in glucose metabolism (decreased glycolysis and pentose phosphate pathway; increased polyol pathway), perturbation of biochemical and signaling pathways (increased production of advanced glycation end products (AGEs), activation of protein kinase C), increased inflammation and oxidative stress (Bierhansl et al., 2017). Such changes can be synergistic, for instance oxidative stress promotes inflammation and vice-a-versa. Eventually they damage and thereby compromise the function of both the vascular and neural compartments of the retina. The current understanding of the pathogenesis of DR has been comprehensively presented in a number of excellent review articles (Antonetti et al., 2012; Antonetti et al., 2021; Cai and Boulton, 2002; Hammer et al., 2017; Kowluru et al., 2015; Noh and King, 2007; Robinson et al., 2012; Stitt et al., 2016; Wu and Zou, 2022). In the sections below we will focus on HG-mediated oxidative stress; Figure 1 provides an overview of how HG-driven oxidative stress underlies damage which occurs to the retina in diabetic individuals.

DM increases oxidative stress in two subcellular compartments (the cytoplasm and mitochondria) in numerous retinal cell types, including photoreceptors, which are the majors producers of ROS in the retina (Arden and Sivaprasad, 2012; de Gooyer et al., 2006;

Du et al., 2013). Hyperglycemia increases the level of oxidative stress in the cytoplasm by a process the includes increasing the level of diacylglycerol and thereby activating protein kinase C family members, which in turn activate NADPH-dependent oxidases (NOX) (Bierhansl et al., 2017; Drummond and Sobey, 2014; Jiang et al., 2011; Laddha and Kulkarni, 2020; Noh and King, 2007; Peng et al., 2019). In endothelial cells, the resultant increase in reactive oxygen species (ROSs) can uncouple endothelial cells nitric oxide synthase (eNOS) so that instead of producing nitric oxide (NO), it generates superoxide, a highly reactive ROS species (de Zeeuw et al., 2015; Meza et al., 2019; Sasaki et al., 2008).

A rise in the level of ROS within the mitochondrial is initially driven by increased flux through the TCA cycle, which is coupled to the ROS-producing electron transport chain (ETC) (Nissanka and Moraes, 2018; Scialo et al., 2017; Stowe and Camara, 2009; Zorov et al., 2014). Overwhelming the mitochondria's innate ability to control ROS results in damage of mitochondrial DNA, which encodes some of the members of the ETC. Failure to produce a complete set of ETC proteins/subunits results in ETC dysfunction that drives progressive self-amplifying damage (Ferrington et al., 2020; Kowluru et al., 2015). As outlined below, increasing clearance of dysfunctional (ROS-producing) mitochondria and a compensatory increase in biogenesis enables cells to remain healthy in the face of ongoing HG-induced damage. As mitophagy fails, damaged mitochondria accumulate, oxidative stress rises and retinal damage ensues (Hombrebueno et al., 2019). Together these studies show that DM increases oxidative stress within multiple subcellular compartments of retinal cells and thereby damages the retina.

There is a growing appreciation, that the endothelium, which is in direct contact with noxious agents that are present in the circulation, is resistant to their damage. For instance, retinal endothelial cells are resistant to HG-induced damage such as senescence, oxidative stress and mitochondrial dysfunction (Bertelli et al., 2022; Busik et al., 2008; Serikbaeva et al., 2022). Similarly, the endothelium of the aorta resists damage caused by pro-atherogenic insults such as elevated cholesterol (Berk et al., 2001; Cunningham and Gotlieb, 2005; Jain et al., 2014). The endothelium within the aorta, which experiences laminar shear stress organizes components of signaling pathways (Notch, VE-PTP, Ca channels, scaffolds) in a way that activates expression of genes (*Klf2, Nos3*) that provide protection (via eNOS) for all of the cell types that are at risk for plaque formation (Coon et al., 2022; Hong et al., 2023; Mack et al., 2017; Mantilidewi et al., 2014; Shirakura et al., 2023). By comparison with this exhaustively-studied endothelial cell-based system that protects the aorta from atherosclerosis, the mechanism by which the retinal endothelium resist hyperglycemia remains largely unexplored.

In light of the intimate functional relationship between the various compartments of the retina (e.g. the neural vascular unit), it seems plausible that the benefit of endothelial cell-based protection could extend to the neural retina. This concept has been observed in the context of retinal damage (instead of protection). Mice that express a mutated tight junction protein (occludin), which prevents DM-driven leakage of retinal blood vessels, also attenuates the DM-mediated decline in vision (Goncalves et al., 2021). Similarly, retinal degeneration can be prevented with endothelial cell precursors, which promote vascularization of the retina (Otani et al., 2004; Ritter et al., 2006). Furthermore, expression

of Notch3 on vascular cells prevents neural degeneration of the retina (Romay et al., 2024). These studies demonstrate that the benefit of preventing damage and/or facilitating repair of the vasculature extends to the neural retina. Whether this same principle applies to resilience, i.e. if there is a bystander effect of resilience, have yet to be determined.

Chronic HG rewires metabolism in ways that elevate oxidative stress

Chronic elevation of blood sugar rewires metabolism in a variety of ways, and some of these changes result in increased oxidative stress (Brownlee, 2001; de Zeeuw et al., 2015). For instance, HG suppresses the pentose phosphate pathway (PPP), a glycolytic side pathway, (Zhang et al., 2000; Zhang et al., 2010) and elevates oxidative stress in at least two ways. Inhibiting PPP reduces its output of NADPH, which is required for the reduction of oxidized glutathione (GSSG) to regenerate the anti-oxidant glutathione (GSH). Similarly, the level of ribose-5-phosphate, which is required for nucleotide synthesis, declines as a result of suppressing the PPP. Both of these changes compromise mitochondrial redox. The mitochondria depend on GSH to limit oxidative stress, and on nucleotide synthesis to make new mitochondrial DNA to offset its loss via mitophagy (Mari et al., 2020). These are two examples of how HG-driven metabolic perturbation impairs endogenous systems to prevent mitochondrial oxidative stress. Other examples of how chronic hyperglycemia affects metabolism in ways that result in elevated oxidative stress have been detailed in a number of review articles (Brownlee, 2001; Obrosova et al., 2001).

Evidence that oxidative stress is required for DM to damage the retina

The central role of oxidative stress in DR pathogenesis is revealed by observations that suppressing oxidative stress by genetic or pharmacological approaches protects the retina from DM-driven damage in experimental animals. Pharmacological agents that normalize mitochondrial oxidative stress suppress HG-mediated pathways that damage the retina (Du et al., 2003; Nishikawa et al., 2000). Furthermore, overexpressing *Sod2* prevents DM-induced mitochondrial oxidative stress and dysfunction, and also protects mice from developing DR in the face of DM (Goto et al., 2008; Kowluru et al., 2006). Recent studies indicate that an NOX4 inhibitor (GLX7013114) protects diabetic rats from retinopathy (Dionysopoulou et al., 2023). Together these studies indicate that the DM-mediated increase in oxidative stress in both subcellular compartments are required for DM to damage the retina.

The importance of elevated oxidative stress for developing DR is reinforced by observations from many laboratories that anti-oxidant therapy protects experimental animals from DR. Supplementing the diet of Goto-Kakizaki rats (a T2D model) with a combination of vitamins C and E for 36 weeks prevents hallmarks of DR, including acellular capillaries and pericyte ghosts (Yatoh et al., 2006). Similarly, supplementing the diet of T1D rats with lipoic acid for 44 weeks inhibits mitochondrial dysfunction and capillary cell apoptosis (Kowluru and Odenbach, 2004). Furthermore, the administration of the polyphenol antioxidants found in green tea suppresses the DM-driven decline of the endogenous antioxidant defense system (including SOD, GSH and CAT) and protects from DR (Kumar et al., 2012). Moreover, treating diabetic rats with melatonin, a strong antioxidant naturally secreted by the pineal gland, suppresses VEGF and oxidation of protein (Djordjevic et al., 2018) and

reduces leakage of retinal vessels (Mehrzadi et al., 2018). Notably, successful antioxidant interventions in animal models often involve initiating treatment close to the onset of DM, before development of DR and administering treatment for extended durations.

While the efficacy of antioxidant supplements in protecting patients from DR remains controversial, there have been promising results, especially those using a combination of antioxidants. For example, a 5-year follow-up study by García-Medina et al. (Garcia-Medina et al., 2011) showed that oral administration of Vitalux Forte®, containing vitamins C and E, lutein, b-carotene and trace elements, significantly reduced plasma lipid peroxidation end products and slowed the progression of DR in patients with T2D with NPDR. Another study using Nutrof Omega®, containing vitamins C, D, B and E, omega-3, lutein, glutathione and trace elements, for 18 months showed a significant reduction in total oxidative stress, plasma lipid peroxidation by-products and decreased DR progression and onset (Roig-Revert et al., 2015). Similarly, oral administration of Nutrof Omega® for 38 months significantly reduced pro-oxidants, increased antioxidants and slowed DR progression in patients with T2D with NPDR (Sanz-González et al., 2020). The profiles of patients who benefit from these types of antioxidant supplements are T2D without DR or mild-to-moderate NPDR without DME or with DME but without the thickening of the retina. These findings suggest that antioxidants have the potential to be beneficial when administered during the early stages of DR, when anatomical damage is not excessive, and that they may not be able to reverse retinal damage once it has occurred (Chen et al., 2016; Kowluru, 2019).

Taken together, these studies indicate that oxidative stress is a viable therapeutic target. Furthermore, since such approaches are best when used prior to the onset of retinal damage. A plausible explanation for why prevention is more effective than treatment is that prolonged hyperglycemia induced epigenetic changes. Such changes are enduring and the reason for metabolic memory (Natarajan, 2021). Consequently, it is critical to intervene before such durable and pathogenic modifications occur. This concept is illustrated in a publication by Biswal et al. (Biswal et al., 2017), who demonstrated that the timing of antioxidant gene therapy administration is critical for efficacy; the therapy failed to be beneficial if is provided after tissue damage has occurred. Consequently, clinical trials should prioritize prevention strategies, specifically targeting patients in the nascent stages of DR. By doing so, interventions will prevent accumulation of irreversible damage.

Metabolism-independent effects of HG (osmolarity)

Elevated blood glucose not only alters metabolism of cells, but also increases the osmolarity of their extracellular environment. Recent studies indicate that while increased osmolarity is not without consequence, metabolism-related changes are required for hyperglycemia-mediated damage.

In vitro studies with primary mouse Muller cells and a human Muller cell line (MIO-M1) demonstrated that clearance of mitochondria (mitophagy) increased when cells were cultured in medium containing an elevated level of either D-glucose or L-glucose, which can, or cannot be metabolized, respectively. In cells cultured in L-glucose, increased mitophagy was accompanied by an increase in mitochondrial biogenesis and hence the mitochondrial content remained unchanged. In contrast, the amount of mitochondria

declined in cells cultured in D-glucose because the increase in mitophagy was not accompanied by a corresponding increase in mitochondrial biogenesis (Hombrebueno et al., 2019). These observations demonstrate that increasing the osmolarity can alter mitochondrial homeostasis. Furthermore, when the boost in osmolarity is driven by D-glucose, it elicits a distinct response, presumably because D-glucose affects both osmolarity and glucose-related metabolism. Thus, the metabolism-mediated aspect of the response is both dominant, and detrimental.

Studies with Slc2a1knock-out mice shed light on the hyperglycemia-driven increase in osmolarity in the context of a T1D mouse model of DR (Holoman et al., 2021). The Slc2a1 gene encodes GLUT1, the primary glucose transporter in the retina (Kumagai et al., 1994; Rizzolo, 1997). While control mice (expressing two alleles of Slc2a1) experience both hyperglycemia-induced changes in metabolism, and increased osmolarity, in Slc2a1+/− mice the effect of hyperglycemia on metabolism is reduced, without changing the impact on osmolarity. Such mice are resistant to DR (Holoman et al., 2021). These in vivo studies align with the in vitro findings that it is the metabolism-dependent effect of hyperglycemia that is essential for DM-driven damage to the retina.

While the section above was written in a way that separated the impact of elevated glucose on osmolarity from its effect on metabolism, this distinction is blurred by the fact that some of the ways in which glucose can be metabolized can also contribute to hyperosmolarity. The polyol pathway coverts glucose to sorbitol and then to fructose. DM-driven inhibition of this pathway leads to an accumulation of sorbitol, which contributes to the rise in osmolarity (Lorenzi, 2007). If accumulation of sorbitol approaches the level of the glucose concentration, then this metabolic product may also contribute to the overall effect of osmolarity.

4. DM-induces mitochondrial dysfunction, which sets the stage for DM to damage the retina

Mitochondrial homeostasis

Mitochondrial homeostasis is essential for mitochondrial function, which is to respond to energetic demands via metabolism-related biochemical processes that they govern: oxidative phosphorylation (OXPHOS), the Krebs cycle and β-oxidation of fatty acids (Trefts and Shaw, 2021). Mitochondrial homeostasis is an interconnected process that can be organized into 4 phases: biogenesis, fusion, fission and mitophagy (Figure 2). Biogenesis generates new mitochondria from existing mitochondria, and this process involves synthesis of new DNA, proteins and lipids (Popov, 2020). Fusion incorporates newly minted mitochondria into the mitochondrial network, and also mixes the contents of the entire mitochondrial network (Chen et al., 2007; Detmer and Chan, 2007). This process monitors and identifies dysfunctional regions of mitochondria, and subjects them to fission, which physically isolates them. “Dysfunction” within the mitochondria can include production of excess reactive oxygen species and depolarization of the mitochondrial membrane potential. Finally, mitophagy is the process by which mitochondrial fragments are sent into the lysosome for disposal (Herhaus and Dikic, 2015; Pickles et al., 2018). The inter-relatedness

of the 4 phases of mitochondrial homeostasis is underscored by the recent observation that fission, which is a prerequisite for mitophagy, can also promote biogenesis (Burman et al., 2017; Kleele et al., 2021; Twig et al., 2008). In light of the central role of mitochondria in energy homeostasis, it is no surprise that each step of mitochondrial homeostasis is controlled by metabolism (Mishra and Chan, 2016). Figure 2 lists some of the key liaisons between metabolism and the transcription factors that govern mitochondrial biogenesis (Amorim et al., 2022; Herzig and Shaw, 2018)

Enhanced mitophagy can protect from a variety of pathologies (Gao et al., 2020; Nah et al., 2022; Sidarala et al., 2020; Xu et al., 2021), however, enforcing mitochondrial functionality involves more than just eliminating damaged fragments of the mitochondrial network. While increasing mitophagy will have an immediate benefit on mitochondrial functionality, if this is the only change, then the long-term consequence will be detrimental because the quantity of mitochondria within a cell will decline. Enforcing mitochondrial functionality requires harmonizing the rate of all 4 component of mitochondrial homeostasis (Figure 2) (Palikaras et al., 2015; Van Huynh et al., 2023). If these processes are unbalanced, then the cell's vulnerability to death-inducing insults increases (Zacharioudakis et al., 2022). There appear to be multiple contributors to the balance between biogenesis and mitophagy, and they often include PGC1α, the transcription factor that regulates biogenesis, and components of the mitophagy machine (Parkin, Pink1, Bnip3) (Liu et al., 2023). The exact mechanism by which the various contributors to the balance between biogenesis and mitophagy are monitored and coordinated remains incompletely understood.

Features of mitochondria that enable resistance to adversity

The mitochondria are designed to mitigate and tolerate dysfunction (Herzig and Shaw, 2018). Enzymes, which reduce the level of reactive oxygen species that damage mtDNA (e.g. SOD2; manganese-dependent superoxide dismutase) are localized in the mitochondria. Furthermore, in contrast to nuclear DNA, of which there is a single unit/cell, each cell contains many copies of mitochondrial DNA (mtDNA), which are housed in nucleoids. These back-up copies of mtDNA reduce the impact of damage to mtDNA.

In addition, damaged mtDNA can be repaired (Kazak et al., 2012; Santos et al., 2012). Santos et al. (Santos et al., 2012) found that after 15 days of DM, expression of enzymes that repair/replicate mitochondria within the retina increased, and then declined by 2 months. After 6 months, when the mitochondrial copy number had declined, the level of these enzymes had fallen below the level of non-DM counterparts. These studies reveal an apparent attempt by the retina to prevent DM-driven mitochondrial damage. Furthermore, suppression of such systems preceded the development of DR.

Moreover, mitochondrial functionality persists in the face of extensive damage of the mtDNA. In both cultured cells and mouse models, cells engineered to contain a mixture of wild-type and pathogenic mtDNA do not succumb to dysfunction until a high threshold of pathogenic mtDNA is breached (Nakada et al., 2009; Ono et al., 2001). Depending on the mutation, heteroplasmic cells (containing both mutant and wild-type mtDNA) can accumulate up to 60%–90% pathogenic mtDNA molecules without a noticeable decline in respiratory activity (Chomyn, 1998; Rossignol et al., 2003).

The design features of the mitochondria that enforces its integrity and thereby enable resistance to adversity include the presence of systems to prevent damage, the existence of many copies of mtDNA, systems to repair damage, the capability to eliminate mtDNA that has not been repaired, and the ability to retain functionality with only a minor fraction of the mtDNA intact.

DR is associated with aberrant mitochondrial homeostasis

There is unequivocal evidence that mitochondrial dysfunction within the retina is associated with early DR in both patients and experimental animals, and a growing appreciation that this damage is caused by HG-driven elevation of mitochondrial oxidative stress. The section below highlights some of the topics that are covered in detail in several recent review articles (Alka et al., 2023; Miller et al., 2020; Skeie et al., 2021; Wu and Zou, 2022).

Studies with cultured cells show that culturing them in HG perturbs mitochondrial homeostasis, and that such changes are required for the deleterious effects of HG (Antonetti et al., 2021; Devi et al., 2019; Duraisamy et al., 2019; Kang et al., 2010; Kim and Roy, 2020; Kim et al., 2020; Li et al., 2024; Roy et al., 2019; Trudeau et al., 2010; Trudeau et al., 2012; Wu et al., 2022; Xie et al., 2021; Zhang et al., 2022). Furthermore, the progressive damage to the retina that occurs in diabetic animals is associated with uncoupling of biogenesis and mitophagy (Hombrebueno et al., 2019). Increased retinal damage (accumulation of senescent cells and AGEs (advanced glycation end products) is associated with a decline in mitophagy that causes accumulation of dysfunctional mitochondria (Hombrebueno et al., 2019). Furthermore, mitochondrial hyperfusion, is associated with damage of the retina (Mueller cells) in both experimental animals and patients who have progressed to early DR (Anderson et al., 2024).Together these studies indicate that mitochondrial homeostasis is perturbed in the damaged retina of patients and experimental animals that have developed early stages of DR. This concept is re-enforced by the emerging appreciation that resilience – the innate ability of the retina to remain healthy in the face of DM – is associated with mitochondrial-based adaptation to hyperglycemia (Serikbaeva et al., 2022).

Mitochondria-based therapy to prevent DR

The observation that aberrant mitochondrial homeostasis is strongly associated with DM-induced damage of the retina raises the possibility that preventing or correcting it would protect from DR (Kaikini et al., 2017). Indeed, pharmacological suppression of DM-driven mitochondrial hyperfusion improves mitochondrial homeostasis, which includes increased mitophagy, and thereby allows the retina to remain healthy in the face of DM (Anderson et al., 2024). Others have also reported that mitochondrial homeostasis-directed therapy protects from DR. Notoginsenoside R1, which boosts mitophagy, protects mice from DR (Zhou et al., 2019). Experimental animals that lack genes, which drive fission (*Dnm1l*) are resistant to DM-induced damage of the retina (Kim et al., 2021). Similarly, pharmacological inhibition of Drp1, which is encoded by *Dnm1l*, prevents DR (Zhang et al., 2022). Curiously, antagonizing fission should prevent mitophagy and promote fusion (Figure 2). While these studies provide compelling evidence that DM perturbs mitochondrial homeostasis, and thereby causes damage to the retina, they do not provide a unified guide for how to enforce mitochondrial homeostasis in the face of DM. Alternative attempts, such

as global mitochondrial uncoupling with niclosamide ethanolamine did not protect diabetic mice from several complications including DR (Hinder et al., 2019). A better understanding of the mechanism by which the various phases of mitochondrial homeostasis remain in harmony as the mitochondria responses to changes in the quantity and nature of nutrients is essential to developing mitochondria-based therapy to prevent DR.

