

Special Issue: Mitochondria - From Diagnosis to Treatment

Review

Mitochondrial Defects Drive Degenerative Retinal Diseases

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Mitochondrial dysfunction is involved in the pathology of two major blinding retinal diseases, diabetic retinopathy (DR) and age-related macular degeneration (AMD). These diseases accumulate mitochondrial defects in distinct retinal subcellular structures, the vascular/neural network in DR and the retinal pigment epithelium (RPE) in AMD. These mitochondrial defects cause a metabolic crisis that drives disease. With no treatments to stop these diseases, coupled with an increasing population suffering from AMD and DR, there is an urgent need to develop new therapeutics targeting the mitochondria to prevent or reverse disease-specific pathology.

Cell-Specific Mitochondrial Defects Cause Distinct Diseases

DR and AMD, covering the age spectrum from working-age adults (DR) through the elderly (AMD), are responsible for the majority of patients with vision loss in the industrialized world [1,2]. The global prevalence of DR was approximately 126.6 million in 2010 and this number is expected to escalate to 191 million by 2030 [1]. The global estimate for the number of people with AMD in 2020 is 196 million and will increase to 288 million by 2040 [2]. These blinding diseases affect different retinal cells and manifest vision changes that are distinct from each other.

Persistent, fluctuating hyperglycemia is the major cause of morbidity and mortality in diabetes, with the deleterious effects categorized into macrovascular (cardiovascular, cerebrovascular, and peripheral artery diseases) and microvascular (retinopathy, nephropathy, and neuropathy) complications [3]. DR is the most common microvascular complication of diabetes. The earliest clinical signs of DR (non-proliferative) are mainly microaneurysms and intraretinal hemorrhages, which can lead to fluid accumulation in the macula (see [Glossary](#)) [3]. Macular swelling that leads to blurred vision is known as diabetic macular edema (DME) and is a complication of DR. In late-stage DR (proliferative), the number and size of hemorrhages increase and cause disruptions in the visual field ([Figure 1](#)). Hypoxia in the capillaries leads to neovascularization that can cause permanent vision loss without prompt treatment with either laser surgery or ocular injections of **vascular endothelial growth factor (VEGF)**.

AMD includes two forms of the disease. The less common 'wet' AMD involves the abnormal growth of blood vessels into the retina from the choroid, the outer retina blood supply ([Figure 1](#)) [4]. Leakage of vascular fluid causes rapid vision loss that can be reversed by anti-VEGF ocular injections. Approximately 80% of AMD patients have the dry form of the disease, for which there are no effective therapies and which is the focus of this review. In contrast to DR, the primary defect in dry AMD occurs in the RPE ([Figure 1](#)). The RPE supports the health and function of the retina; therefore, RPE dysfunction precipitates the death of **photoreceptors (PRs)**, the light-sensing neurons of the retina. Vision changes associated with AMD include distortion of vertical and horizontal lines and a scotoma, or black spot, in the area of central vision. The loss of central vision is due to the death of RPE and PRs primarily in the macula, which is a small, centrally located region of the retina responsible for high-resolution and color vision.

Vision loss due to either DR or AMD has a significant negative impact on activities of daily living, including reading, driving, and recognizing faces. Thus, there is an urgent need to develop new therapies that can prevent or reverse cellular changes that cause disease-specific pathology. Recent evidence points to mitochondrial dysfunction in the microvasculature and retina in DR and the RPE in AMD as newly emerging hypotheses for their pathologies and offers potential novel targets for intervention. This review discusses the use of model systems to study DR and AMD as well as the recent findings that support the hypothesis that mitochondrial defects drive both diseases. Finally, current

Highlights

In diabetic retinopathy (DR), cytosolic reactive oxygen species (ROS) accumulation in retinal vasculature occurs before mitochondrial damage and mitochondrial damage precedes capillary cell loss.

In DR, mitochondrial fission is increased and mitochondrial DNA has increased damage and lower copy numbers and is hypermethylated.

The retina and retinal pigment epithelium (RPE) are codependent tissues that exchange the energy substrates required for their survival.

RPE mitochondrial damage occurs early in age-related macular degeneration (AMD) and leads to a bioenergetic crisis due to an imbalance in available energy substrates.