5. Evidence for the existence of resilience to pathology

The immune system is an example of an endogenous system that confers resilience to pathology. While the relative rare frequency of disease in individuals with a functional immune system strongly supports this concept, even more convincing is the sharp increase in susceptibility to a plethora of afflictions that results when the immune system is compromised.

Similarly, the incidence of disease increases sharply with age (Belikov, 2019; Guo et al., 2022; Niccoli and Partridge, 2012). Although a decline in the potency of the immune system is one of the changes that correlates with this increased vulnerability, additional age-related changes, such as compromised mitochondrial homeostasis are also likely to contribute. Similarly, VEGF signaling declines with age, and enforcing it promotes healthy aging and increases life span (Grunewald et al., 2021; Ungvari et al., 2018). The aging process and how it affect resilience to disease has been extensively investigated and this body of research has been summarized in a number of excellent review articles (Haigis and Yankner, 2010; Lopez-Otin et al., 2013, 2023).

Another approach to understand resistance to disease has emerged from attempts to define health (Lopez-Otin and Kroemer, 2021; Luu and Palczewski, 2018). A recent review article articulates eight hallmarks of health, which are organized into three categories (Table 3 (Lopez-Otin and Kroemer, 2021)). Of these eight, the four that are written in red are the ones that are particularly relevant to DR because their deterioration is a likely prerequisite for damage to accumulate in response to DM-related stress such as hyperglycemia. Those systems whose failure results in quintessential features of DR are likely to be the cornerstones of resilience to DR.

Recent publications have contributed to the growing realization that enforcing hallmarks of health such as those in the “Response to stress” category (Table 3) can protect from disease (Chen et al., 2023; Luu et al., 2023). A comprehensive, omics-based approach to identify druggable enzymes that prevent stress-induced retinal degeneration identified phosphodiesterases (PDEs) (Luu et al., 2023). Inhibition of PDEs suppressed bright light-induced retinal degeneration in *Abca4*$^{-/-}$*Rdh8*$^{-/-}$ double knock-out mice. Inhibition of PDEs, which regulate cyclic nucleotide-dependent signaling, was beneficial in two complementary ways: by enforcing somatic maintenance and preventing apoptosis. These discoveries indicate that health can be enforced via pharmacological approaches. More importantly, it shows that fortifying health prevents disease.

Luu et al. also reported that inhibiting PDEs protected mice from additional diseases; namely, genetic mutation-driven retinal degeneration and DR. Since many diseases can arise

from failure of a given health category (e.g. being overwhelmed by stress), enhancing such processes has the potential to protect from all diseases that manifest upon failure of a specific hallmark of health.

Resilience to DR is reminiscent of pre-conditioning/hormesis

Preconditioning/hormesis, a brief exposure to a sub-threshold level of injury, protects organs such as the brain and heart from subsequent insult (Calabrese, 2018; Gems and Partridge, 2008; Granger and Kvietys, 2015; Lopez-Otin and Kroemer, 2021; Murry et al., 1986). The mitochondria play a central role in this response. The initial insult elevates the level of mitochondrial reactive oxygen species thereby promotes expression of protective genes including BCL2 and SOD2 (Sivandzade et al., 2019). In addition, the functionality of the mitochondria is improved by increasing mitophagy to reduce the level of damaged mitochondria (Correia et al., 2010; Gottlieb and Gustafsson, 2011). More recent studies have elucidated molecular mediators of hormesis within specific organs. For instance, the exercise capacity of skeletal muscle is dependent on autophagy to clear dysfunctional mitochondria. These events are driven by the REDD1/TXNIP complex, which increases oxidative stress that boosts mitophagy (Qiao et al., 2015). Similarly, ischemic post-conditioning of the retina improves the retina's neural function and is dependent on autophagy, which also strongly suggests a mitochondrial involvement (Mathew et al., 2020). Together these findings indicate that mitochondria adapt to environmental conditions in ways that protect from pathology.

In contrast to the extensive literature focused on preconditioning-mediated protection of patients from cardiovascular disease, there are only a handful of publications investigating whether DM/HG-mediated stress induces hormesis. Patients with T1D are protected from ischemia-induced injury of skeletal muscle (Engbersen et al., 2012). Similarly, one week of DM protected mice from ischemia/reperfusion-induced heart injury as compared with non-DM mice (Ravingerova et al., 2010). The underlying mechanism of this phenomenon has not been addressed. As compared with ischemia- or hypoxia-induced preconditioning, HG-induced pre-conditioning is a largely unexplored area of research.

6. Resilience to DR

While the preceding sections focus on DM/HG-induced oxidative stress, this is not the only change that accompanies DM. Work from multiple investigators has contributed to our current appreciation that prolonged hyperglycemia triggers many interrelated drivers of pathology (inflammation, oxidative stress, mitochondrial dysfunction, senescence, etc.). Furthermore, targeting a single one of these changes has often been reported as an effective approach to prevent DR. Taken at face value the sum of these published studies suggest that DR requires a "perfect storm", i.e. that the entire plethora of DM-triggered drivers of pathology are necessary for retinopathy. This concept suggests that preventing any one of the pathogenic drivers of DR would suffice for resilience. In the context of this review, we chose a single putative driver of DR (mitochondrial oxidative stress) to facilitate the presentation of the key concept of resilience: suppression of those events and processes that damage the retina is a plausible explanation for how the retina resists injury in the face of DM.

After developing DM patients are initially resilient to DR

Patients who develop DM (either T1D or T2D) are initially resistant to DR, which develops slowly and progressively. It takes several decades for the proliferative form of DR (PDR) to manifest (1993; 1997; Aiello et al., 1998; Cruickshanks et al., 1992; Klein et al., 1984). It is only after the loss of the innate ability to remain healthy in the face of DM that the retina progressively accumulates damage (Figure 3). While understanding the underlying mechanism of resilience has enormous therapeutic potential (e.g. indefinitely delaying vision-threatening DR), this topic is understudied as compared with investigation of DM-driven damage of the retina (Yu et al., 2024).

In a small subset of patients with T1D, DR is delayed for 50 or more years. The exceptional resistance to DR of these participants of the Joslin 50-Year Medalist Study (Sun et al., 2011) is not associated with strict glycemic control; the average HbA1c was 7.3±1.0%. Thus, the retina remains healthy in the face of hyperglycemia. Further investigation revealed that durable resistance to DR is associated with elevated expression of retinol binding protein 3 (RBP3), a retinol transport protein expressed mainly by photoreceptors. A higher level of RBP3 in the vitreous was associated with less severe DR and a reduced risk of developing PDR in participants of the Medalist Study (Fickweiler et al., 2022). RBP3 associates with glucose transporter 1 (GLUT1) to decrease glucose uptake and thereby attenuate hyperglycemia-driven expression of inflammatory cytokines (Fickweiler et al., 2022). Curiously, RBP3 is an anti-oxidant (Chen et al., 2021; Gonzalez-Fernandez et al., 2014; Lee et al., 2016), which raises the possibility that RBP3-mediated protection also involves suppression of oxidative stress. Regardless of the underlying mechanism by which Medalists remain retinopathy-free for many decades, their existence demonstrates that it is possible for the retina to stay healthy in the face of DM for a very long time.

There is a growing appreciation that sleep enforces health by preventing a plethora of pathologies (Buysse, 2014; Lopez-Otin and Kroemer, 2021). Sleep disturbances such as short or long sleep duration, poor sleep quality or mistimed sleep compromise glycemic control and increases an individual’s risk of developing DM (Anothaisintawee et al., 2016). Similarly, poor sleep quality is associated with both activation of process that drive DR, and the presence of DR (Besedovsky et al., 2012; Joussen et al., 2004; Mullington et al., 2010). For instance, obstructive sleep apnea contributes to insulin resistance, elevated blood pressure, endothelial dysfunction, increased systemic inflammation and oxidative stress (Anothaisintawee et al., 2016; Garcia-Sanchez et al., 2022; Han et al., 2020; Lee et al., 2017; Reutrakul and Mokhlesi, 2017). A plausible mechanism by which sleep protects from DR has emerged from the discovery that the circadian clock governs mitophagy via SIRT1 (Ramsey et al., 2009). Perhaps sleep enforces mitochondrial homeostasis and thereby render the retina capable of withstanding stress caused by chronic HG.

Resilience is the innate ability of the retina to resist DM/HG-driven damage

Resilience is the innate ability of the retina to remain healthy in the face of DM (Figure 3). This retinopathy-resistant period occurs in both patients and experimental animals. Resilience to the HG-mediated damage is transient; as resilience deteriorates, damage of the retina progressively accumulates.

A plausible explanation for the retinopathy-resistant period of DM is that DM triggers protection from the DM-related damage. The results of the Medalist study (see above), as well as recent publications from our group (see below) provide support for this intriguing possibility.

Animal models of resilience to DR

Mouse models of DR (both T1D and T2D) recapitulate key steps of DR pathogenesis in humans (Engerman and Kern, 1995; Quiroz and Yazdanyar, 2021; Robinson et al., 2012). This includes a period of resilience that is followed by a progressive accumulation of damage to the neural and vascular compartments of the retina. However, the time course (both duration of resilience and onset of subsequent damage) is shorter in mice, and regardless of the duration of DM, they only develop the early stages of DR; they do not advance to PDR (Engerman and Kern, 1995; Quiroz and Yazdanyar, 2021; Robinson et al., 2012; Samuels et al., 2015; Sergeys et al., 2019). Importantly, the underlying mechanism of pathogenesis in mice reflects what occurs in the eyes of humans (Antonetti et al., 2012; Brownlee, 2001; Ferrington et al., 2020; Kowluru et al., 2015; Stitt et al., 2016). Following the loss of resilience, HG increases oxidative stress, which damages the retinal vasculature and thereby sets the stage for additional drivers of retinal damage (Figure 1) (Kanwar et al., 2007; Kowluru and Abbas, 2003; Kowluru et al., 2015; Zhong and Kowluru, 2011). Such mouse models have been successfully used to identify therapeutic approaches to prevent DR. For instance, overexpressing *Sod2* prevents both DM-induced mitochondrial oxidative stress and dysfunction, and protects mice from developing DR in the face of DM (Goto et al., 2008; Kowluru et al., 2006). Similarly, anti-oxidant-based therapies shield DM mice from developing DR (Dionysopoulou et al., 2023; Kowluru and Mishra, 2015). Thus, existing mouse models of DR have a proven track record of providing translationally relevant information. These same models can be used to investigate resilience.

We used both T1D (induced by injection of streptozotocin) and T2D (db/db) mouse models to investigate the mechanism of resilience. We reasoned that the retina was resistant to HG-driven oxidative stress because it had activated an endogenous anti-oxidative defense system. Indeed, we found that this was the case. Expression of anti-oxidant defense genes increased coincident with resilience (Li et al., 2023). This gene set included transcription factors (*Nrf2*) that govern anti-oxidant programs (He et al., 2020), NAD(P)H Quinone Dehydrogenase 1 (*Nqo1*), which encodes a cytoplasmic 2-electron reductase (Ross and Siegel, 2021), mitochondrially-localized enzymes that suppress oxidative stress (*Sod2*) (Kasai et al., 2020) and the first rate-limiting enzyme responsible for the synthesis of the anti-oxidant glutathione (*Gclc*) (Deneke and Fanburg, 1989). Focusing on the retinal vasculature, we found that cells within the retinal vessels that were isolated from mice that were within the resilience phase of DM had acquired resistance to ischemia/oxidative stress- and cytokine-induced death. As the duration of DM was extended and the resilience phase ended, expression of anti-oxidative defense genes declined and the retinal vessels from DM mice were no longer superior to vessels from non-DM mice in resisting death caused by DM-related insults. Furthermore, the DM vasculature became more vulnerable to death after a duration of DM that caused accumulation of detectable vascular and neural damage to the retina (Li et al., 2023).

The resilience described above appears to be present in both the neural and vascular compartments of the retina. The resilience bioactivity (resistance to oxidative stress- or cytokine-induced death) was observed in the vasculature, whereas the resilience biomarker (increased expression of anti-oxidative stress defense genes) was detected by PCR analysis of the whole retina, of which 99% (based on cell #) is non-vascular.

In vitro models of resilience to hyperglycemia-induced damage

Many investigators report that increasing the glucose concentration with the medium of cultured retinal endothelial cells quickly (within hours or days) induces quintessential drivers of DR such as elevated oxidative stress and mitochondrial dysfunction (Ferrington et al., 2020; Zheng and Kern, 2009). Consequently, such experimental systems have been used to determine the various mechanisms by which hyperglycemia damages retinal endothelial cells.

Not all investigators find this to be the case (Ghanian et al., 2018; Huang and Sheibani, 2008). Some report no retinopathy-related damage even after culturing primary human retinal endothelial cells (HRECs) for 6 days in HG-containing medium (Busik et al., 2008). Similarly, senescence was induced in HRECs only after 4 or 5 weeks of HG (Bertelli et al., 2022; Crespo-Garcia et al., 2024). In contrast to the commonplace observation that HG induces oxidative stress in human umbilical vein endothelial cells within days, one group recently reported no change in the level of reactive oxygen species even after 4 weeks of HG (Khapchaev et al., 2023). The reason for this non-uniform response of primary endothelial cells when they are cultured in high glucose-containing medium has not been systematically investigated.

We recently observed that exposing primary human retinal endothelial cells to high glucose at first induced death and compromised mitochondrial respiration and functionality, but as the duration of exposure was prolonged, cells adapted and became resistant to the deleterious effect of hyperglycemia (Serikbaeva et al., 2022). Thus, prolonged exposure to HG induced protection. Taken together, the work from multiple labs indicates that the simple experimental setting that involves culturing retinal endothelial cells in elevated glucose can be used to model both resilience to DR as well as HG-induced damage. The determinants of how cells will respond to HG has not been elucidated.

We used this in vitro experimental system to investigate the mechanism by which cells adapt, i.e. become capable of resisting the deleterious effects of HG (Serikbaeva et al., 2022). As detailed below, mitochondrial oxidative stress remained unchanged even after 10 days of HG (Table 5). In contrast, certain types of reactive oxygen species increased in other subcellular compartments: hydrogen peroxide (H_2O_2) in the Golgi and oxidized glutathione (oxGSH) in the vicinity of the plasma membrane. Short exposure to HG (1 instead of 10 days) had no effect on any of the reactive oxygen species that were measured (data not shown). These data indicate the existence of a system that protects the mitochondria from oxidative stress in the face of HG. Figure 4 illustrates the key features of HIMA (hyperglycemia-induced mitochondrial adaptation)

We considered the contribution of osmolarity to HIMA and found that it had a partial effect (Serikbaeva et al., 2022). Culturing cells in 5 mM D-glucose + 25 mM L-glucose made them resistant to oxidative stress-induced death, however it did not increase mitophagy or improve mitochondrial functionality as was seen with cells cultured in 30 mM D-glucose.

HIMA is associated with protection from mitochondrial oxidative stress

A combination of experimental approaches revealed that acquisition of HIMA (hyperglycemia-induced mitochondrial adaptation) prevented mitochondrial oxidative stress (Serikbaeva et al., 2022). We evaluated overall oxidative stress within cells using traditional redox sensitive dyes such as DHE (dihydroethidium) and DCFDA (2',7' –dichlorofluorescin diacetate). In addition, we used roGFP sensors which are mutant versions of green fluorescent protein (GFP) that are redox sensitive. Such sensors contain a tag that determines their subcellular location, and are fused with either yeast peroxidase ORP1, or with human glutaredoxin-1 in order to impart preference for hydrogen peroxide or oxidized glutathione, respectively. Finally, they are reversible and therefore can be used to observe both an increase, and subsequent decrease in oxidative stress in living cells and in real time. Such roGFP sensors have been characterized extensively and used to evaluate changes in oxidative stress in a variety of experimental conditions (Albrecht et al., 2011; Meyer and Dick, 2010; Roma et al., 2018).

The subcellular location of the sensors listed in Table 4 was assessed in the experiments shown in Figures 5 and 6.

The results of this series of experiments are listed in Table 5. While culturing cells for 10 days in HG-containing medium did not cause a global change in the level of H2O2 or superoxide, oxidative stress did increase in certain subcellular compartments. More specifically, the level of oxidized GSH increased in the vicinity of the plasma membrane (Table 5), which is consistent, with reports by other investigators (de Zeeuw et al., 2015). Furthermore, H2O2 increased in the Golgi (Table 5). In contrast, there was no change in the level of three different reactive oxygen species (superoxide, H2O2 and oxGSH) in the mitochondria (Serikbaeva et al., 2022) (Table 5). We conclude that while culturing cells in HG for 10 days increased oxidative stress in certain subcellular compartments, the mitochondria was not one of them.

HG-induced protection from mitochondrial oxidative stress is independent of NRF2

We considered if protection from HG-driven oxidative stress involved nuclear factor erythroid 2–related factor 2 (NRF2), a transcription factor that increases expression of a plethora of anti-oxidant genes. Other groups have reported that overexpression of NRF2 (Itoh et al., 2015; Kiyama et al., 2018; Li et al., 2008; Santos and Kowluru, 2011; Strom et al., 2016) protected the retina and prevented development of DR in animals (Wang et al., 2020). As shown in Figure 7A, the mRNA expression of NRF2 and genes that it regulates (HMOX1, SOD1, NQO1, GSTP1, GPX2, CAT) was not changed in cells that had undergone HIMA (10 days of HG). Furthermore, siRNA-mediated suppression of NRF2 (Figure 7B) had no effect on HG-induced death, or the level of either global, or mitochondrial superoxide (Figure 7C). These results indicate that the mechanism by which

cells that had undergone HIMA resisted the deleterious effects of HG was independent of NRF2.

We also investigated the role of mitochondrial homeostasis in HIMA (Serikbaeva et al., 2022). In cells that had attained HIMA, both biogenesis and mitophagy were increased, while the total mitochondrial mass remained unchanged. Furthermore, perturbing mitochondrial homeostasis by reducing expression of Mfn2 or Drp1 compromised mitochondrial functionality. These data demonstrate that HIMA was associated with a balanced adjustment in both biogenesis and mitophagy. Furthermore, HIMA required persistent mitochondrial homeostasis, which is likely due to the HG-driven increase in the damaged mitochondria, which need to be eliminated (mitophagy) and replaced (biogenesis).

Retinal cell types that are responsible for resilience

The observation that cultured human retinal endothelial cells undergo HIMA indicates that this phenomenon is cell autonomous – manifests without the input of any of the other retinal cell types. However, this does not eliminate the possibility that other cell types contribute. Because endothelial cells work together with other retinal cells types (i.e. ganglion, glial (astrocytes and Müller), immune (microglia), and vascular (endothelial and pericytes) in the context of neurovascular unit), it seems likely that resilience will be governed by the input of these other cell types (Tang et al., 2023). In support of this possibility is the intriguing observation that mesenchymal cells transfer mitochondria to endothelial cells and thereby promote mitophagy of the endothelium (Lin et al., 2024).

Besides other cell types, additional types of extracellular cues may influence resilience within the endothelium. Shear stress, which the blood exerts as it flows across the endothelium of the aorta, can induce resilience to cardiovascular disease (Gao and Galis, 2021). The underlying mechanism involves shear-mediated localization of signaling platforms that govern expression of transcription factors such as Klf2, which mediate expression of atheroprotective genes such as *NOS3*, encoding eNOS (Coon et al., 2022; Hong et al., 2023). Atherosclerotic plaques develop in adjacent regions of the aorta, which are subject to low or disturbed shear stress. Such studies demonstrate that the vascular endothelium harbors endogenous systems that protect it from pathology, and that such systems are responsive to environmental cues. Additional studies are necessary to determine if resilience of the retinal endothelium is under the influence of extracellular cues other than hyperglycemia, which induces it.

Existing biomarkers of DR are not useful to assess resilience

Biomarkers that are used to diagnose DR in patients are based on the presence of abnormalities in the appearance and/or function of the retinal vasculature (Table 1). Such biomarkers appear once resilience is lost, and only indirectly speak to its existence. Resilience biomarkers are likely to reflect features of protection from HG-mediated damage such as increased expression of anti-oxidant defense genes and improved mitochondrial functionality, which are required to limit the HG-driven increase in oxidative stress. Identification of such biomarkers will enable direct assessment of resilience, and thereby catalyze development of resilience-based therapeutics.

7. Conclusions and opportunities

Patients and experimental animals that develop DM enjoy an initial retinopathy-resistant period. Recent discoveries indicate that resilience is a deliberate response, which involves engaging endogenous defense against DM/HG-driven mitochondrial oxidative stress (Figure 8). While such concepts are novel to the field of DR, which is largely focused on damage to the retina, it resonates with hormesis, which is mitochondrial-based protection from pathology.

The appreciation that the retina is capable of mounting resilience to DM-driven damage brings to light intriguing research questions and opportunities. What is the mechanism by which the onset of DM engages resilience, and why is it lost as the duration of DM is extended? Can resilience be re-activated after the onset of detectable damage, and if yes, then can either the neural or vascular compartments of the retina be repaired? Is enduring resilience the reason that some individuals do not develop DR even after 50 or more years of DM? Does fenofibrate, which reduces the onset and slows progression of DR (Chew et al., 2010; Group et al., 2010; Knickelbein et al., 2016) prevent the loss of resilience?

The resilience concept constitutes a novel therapeutic strategy of resisting the deleterious effects of DM. Resistance to HG-induced damage by becoming tolerant to HG is distinct from current approaches of reducing the level of blood sugar in order to reduce the magnitude of the damage-inducing insult. These resilience-oriented therapeutics will complement existing approaches of preventing damage (by curbing blood sugar) and mitigating the symptoms of damage (anti-VEGF, steroids, laser, surgery).

9. Materials and Methods

Tissue culture

Primary HRECs were purchased from Cell Systems (ACBRI 181; Kirkland, WA, USA). They were isolated from donor A, a 26-year-old Caucasian male. Cells were authenticated for cytoplasmic VWF/Factor VIII, cytoplasmic uptake of Di-I-AC-LDL, cytoplasmic CD31, GFAP, NG2 and PDGFRb by immunofluorescence. Mycoplasma, fungal and bacterial sterility was confirmed using a culture method. Cells were cultured in endothelial cell basal medium-2 (Lonza, EBM-2, CC3156) supplemented with microvascular endothelial SingleQuots kit (Lonza, EGM-2MV, CC4147). The media was refreshed daily and the cells were passaged within a day or two of reaching confluence. The glucose concentration in normal glucose (NG) and high glucose (HG) medium was 5 mM and 30 mM D-glucose, respectively. For osmotic control, cells were cultured in 30mM glucose by adding 25mM L-glucose (LG) into 5mM NG media.

293T cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). These cells were cultured in DMEM with L-glutamine and sodium bicarbonate, supplemented with 10% fetal bovine serum (ThermoFisherScientific, MT35010CV) and penicillin/streptomycin in a 5% CO2 tissue culture incubator.

Lentivirus production and infection

70% confluent 293T cells were transfected with Lipofectamine 2000 (Invitrogen, Cat: 11668019) complexed with the packaging plasmid (psPAX2), envelope plasmid (pVSVg) and lenti plasmid of interest (e.g. roGFP sensors). The supernatant containing the virus was collected for three consecutive days, aliquoted and stored at −80C. Primary human retinal endothelial cells were infected with lentivirus harboring the GFP plasmid with 8 ug/ml polybrene reagent added to the media. On the following day, the media was replaced complete growth medium. The infection efficiency was routinely >80% across all experiments.

Immunofluorescent staining

Cells were plated on a gelatin-coated glass surface plates and fixed with 4% paraformaldehyde. After permeabilization with 0.25% TritonX100 in PBS solution, cells were washed with PBS and blocked in a solution containing 10% serum (goat, since secondary antibody is from a goat), 1% BSA in PBST (0.05% Tween). Samples were incubated in primary antibody overnight. Next day, cells were washed and incubated in fluorescent secondary antibody. Primary antibodies used: MnSOD2 (Cat #13533, Abcam, USA), catalase (D4P7B, Cat #12980, Cell Signaling, USA). Secondary antibodies used: goat anti-mouse IgG H&L Alexa Fluor 555 (Cat#ab150114, Abcam, USA).

Fluorescent staining with dyes

Cells were stained with WGA (Cat# W32466, ThermoFisher, USA) and BODIPY-ceramide (Cat# D7540, ThermoFisher, USA) dyes following manufacturer’s protocol.

siRNA

Confluent HRECs, plated onto a 6-well tissue culture plate, were transfected with siRNA in an antibiotic free complete endothelial cell medium (Lonza). To this end, 10 nM of ON-TARGETplus Human siRNA SMARTpool (Horizon Discovery) targeting NFE2L2 (NRF2) (L-003755-00) or non-targeting pool (Scr, D-001810-10-05) was complexed with DharmaFECT 1 transfection reagent (Horizon Discovery, Catalog ID: T-2001) at 1:2 ratio in reduced serum Opti-MEM medium (Gibco, 31985070) and added to the media. The next day, transfected cells were trypsinized and plated into a 96-well tissue culture plate (for LDH-assay) in complete endothelial cell media. At 48-hour time point post-transfection, transfected cells were subjected to the designated experimental assay. The extent of silencing was determined on both the protein, and mRNA level using Western blot and qRT-PCR, respectively. The sequences of the primers used to measure mRNA of Nrf2-targeting enzymes with qRT-PCR are listed in Table 6.

LDH to assess cell death:

LDH, a measure of membrane integrity for cell-mediated cytotoxicity, was quantified using colorimetric CytoTox96 non-radioactive cytotoxicity assay (Promega, G1780). LDH is a stable cytosolic enzyme that is released upon cell lysis. The LDH activity that was released in the culture supernatant was measured with a coupled enzymatic assay following the manufacturer’s instructions. The optical density was determined using a Synergy H1

spectrophotometer (Agilent, CA, USA). The amount of color formed is proportional to the number of lysed cells. For each experimental group, the amount of released LDH was normalized to the total LDH level, which was obtained by lysing cells using the lysation buffer supplied in the kit.

Oxidative stress

NG- and HG-HRECs were plated at full confluency into 96-well plate and cultured in compete Lonza media. Media was aspirated and cells were stained with 10 uM DHE (D23107, ThermoFisher, USA), 5 uM mitosox (M36008, ThermoFisher, USA), 10uM DCF-DA (D6883, Sigma) (resuspended in DMSO according to the manufacturer's protocol) in Hank's balanced salt solution (HBSS) supplemented with 1% fetal bovine serum (FBS) for 30 min. The staining solution was further replaced with HBSS + 1% FBS, and fluorescent intensity was measured on spectroscopy with ex./em. at 510/580, 518/606, and 495/527 for each dye. Fluorescent values from unstained wells were subtracted from stained wells, and the data was normalized to the total protein amount per each condition. Total protein amount was measured using BCA described above.

Plasmid design

The mito-roGFP2-Orp1 (H_2O_2) sensor was made from pLPCX mito-roGFP2-Orp1, which was a gift from Tobias Dick (64992, Addgene, Watertown, MA, USA). The mito-roGFP2-Orp1 in the pLPCX plasmid was cut with ClaI, blunted with T4DNA polymerase, then cut with Bgl2. The resulting 1.5 kb DNA fragment was ligated into the Hpa1/BamH1-cut pLV-EF1a vector. Insert-containing constructs were detected based on diagnostic BamH1/EcoR1 restriction fragments. Because the ClaI site in the pLPCX mito-roGFP2-Orp1 plasmid is blocked by methylation this construct was propagated in dam-/dcm- bacteria. The mito-roGFP2-Orp1-pLV-EF1a plasmid was used to make lentivirus (as described previously(Serikbaeva et al., 2022)), which was used to stably express mito-roGFP2-Orp1 in HRECs. Mito-Grx1-roGFP2 has been generated in the similar way as Orp1 version of the sensor.

PRX-Grx1-roGFP2 was provided by Dr. Yuta Hatori (Hatori et al., 2018) and was cut with Bgl2 and Not1, and the resulting 1.8 kb fragment was gel purified and ligated into gel purified BamH1/Not1-cut pLV.AS. pLV.AS is pLV-EF1a-IRES-Neo is a lentiviral vector that was modified in the Kazlauskas lab to improve the multiple cloning site (MCS). The resulting construct (pLV-PRX-Grx1) was analyzed by restriction digestion and DNA sequencing. The results confirmed that the inserts were successfully cloned into pLV.AS.

G-Grx1-roGFP2 was provided by Dr. Yuta Hatori (Hatori et al., 2018) and subcloned into pLV-AS lentiviral vector. G-Grx1-roGFP2 was PCR amplified with primers that generated a 5' BamH1 site and a 3' Nsi1 site. The resulting PCR product was cut with BamH1 and Nsi1 and subcloned into pLV.AS that had been cut with BamH1 and Nsi1. DNA sequencing of the resulting construct indicated that it was error-free.

PM-Grx1-roGFP2 and PM-Orp1-roGFP2 constructs provided by Dr. Yuta Hatori (Hatori et al., 2018) were targeting PM. Hatori's group published that adding the CAAX sequence of p63/Rho guanine nucleotide exchange factor 25 (ARHGEF25) targets roGFP2 to both

plasma membrane and intracellular vesicles of HeLa cells. We generated lentiviral version of these constructs. Specifically, pPalmitoyl-Grx1-roGFP2 and pPalmitoyl-roGFP2- Orp1 constructs were cut with Bgl2 and Not1, and the resulting 1.3 kb (Grx1) or 1.5 (Orp1) fragments were gel purified and ligated into BamH1/Not1-cut pLV.AS. The resulting constructs (pLV-PM-Grx1 and pLV-PM-Orp1) were analyzed by restriction digestion and DNA sequencing. The results confirmed that the inserts were successfully cloned into pLV.AS lentiviral vector. All plasmids of the roGFP sensors that were used in the study are listed in Table 4 and representative pictures of cellular localizations are depicted in Figures 5 and 6.

Acknowledgments

We thank our colleagues Jonathan Coloff, Michael Grassi, Brian Layden, Jason MacAny, Max McCann and Steven Roth for constructive criticism of this review's content. In addition, we are grateful to Lauren Kalinoski for creating Figures 1-4, 8. This project was supported by grants from the Juvenile Diabetes Research Foundation, Illinois Society to Prevent Blindness, Retina Research Foundation, National Institute of Health (EY031350 and EY001792) and an unrestricted grant from the Research to Prevent Blindness Foundation.

10. References

1991. UK Prospective Diabetes Study (UKPDS). VIII. Study design, progress and performance. Diabetologia 34, 877–890. [PubMed: 1778353]

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, 977–986. [PubMed: 8366922]

1997. Clustering of long-term complications in families with diabetes in the diabetes control and complications trial. The Diabetes Control and Complications Trial Research Group. Diabetes 46, 1829–1839. [PubMed: 9356033]

1999. Epidemiology of Diabetes Interventions and Complications (EDIC). Design, implementation, and preliminary results of a long-term follow-up of the Diabetes Control and Complications Trial cohort. Diabetes Care 22, 99–111. [PubMed: 10333910]

Abramoff MD, Folk JC, Han DP, Walker JD, Williams DF, Russell SR, Massin P, Cochener B, Gain P, Tang L, Lamard M, Moga DC, Quellec G, Niemeijer M, 2013. Automated analysis of retinal images for detection of referable diabetic retinopathy. JAMA Ophthalmol 131, 351–357. [PubMed: 23494039]

Abramoff MD, Lavin PT, Birch M, Shah N, Folk JC, 2018. Pivotal trial of an autonomous AI-based diagnostic system for detection of diabetic retinopathy in primary care offices. NPJ Digit Med 1, 39. [PubMed: 31304320]

Abramoff MD, Lou Y, Erginay A, Clarida W, Amelon R, Folk JC, Niemeijer M, 2016. Improved Automated Detection of Diabetic Retinopathy on a Publicly Available Dataset Through Integration of Deep Learning. Invest Ophthalmol Vis Sci 57, 5200–5206. [PubMed: 27701631]

Abramoff MD, Niemeijer M, Suttorp-Schulten MS, Viergever MA, Russell SR, van Ginneken B, 2008. Evaluation of a system for automatic detection of diabetic retinopathy from color fundus photographs in a large population of patients with diabetes. Diabetes Care 31, 193–198. [PubMed: 18024852]

Abramoff MD, Reinhardt JM, Russell SR, Folk JC, Mahajan VB, Niemeijer M, Quellec G, 2010. Automated early detection of diabetic retinopathy. Ophthalmology 117, 1147–1154. [PubMed: 20399502]

Administration, U.F.a.D., 2018. FDA permits marketing of artificial intelligence-based device to detect certain diabetes-related eye problems.

Aiello LP, Gardner TW, King GL, Blankenship G, Cavallerano JD, Ferris FL 3rd, Klein R, 1998. Diabetic retinopathy. Diabetes Care 21, 143–156. [PubMed: 9538986]

Albrecht SC, Barata AG, Grosshans J, Teleman AA, Dick TP, 2011. In vivo mapping of hydrogen peroxide and oxidized glutathione reveals chemical and regional specificity of redox homeostasis. Cell Metab 14, 819–829. [PubMed: 22100409]

Alka K, Kumar J, Kowluru RA, 2023. Impaired mitochondrial dynamics and removal of the damaged mitochondria in diabetic retinopathy. Front Endocrinol (Lausanne) 14, 1160155. [PubMed: 37415667]

American Diabetes, A., 2014. Diagnosis and classification of diabetes mellitus. Diabetes Care 37 Suppl 1, S81–90. [PubMed: 24357215]

American Diabetes, A., 2020. 11. Microvascular Complications and Foot Care: Standards of Medical Care in Diabetes-2020. Diabetes Care 43, S135–S151. [PubMed: 31862754]

American Diabetes Association Professional Practice, C., 2024. 2. Diagnosis and Classification of Diabetes: Standards of Care in Diabetes-2024. Diabetes Care 47, S20–S42. [PubMed: 38078589]

Amorim JA, Coppotelli G, Rolo AP, Palmeira CM, Ross JM, Sinclair DA, 2022. Mitochondrial and metabolic dysfunction in ageing and age-related diseases. Nat Rev Endocrinol 18, 243–258. [PubMed: 35145250]

Anderson A, Alfahad N, Wimalachandra D, Bouzinab K, Rudzinska P, Wood H, Fazey I, Xu H, Lyons TJ, Barnes NM, Narendran P, Lord JM, Rauz S, Ganley IG, Curtis TM, Wallace GR, Hombrebueno JR, 2024. Relaxation of mitochondrial hyperfusion in the diabetic retina via N6-furfuryladenosine confers neuroprotection regardless of glycaemic status. Nat Commun 15, 1124. [PubMed: 38321058]

Anothaisintawee T, Reutrakul S, Van Cauter E, Thakkinstian A, 2016. Sleep disturbances compared to traditional risk factors for diabetes development: Systematic review and meta-analysis. Sleep Med Rev 30, 11–24. [PubMed: 26687279]

Antonetti DA, Klein R, Gardner TW, 2012. Diabetic retinopathy. N Engl J Med 366, 1227–1239. [PubMed: 22455417]

Antonetti DA, Silva PS, Stitt AW, 2021. Current understanding of the molecular and cellular pathology of diabetic retinopathy. Nat Rev Endocrinol 17, 195–206. [PubMed: 33469209]

Arden GB, Sivaprasad S, 2012. The pathogenesis of early retinal changes of diabetic retinopathy. Doc Ophthalmol 124, 15–26. [PubMed: 22302291]

Ashraf M, Nesper PL, Jampol LM, Yu F, Fawzi AA, 2018. Statistical Model of Optical Coherence Tomography Angiography Parameters That Correlate With Severity of Diabetic Retinopathy. Invest Ophthalmol Vis Sci 59, 4292–4298. [PubMed: 30167660]

Babenko B, Mitani A, Traynis I, Kitade N, Singh P, Maa AY, Cuadros J, Corrado GS, Peng L, Webster DR, Varadarajan A, Hammel N, Liu Y, 2022. Detection of signs of disease in external photographs of the eyes via deep learning. Nat Biomed Eng, 1–14. [PubMed: 35064246]

Badrinarayanan V, Kendall A, Cipolla R, 2017. SegNet: A Deep Convolutional Encoder-Decoder Architecture for Image Segmentation. IEEE Trans Pattern Anal Mach Intell 39, 2481–2495. [PubMed: 28060704]

Bain SC, Klufas MA, Ho A, Matthews DR, 2019. Worsening of diabetic retinopathy with rapid improvement in systemic glucose control: A review. Diabetes Obes Metab 21, 454–466. [PubMed: 30226298]

Banday MZ, Sameer AS, Nissar S, 2020. Pathophysiology of diabetes: An overview. Avicenna J Med 10, 174–188. [PubMed: 33437689]

Barber AJ, Gardner TW, Abcouwer SF, 2011. The significance of vascular and neural apoptosis to the pathology of diabetic retinopathy. Invest Ophthalmol Vis Sci 52, 1156–1163. [PubMed: 21357409]

Barouch FC, Miyamoto K, Allport JR, Fujita K, Bursell SE, Aiello LP, Luscinskas FW, Adamis AP, 2000. Integrin-mediated neutrophil adhesion and retinal leukostasis in diabetes. Invest Ophthalmol Vis Sci 41, 1153–1158. [PubMed: 10752954]

Belikov AV, 2019. Age-related diseases as vicious cycles. Ageing Res Rev 49, 11–26. [PubMed: 30458244]

Berk BC, Abe JI, Min W, Surapisitchat J, Yan C, 2001. Endothelial atheroprotective and anti-inflammatory mechanisms. Ann N Y Acad Sci 947, 93–109; discussion 109-111. [PubMed: 11795313]

Bertelli PM, Pedrini E, Hughes D, McDonnell S, Pathak V, Peixoto E, Guduric-Fuchs J, Stitt AW, Medina RJ, 2022. Long term high glucose exposure induces premature senescence in retinal endothelial cells. Front Physiol 13, 929118. [PubMed: 36091370]

Besedovsky L, Lange T, Born J, 2012. Sleep and immune function. Pflugers Arch 463, 121–137. [PubMed: 22071480]

Bhatwadekar AD, Duan Y, Korah M, Thinschmidt JS, Hu P, Leley SP, Caballero S, Shaw L, Busik J, Grant MB, 2017. Hematopoietic stem/progenitor involvement in retinal microvascular repair during diabetes: Implications for bone marrow rejuvenation. Vision Res 139, 211–220. [PubMed: 29042190]

Bierhansl L, Conradi LC, Treps L, Dewerchin M, Carmeliet P, 2017. Central Role of Metabolism in Endothelial Cell Function and Vascular Disease. Physiology (Bethesda) 32, 126–140. [PubMed: 28202623]

Biswal MR, Han P, Zhu P, Wang Z, Li H, Ildefonso CJ, Lewin AS, 2017. Timing of Antioxidant Gene Therapy: Implications for Treating Dry AMD. Invest Ophthalmol Vis Sci 58, 1237–1245. [PubMed: 28241311]

Brownlee M, 2001. Biochemistry and molecular cell biology of diabetic complications. Nature 414, 813–820. [PubMed: 11742414]

Bryl A, Mrugacz M, Falkowski M, Zorena K, 2022. The Effect of Hyperlipidemia on the Course of Diabetic Retinopathy-Literature Review. J Clin Med 11.

Brys K, Castelein N, Matthijssens F, Vanfleteren JR, Braeckman BP, 2010. Disruption of insulin signalling preserves bioenergetic competence of mitochondria in ageing Caenorhabditis elegans. BMC Biol 8, 91. [PubMed: 20584279]

Burman JL, Pickles S, Wang C, Sekine S, Vargas JNS, Zhang Z, Youle AM, Nezich CL, Wu X, Hammer JA, Youle RJ, 2017. Mitochondrial fission facilitates the selective mitophagy of protein aggregates. J Cell Biol 216, 3231–3247. [PubMed: 28893839]

Busik JV, Mohr S, Grant MB, 2008. Hyperglycemia-induced reactive oxygen species toxicity to endothelial cells is dependent on paracrine mediators. Diabetes 57, 1952–1965. [PubMed: 18420487]

Buysse DJ, 2014. Sleep health: can we define it? Does it matter? Sleep 37, 9–17. [PubMed: 24470692]

Cai J, Boulton M, 2002. The pathogenesis of diabetic retinopathy: old concepts and new questions. Eye (Lond) 16, 242–260. [PubMed: 12032713]

Calabrese EJ, 2018. Hormesis: Path and Progression to Significance. Int J Mol Sci 19.