Therapeutic strategies targeted towards maintaining mitochondrial homeostasis (structural, functional, and genomic) have the potential to prevent the development and progression of both DR and AMD.

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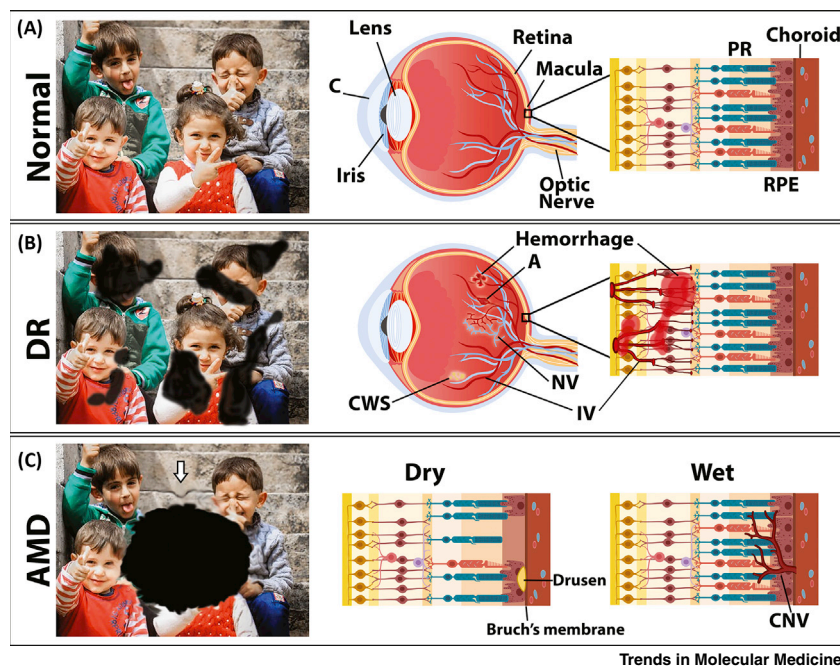


Figure 1. Disease-Dependent Changes in Vision and Retinal Anatomy.

Examples of vision (left panels) in patients with normal vision (A), with diabetic retinopathy (DR) (B), and with age-related macular degeneration (AMD) (C). With normal vision an individual can easily recognize faces. DR causes visual blurring and black patches throughout the visual field. Vision loss with AMD includes distortion of horizontal and vertical lines (arrow) and a black spot (scotoma) in the central vision. Schematic of the healthy eye (A) indicates structures associated with vision. Light passes through the cornea (C) and iris and is focused by the lens on the back of the eye at the macula, a small, central retinal region responsible for high-acuity central vision. Light is sensed by photoreceptors (PRs) in the retina and the signal is sent through the secondary neurons (bipolar, horizontal, and ganglion cells) to the optic nerve. Clinical symptoms of DR (B) include hemorrhages, aneurysms (A), neovascularization (NV), and 'cotton-wool' spots (CWS). Hemorrhaging from the inner retinal vessels (IV) of the neural retina is depicted. AMD exists in a 'dry' and a 'wet' form (C). The dry form is characterized by the presence of drusen – deposits of lipids and proteins that form between the retinal pigment epithelium (RPE) and Bruch's membrane. Loss of the RPE and PRs occurs with advanced dry AMD. In wet AMD, choroidal neovascularization (CNV), which is the abnormal growth of blood vessels from the choroid into the retina, results in leakage of fluid that can cause rapid vision loss.

and emerging strategies to treat the diseases will provide insight into future directions for improved therapies.

Model Systems to Study Degenerative Retinal Diseases

In humans, DR is a slow, progressive disease with characteristics that can be partially replicated in animal models and cultured cells. Development of DR in animal models is accomplished through genetic mutation or induction via laser or chemical damage, surgery, diet, or drugs [5]. These different models in a range of species, including mice, rats, cats, dogs, pigs, and non-human primates, exhibit many retinal changes that mimic the human disease. However, the replication of specific aspects of DR pathology is dependent on the model system ([6], Table 1). DR pathology mainly includes damage to the microvasculature but can also effect retinal neurons, as evidenced by changes in the electroretinogram, visual acuity, or color sensitivity and damage to PRs [6–8]. Culture models of DR include the use of primary retinal endothelial cells and pericytes, RPE, Müller cells, or ganglion cells cultured in high glucose. This review focuses on findings from a variety of tissues and cells, including human and rodent models. Most studies of DR discussed herein utilize streptozotocin-induced diabetic rats or mice or isolated retinal cells in culture. A caveat of these model systems is their applicability

Glossary

Cristae: folds of the IMM that increase the surface area, allowing efficient packing of the ETC and the isolated generation of proton gradients to fuel ATP generation.
Displacement loop (D-loop): a noncoding region of mtDNA and the initiation site of mtDNA replication.