Channa R, Lee K, Staggers KA, Mehta N, Zafar S, Gao J, Frankfort BJ, Chua SYL, Khawaja AP, Foster PJ, Patel PJ, Minard CG, Amos C, Abramoff MD, 2021. Detecting retinal neurodegeneration in people with diabetes: Findings from the UK Biobank. PLoS One 16, e0257836. [PubMed: 34587216]

Chen H, McCaffery JM, Chan DC, 2007. Mitochondrial fusion protects against neurodegeneration in the cerebellum. Cell 130, 548–562. [PubMed: 17693261]

Chen HY, Swaroop M, Papal S, Mondal AK, Song HB, Campello L, Tawa GJ, Regent F, Shimada H, Nagashima K, de Val N, Jacobson SG, Zheng W, Swaroop A, 2023. Reserpine maintains photoreceptor survival in retinal ciliopathy by resolving proteostasis imbalance and ciliogenesis defects. Elife 12.

Chen J, Shao Y, Sasore T, Moiseyev G, Zhou K, Ma X, Du Y, Ma JX, 2021. Interphotoreceptor Retinol-Binding Protein Ameliorates Diabetes-Induced Retinal Dysfunction and Neurodegeneration Through Rhodopsin. Diabetes 70, 788–799. [PubMed: 33334874]

Chen L-C, Papandreou G, Schroff F, Adam H, 2017a. Rethinking Atrous Convolution for Semantic Image Segmentation, p. arXiv:1706.05587.

Chen Q, Ma Q, Wu C, Tan F, Chen F, Wu Q, Zhou R, Zhuang X, Lu F, Qu J, Shen M, 2017b. Macular Vascular Fractal Dimension in the Deep Capillary Layer as an Early Indicator of Microvascular Loss for Retinopathy in Type 2 Diabetic Patients. Invest Ophthalmol Vis Sci 58, 3785–3794. [PubMed: 28744552]

Chen Z, Miao F, Paterson AD, Lachin JM, Zhang L, Schones DE, Wu X, Wang J, Tompkins JD, Genuth S, Braffett BH, Riggs AD, Group DER, Natarajan R, 2016. Epigenomic profiling reveals

an association between persistence of DNA methylation and metabolic memory in the DCCT/EDIC type 1 diabetes cohort. Proc Natl Acad Sci U S A 113, E3002–3011. [PubMed: 27162351]

Cheung CY, Ikram MK, Klein R, Wong TY, 2015. The clinical implications of recent studies on the structure and function of the retinal microvasculature in diabetes. Diabetologia 58, 871–885. [PubMed: 25669631]

Cheung N, Mitchell P, Wong TY, 2010. Diabetic retinopathy. Lancet 376, 124–136. [PubMed: 20580421]

Chew EY, Ambrosius WT, Davis MD, Danis RP, Gangaputra S, Greven CM, Hubbard L, Esser BA, Lovato JF, Perdue LH, Goff DC Jr., Cushman WC, Ginsberg HN, Elam MB, Genuth S, Gerstein HC, Schubart U, Fine LJ, 2010. Effects of medical therapies on retinopathy progression in type 2 diabetes. N Engl J Med 363, 233–244. [PubMed: 20587587]

Chomyn A, 1998. The myoclonic epilepsy and ragged-red fiber mutation provides new insights into human mitochondrial function and genetics. Am J Hum Genet 62, 745–751. [PubMed: 9529371]

Collaborators, G.B.D.D., 2023. Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: a systematic analysis for the Global Burden of Disease Study 2021. Lancet 402, 203–234. [PubMed: 37356446]

Coon BG, Timalsina S, Astone M, Zhuang ZW, Fang J, Han J, Themen J, Chung M, Yang-Klingler YJ, Jain M, Hirschi KK, Yamamato A, Trudeau LE, Santoro M, Schwartz MA, 2022. A mitochondrial contribution to anti-inflammatory shear stress signaling in vascular endothelial cells. J Cell Biol 221.

Correia SC, Santos RX, Perry G, Zhu X, Moreira PI, Smith MA, 2010. Mitochondria: the missing link between preconditioning and neuroprotection. J Alzheimers Dis 20 Suppl 2, S475–485. [PubMed: 20463394]

Crespo-Garcia S, Fournier F, Diaz-Marin R, Klier S, Ragusa D, Masaki L, Cagnone G, Blot G, Hafiane I, Dejda A, Rizk R, Juneau R, Buscarlet M, Chorfi S, Patel P, Beltran PJ, Joyal JS, Rezende FA, Hata M, Nguyen A, Sullivan L, Damiano J, Wilson AM, Mallette FA, David NE, Ghosh A, Tsuruda PR, Dananberg J, Sapieha P, 2024. Therapeutic targeting of cellular senescence in diabetic macular edema: preclinical and phase 1 trial results. Nat Med 30, 443–454. [PubMed: 38321220]

Cruickshanks KJ, Moss SE, Klein R, Klein BE, 1992. Physical activity and proliferative retinopathy in people diagnosed with diabetes before age 30 yr. Diabetes Care 15, 1267–1272. [PubMed: 1425087]

Cunningham KS, Gotlieb AI, 2005. The role of shear stress in the pathogenesis of atherosclerosis. Lab Invest 85, 9–23. [PubMed: 15568038]

Dai L, Wu L, Li H, Cai C, Wu Q, Kong H, Liu R, Wang X, Hou X, Liu Y, Long X, Wen Y, Lu L, Shen Y, Chen Y, Shen D, Yang X, Zou H, Sheng B, Jia W, 2021. A deep learning system for detecting diabetic retinopathy across the disease spectrum. Nat Commun 12, 3242. [PubMed: 34050158]

Dalal N, Triggs B, 2005. Histograms of oriented gradients for human detection, IEEE Computer Society Conference on Computer Vision and Pattern Recognition. IEEE, San Diego, CA, USA.

de Gooyer TE, Stevenson KA, Humphries P, Simpson DA, Gardiner TA, Stitt AW, 2006. Retinopathy is reduced during experimental diabetes in a mouse model of outer retinal degeneration. Invest Ophthalmol Vis Sci 47, 5561–5568. [PubMed: 17122149]

de Zeeuw P, Wong BW, Carmeliet P, 2015. Metabolic adaptations in diabetic endothelial cells. Circ J 79, 934–941. [PubMed: 25787231]

Delpino FM, Figueiredo LM, Bielemann RM, da Silva BGC, Dos Santos FS, Mintem GC, Flores TR, Arcencio RA, Nunes BP, 2022. Ultra-processed food and risk of type 2 diabetes: a systematic review and meta-analysis of longitudinal studies. Int J Epidemiol 51, 1120–1141. [PubMed: 34904160]

Deneke SM, Fanburg BL, 1989. Regulation of cellular glutathione. Am J Physiol 257, L163–173. [PubMed: 2572174]

Deshpande AD, Harris-Hayes M, Schootman M, 2008. Epidemiology of diabetes and diabetes-related complications. Phys Ther 88, 1254–1264. [PubMed: 18801858]

Detmer SA, Chan DC, 2007. Functions and dysfunctions of mitochondrial dynamics. Nat Rev Mol Cell Biol 8, 870–879. [PubMed: 17928812]

Devi TS, Yumnamcha T, Yao F, Somayajulu M, Kowluru RA, Singh LP, 2019. TXNIP mediates high glucose-induced mitophagic flux and lysosome enlargement in human retinal pigment epithelial cells. Biol Open 8.

Dimitrova G, Chihara E, Takahashi H, Amano H, Okazaki K, 2017. Quantitative Retinal Optical Coherence Tomography Angiography in Patients With Diabetes Without Diabetic Retinopathy. Invest Ophthalmol Vis Sci 58, 190–196. [PubMed: 28114579]

Dionysopoulou S, Wikstrom P, Bucolo C, Romano GL, Micale V, Svensson R, Spyridakos D, Mastrodimou N, Georgakis S, Verginis P, Walum E, Thermos K, 2023. Topically Administered NOX4 Inhibitor, GLX7013114, Is Efficacious in Treating the Early Pathological Events of Diabetic Retinopathy. Diabetes 72, 638–652. [PubMed: 36821829]

Djordjevic B, Cvetkovic T, Stoimenov TJ, Despotovic M, Zivanovic S, Basic J, Veljkovic A, Velickov A, Kocic G, Pavlovic D, Sokolovic D, 2018. Oral supplementation with melatonin reduces oxidative damage and concentrations of inducible nitric oxide synthase, VEGF and matrix metalloproteinase 9 in the retina of rats with streptozotocin/nicotinamide induced pre-diabetes. Eur J Pharmacol 833, 290–297. [PubMed: 29890158]

Drummond GR, Sobey CG, 2014. Endothelial NADPH oxidases: which NOX to target in vascular disease? Trends Endocrinol Metab 25, 452–463. [PubMed: 25066192]

Du X, Matsumura T, Edelstein D, Rossetti L, Zsengeller Z, Szabo C, Brownlee M, 2003. Inhibition of GAPDH activity by poly(ADP-ribose) polymerase activates three major pathways of hyperglycemic damage in endothelial cells. J Clin Invest 112, 1049–1057. [PubMed: 14523042]

Du Y, Veenstra A, Palczewski K, Kern TS, 2013. Photoreceptor cells are major contributors to diabetes-induced oxidative stress and local inflammation in the retina. Proc Natl Acad Sci U S A 110, 16586–16591. [PubMed: 24067647]

Dunnmon JA, Yi D, Langlotz CP, Re C, Rubin DL, Lungren MP, 2019. Assessment of Convolutional Neural Networks for Automated Classification of Chest Radiographs. Radiology 290, 537–544. [PubMed: 30422093]

Duraisamy AJ, Mohammad G, Kowluru RA, 2019. Mitochondrial fusion and maintenance of mitochondrial homeostasis in diabetic retinopathy. Biochim Biophys Acta Mol Basis Dis 1865, 1617–1626. [PubMed: 30922813]

Engbersen R, Riksen NP, Mol MJ, Bravenboer B, Boerman OC, Meijer P, Oyen WJ, Tack C, Rongen GA, Smits P, 2012. Improved resistance to ischemia and reperfusion, but impaired protection by ischemic preconditioning in patients with type 1 diabetes mellitus: a pilot study. Cardiovasc Diabetol 11, 124. [PubMed: 23051145]

Engerman RL, Kern TS, 1995. Retinopathy in animal models of diabetes. Diabetes Metab Rev 11, 109–120. [PubMed: 7555563]

Esteva A, Kuprel B, Novoa RA, Ko J, Swetter SM, Blau HM, Thrun S, 2017. Dermatologist-level classification of skin cancer with deep neural networks. Nature 542, 115–118. [PubMed: 28117445]

Ferrington DA, Fisher CR, Kowluru RA, 2020. Mitochondrial Defects Drive Degenerative Retinal Diseases. Trends Mol Med 26, 105–118. [PubMed: 31771932]

Fickweiler W, Park H, Park K, Mitzner MG, Chokshi T, Boumenna T, Gautier J, Zaitsu Y, Wu IH, Cavallerano J, Aiello LP, Sun JK, King GL, 2022. Elevated Retinol Binding Protein 3 Concentrations Are Associated With Decreased Vitreous Inflammatory Cytokines, VEGF, and Progression of Diabetic Retinopathy. Diabetes Care 45, 2159–2162. [PubMed: 35852358]

Foxton RH, Finkelstein A, Vijay S, Dahlmann-Noor A, Khaw PT, Morgan JE, Shima DT, Ng YS, 2013. VEGF-A is necessary and sufficient for retinal neuroprotection in models of experimental glaucoma. Am J Pathol 182, 1379–1390. [PubMed: 23416159]

Gao Y, Dai X, Li Y, Li G, Lin X, Ai C, Cao Y, Li T, Lin B, 2020. Role of Parkin-mediated mitophagy in the protective effect of polydatin in sepsis-induced acute kidney injury. J Transl Med 18, 114. [PubMed: 32131850]

Gao Y, Galis ZS, 2021. Exploring the Role of Endothelial Cell Resilience in Cardiovascular Health and Disease. Arterioscler Thromb Vasc Biol 41, 179–185. [PubMed: 33086867]

Garcia-Medina JJ, Pinazo-Duran MD, Garcia-Medina M, Zanon-Moreno V, Pons-Vazquez S, 2011. A 5-year follow-up of antioxidant supplementation in type 2 diabetic retinopathy. Eur J Ophthalmol 21, 637–643. [PubMed: 21218388]

Garcia-Sanchez A, Villalain I, Asencio M, Garcia J, Garcia-Rio F, 2022. Sleep apnea and eye diseases: evidence of association and potential pathogenic mechanisms. J Clin Sleep Med 18, 265–278. [PubMed: 34283018]

Gardner GG, Keating D, Williamson TH, Elliott AT, 1996. Automatic detection of diabetic retinopathy using an artificial neural network: a screening tool. Br J Ophthalmol 80, 940–944. [PubMed: 8976718]

Gems D, Partridge L, 2008. Stress-response hormesis and aging: "that which does not kill us makes us stronger". Cell Metab 7, 200–203. [PubMed: 18316025]

Ghanian Z, Mehrvar S, Jamali N, Sheibani N, Ranji M, 2018. Time-lapse microscopy of oxidative stress demonstrates metabolic sensitivity of retinal pericytes under high glucose condition. J Biophotonics 11, e201700289. [PubMed: 29577636]

Goncalves A, Dreffs A, Lin CM, Sheskey S, Hudson N, Keil J, Campbell M, Antonetti DA, 2021. Vascular Expression of Permeability-Resistant Occludin Mutant Preserves Visual Function in Diabetes. Diabetes 70, 1549–1560. [PubMed: 33883214]

Gong Q, Zhang P, Wang J, Ma J, An Y, Chen Y, Zhang B, Feng X, Li H, Chen X, Cheng YJ, Gregg EW, Hu Y, Bennett PH, Li G, Da Qing Diabetes Prevention Study, G., 2019. Morbidity and mortality after lifestyle intervention for people with impaired glucose tolerance: 30-year results of the Da Qing Diabetes Prevention Outcome Study. Lancet Diabetes Endocrinol 7, 452–461. [PubMed: 31036503]

Gonzalez-Fernandez F, Sung D, Haswell KM, Tsin A, Ghosh D, 2014. Thiol-dependent antioxidant activity of interphotoreceptor retinoid-binding protein. Exp Eye Res 120, 167–174. [PubMed: 24424263]

Goto H, Nishikawa T, Sonoda K, Kondo T, Kukidome D, Fujisawa K, Yamashiro T, Motoshima H, Matsumura T, Tsuruzoe K, Araki E, 2008. Endothelial MnSOD overexpression prevents retinal VEGF expression in diabetic mice. Biochem Biophys Res Commun 366, 814–820. [PubMed: 18083119]

Gottlieb RA, Gustafsson AB, 2011. Mitochondrial turnover in the heart. Biochim Biophys Acta 1813, 1295–1301. [PubMed: 21147177]

Granger DN, Kvietys PR, 2015. Reperfusion injury and reactive oxygen species: The evolution of a concept. Redox Biol 6, 524–551. [PubMed: 26484802]

Group, A.S., Group, A.E.S., Chew EY, Ambrosius WT, Davis MD, Danis RP, Gangaputra S, Greven CM, Hubbard L, Esser BA, Lovato JF, Perdue LH, Goff DC Jr., Cushman WC, Ginsberg HN, Elam MB, Genuth S, Gerstein HC, Schubart U, Fine LJ, 2010. Effects of medical therapies on retinopathy progression in type 2 diabetes. N Engl J Med 363, 233–244. [PubMed: 20587587]

Grunewald M, Kumar S, Sharife H, Volinsky E, Gileles-Hillel A, Licht T, Permyakova A, Hinden L, Azar S, Friedmann Y, Kupetz P, Tzuberi R, Anisimov A, Alitalo K, Horwitz M, Leebhoff S, Khoma OZ, Hlushchuk R, Djonov V, Abramovitch R, Tam J, Keshet E, 2021. Counteracting age-related VEGF signaling insufficiency promotes healthy aging and extends life span. Science 373.

Gulshan V, Peng L, Coram M, Stumpe MC, Wu D, Narayanaswamy A, Venugopalan S, Widner K, Madams T, Cuadros J, Kim R, Raman R, Nelson PC, Mega JL, Webster DR, 2016. Development and Validation of a Deep Learning Algorithm for Detection of Diabetic Retinopathy in Retinal Fundus Photographs. JAMA 316, 2402–2410. [PubMed: 27898976]

Gulshan V, Rajan RP, Widner K, Wu D, Wubbels P, Rhodes T, Whitehouse K, Coram M, Corrado G, Ramasamy K, Raman R, Peng L, Webster DR, 2019. Performance of a Deep-Learning Algorithm vs Manual Grading for Detecting Diabetic Retinopathy in India. JAMA Ophthalmol 137, 987–993. [PubMed: 31194246]

Guo J, Huang X, Dou L, Yan M, Shen T, Tang W, Li J, 2022. Aging and aging-related diseases: from molecular mechanisms to interventions and treatments. Signal Transduct Target Ther 7, 391. [PubMed: 36522308]

Haigis MC, Yankner BA, 2010. The aging stress response. Mol Cell 40, 333–344. [PubMed: 20965426]

Hammer SS, Beli E, Kady N, Wang Q, Wood K, Lydic TA, Malek G, Saban DR, Wang XX, Hazra S, Levi M, Busik JV, Grant MB, 2017. The Mechanism of Diabetic Retinopathy Pathogenesis Unifying Key Lipid Regulators, Sirtuin 1 and Liver X Receptor. EBioMedicine 22, 181–190. [PubMed: 28774737]

Han B, Chen WZ, Li YC, Chen J, Zeng ZQ, 2020. Sleep and hypertension. Sleep Breath 24, 351–356. [PubMed: 31402441]

Hatori Y, Inouye S, Akagi R, Seyama T, 2018. Local redox environment beneath biological membranes probed by palmitoylated-roGFP. Redox Biol 14, 679–685. [PubMed: 29179107]

He F, Ru X, Wen T, 2020. NRF2, a Transcription Factor for Stress Response and Beyond. Int J Mol Sci 21.

He K, Zhang X, Ren S, Sun J, 2015. Spatial Pyramid Pooling in Deep Convolutional Networks for Visual Recognition. IEEE Trans Pattern Anal Mach Intell 37, 1904–1916. [PubMed: 26353135]

He KG, Dollár Georgia, Piotr Girshick Ross, 2017. Mask R-CNN, IEEE Conf Comput Vis Pattern Recognit, pp. 2980–2988.

He KZ, Ren Xiangyu, Shaoqing Sun Jian, 2016. Deep Residual Learning for Image Recognition, IEEE Conf Comput Vis Pattern Recognit, pp. 770–778.

Herhaus L, Dikic I, 2015. Expanding the ubiquitin code through post-translational modification. EMBO Rep 16, 1071–1083. [PubMed: 26268526]

Herold KC, Delong T, Perdigoto AL, Biru N, Brusko TM, Walker LSK, 2024. The immunology of type 1 diabetes. Nat Rev Immunol.

Herzig S, Shaw RJ, 2018. AMPK: guardian of metabolism and mitochondrial homeostasis. Nat Rev Mol Cell Biol 19, 121–135. [PubMed: 28974774]

Hinder LM, Sas KM, O'Brien PD, Backus C, Kayampilly P, Hayes JM, Lin CM, Zhang H, Shanmugam S, Rumora AE, Abcouwer SF, Brosius FC 3rd, Pennathur S, Feldman EL, 2019. Mitochondrial uncoupling has no effect on microvascular complications in type 2 diabetes. Sci Rep 9, 881. [PubMed: 30696927]

Holoman NC, Aiello JJ, Trobenter TD, Tarchick MJ, Kozlowski MR, Makowski ER, De Vivo DC, Singh C, Sears JE, Samuels IS, 2021. Reduction of Glut1 in the Neural Retina But Not the RPE Alleviates Polyol Accumulation and Normalizes Early Characteristics of Diabetic Retinopathy. J Neurosci 41, 3275–3299. [PubMed: 33622781]

Hombrebueno JR, Cairns L, Dutton LR, Lyons TJ, Brazil DP, Moynagh P, Curtis TM, Xu H, 2019. Uncoupled turnover disrupts mitochondrial quality control in diabetic retinopathy. JCI Insight 4.

Hong SG, Ashby JW, Kennelly JP, Wu M, Chattopadhyay E, Foreman R, Tontonoz P, Turowski P, Gallagher-Jones M, Mack JJ, 2023. Polarized Mechanosensitive Signaling Domains Protect Arterial Endothelial Cells Against Inflammation. bioRxiv.

Huang BB, Fukuyama H, Burns SA, Fawzi AA, 2024. Imaging the Retinal Vascular Mural Cells In Vivo: Elucidating the Timeline of Their Loss in Diabetic Retinopathy. Arterioscler Thromb Vasc Biol 44, 465–476. [PubMed: 38152885]

Huang C, Rosencrans RF, Bugescu R, Vieira CP, Hu P, Adu-Agyeiwaah Y, Gamble KL, Longhini ALF, Fuller PM, Leinninger GM, Grant MB, 2021. Depleting hypothalamic somatostatinergic neurons recapitulates diabetic phenotypes in mouse brain, bone marrow, adipose and retina. Diabetologia 64, 2575–2588. [PubMed: 34430981]

Huang GL, Zhuang Laurens, van der Maaten Weinberger Kilian Q. , 2017. Densely Connected Convolutional Networks, IEEE Conf Comput Vis Pattern Recognit, pp. 2261–2269.

Huang Q, Sheibani N, 2008. High glucose promotes retinal endothelial cell migration through activation of Src, PI3K/Akt1/eNOS, and ERKs. Am J Physiol Cell Physiol 295, C1647–1657. [PubMed: 18945941]

Ipp E, Liljenquist D, Bode B, Shah VN, Silverstein S, Regillo CD, Lim JI, Sadda S, Domalpally A, Gray G, Bhaskaranand M, Ramachandra C, Solanki K, EyeArt Study, G., 2021. Pivotal Evaluation of an Artificial Intelligence System for Autonomous Detection of Referrable and Vision-Threatening Diabetic Retinopathy. JAMA Netw Open 4, e2134254. [PubMed: 34779843]

Itoh K, Ye P, Matsumiya T, Tanji K, Ozaki T, 2015. Emerging functional cross-talk between the Keap1-Nrf2 system and mitochondria. J Clin Biochem Nutr 56, 91–97. [PubMed: 25759513]

Jain MK, Sangwung P, Hamik A, 2014. Regulation of an inflammatory disease: Kruppel-like factors and atherosclerosis. Arterioscler Thromb Vasc Biol 34, 499–508. [PubMed: 24526695]

Jiang F, Zhang Y, Dusting GJ, 2011. NADPH oxidase-mediated redox signaling: roles in cellular stress response, stress tolerance, and tissue repair. Pharmacol Rev 63, 218–242. [PubMed: 21228261]

Joesch M, Meister M, 2016. A neuronal circuit for colour vision based on rod-cone opponency. Nature 532, 236–239. [PubMed: 27049951]

Joussen AM, Murata T, Tsujikawa A, Kirchhof B, Bursell SE, Adamis AP, 2001. Leukocyte-mediated endothelial cell injury and death in the diabetic retina. Am J Pathol 158, 147–152. [PubMed: 11141487]

Joussen AM, Poulaki V, Le ML, Koizumi K, Esser C, Janicki H, Schraermeyer U, Kociok N, Fauser S, Kirchhof B, Kern TS, Adamis AP, 2004. A central role for inflammation in the pathogenesis of diabetic retinopathy. FASEB J 18, 1450–1452. [PubMed: 15231732]

Joussen AM, Poulaki V, Mitsiades N, Cai WY, Suzuma I, Pak J, Ju ST, Rook SL, Esser P, Mitsiades CS, Kirchhof B, Adamis AP, Aiello LP, 2003. Suppression of Fas-FasL-induced endothelial cell apoptosis prevents diabetic blood-retinal barrier breakdown in a model of streptozotocin-induced diabetes. FASEB J 17, 76–78. [PubMed: 12475915]

Kahaly GJ, Hansen MP, 2016. Type 1 diabetes associated autoimmunity. Autoimmun Rev 15, 644–648. [PubMed: 26903475]

Kaikini AA, Kanchan DM, Nerurkar UN, Sathaye S, 2017. Targeting Mitochondrial Dysfunction for the Treatment of Diabetic Complications: Pharmacological Interventions through Natural Products. Pharmacogn Rev 11, 128–135. [PubMed: 28989247]

Kang Q, Yang C, 2020. Oxidative stress and diabetic retinopathy: Molecular mechanisms, pathogenetic role and therapeutic implications. Redox Biol 37, 101799. [PubMed: 33248932]

Kang R, Livesey KM, Zeh HJ, Loze MT, Tang D, 2010. HMGB1: a novel Beclin 1-binding protein active in autophagy. Autophagy 6, 1209–1211. [PubMed: 20935509]

Kanwar M, Chan PS, Kern TS, Kowluru RA, 2007. Oxidative damage in the retinal mitochondria of diabetic mice: possible protection by superoxide dismutase. Invest Ophthalmol Vis Sci 48, 3805–3811. [PubMed: 17652755]

Kasai S, Shimizu S, Tatara Y, Mimura J, Itoh K, 2020. Regulation of Nrf2 by Mitochondrial Reactive Oxygen Species in Physiology and Pathology. Biomolecules 10.

Kazak L, Reyes A, Holt IJ, 2012. Minimizing the damage: repair pathways keep mitochondrial DNA intact. Nat Rev Mol Cell Biol 13, 659–671. [PubMed: 22992591]

Khapchaev AY, Antonova OA, Kazakova OA, Samsonov MV, Vorotnikov AV, Shirinsky VP, 2023. Long-Term Experimental Hyperglycemia Does Not Impair Macrovascular Endothelial Barrier Integrity and Function in vitro. Biochemistry (Mosc) 88, 1126–1138. [PubMed: 37758312]

Kim D, Roy S, 2020. Effects of Diabetes on Mitochondrial Morphology and Its Implications in Diabetic Retinopathy. Invest Ophthalmol Vis Sci 61, 10.

Kim D, Sankaramoorthy A, Roy S, 2020. Downregulation of Drp1 and Fis1 Inhibits Mitochondrial Fission and Prevents High Glucose-Induced Apoptosis in Retinal Endothelial Cells. Cells 9.

Kim D, Sesaki H, Roy S, 2021. Reduced Levels of Drp1 Protect against Development of Retinal Vascular Lesions in Diabetic Retinopathy. Cells 10.

Kim IJ, Zhang Y, Yamagata M, Meister M, Sanes JR, 2008. Molecular identification of a retinal cell type that responds to upward motion. Nature 452, 478–482. [PubMed: 18368118]

Kiyama T, Chen CK, Wang SW, Pan P, Ju Z, Wang J, Takada S, Klein WH, Mao CA, 2018. Essential roles of mitochondrial biogenesis regulator Nrf1 in retinal development and homeostasis. Mol Neurodegener 13, 56. [PubMed: 30333037]

Kleele T, Rey T, Winter J, Zaganelli S, Mahecic D, Perreten Lambert H, Ruberto FP, Nemir M, Wai T, Pedrazzini T, Manley S, 2021. Distinct fission signatures predict mitochondrial degradation or biogenesis. Nature 593, 435–439. [PubMed: 33953403]

Klein R, Klein BE, Moss SE, Davis MD, DeMets DL, 1984. The Wisconsin epidemiologic study of diabetic retinopathy. II. Prevalence and risk of diabetic retinopathy when age at diagnosis is less than 30 years. Arch Ophthalmol 102, 520–526. [PubMed: 6367724]

Knickelbein JE, Abbott AB, Chew EY, 2016. Fenofibrate and Diabetic Retinopathy. Curr Diab Rep 16, 90. [PubMed: 27525681]

Knip M, Siljander H, 2008. Autoimmune mechanisms in type 1 diabetes. Autoimmun Rev 7, 550–557. [PubMed: 18625444]

Korot E, Pontikos N, Liu X, Wagner SK, Faes L, Huemer J, Balaskas K, Denniston AK, Khawaja A, Keane PA, 2021. Predicting sex from retinal fundus photographs using automated deep learning. Sci Rep 11, 10286. [PubMed: 33986429]

Kowluru RA, 2019. Mitochondrial Stability in Diabetic Retinopathy: Lessons Learned From Epigenetics. Diabetes 68, 241–247. [PubMed: 30665952]

Kowluru RA, Abbas SN, 2003. Diabetes-induced mitochondrial dysfunction in the retina. Invest Ophthalmol Vis Sci 44, 5327–5334. [PubMed: 14638734]

Kowluru RA, Kowluru A, Mishra M, Kumar B, 2015. Oxidative stress and epigenetic modifications in the pathogenesis of diabetic retinopathy. Prog Retin Eye Res 48, 40–61. [PubMed: 25975734]

Kowluru RA, Kowluru V, Xiong Y, Ho YS, 2006. Overexpression of mitochondrial superoxide dismutase in mice protects the retina from diabetes-induced oxidative stress. Free Radic Biol Med 41, 1191–1196. [PubMed: 17015165]

Kowluru RA, Mishra M, 2015. Oxidative stress, mitochondrial damage and diabetic retinopathy. Biochim Biophys Acta 1852, 2474–2483. [PubMed: 26248057]

Kowluru RA, Odenbach S, 2004. Effect of long-term administration of alpha-lipoic acid on retinal capillary cell death and the development of retinopathy in diabetic rats. Diabetes 53, 3233–3238. [PubMed: 15561955]

Krizhevsky AS, Hinton Ilya, Geoffrey E, 2012. ImageNet classification with deep convolutional neural networks, Adv Neural Inf Process Syst, pp. 1097–1105.

Kumagai AK, Glasgow BJ, Pardridge WM, 1994. GLUT1 glucose transporter expression in the diabetic and nondiabetic human eye. Invest Ophthalmol Vis Sci 35, 2887–2894. [PubMed: 8188484]

Kumar B, Gupta SK, Nag TC, Srivastava S, Saxena R, 2012. Green tea prevents hyperglycemia-induced retinal oxidative stress and inflammation in streptozotocin-induced diabetic rats. Ophthalmic Res 47, 103–108. [PubMed: 21997135]

Laddha AP, Kulkarni YA, 2020. NADPH oxidase: A membrane-bound enzyme and its inhibitors in diabetic complications. Eur J Pharmacol 881, 173206. [PubMed: 32442539]

Lee M, Li S, Sato K, Jin M, 2016. Interphotoreceptor Retinoid-Binding Protein Mitigates Cellular Oxidative Stress and Mitochondrial Dysfunction Induced by All-trans-Retinal. Invest Ophthalmol Vis Sci 57, 1553–1562. [PubMed: 27046120]

Lee SWH, Ng KY, Chin WK, 2017. The impact of sleep amount and sleep quality on glycemic control in type 2 diabetes: A systematic review and meta-analysis. Sleep Med Rev 31, 91–101. [PubMed: 26944909]

Lessieur EM, Liu H, Saadane A, Du Y, Kiser J, Kern TS, 2022. ICAM-1 on the luminal surface of endothelial cells is induced to a greater extent in mouse retina than in other tissues in diabetes. Diabetologia 65, 1734–1744. [PubMed: 35852587]

Lessieur EM, Liu H, Saadane A, Du Y, Tang J, Kiser J, Kern TS, 2021. Neutrophil-Derived Proteases Contribute to the Pathogenesis of Early Diabetic Retinopathy. Invest Ophthalmol Vis Sci 62, 7.

Li S, Sun D, Chen S, Zhang S, Gu Q, Shen Y, Xu L, Xu X, Wei F, Wang N, 2024. UCP2-SIRT3 Signaling Relieved Hyperglycemia-Induced Oxidative Stress and Senescence in Diabetic Retinopathy. Invest Ophthalmol Vis Sci 65, 14.

Li W, Khor TO, Xu C, Shen G, Jeong WS, Yu S, Kong AN, 2008. Activation of Nrf2-antioxidant signaling attenuates NFkappaB-inflammatory response and elicits apoptosis. Biochem Pharmacol 76, 1485–1489. [PubMed: 18694732]

Li Y, Baccouche B, Del-Risco N, Park J, Song A, McAnany JJ, Kazlauskas A, 2023. The Slow Progression of Diabetic Retinopathy Is Associated with Transient Protection of Retinal Vessels from Death. Int J Mol Sci 24.

Lim JI, Regillo CD, Sadda SR, Ipp E, Bhaskaranand M, Ramachandra C, Solanki K, 2023. Artificial Intelligence Detection of Diabetic Retinopathy: Subgroup Comparison of the EyeArt System with Ophthalmologists' Dilated Examinations. Ophthalmol Sci 3, 100228. [PubMed: 36345378]

Lin RZ, Im GB, Luo AC, Zhu Y, Hong X, Neumeyer J, Tang HW, Perrimon N, Melero-Martin JM, 2024. Mitochondrial transfer mediates endothelial cell engraftment through mitophagy. Nature.

Lin TY, Goyal P, Girshick R, He K, Dollar P, 2020. Focal Loss for Dense Object Detection. IEEE Trans Pattern Anal Mach Intell 42, 318–327. [PubMed: 30040631]

Liu L, Li Y, Chen G, Chen Q, 2023. Crosstalk between mitochondrial biogenesis and mitophagy to maintain mitochondrial homeostasis. J Biomed Sci 30, 86. [PubMed: 37821940]

Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G, 2013. The hallmarks of aging. Cell 153, 1194–1217. [PubMed: 23746838]

Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G, 2023. Hallmarks of aging: An expanding universe. Cell 186, 243–278. [PubMed: 36599349]

Lopez-Otin C, Kroemer G, 2021. Hallmarks of Health. Cell 184, 33–63. [PubMed: 33340459]

Lorenzi M, 2007. The polyol pathway as a mechanism for diabetic retinopathy: attractive, elusive, and resilient. Exp Diabetes Res 2007, 61038. [PubMed: 18224243]

Lowe DG, 1999. Object recognition from local scale-invariant features, IEEE Proceedings of the Seventh IEEE International Conference on Computer Vision. IEEE, Kerkyra, Greece, pp. 1150–1157.

Luu J, Palczewski K, 2018. Human aging and disease: Lessons from age-related macular degeneration. Proc Natl Acad Sci U S A 115, 2866–2872. [PubMed: 29483257]

Luu JC, Saadane A, Leinonen H, Choi EH, Gao F, Lewandowski D, Halabi M, Sander CL, Wu A, Wang JM, Singh R, Gao S, Lessieur EM, Dong Z, Palczewska G, Mullins RF, Peachey NS, Kiser PD, Tabaka M, Kern TS, Palczewski K, 2023. Stress resilience-enhancing drugs preserve tissue structure and function in degenerating retina via phosphodiesterase inhibition. Proc Natl Acad Sci U S A 120, e2221045120. [PubMed: 37126699]

Mack JJ, Mosqueiro TS, Archer BJ, Jones WM, Sunshine H, Faas GC, Briot A, Aragon RL, Su T, Romay MC, McDonald AI, Kuo CH, Lizama CO, Lane TF, Zovein AC, Fang Y, Tarling EJ, de Aguiar Vallim TQ, Navab M, Fogelman AM, Bouchard LS, Iruela-Arispe ML, 2017. NOTCH1 is a mechanosensor in adult arteries. Nat Commun 8, 1620. [PubMed: 29158473]

Mantilidewi KI, Murata Y, Mori M, Otsubo C, Kotani T, Kusakari S, Ohnishi H, Matozaki T, 2014. Shear stress-induced redistribution of vascular endothelial-protein-tyrosine phosphatase (VE-PTP) in endothelial cells and its role in cell elongation. J Biol Chem 289, 6451–6461. [PubMed: 24451369]

Mari M, de Gregorio E, de Dios C, Roca-Agujetas V, Cucarull B, Tutusaus A, Morales A, Colell A, 2020. Mitochondrial Glutathione: Recent Insights and Role in Disease. Antioxidants (Basel) 9.

Mathew B, Chennakesavalu M, Sharma M, Torres LA, Stelman CR, Tran S, Patel R, Burg N, Salkovski M, Kadzielawa K, Seiler F, Aldrich LN, Roth S, 2020. Autophagy and post-ischemic conditioning in retinal ischemia. Autophagy, 1–21. [PubMed: 31516068]

Mehrzadi S, Motevalian M, Rezaei Kanavi M, Fatemi I, Ghaznavi H, Shahriari M, 2018. Protective effect of melatonin in the diabetic rat retina. Fundam Clin Pharmacol 32, 414–421. [PubMed: 29495082]

Metascreen Writing, C., Bonadonna R, Cucinotta D, Fedele D, Riccardi G, Tiengo A, 2006. The metabolic syndrome is a risk indicator of microvascular and macrovascular complications in diabetes: results from Metascreen, a multicenter diabetes clinic-based survey. Diabetes Care 29, 2701–2707. [PubMed: 17130208]

Meyer AJ, Dick TP, 2010. Fluorescent protein-based redox probes. Antioxid Redox Signal 13, 621–650. [PubMed: 20088706]

Meza CA, La Favor JD, Kim DH, Hickner RC, 2019. Endothelial Dysfunction: Is There a Hyperglycemia-Induced Imbalance of NOX and NOS? Int J Mol Sci 20.

Miller DJ, Cascio MA, Rosca MG, 2020. Diabetic Retinopathy: The Role of Mitochondria in the Neural Retina and Microvascular Disease. Antioxidants (Basel) 9.