Electron transport chain (ETC): comprises multisubunit complexes contained within the IMM. Complexes I, II, III, and IV consume NADH and FADH₂ to generate a proton gradient. This proton gradient is utilized by complex V to produce ATP.

Macula: a small, pea-sized region adjacent to the optic nerve where light is focused; responsible for high-acuity central vision.

Nicotinamide adenine dinucleotide (phosphate) [NAD(P)H] oxidase 2 (Nox2): a cytosolic, membrane-bound enzyme that generates ROS.

Oxidative phosphorylation (Ox-Phos): process of producing ATP via the mitochondrial ETC, involving the transfer of electrons from NADH or FADH₂ to oxygen.
Peroxisome proliferator-activated receptor gamma co-activator 1 alpha (PGC-1 α): regulates genes involved in energy metabolism. Importantly, this transcriptional coactivator is known as the master regulator of mitochondrial biogenesis.

Photoreceptors (PRs): the light-sensing neurons of the retina comprising two main subpopulations. Cones are responsible for color vision while rods are responsible for vision under low-light conditions.

Ras-related C3 botulinum toxin substrate 1 (Rac1): small G protein required for the assembly of the Nox2 holoenzyme.

Reactive oxygen species (ROS): include any chemically reactive species containing oxygen, including peroxide, superoxide, and hydroxyl radicals. ROS are natural byproducts of the metabolism of oxygen and act as signaling molecules within the cell. However, ROS are able to damage cell structures, including lipids, protein, and DNA.

Retina: the light-sensitive layer of the eye, comprising neurons, glia, and RPE, that converts light

Table 1. Summary of Mitochondrial Changes in DR and AMD

Disease	Change	Model system	Refs
DR	Decreased mitochondrial content	Rodents, primary retinal endothelial cells (RECs)	[19,30]
	Mitochondrial morphology (cristae and fragmentation)	Rodents, primary RECs, Müller cells	[19,74,76,77]
	Increased mtDNA damage	Rodents, human donors with DR, primary RECs	[31,44,45]
	Decreased mitochondrial function	Rodents, primary RECs, Müller cells	[8,18,76]
	Mitochondrial membrane damage, transporters and protein transport	Rodents, primary RECs	[45,74,78]
	ETC components	Rodents, primary RECs	[44,79]
	Fusion–fission, autophagy, biogenesis	Rodents, primary RECs, Müller cells	[17,19,30,33,74]
	Mitochondrial superoxide scavengers	Rodents, primary RECs	[18,19]
	Epigenetic modification of mtDNA	Rodents, human donors with DR, primary RECs	[72,73]
AMD	Decreased mitochondrial content	Human RPE tissue (hRPE-T)	[36]
	Decreased mitochondrial surface area	hRPE-T, primary human RPE cell culture (hRPE)	[36,41]
	Mitochondrial morphology(cristae)	hRPE-T, primary hRPE	[36,41]
	Increased mtDNA damage	hRPE-T	[39,47]
	Decreased mitochondrial function	Primary hRPE	[41,42]
	Mitochondrial protein trafficking and refolding, apoptosis	hRPE-T	[37]
	ETC components, cristae morphology, protein import	hRPE-T	[38]
	Metabolism, chaperones, protein homeostasis	hRPE-T	[80]
	Regulators of metabolism, oxidative stress response	Primary hRPE	[42]
	Autophagy	Primary hRPE	[41]
	Altered response to drugs	Primary hRPE	[47]

signals into electrical neural impulses.

Retinal pigment epithelium (RPE): performs multiple functions to maintain the health of the retina, including acting as the blood–retina barrier and transporting essential nutrients and oxygen from the choroid.

Vascular endothelial growth factor (VEGF): a signaling molecule that stimulates the formation of blood vessels.

to human disease. However, human donors with established DR and these model systems exhibit similar mitochondrial defects. While no model replicates all aspects of DR, the advantage of model systems is that they allow investigations into specific mechanisms of the human disease.

The unique characteristics of AMD present several challenges in selecting a model system to study this disease. AMD affects the macula, a retinal structure found only in primates. The age-dependent onset suggests that aging is an important contributing factor that is difficult to replicate in more traditional cell culture and mouse models. Animal models for AMD have been developed by manipulation of both genetic and environmental factors; however, no animal model recapitulates all disease phenotypes [9,10]. The use of human donor tissue circumvents these challenges and provides the most

accurate insight into AMD pathobiology. Thus, this review focuses on information provided by human retinal tissue and primary RPE cell cultures comparing donors with and without AMD.

Altered Metabolism Is a Shared Mechanism

Central to both diseases is the accumulation of **reactive oxygen species (ROS)**-induced damage (Figure 2). While our discussion focuses on the damaging effects of ROS overproduction on mitochondrial function, it is important to note that ROS make positive contributions; for example, in cell signaling. Overproduction and imbalance of ROS can have serious consequences for cellular signaling and other processes as discussed in Box 1.

In diabetes, saturated glycolysis, increased flux through the polyol and hexosamine pathways, impaired protein kinase C activation, increased formation of advanced glycation end products, and elevated oxidative stress are some of the biochemical changes contributing to DR pathology [11]. The accelerated production of the ROS superoxide is initiated by hyperglycemia-induced activation of **Ras-related C3 botulinum toxin substrate 1 (Rac1)** and **NADPH oxidase 2 (Nox2)** and by other metabolic abnormalities including activation of the polyol pathway [12]. Importantly, Nox2 stimulation and a cytosolic increase in ROS precedes mitochondrial damage in the retinal capillaries [13,14] (Figure 2A).

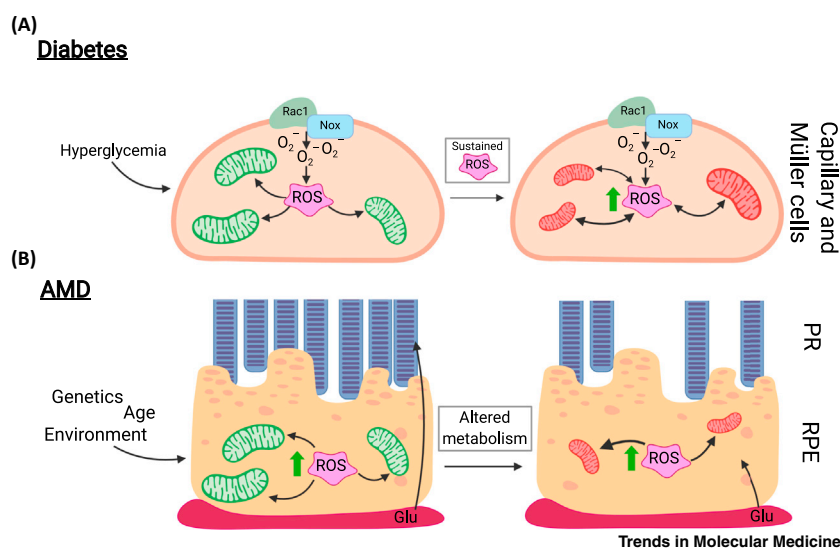


Figure 2. Metabolic Changes Drive Diabetic Retinopathy (DR) and Age-Related Macular Degeneration (AMD).