Mishra P, Chan DC, 2016. Metabolic regulation of mitochondrial dynamics. J Cell Biol 212, 379–387. [PubMed: 26858267]

Miyamoto K, Hiroshiba N, Tsujikawa A, Ogura Y, 1998. In vivo demonstration of increased leukocyte entrapment in retinal microcirculation of diabetic rats. Invest Ophthalmol Vis Sci 39, 2190–2194. [PubMed: 9761301]

Morrish NJ, Wang SL, Stevens LK, Fuller JH, Keen H, 2001. Mortality and causes of death in the WHO Multinational Study of Vascular Disease in Diabetes. Diabetologia 44 Suppl 2, S14–21. [PubMed: 11587045]

Motz CT, Chesler KC, Allen RS, Bales KL, Mees LM, Feola AJ, Maa AY, Olson DE, Thule PM, Iuvone PM, Hendrick AM, Pardue MT, 2020. Novel Detection and Restorative Levodopa Treatment for Preclinical Diabetic Retinopathy. Diabetes 69, 1518–1527. [PubMed: 32051147]

Mullington JM, Simpson NS, Meier-Ewert HK, Haack M, 2010. Sleep loss and inflammation. Best Pract Res Clin Endocrinol Metab 24, 775–784. [PubMed: 21112025]

Murry CE, Jennings RB, Reimer KA, 1986. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 74, 1124–1136. [PubMed: 3769170]

Nah J, Shirakabe A, Mukai R, Zhai P, Sung EA, Ivessa A, Mizushima W, Nakada Y, Saito T, Hu C, Jung YK, Sadoshima J, 2022. Ulk1-dependent alternative mitophagy plays a protective role during pressure overload in the heart. Cardiovasc Res 118, 2638–2651. [PubMed: 35018428]

Nakada K, Sato A, Hayashi J, 2009. Mitochondrial functional complementation in mitochondrial DNA-based diseases. Int J Biochem Cell Biol 41, 1907–1913. [PubMed: 19464386]

Natarajan R, 2021. Epigenetic Mechanisms in Diabetic Vascular Complications and Metabolic Memory: The 2020 Edwin Bierman Award Lecture. Diabetes 70, 328–337. [PubMed: 33472942]

Nath A, Schwartz GW, 2017. Electrical synapses convey orientation selectivity in the mouse retina. Nat Commun 8, 2025. [PubMed: 29229967]

Nauck MA, Quast DR, Wefers J, Meier JJ, 2021. GLP-1 receptor agonists in the treatment of type 2 diabetes - state-of-the-art. Mol Metab 46, 101102. [PubMed: 33068776]

Niccoli T, Partridge L, 2012. Ageing as a risk factor for disease. Curr Biol 22, R741–752. [PubMed: 22975005]

Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, Brownlee M, 2000. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature 404, 787–790. [PubMed: 10783895]

Nissanka N, Moraes CT, 2018. Mitochondrial DNA damage and reactive oxygen species in neurodegenerative disease. FEBS Lett 592, 728–742. [PubMed: 29281123]

Noh H, King GL, 2007. The role of protein kinase C activation in diabetic nephropathy. Kidney Int Suppl, S49–53. [PubMed: 17653211]

Obrosova IG, Stevens MJ, Lang HJ, 2001. Diabetes-induced changes in retinal NAD-redox status: pharmacological modulation and implications for pathogenesis of diabetic retinopathy. Pharmacology 62, 172–180. [PubMed: 11287819]

Ono T, Isobe K, Nakada K, Hayashi JI, 2001. Human cells are protected from mitochondrial dysfunction by complementation of DNA products in fused mitochondria. Nat Genet 28, 272–275. [PubMed: 11431699]

Otani A, Dorrell MI, Kinder K, Moreno SK, Nusinowitz S, Banin E, Heckenlively J, Friedlander M, 2004. Rescue of retinal degeneration by intravitreally injected adult bone marrow-derived lineage-negative hematopoietic stem cells. J Clin Invest 114, 765–774. [PubMed: 15372100]

Palikaras K, Lionaki E, Tavernarakis N, 2015. Coordination of mitophagy and mitochondrial biogenesis during ageing in C. elegans. Nature 521, 525–528. [PubMed: 25896323]

Park A, Chute C, Rajpurkar P, Lou J, Ball RL, Shpanskaya K, Jabarkheel R, Kim LH, McKenna E, Tseng J, Ni J, Wishah F, Wittber F, Hong DS, Wilson TJ, Halabi S, Basu S, Patel BN, Lungren MP, Ng AY, Yeom KW, 2019. Deep Learning-Assisted Diagnosis of Cerebral Aneurysms Using the HeadXNet Model. JAMA Netw Open 2, e195600. [PubMed: 31173130]

Peng JJ, Xiong SQ, Ding LX, Peng J, Xia XB, 2019. Diabetic retinopathy: Focus on NADPH oxidase and its potential as therapeutic target. Eur J Pharmacol 853, 381–387. [PubMed: 31009636]

Pickles S, Vigie P, Youle RJ, 2018. Mitophagy and Quality Control Mechanisms in Mitochondrial Maintenance. Curr Biol 28, R170–R185. [PubMed: 29462587]

Popkin BM, Adair LS, Ng SW, 2012. Global nutrition transition and the pandemic of obesity in developing countries. Nutr Rev 70, 3–21. [PubMed: 22221213]

Popov LD, 2020. Mitochondrial biogenesis: An update. J Cell Mol Med 24, 4892–4899. [PubMed: 32279443]

Qiao S, Dennis M, Song X, Vadysirisack DD, Salunke D, Nash Z, Yang Z, Liesa M, Yoshioka J, Matsuzawa S, Shirihai OS, Lee RT, Reed JC, Ellisen LW, 2015. A REDD1/TXNIP pro-oxidant complex regulates ATG4B activity to control stress-induced autophagy and sustain exercise capacity. Nat Commun 6, 7014. [PubMed: 25916556]

Quellec G, Lamard M, Cazuguel G, Bekri L, Daccache W, Roux C, Cochener B, 2011. Automated assessment of diabetic retinopathy severity using content-based image retrieval in multimodal fundus photographs. Invest Ophthalmol Vis Sci 52, 8342–8348. [PubMed: 21896872]

Quiroz J, Yazdanyar A, 2021. Animal models of diabetic retinopathy. Ann Transl Med 9, 1272. [PubMed: 34532409]

Ramsey KM, Yoshino J, Brace CS, Abrassart D, Kobayashi Y, Marcheva B, Hong HK, Chong JL, Buhr ED, Lee C, Takahashi JS, Imai S, Bass J, 2009. Circadian clock feedback cycle through NAMPT-mediated NAD+ biosynthesis. Science 324, 651–654. [PubMed: 19299583]

Rashidisabet H, Sethi A, Jindarak P, Edmonds J, Chan RVP, Leiderman YI, Vajaranant TS, Yi D, 2023. Validating the Generalizability of Ophthalmic Artificial Intelligence Models on Real-World Clinical Data. Transl Vis Sci Technol 12, 8.

Ravingerova T, Adameova A, Matejikova J, Kelly T, Nemcekova M, Kucharska J, Pechanova O, Lazou A, 2010. Subcellular mechanisms of adaptation in the diabetic myocardium: Relevance to ischemic preconditioning in the nondiseased heart. Exp Clin Cardiol 15, 68–76. [PubMed: 21264077]

Redmon JF, Ali, 2017. YOLO9000: Better, Faster, Stronger, IEEE Conf Comput Vis Pattern Recognit.

Ren S, He K, Girshick R, Sun J, 2017. Faster R-CNN: Towards Real-Time Object Detection with Region Proposal Networks. IEEE Trans Pattern Anal Mach Intell 39, 1137–1149. [PubMed: 27295650]

Reutrakul S, Mokhlesi B, 2017. Obstructive Sleep Apnea and Diabetes: A State of the Art Review. Chest 152, 1070–1086. [PubMed: 28527878]

Ritter MR, Banin E, Moreno SK, Aguilar E, Dorrell MI, Friedlander M, 2006. Myeloid progenitors differentiate into microglia and promote vascular repair in a model of ischemic retinopathy. J Clin Invest 116, 3266–3276. [PubMed: 17111048]

Rizzolo LJ, 1997. Polarity and the development of the outer blood-retinal barrier. Histol Histopathol 12, 1057–1067. [PubMed: 9302567]

Robinson R, Barathi VA, Chaurasia SS, Wong TY, Kern TS, 2012. Update on animal models of diabetic retinopathy: from molecular approaches to mice and higher mammals. Dis Model Mech 5, 444–456. [PubMed: 22730475]

Roden M, Shulman GI, 2019. The integrative biology of type 2 diabetes. Nature 576, 51–60. [PubMed: 31802013]

Roig-Revert MJ, Lleo-Perez A, Zanon-Moreno V, Vivar-Llopis B, Marin-Montiel J, Dolz-Marco R, Alonso-Munoz L, Albert-Fort M, Lopez-Galvez MI, Galarreta-Mira D, Garcia-Esparza MF, Galbis-Estrada C, Marco-Ramirez C, Shoaie-Nia K, Sanz-Gonzalez SM, Vila-Bou V, Bendala-Tufanisco E, Garcia-Medina JJ, Nucci C, Gallego-Pinazo R, Arevalo JF, Pinazo-Duran MD, Valencia Study On Diabetic, R., 2015. Enhanced Oxidative Stress and Other Potential Biomarkers for Retinopathy in Type 2 Diabetics: Beneficial Effects of the Nutraceutic Supplements. Biomed Res Int 2015, 408180. [PubMed: 26618168]

Roma LP, Deponte M, Riemer J, Morgan B, 2018. Mechanisms and Applications of Redox-Sensitive Green Fluorescent Protein-Based Hydrogen Peroxide Probes. Antioxid Redox Signal 29, 552–568. [PubMed: 29160083]

Romay MC, Knutsen RH, Ma F, Mompeon A, Hernandez GE, Salvador J, Mirkov S, Batra A, Sullivan DP, Procissi D, Buchanan S, Kronquist E, Ferrante EA, Muller WA, Walshon J, Steffens A, McCortney K, Horbinski C, Tournier-Lasserve E, Sonabend AM, Sorond FA, Wang MM, Boehm M, Kozel BA, Iruela-Arispe ML, 2024. Age-related loss of Notch3 underlies brain vascular contractility deficiencies, glymphatic dysfunction, and neurodegeneration in mice. J Clin Invest 134.

Ronneberger O, Fischer P, Brox T, 2015. U-Net: Convolutional Networks for Biomedical Image Segmentation, Med Image Comput Comput Assist Interv, p. arXiv:1505.04597.

Rosen RB, Andrade Romo JS, Krawitz BD, Mo S, Fawzi AA, Linderman RE, Carroll J, Pinhas A, Chui TYP, 2019. Earliest Evidence of Preclinical Diabetic Retinopathy Revealed Using Optical Coherence Tomography Angiography Perfused Capillary Density. Am J Ophthalmol 203, 103–115. [PubMed: 30689991]

Ross D, Siegel D, 2021. The diverse functionality of NQO1 and its roles in redox control. Redox Biol 41, 101950. [PubMed: 33774477]

Rossignol R, Faustin B, Rocher C, Malgat M, Mazat JP, Letellier T, 2003. Mitochondrial threshold effects. Biochem J 370, 751–762. [PubMed: 12467494]

Roy S, Kim D, Sankaramoorthy A, 2019. Mitochondrial Structural Changes in the Pathogenesis of Diabetic Retinopathy. J Clin Med 8.

Ruamviboonsuk P, Tiwari R, Sayres R, Nganthavee V, Hemarat K, Kongprayoon A, Raman R, Levinstein B, Liu Y, Schaekermann M, Lee R, Virmani S, Widner K, Chambers J, Hersch F, Peng L, Webster DR, 2022. Real-time diabetic retinopathy screening by deep learning in a multisite national screening programme: a prospective interventional cohort study. Lancet Digit Health 4, e235–e244. [PubMed: 35272972]

Samuels IS, Bell BA, Pereira A, Saxon J, Peachey NS, 2015. Early retinal pigment epithelium dysfunction is concomitant with hyperglycemia in mouse models of type 1 and type 2 diabetes. J Neurophysiol 113, 1085–1099. [PubMed: 25429122]

Sang D, Lin K, Yang Y, Ran G, Li B, Chen C, Li Q, Ma Y, Lu L, Cui XY, Liu Z, Lv SQ, Luo M, Liu Q, Li Y, Zhang EE, 2023. Prolonged sleep deprivation induces a cytokine-storm-like syndrome in mammals. Cell 186, 5500–5516 e5521. [PubMed: 38016470]

Santos JM, Kowluru RA, 2011. Role of mitochondria biogenesis in the metabolic memory associated with the continued progression of diabetic retinopathy and its regulation by lipoic acid. Invest Ophthalmol Vis Sci 52, 8791–8798. [PubMed: 22003111]

Santos JM, Tewari S, Kowluru RA, 2012. A compensatory mechanism protects retinal mitochondria from initial insult in diabetic retinopathy. Free Radic Biol Med 53, 1729–1737. [PubMed: 22982046]

Sanz-González SM, García-Medina JJ, Zanón-Moreno V, López-Gálvez MI, Galarreta-Mira D, Duarte L, Valero-Velló M, Ramírez AI, Arévalo JF, Pinazo-Durán MD, On Behalf Of The Valencia Study Group On Diabetic Retinopathy Vsdr Report, N., 2020. Clinical and Molecular-Genetic Insights into the Role of Oxidative Stress in Diabetic Retinopathy: Antioxidant Strategies and Future Avenues. Antioxidants (Basel) 9.

Sasaki N, Yamashita T, Takaya T, Shinohara M, Shiraki R, Takeda M, Emoto N, Fukatsu A, Hayashi T, Ikemoto K, Nomura T, Yokoyama M, Hirata K, Kawashima S, 2008. Augmentation of vascular remodeling by uncoupled endothelial nitric oxide synthase in a mouse model of diabetes mellitus. Arterioscler Thromb Vasc Biol 28, 1068–1076. [PubMed: 18403727]

Scarinci F, Picconi F, Giorno P, Boccassini B, De Geronimo D, Varano M, Frontoni S, Parravano M, 2018. Deep capillary plexus impairment in patients with type 1 diabetes mellitus with no signs of diabetic retinopathy revealed using optical coherence tomography angiography. Acta Ophthalmol 96, e264–e265. [PubMed: 28887911]

Schauer PR, Bhatt DL, Kirwan JP, Wolski K, Aminian A, Brethauer SA, Navaneethan SD, Singh RP, Pothier CE, Nissen SE, Kashyap SR, Investigators, S., 2017. Bariatric Surgery versus Intensive Medical Therapy for Diabetes - 5-Year Outcomes. N Engl J Med 376, 641–651. [PubMed: 28199805]

Schroder S, Palinski W, Schmid-Schonbein GW, 1991. Activated monocytes and granulocytes, capillary nonperfusion, and neovascularization in diabetic retinopathy. Am J Pathol 139, 81–100. [PubMed: 1713023]

Scialo F, Fernandez-Ayala DJ, Sanz A, 2017. Role of Mitochondrial Reverse Electron Transport in ROS Signaling: Potential Roles in Health and Disease. Front Physiol 8, 428. [PubMed: 28701960]

Sergeys J, Etienne I, Van Hove I, Lefevere E, Stalmans I, Feyen JHM, Moons L, Van Bergen T, 2019. Longitudinal In Vivo Characterization of the Streptozotocin-Induced Diabetic Mouse Model: Focus on Early Inner Retinal Responses. Invest Ophthalmol Vis Sci 60, 807–822. [PubMed: 30811545]

Serikbaeva A, Li Y, Ganesh B, Zelkha R, Kazlauskas A, 2022. Hyperglycemia Promotes Mitophagy and Thereby Mitigates Hyperglycemia-Induced Damage. Am J Pathol 192, 1779–1794. [PubMed: 36063899]

Serra AM, Waddell J, Manivannan A, Xu H, Cotter M, Forrester JV, 2012. CD11b+ bone marrow-derived monocytes are the major leukocyte subset responsible for retinal capillary leukostasis in experimental diabetes in mouse and express high levels of CCR5 in the circulation. Am J Pathol 181, 719–727. [PubMed: 22677420]

Shah AL, Niemeijer S, Amelon M, Clarida R, Folk W, Russell J, Wu S, Abràmoff X, D. M, 2018. Susceptibility to misdiagnosis of adversarial images by deep learning based retinal image analysis algorithms, IEEE 15th International Symposium on Biomedical Imaging. IEEE, Washington, D.C., USA.

Shelhamer E, Long J, Darrell T, 2017. Fully Convolutional Networks for Semantic Segmentation. IEEE Trans Pattern Anal Mach Intell 39, 640–651. [PubMed: 27244717]

Shirakura K, Baluk P, Nottebaum AF, Ipe U, Peters KG, McDonald DM, Vestweber D, 2023. Shear stress control of vascular leaks and atheromas through Tie2 activation by VE-PTP sequestration. EMBO Mol Med 15, e16128. [PubMed: 36740996]

Sidarala V, Pearson GL, Parekh VS, Thompson B, Christen L, Gingerich MA, Zhu J, Stromer T, Ren J, Reck EC, Chai B, Corbett JA, Mandrup-Poulsen T, Satin LS, Soleimanpour SA, 2020. Mitophagy protects beta cells from inflammatory damage in diabetes. JCI Insight 5.

Simo R, Hernandez C, European Consortium for the Early Treatment of Diabetic, R., 2014. Neurodegeneration in the diabetic eye: new insights and therapeutic perspectives. Trends Endocrinol Metab 25, 23–33. [PubMed: 24183659]

Simo R, Stitt AW, Gardner TW, 2018. Neurodegeneration in diabetic retinopathy: does it really matter? Diabetologia 61, 1902–1912. [PubMed: 30030554]

Simonyan K, Zisserman A, 2014. Very Deep Convolutional Networks for Large-Scale Image Recognition, p. arXiv:1409.1556.

Skeie JM, Nishimura DY, Wang CL, Schmidt GA, Aldrich BT, Greiner MA, 2021. Mitophagy: An Emerging Target in Ocular Pathology. Invest Ophthalmol Vis Sci 62, 22.

Skyler JS, Bakris GL, Bonifacio E, Darsow T, Eckel RH, Groop L, Groop PH, Handelsman Y, Insel RA, Mathieu C, McElvaine AT, Palmer JP, Pugliese A, Schatz DA, Sosenko JM, Wilding JP, Ratner RE, 2017. Differentiation of Diabetes by Pathophysiology, Natural History, and Prognosis. Diabetes 66, 241–255. [PubMed: 27980006]

Sohn EH, van Dijk HW, Jiao C, Kok PH, Jeong W, Demirkaya N, Garmager A, Wit F, Kucukevcilioglu M, van Velthoven ME, DeVries JH, Mullins RF, Kuehn MH, Schlingemann RO, Sonka M, Verbraak FD, Abramoff MD, 2016. Retinal neurodegeneration may precede microvascular changes characteristic of diabetic retinopathy in diabetes mellitus. Proc Natl Acad Sci U S A 113, E2655–2664. [PubMed: 27114552]

Srinivasan S, Sivaprasad S, Rajalakshmi R, Anjana RM, Malik RA, Kulothungan V, Natarajan V, Raman R, Bhende M, 2022. Early retinal functional alteration in relation to diabetes duration in patients with type 2 diabetes without diabetic retinopathy. Sci Rep 12, 11422. [PubMed: 35794201]

Stene LC, Norris JM, Rewers MJ, 2023. Risk Factors for Type 1 Diabetes, in: Lawrence JM, Casagrande SS, Herman WH, Wexler DJ, Cefalu WT (Eds.), Diabetes in America, Bethesda (MD).

Stitt AW, Curtis TM, Chen M, Medina RJ, McKay GJ, Jenkins A, Gardiner TA, Lyons TJ, Hammes HP, Simo R, Lois N, 2016. The progress in understanding and treatment of diabetic retinopathy. Prog Retin Eye Res 51, 156–186. [PubMed: 26297071]

Stowe DF, Camara AK, 2009. Mitochondrial reactive oxygen species production in excitable cells: modulators of mitochondrial and cell function. Antioxid Redox Signal 11, 1373–1414. [PubMed: 19187004]

Strom J, Xu B, Tian X, Chen QM, 2016. Nrf2 protects mitochondrial decay by oxidative stress. FASEB J 30, 66–80. [PubMed: 26340923]

Sun JK, Keenan HA, Cavallerano JD, Asztalos BF, Schaefer EJ, Sell DR, Strauch CM, Monnier VM, Doria A, Aiello LP, King GL, 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, 968–974. [PubMed: 21447665]

Szegedy CV, Ioffe Vincent, Sergey Shlens Jon, 2016. Rethinking the Inception Architecture for Computer Vision, IEEE Conf Comput Vis Pattern Recognit, pp. 2818–2826.