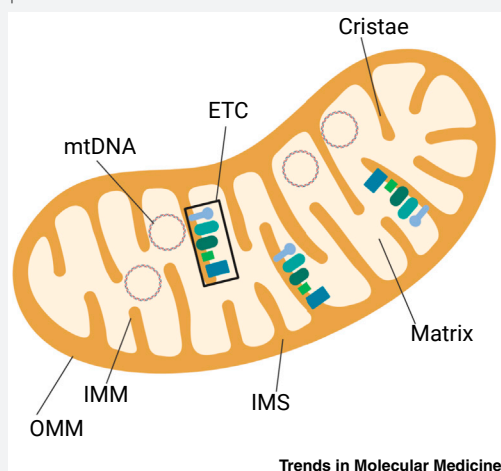
(A) Hyperglycemia is considered the major causal factor in the development of DR. The hyperglycemic milieu activates Rac1–Nox2, generating the reactive oxygen species (ROS) superoxide (O₂^{•−}). Overactive Rac1–Nox2, along with an impaired cytosolic antioxidant defense mechanism, leads to the accumulation of cytosolic ROS. Right panel: With diabetes progression, the sustained increase in cytosolic ROS damages mitochondrial membranes, proteins, and DNA, leading to an imbalance in mitochondrial dynamics. Impaired mitochondrial DNA (mtDNA) transcription compromises the electron transport chain, decreasing mitochondrial function while stimulating the production of mitochondrial ROS. Mitochondrial damage can stimulate apoptotic cell death, as observed in the vascular histopathology associated with DR. (B) The retinal pigment epithelium (RPE) supplies oxygen and glucose from the choroid (Ch) to the photoreceptors (PRs). AMD disrupts this system. Advanced age, along with numerous genetic and environmental factors, is thought to contribute to the development of AMD. In early AMD, an imbalance in RPE ROS damages mitochondrial proteins, lipids, and mtDNA and causes mitochondrial dysfunction. Right panel: Without fully functioning RPE mitochondria, the RPE begins to utilize glucose to generate energy via glycolysis, thereby usurping this energy source away from the PRs, which rely heavily on the glycolytic pathway. This alteration of retinal metabolism causes a bioenergetic crisis in the retina that ultimately leads to the death of PRs and the RPE, a hallmark of advanced disease.

Box 1. Mitochondria and the 'Jekyll and Hyde' of Producing Energy

Mitochondria have two membranes [the outer mitochondrial membrane (OMM) and the inner mitochondrial membrane (IMM)] that define functionally distinct regions of the inner mitochondrial space (IMS) and the matrix (M) (Figure 1). The surface area of the IMM is increased by multiple invaginations, called cristae, where proteins of the ETC are embedded. The ETC comprises multisubunit complexes (I to IV) that perform OxPhos, the main pathway for ATP generation. OxPhos involves the reduction of molecular oxygen coupled to the generation of an electrochemical gradient across the IMM, driving the conversion of ADP to ATP by complex V, also known as ATP synthase. Under normal physiological conditions, approximately 2% of the total oxygen is incompletely reduced and forms superoxide radicals (O_2^-) [66]. These ROS are continuously formed in the mitochondria, with complexes I and III identified as the major sites of ROS leakage [67]. ROS are important signaling molecules that allow communication between the mitochondria and the nucleus through numerous transcription factors regulated by ROS [67]. In addition to ROS, changes in mitochondrial membrane potential, the quantity of ATP, calcium, and unfolded proteins are other signals that initiate an adjustment in gene expression to accommodate changes in energy demands and cellular environment.

While ROS provide an important means of communication between the mitochondria and nucleus, overproduction of ROS can damage mtDNA, proteins, and lipids. mtDNA, located in the matrix, is particularly prone to ROS damage due to several factors, including its close proximity to the ETC, the site of ROS production. Unlike the nuclear genome, mtDNA is not protected from ROS-induced damage by histones. However, some protection is provided by TFAM and the nucleoid complexes of multiple proteins [68]. There is only limited DNA repair available in the mitochondria, so mtDNA damage can be long lasting and accumulate under conditions of increased oxidative stress, a hallmark of both DR and AMD.

In summary, ATP generation produces ROS, which has both positive and negative consequences. The positive, 'Jekyll' effect includes their role as critical signaling molecules. The negative, 'Hyde' aspect involves molecular damage due to ROS overproduction.

**Figure 1. Mitochondrial Structure**

Abbreviations: ETC, electron transport chain; IMM, inner mitochondrial membrane; IMS, inner mitochondrial space; OMM, outer mitochondrial membrane; mtDNA, mitochondrial DNA.

Another major source of ROS are mitochondria, which generate ROS as a byproduct of **oxidative phosphorylation (OxPhos)**. Under conditions of hyperglycemia and saturated glycolysis, the flux through OxPhos is increased. When the proton gradient reaches capacity, slower transfer of electrons through complex III of the **electron transport chain (ETC)** increases ROS generation [15] (see Box 1 for an in-depth discussion of mitochondrial architecture). The increased concentration of mitochondrial ROS damages mitochondria and, combined with damage from cytosolic ROS, leads to mitochondrial dysfunction.