Taheri S, Zaghloul H, Chagoury O, Elhadad S, Ahmed SH, El Khatib N, Amona RA, El Nahas K, Suleiman N, Alnaama A, Al-Hamaq A, Charlson M, Wells MT, Al-Abdulla S, Abou-Samra AB, 2020. Effect of intensive lifestyle intervention on bodyweight and glycaemia in early type 2 diabetes (DIADEM-I): an open-label, parallel-group, randomised controlled trial. Lancet Diabetes Endocrinol 8, 477–489. [PubMed: 32445735]

Tang L, Xu GT, Zhang JF, 2023. Inflammation in diabetic retinopathy: possible roles in pathogenesis and potential implications for therapy. Neural Regen Res 18, 976–982. [PubMed: 36254977]

Teo ZL, Tham YC, Yu M, Chee ML, Rim TH, Cheung N, Bikbov MM, Wang YX, Tang Y, Lu Y, Wong IY, Ting DSW, Tan GSW, Jonas JB, Sabanayagam C, Wong TY, Cheng CY, 2021. Global Prevalence of Diabetic Retinopathy and Projection of Burden through 2045: Systematic Review and Meta-analysis. Ophthalmology 128, 1580–1591. [PubMed: 33940045]

Tichauer KM, Guthrie M, Hones L, Sinha L, St Lawrence K, Kang-Mieler JJ, 2015. Quantitative retinal blood flow mapping from fluorescein videoangiography using tracer kinetic modeling. Opt Lett 40, 2169–2172. [PubMed: 26393691]

Ting DSW, Cheung CY, Lim G, Tan GSW, Quang ND, Gan A, Hamzah H, Garcia-Franco R, San Yeo IY, Lee SY, Wong EYM, Sabanayagam C, Baskaran M, Ibrahim F, Tan NC, Finkelstein EA, Lamoureux EL, Wong IY, Bressler NM, Sivaprasad S, Varma R, Jonas JB, He MG, Cheng CY, Cheung GCM, Aung T, Hsu W, Lee ML, Wong TY, 2017. Development and Validation of a Deep Learning System for Diabetic Retinopathy and Related Eye Diseases Using Retinal Images From Multiethnic Populations With Diabetes. JAMA 318, 2211–2223. [PubMed: 29234807]

Trefts E, Shaw RJ, 2021. AMPK: restoring metabolic homeostasis over space and time. Mol Cell 81, 3677–3690. [PubMed: 34547233]

Trudeau K, Molina AJ, Guo W, Roy S, 2010. High glucose disrupts mitochondrial morphology in retinal endothelial cells: implications for diabetic retinopathy. Am J Pathol 177, 447–455. [PubMed: 20522647]

Trudeau K, Muto T, Roy S, 2012. Downregulation of mitochondrial connexin 43 by high glucose triggers mitochondrial shape change and cytochrome C release in retinal endothelial cells. Invest Ophthalmol Vis Sci 53, 6675–6681. [PubMed: 22915032]

Turtinen M, Harkonen T, Parkkola A, Ilonen J, Knip M, Finnish Pediatric Diabetes, R., 2019. Characteristics of familial type 1 diabetes: effects of the relationship to the affected family member on phenotype and genotype at diagnosis. Diabetologia 62, 2025–2039. [PubMed: 31346657]

Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G, Stiles L, Haigh SE, Katz S, Las G, Alroy J, Wu M, Py BF, Yuan J, Deeney JT, Corkey BE, Shirihai OS, 2008. Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. EMBO J 27, 433–446. [PubMed: 18200046]

Ungvari Z, Tarantini S, Kiss T, Wren JD, Giles CB, Griffin CT, Murfee WL, Pacher P, Csiszar A, 2018. Endothelial dysfunction and angiogenesis impairment in the ageing vasculature. Nat Rev Cardiol 15, 555–565. [PubMed: 29795441]

Van Huynh T, Rethi L, Rethi L, Chen CH, Chen YJ, Kao YH, 2023. The Complex Interplay between Imbalanced Mitochondrial Dynamics and Metabolic Disorders in Type 2 Diabetes. Cells 12.

Vlasits AL, Euler T, Franke K, 2019. Function first: classifying cell types and circuits of the retina. Curr Opin Neurobiol 56, 8–15. [PubMed: 30447507]

Vujosevic S, Aldington SJ, Silva P, Hernandez C, Scanlon P, Peto T, Simo R, 2020. Screening for diabetic retinopathy: new perspectives and challenges. Lancet Diabetes Endocrinol 8, 337–347. [PubMed: 32113513]

Wang W, Zhao H, Chen B, 2020. DJ-1 protects retinal pericytes against high glucose-induced oxidative stress through the Nrf2 signaling pathway. Sci Rep 10, 2477. [PubMed: 32051471]

Wong TY, Cheung CM, Larsen M, Sharma S, Simo R, 2016. Diabetic retinopathy. Nat Rev Dis Primers 2, 16012. [PubMed: 27159554]

Wong TY, Sun J, Kawasaki R, Ruamviboonsuk P, Gupta N, Lansingh VC, Maia M, Mathenge W, Moreker S, Muqit MMK, Resnikoff S, Verdaguer J, Zhao P, Ferris F, Aiello LP, Taylor HR, 2018. Guidelines on Diabetic Eye Care: The International Council of Ophthalmology Recommendations for Screening, Follow-up, Referral, and Treatment Based on Resource Settings. Ophthalmology 125, 1608–1622. [PubMed: 29776671]

Wu H, Li G, Chen W, Luo W, Yang Z, You Z, Zou Y, 2022. Drp1 knockdown represses apoptosis of rat retinal endothelial cells by inhibiting mitophagy. Acta Histochem 124, 151837. [PubMed: 34959219]

Wu Y, Zou H, 2022. Research Progress on Mitochondrial Dysfunction in Diabetic Retinopathy. Antioxidants (Basel) 11.

Wykoff CC, 2017. Impact of intravitreal pharmacotherapies including antivascular endothelial growth factor and corticosteroid agents on diabetic retinopathy. Curr Opin Ophthalmol 28, 213–218. [PubMed: 28376510]

Wykoff CC, Elman MJ, Regillo CD, Ding B, Lu N, Stoilov I, 2016. Predictors of Diabetic Macular Edema Treatment Frequency with Ranibizumab During the Open-Label Extension of the RIDE and RISE Trials. Ophthalmology 123, 1716–1721. [PubMed: 27208982]

Xie J, Cui Y, Chen X, Yu H, Chen J, Huang T, He M, Zhang L, Meng Q, 2021. VDAC1 regulates mitophagy in NLRP3 inflammasome activation in retinal capillary endothelial cells under high-glucose conditions. Exp Eye Res 209, 108640. [PubMed: 34058229]

Xu L, Humphries F, Delagic N, Wang B, Holland A, Edgar KS, Hombrebueno JR, Stolz DB, Oleszycka E, Rodgers AM, Glezeva N, McDonald K, Watson CJ, Ledwidge MT, Ingram RJ, Grieve DJ, Moynagh PN, 2021. ECSIT is a critical limiting factor for cardiac function. JCI Insight 6.

Yatoh S, Mizutani M, Yokoo T, Kozawa T, Sone H, Toyoshima H, Suzuki S, Shimano H, Kawakami Y, Okuda Y, Yamada N, 2006. Antioxidants and an inhibitor of advanced glycation ameliorate death of retinal microvascular cells in diabetic retinopathy. Diabetes Metab Res Rev 22, 38–45. [PubMed: 15892182]

Yau JW, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, Chen SJ, Dekker JM, Fletcher A, Grauslund J, Haffner S, Hamman RF, Ikram MK, Kayama T, Klein BE, Klein R, Krishnaiah S, Mayurasakorn K, O'Hare JP, Orchard TJ, Porta M, Rema M, Roy MS, Sharma T, Shaw J, Taylor H, Tielsch JM, Varma R, Wang JJ, Wang N, West S, Xu L, Yasuda M, Zhang X, Mitchell P, Wong TY, Meta-Analysis for Eye Disease Study, G., 2012. Global prevalence and major risk factors of diabetic retinopathy. Diabetes Care 35, 556–564. [PubMed: 22301125]

Yu MG, Gordin D, Fu J, Park K, Li Q, King GL, 2024. Protective Factors and the Pathogenesis of Complications in Diabetes. Endocr Rev 45, 227–252. [PubMed: 37638875]

Zacharioudakis E, Agianian B, Kumar Mv V, Biris N, Garner TP, Rabinovich-Nikitin I, Ouchida AT, Margulets V, Nordstrom LU, Riley JS, Dolgalev I, Chen Y, Wittig AJH, Pekson R, Mathew C, Wei P, Tsirigos A, Tait SWG, Kirshenbaum LA, Kitsis RN, Gavathiotis E, 2022. Modulating mitofusins to control mitochondrial function and signaling. Nat Commun 13, 3775. [PubMed: 35798717]

Zarse K, Schmeisser S, Groth M, Priebe S, Beuster G, Kuhlow D, Guthke R, Platzer M, Kahn CR, Ristow M, 2012. Impaired insulin/IGF1 signaling extends life span by promoting mitochondrial L-proline catabolism to induce a transient ROS signal. Cell Metab 15, 451–465. [PubMed: 22482728]

Zhang MY, Zhu L, Bao X, Xie TH, Cai J, Zou J, Wang W, Gu S, Li Y, Li HY, Yao Y, Wei TT, 2022. Inhibition of Drp1 ameliorates diabetic retinopathy by regulating mitochondrial homeostasis. Exp Eye Res 220, 109095. [PubMed: 35490835]

Zhang Z, Apse K, Pang J, Stanton RC, 2000. High glucose inhibits glucose-6-phosphate dehydrogenase via cAMP in aortic endothelial cells. J Biol Chem 275, 40042–40047. [PubMed: 11007790]

Zhang Z, Liew CW, Handy DE, Zhang Y, Leopold JA, Hu J, Guo L, Kulkarni RN, Loscalzo J, Stanton RC, 2010. High glucose inhibits glucose-6-phosphate dehydrogenase, leading to increased oxidative stress and beta-cell apoptosis. FASEB J 24, 1497–1505. [PubMed: 20032314]

Zheng L, Kern TS, 2009. Role of nitric oxide, superoxide, peroxynitrite and PARP in diabetic retinopathy. Front Biosci (Landmark Ed) 14, 3974–3987. [PubMed: 19273327]

Zhong Q, Kowluru RA, 2011. Epigenetic changes in mitochondrial superoxide dismutase in the retina and the development of diabetic retinopathy. Diabetes 60, 1304–1313. [PubMed: 21357467]

Zhou P, Xie W, Meng X, Zhai Y, Dong X, Zhang X, Sun G, Sun X, 2019. Notoginsenoside R1 Ameliorates Diabetic Retinopathy through PINK1-Dependent Activation of Mitophagy. Cells 8.

Zorov DB, Juhaszova M, Sollott SJ, 2014. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. Physiol Rev 94, 909–950. [PubMed: 24987008]

Zoungas S, Arima H, Gerstein HC, Holman RR, Woodward M, Reaven P, Hayward RA, Craven T, Coleman RL, Chalmers J, Collaborators on Trials of Lowering Glucose, g., 2017. Effects of intensive glucose control on microvascular outcomes in patients with type 2 diabetes: a meta-analysis of individual participant data from randomised controlled trials. Lancet Diabetes Endocrinol 5, 431–437. [PubMed: 28365411]

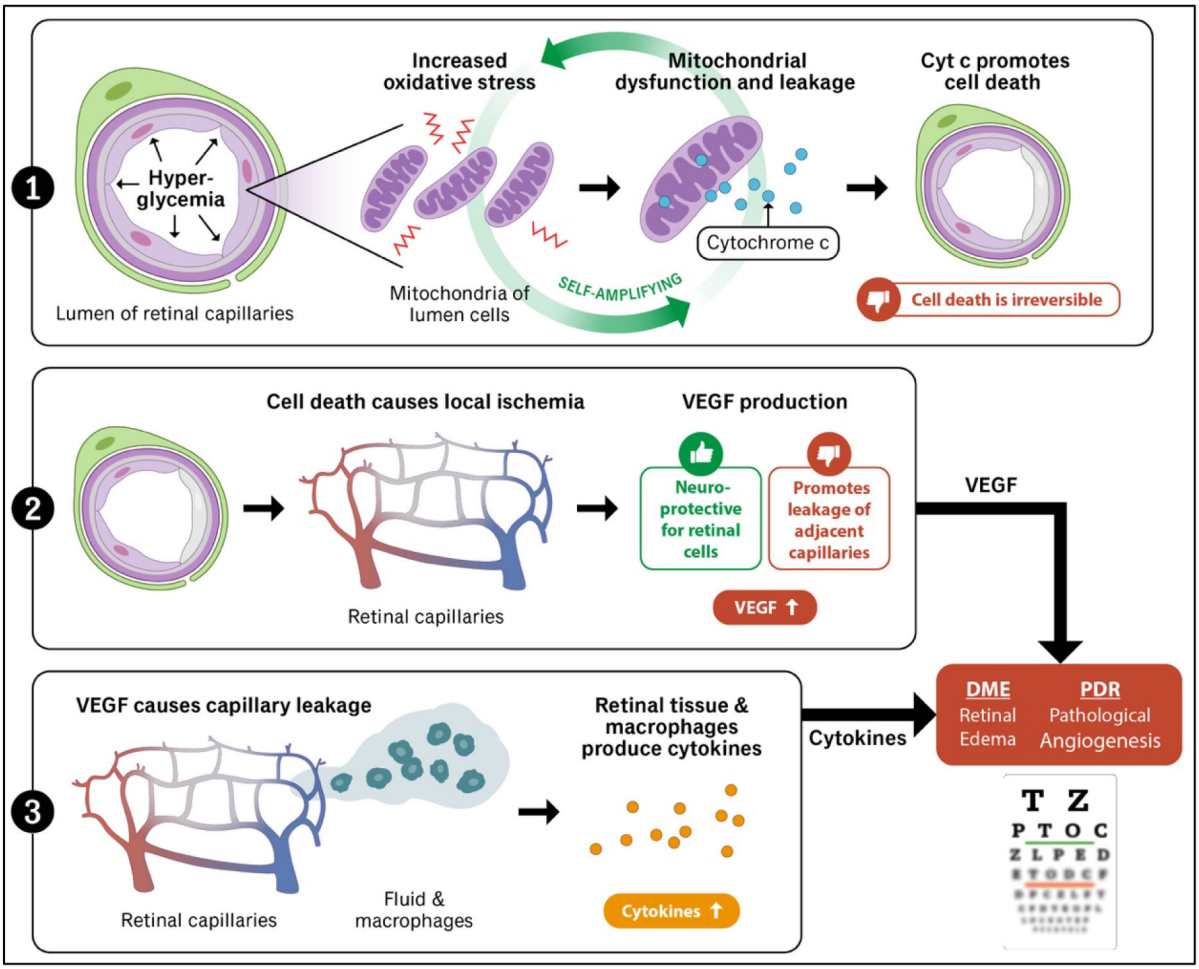

Figure 1. The role of HG-induced mitochondrial oxidative stress and subsequent damage of the retina.

Panel 1; focus on cells. Exposure of endothelial cells to hyperglycemia promotes oxidative stress within the cytoplasm and mitochondria. The ensuing mitochondrial dysfunction allows leakage of cytochrome c from the mitochondria into the cytoplasm, and thereby promotes death of the endothelium.

Panel 2; focus on capillaries. Death of cells within capillaries results in ischemia of the surrounding tissue, which responds by producing VEGF. VEGF is both neuroprotective (Foxton et al., 2013) (beneficial for the neural cells within the retina) but also promotes leakage of the adjacent capillaries that have not died. Accumulation of acellular capillaries and the resulting hypoxia is likely to drive progression to sight-threatening forms of DR.

Panel 3; focus on tissue and symptoms. Leakage of fluid from the capillaries into the neural retina, as well as influx of macrophages from the circulation into the retinal tissue triggers cytokine production (by both the retinal tissue and macrophages that have entered into the tissue and become activated). The increased level of VEGF and cytokines, drive progression to sight-threatening forms of DR, namely diabetic macular edema (DME) and proliferative diabetic retinopathy (PDR).

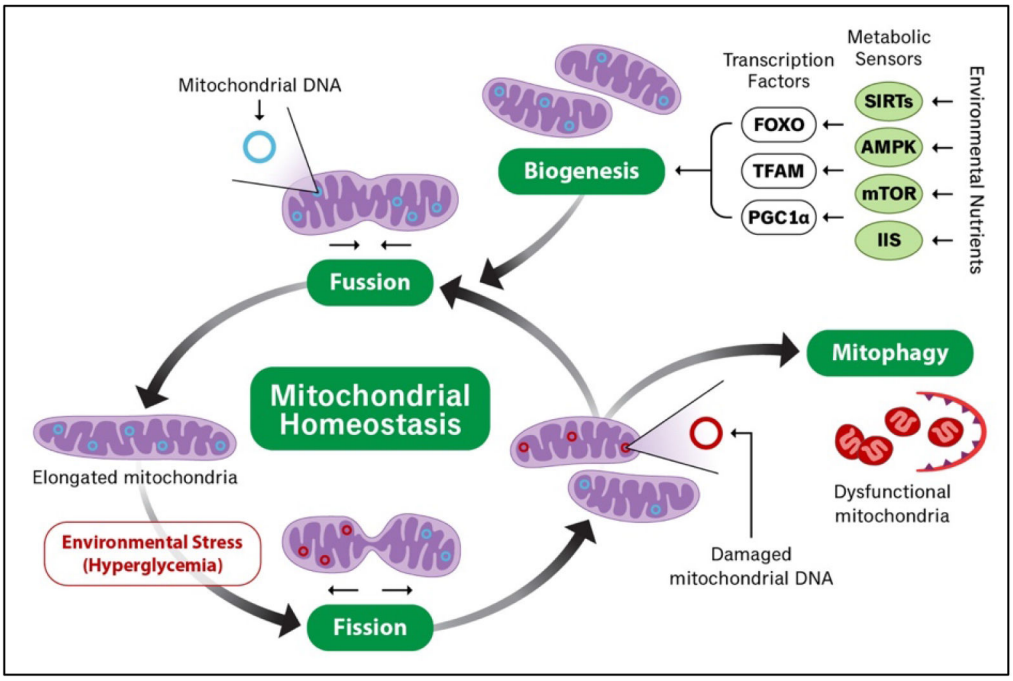

Figure 2: Essential elements of mitochondrial homeostasis.

Changes in nutrient demands are detected by metabolic sensors that regulate biogenesis of mitochondrial. New mitochondria fuse and thereby integrate into the existing mitochondrial network. Environmental stress such as chronic hyperglycemia elevate oxidative stress, which damages the mitochondrial DNA and thereby further increases oxidative stress and compromises the functionality of the mitochondria. These damaged regions of mitochondria are detected, sequestered, physically removed by the process of fission and then eliminated via the lysosome in a process called mitophagy. Mitochondrial dynamics (fusion and fission), biogenesis and mitophagy must respond harmoniously to changes in the level and nature of environmental nutrients. SIRTs: sirtuins; AMPK: AMP-dependent protein kinase; mTOR: mammalian target of rapamycin (a ser/thr protein tyrosine kinase); IIS: insulin/IGF (insulin-like growth factor) signaling pathway.

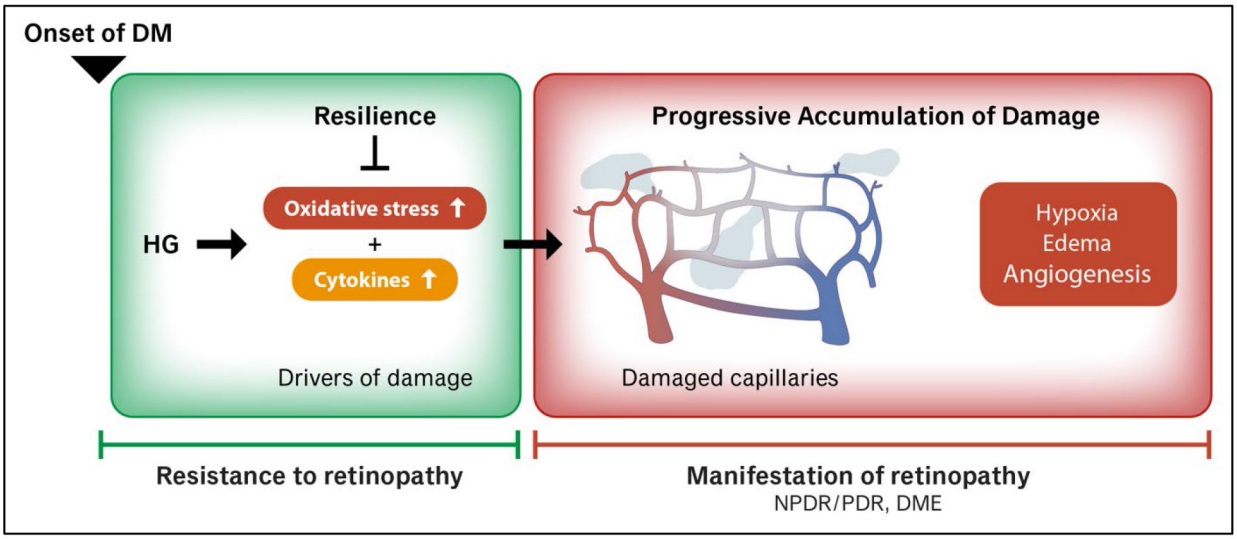

Figure 3. The two phases that the retina undergoes following the onset of DM.

In both humans and experimental animals, a retinopathy-resistant period precedes the appearance of DR. Recent discoveries in both T1D, and T2D mice indicate that this retinopathy-resistant period is associated with enhanced tolerance of insults that cause damage to the retina (Li et al., 2023). Loss of resilience is a prerequisite for accumulation and progression of overt damage to the retina, which is clinically recognized as DR.