Hyperglycemia in many retinal cells, including microvascular cells, PRs, and ganglion cells leads to ROS-induced mitochondrial dysfunction [16,17]. As reported in rodent retina and endothelial cells

isolated from humans and bovines, mitochondria become swollen, their membrane potential is decreased, and membrane permeability is increased [17]. Damaged membranes allow cytochrome c to escape from the mitochondria into the cytosol, inducing apoptosis, a phenomenon that precedes the histopathologic development of DR. This process is the apparent link between mitochondrial oxidative stress, mitochondrial dysfunction, and DR pathology [18,19].

While hyperglycemia-induced metabolic changes play a major role in the development of DR, no single cause for AMD has been identified. Instead, AMD is a multifactorial disease that is associated with at least 34 genetic loci and numerous environmental factors that place individuals at greater risk for the disease [20] (Figure 2B). These genetic and environmental factors increase oxidative stress and inflammation, two stressors that are clearly linked to mitochondrial damage [21]. Recent studies investigating retinal metabolism have demonstrated the metabolic codependence of the RPE and retina [22–26]. Together, these studies have developed a new model for a ‘metabolic ecosystem’ [24]. In the healthy eye, glucose from the choroidal circulation remains largely unused by the RPE and is transported to the PRs. PRs and Müller glia cells utilize glucose to generate ATP via glycolysis [24,25]. By contrast, the RPE relies almost exclusively on the mitochondria, which generate energy via OxPhos using a number of substrates such as lipids, ketone bodies, and pyruvate to satisfy its energy requirements [22,23,26].

In AMD, mitochondrial damage and dysfunction disrupts this delicate metabolic ecosystem by causing the RPE to rely on glucose and glycolysis to supply its energy requirements (Figure 2B) [27]. With limited availability of glucose, the PRs starve. The RPE’s reliance on glycolysis eventually leads to the death of both PRs and RPE, a characteristic associated with late-stage AMD. This concept of a bioenergetic crisis provides a potential explanation for how reduced RPE mitochondrial function could have a global effect on multiple cells in the retina.

Mitochondria at the Nexus of Both Diseases

In addition to generating energy, mitochondria perform a number of other functions essential for cell survival such as calcium buffering and the metabolism of cholesterol and iron. Importantly, mitochondria must maintain all of these processes while adjusting to changing cellular conditions. Mitochondria form dynamic and extensive networks that maintain homeostasis through fusion, fission, mitophagy, and biogenesis (Figure 3). Fission culls damaged fragments from the mitochondrial network, which are subsequently degraded through mitochondrial autophagy, called mitophagy [28]. With fusion, healthy mitochondria merge and mix their lipids, proteins, and mitochondrial DNA (mtDNA) [28]. Biogenesis replenishes the mitochondrial pool by generating new mitochondrial membranes, proteins, and mtDNA through the coordinated effort of nuclear and mtDNA gene expression [29]. Epigenetic modifications also regulate nuclear and mitochondrial gene expression, influencing mitochondrial homeostasis (Box 2). The process of mitochondrial homeostasis is a delicate balance and disruptions to any of the pathways involved could lead to disease.

Many facets involved in the maintenance of mitochondrial homeostasis are altered in diabetes. Direct evidence for impaired mitochondrial biogenesis in retinal endothelial cells and microvasculature from streptozotocin-induced diabetic rats and mice includes a significant decrease in mtDNA copy number [30]. Other changes include a 50% reduction in the mtDNA replication enzymes DNA polymerase gamma and mtDNA helicase (Twinkle), which may be caused by the sustained increase in ROS associated with hyperglycemic conditions [30,31]. Additionally, the import of the nuclear-encoded mtDNA replication enzyme polymerase gamma and mitochondrial transcription factor A (TFAM) inside the retinal mitochondria is significantly reduced by 35–45% [31,32].

Fission and fusion are also affected, as evidenced by the ~50% increase in Drp1 (fission) protein and a similar decrease in Mfn2 (fusion) content in the retina and its vasculature from streptozotocin-induced diabetic rats and from human donors with established DR [17]. Decreased Mfn2 not only affects mitochondrial morphology but also causes a loss in OxPhos function [33]. Mitophagy is increased under high-glucose conditions in cultured RPE and Müller cell lines, as observed by the colocalization of

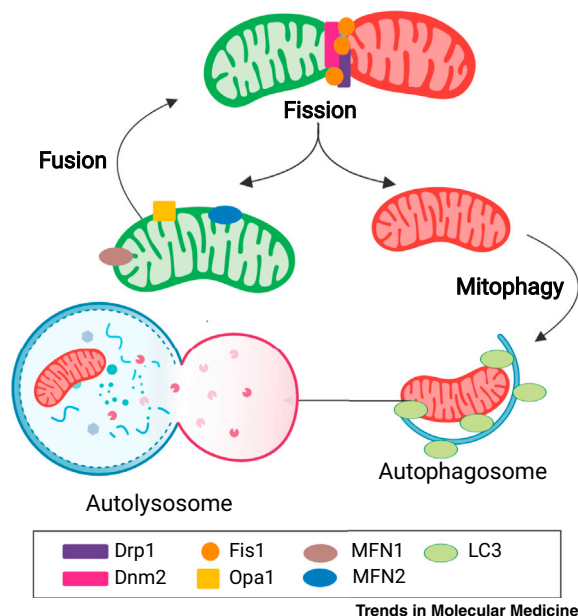


Figure 3. Overview of Mitochondrial Homeostasis.

Mitochondrial homeostasis is regulated through multiple steps that preserve a healthy network of mitochondria (green). Mitochondrial damage causes fragmentation (fission), regulated by mitochondrial fission protein 1 (Fis1), the large GTPases dynamin-like protein 1 (Drp1), and dynamin 2 (Dnm2). Importantly, fission results in segregation of the damaged mitochondrial remnants (red). If not repaired, these remnants are isolated for degradation through mitochondrial autophagy (mitophagy). The mitochondria are encapsulated by a double-membrane autophagosome (A) containing microtubule-associated protein 1A/1B (LC3). These autophagosomes merge with a lysosome (L) where the acidic environment and lysosomal enzymes degrade and recycle the mitochondria. Healthy mitochondria continually fuse, mixing lipids, proteins, and mitochondrial DNA (mtDNA) using mitofusin 1 and 2 (Mfn1 and Mfn2) for outer mitochondrial membrane fusion and Opa1 for inner mitochondrial membrane fusion. New components are added to expand and replenish mitochondrial networks through mitochondrial biogenesis, a process that involves both mitochondrial and nuclear transcription and translation, lipid synthesis, and replication of the mitochondrial genome.

fragmented mitochondria with the autophagosome and lysosomal markers [33,34]. However, in cultured retinal capillary pericytes, the effect of accelerated autophagy is dependent on the stage of the disease. Autophagy induction under mild stress induced by low concentrations of heavily oxidized low-density lipoproteins is protective [35]. By contrast, conditions of severe stress induced by high concentrations of oxidized lipoproteins upregulate autophagy and promote cell death, suggesting that autophagy could be cytoprotective for vascular cells in the early stages of DR [35]. Regardless of the mechanism, mitochondrial damage and dysfunctional mitochondrial homeostasis (summarized in Table 1) occupy a central role in the pathogenesis of DR.

RPE from AMD human donor tissue also shows mitochondrial damage potentially due to disrupted mitochondrial homeostasis. Data that support diminished mitochondrial biogenesis include a decrease in mitochondrial number in RPE from AMD compared with age-matched donors [36] and lower content of proteins in the ETC, such as ~55% decrease in subunits of complex IV and V [37,38]. A 60% reduction in mitochondrial heat shock protein 70, a protein involved in the import of nuclear-encoded proteins that reside in the mitochondria, has also been reported [37,38]. Protein import is integral to mitochondrial biogenesis since the vast majority of mitochondrial proteins are nucleus encoded, produced in the cytosol, and imported into the mitochondria.

Evidence for defects in the isolation and removal of damaged mitochondria include a significant increase of mtDNA damage in the macula of human donor RPE [39,40]. Additional evidence for the

Box 2. Epigenetic Changes Associated with Diabetes

Gene expression can be regulated by external factors and disease states via epigenetic modifications, which includes DNA methylation and modifications such as the acetylation and phosphorylation of histones. Epigenetic modifications can either be passed to the next generation or be erased, thus potentially making them good therapeutic targets [69].