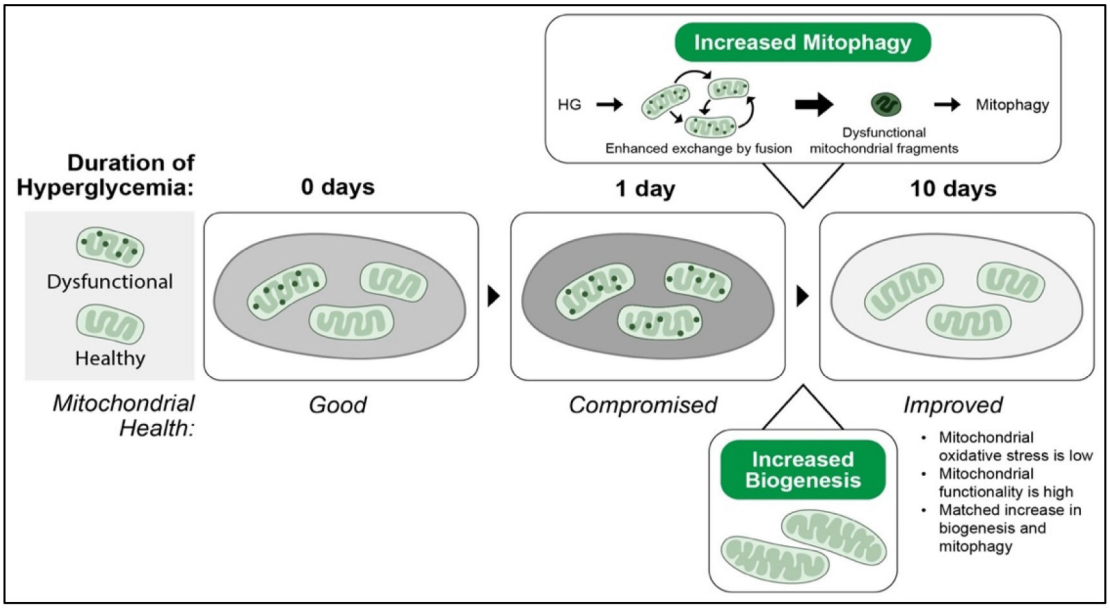

Figure 4. Hyperglycemia-induced mitochondrial adaptation (HIMA).

Hyperglycemia at first compromises the health of the mitochondria, which is followed by a series of changes that enable the mitochondrial health to improve despite the continued presence of hyperglycemia. These changes include a balanced increase of biogenesis and mitophagy, which prevents mitochondrial stress and improves the functionality of the mitochondria.

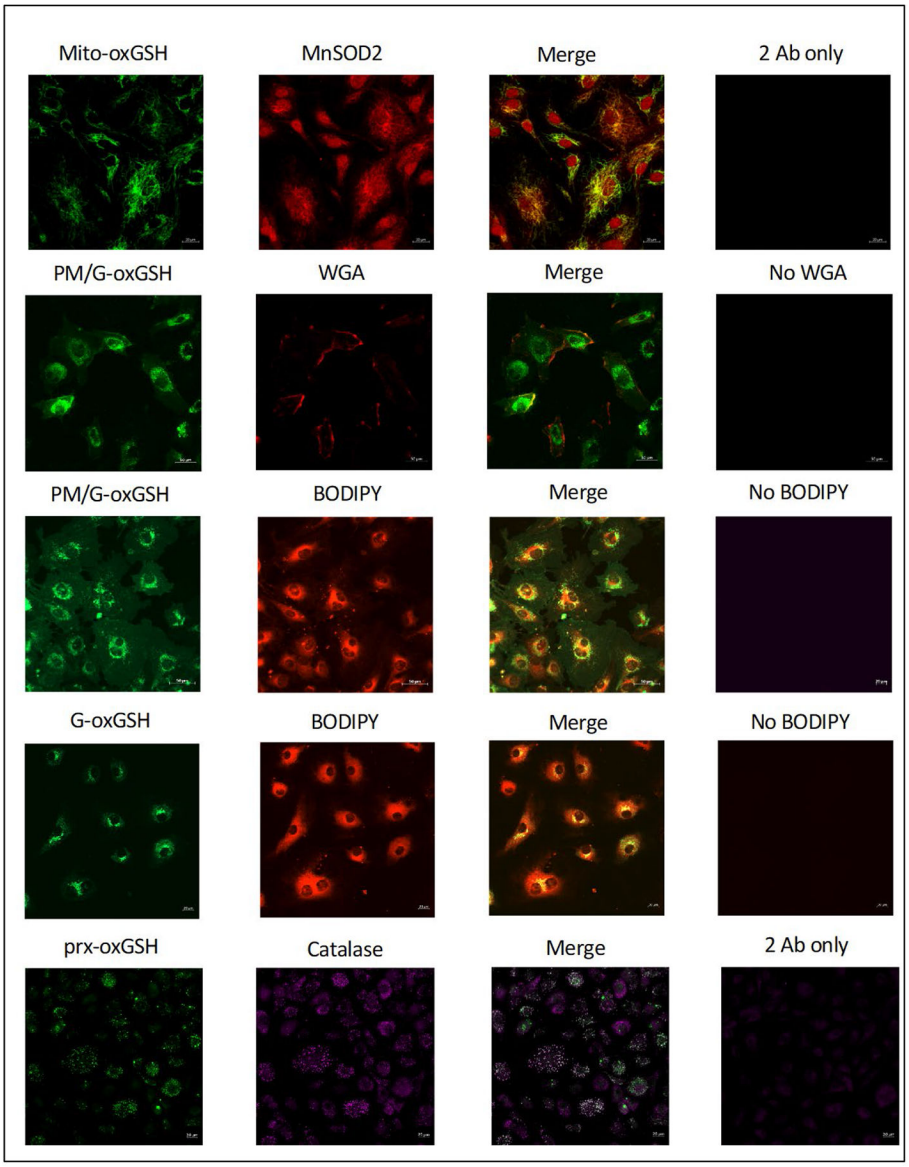

Figure 5: The subcellular location of roGFP sensors.

Representative images of primary human retinal endothelial cells (HRECs) expressing the indicated roGFP sensors. Fixed cells expressing mito-oxGSH or prx-oxGSH colocalized with antibodies recognizing mitochondrial MnSOD2 or peroxisomal catalase, respectively. Living (not fixed) HRECs expressing the CAAX-tagged roGFP sensor (PM/G) colocalized with the plasma membrane (WGA1) and Golgi (BODIPY) markers. The Golgi-targeted sensor (G-oxGSH) colocalized with the Golgi (BODIPY) marker. The subcellular location of the H2O2-selective sensors was indistinguishable from the location of their oxGSH counterparts shown in this figure (data not shown). Scale bar, 20 um.

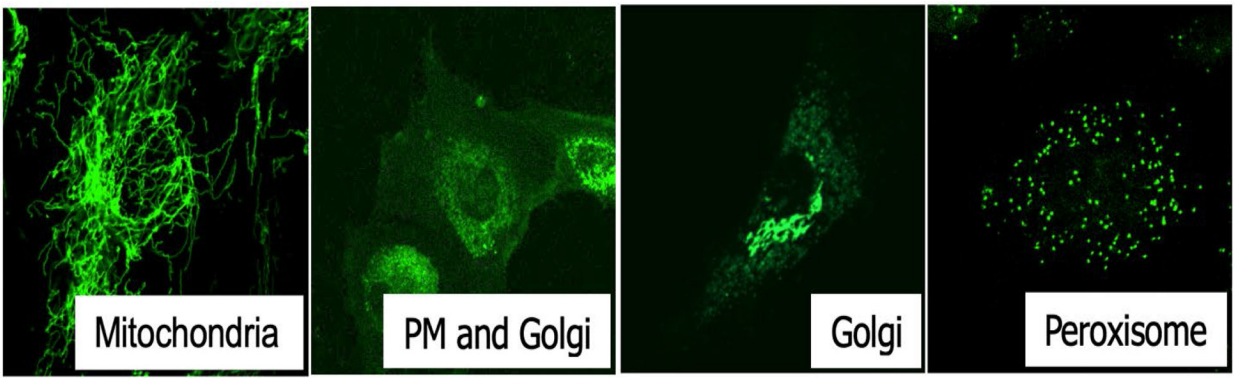

Figure 6: High magnification of cells expressing roGFP sensors targeted to the indicated subcellular location.

Representative images of roGFP-expressing HRECs described in Figure 5.

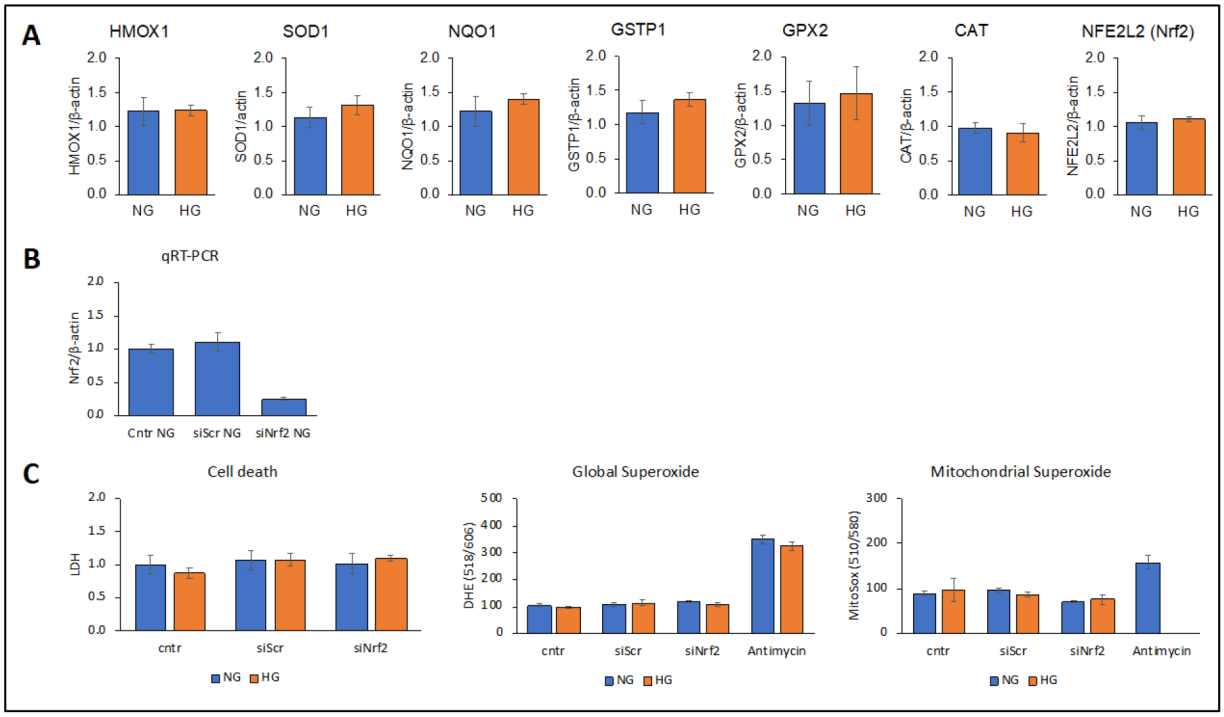

Figure 7: Protection from HG-induced oxidative stress was independent of NRF2.

(A) The mRNA level of HMOX1, SOD1, NQO1, GSTP1, GPX2, CAT and NRF2 in primary human retinal endothelial cells (HRECs) that were cultured for 10 days in either NG- (5 mM) or HG- (30 mM) containing medium. The expression of each mRNA was measured using qRT-PCR and normalized to β-actin. Three independent experiments showed similar results.

(B) The mRNA level of NRF2 in HRECs transfected with 10 nM scrambled and NRF2-targeted siRNA. The control group (Cntr) are non-transfected cells.

(C) NG and HG-HRECs were transfected with 10 nM scrambled or NRF2-targeted siRNA. Cell death was measured using an LDH assay as previously described (Serikbaeva et al., 2022). Oxidative stress was measured by staining with DHE and Mitosox for global and mitochondrial superoxide, respectively. The response of cell to antimycin A was included as a positive control. Statistical significance for all panels was determined using student t-test, *$p<0.05$.

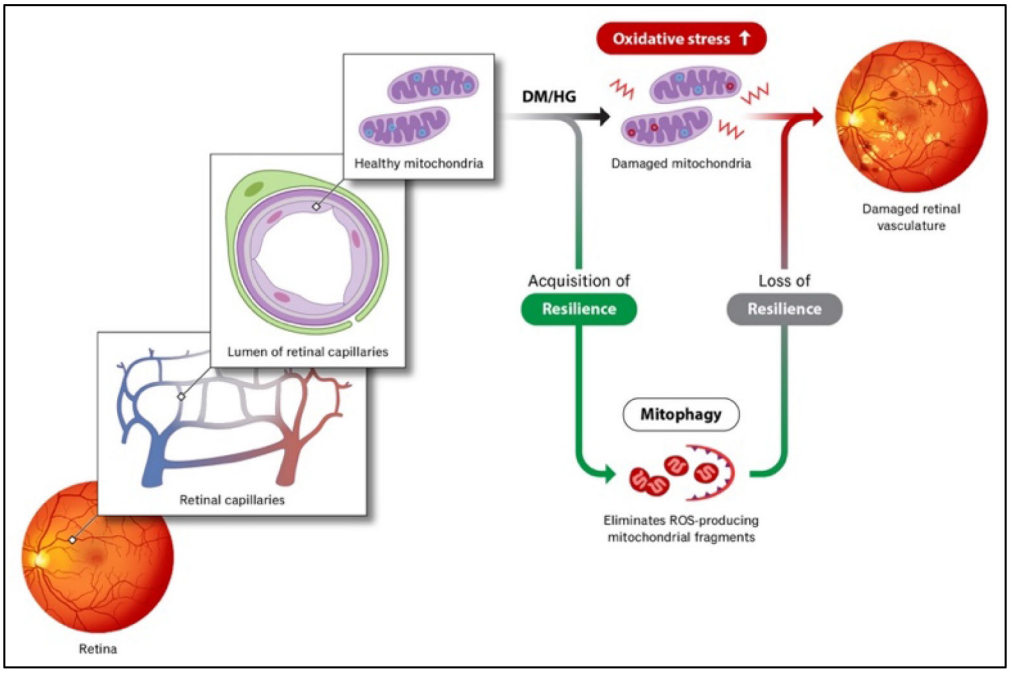

Figure 8. Resilience involves increased clearance of dysfunctional mitochondrial (mitophagy) within the retinal vasculature in the face of DM/HG.

DM/HG increases oxidative stress within the mitochondria and thereby initiates self-amplifying damage leading to dysfunction and subsequent death of cells within retinal capillaries (Kang and Yang, 2020; Kowluru, 2019). Resilience counters these events by increasing mitophagy to eliminate the dysfunctional, ROS-producing mitochondria (Li et al., 2023; Serikbaeva et al., 2022). Loss of resilience, perhaps due to compromised mitochondrial homeostasis, results in accumulation of damage to the retinal vasculature, which is diagnostic of DR.

Table 1

Diagnosis of DR

DR classification	Clinical findings*
No apparent DR	No DR-associated abnormalities
NPDR	
Mild NPDR	Microaneurysms only
Moderate NPDR	Microaneurysms and any of the following: microaneurysms, retinal dot and blot hemorrhages, hard exudates, or cotton wool spots; no signs of severe non-proliferative DR
Severe NPDR	Any of the following: intra-retinal hemorrhages (≥20 in each of 4 quadrants), definite venous beading (in 2 quadrants) or intra-retinal microvascular abnormalities (in 1 quadrant); no signs of proliferative DR
PDR	One or more of the following: neovascularization, vitreous or pre-retinal hemorrhages
Diabetic macular edema	
No DME	No retinal thickening or hard exudates in the macula
None center-involving DME	Retinal thickening in the macula that does not involve the central subfield zone that is 1 mm in diameter
Center-involving DME	Retinal thickening in the macula that does involve the central subfield zone that is 1 mm in diameter

*Findings are based on dilated ophthalmoscopy for DR

Table 2:

Pros and cons of AI-based diagnosis of DR

Pros	Cons
Higher accuracy, consistent disease diagnosis	Less explainability in details
Increase disease detection efficiency	Vulnerable to adversarial attacks from crafted input data
Reduce unnecessary doctor visits	Susceptible to Out-of-Distribution robustness problems
	Concerns of privacy and security

Table 3:
Hallmarks of Health (Lopez-Otin and Kroemer, 2021)

Categories	***Maintenance of homeostasis***	***Spatial compartmentalization***	***Responses to stress***
Hallmarks	Recycling and turnover	Integrity of barriers	Repair and regeneration
	Integration of circuitries	Containment of perturbations	Hormetic regulation
	Rhythmic oscillations		Homeostatic resilience

Table 4:

Grx1- (oxGSH) and Orp1-roGFP2 (H_2O_2) targeted to various subcellular compartments

Name	Targeting approach	Subcellular location
Mito-H_2O_2 Mito-oxGSH	Addition of a fragment from cytochrome oxidase subunit IV	Mitochondria
PM-H_2O_2 PM-oxGSH	Addition of a palmitoylation sequence	Cytoplasmic side of the plasma membrane and the Golgi*
G-oxGSH	Addition of a fragment from giantin	Cytoplasmic side of the Golgi
PRX-oxGSH	Addition of a fragment from peroxisomal membrane protein 2 (PXMP2)	Cytoplasmic side of the peroxisome

*When expressed in Hela cells the PM-roGFP sensors are localized primarily in the plasma membrane (Hatori et al., 2018). We observed that the PM-roGFP sensors were present in both the plasma membrane and Golgi of primary human retinal endothelial cells (Figures 5 and 6).

Table 5:
Summary of the effect of HG on the basal redox status of HRECs

Compartment and reactive oxygen species	Effect of exposure to HG for at least 10 days
Dyes *	
Global Superoxide (DHE)	Unchanged
Global H2O2 (DCF-DA)	Unchanged
Mito; Superoxide (MitoSox)	Unchanged
roGFP sensors	
Mito-H2O2	Unchanged
Mito-oxGSH	Unchanged
G-H2O2	Elevated
G-oxGSH	Unchanged
Deduced PM-oxGSH **	Elevated
PRX-oxGSH	Unchanged

"Unchanged" indicates that there was no statistically significant difference between cells cultured in medium containing NG (5 mM) and HG (30 mM). "Elevated" indicates that a statistically significant difference was observed. The results presented in this Table are a compilation of at least 3 independent experiments.

* Superoxide was assessed using dihydroethidium (DHE). Unmodified DHE was used to detect global superoxide, whereas mitochondrial superoxide was detected with DHE that was modified to target it to the mitochondria (mitoSox).

The prefixes of the roGFP sensors indicate the subcellular compartment to which the roGFP was localized; mito, PM/G, G and prx: mitochondria, plasma membrane/Golgi, Golgi and peroxisome, respectively.

** By comparing the data obtained with the sensors targeted to the PM/G and G we deduced the status in the PM compartment.

Table 6:

Sequence of primers used in qRT-PCR.

Name of the gene, sequence of forward and reverse primer

Sequence Name (Nrf2-targeting enzyme)	Sequence
HMOX1 FWD	5'-TTC TCC GAT GGG TCC TTA CA-3'
HMOX1 REV	5'-CTT CCA CCG GAC AAA GTT CA-3'
NQO1 FWD	5'-GGG ATG AGA CAC CAC TGT ATT T-3'
NQO1 REV	5'-TCT CCT CAT CCT GTA CCT CTT T-3'
CAT FWD	5'-CTG GAG CAC AGC ATC CAA TA-3'
CAT REV	5'-TCA TTC AGC ACG TTC ACA TAG A-3'
SOD1 FWD	5'-GTG CAG GGC ATC ATC AAT TTC-3'
SOD1 REV	5'-GGC CTT CAG TCA GTC CTT TAA T-3'
GPX2 FWD	5'-CCT ACC CTT ATG ATG ACC CAT TT-3'
GPX2 REV	5'-TCA AAG TTC CAG GCC ACA TC-3'
GSTP1 FWD	5'-GGG CAA GGA TGA CTA TGT GAA G-3'
GSTP1 REV	5'-GAT CTG GTC TCC CAC AAT GAA G-3'