In DR, many epigenetic modifications are implicated in the dysregulation of mitochondrial homeostasis. Retinal microvasculature from diabetic rodents and human donors with established DR, and retinal endothelial cells cultured in high glucose, have hypermethylated DNA at the promoters for Mfn2, Mlh1, and POLG, affecting both the structural and the genomic stability of the mitochondria [17,31,70]. Dynamic DNA methylation is also implicated in the transcription of matrix metalloproteinase 9 (MMP-9), a mitochondrion-damaging enzyme, and Rac1, an obligatory component of the Nox2 holoenzyme [14,17]. Increased import of the methyl transfer enzyme Dnmt1 into the mitochondria and subsequent hypermethylated mtDNA have been observed in experimental models of DR [30]. Furthermore, mtDNA is more extensively methylated at its D-loop, the site of transcriptional regulation, compared with the other regions of mtDNA [30]. Due to hypermethylation, the transcription of mtDNA-encoded genes, including some subunits of the ETC, is impaired, and the compromised ETC feeds into the vicious cycle of ROS production and damage [15,71–73]. In DR, DNA methylation and base mismatches show a positive correlation [73], suggesting a close relation between epigenetic modifications and mtDNA damage.

Histone modifications are also implicated in mitochondrial homeostasis. For example, increased methylation at H3K9 and H4K20 reduced transcription factor binding at the promoter for Sod2, a key mitochondrial antioxidant that is downregulated in DR [74]. Histone modifications are also associated with increased expression of the Nrf2 inhibitor Kelch-like ECH-associated protein 1, thus impeding Nrf2 movement into the nucleus and increasing oxidative stress [45]. Thus, the current evidence clearly suggests a major role for epigenetic modifications in the structural, functional, and genomic stability of the mitochondria and the processes of mitochondrial fusion and biogenesis [15,75]. Although the majority of diabetes-induced epigenetic modifications are reported in the retinal vasculature from diabetic rodents, human donors with established DR and primary retinal endothelial cells cultured in high glucose exhibit similar epigenetic modifications in other retinal cells, and their role in the pathogenesis of DR remains to be investigated.

In summary, the diabetic environment favors epigenetic modifications in the retina and its vasculature and these modifications affect many aspects of mitochondrial homeostasis. By contrast, no genome-wide methylation profiles or histone modifications have been published for AMD. Thus, this area of research is largely untouched and should be investigated.

accumulation of damaged mitochondria includes the observed disruption and disorganization of mitochondrial cristae (Box 1) in electron micrographs from AMD donor RPE [36] and the increased content of mitofilin at an early stage of AMD [38]. Mitofilin is involved in stabilizing cristae, so its up-regulation could be a compensatory response to altered mitochondrial remodeling [38]. The removal of damaged mitochondria through mitophagy may be impaired due to the significant decrease in cellular autophagy in cultured RPE from AMD donors [41]. While mitophagy has not been directly measured, this overall decrease in autophagy would lead to decreased mitophagy as well.

Data from primary RPE cultures have shown that the accumulation of damaged mitochondria has functional consequences for metabolism. Two laboratories reported decreased mitochondrial function in cultured RPE from AMD donors [41,42]. Taken together, these results show that mitochondrial damage occurs in the RPE of AMD donors (summarized in Table 1) and support the notion that RPE mitochondrial dysfunction contributes to AMD pathology. Importantly, failures in any pathways of mitochondrial homeostasis could lead to the mitochondrial damage and dysfunction observed in RPE with AMD. This accumulation of dysfunctional mitochondria eventually leads to RPE death, the hallmark of dry AMD.

Mitochondrial Genomic Stability and Damage

Mitochondria contain their own 16.5-kb double-stranded, circular DNA. This genome encodes 13 proteins, including essential components of the ETC [43]. The remaining genome encodes the

machinery (16S and 12S rRNAs, 22 tRNAs) required to produce the 13 mitochondrion-encoded proteins. Additionally, there is a noncoding region containing the **displacement loop (D-loop)** that regulates mtDNA replication and translation (for a more extensive discussion of mtDNA, see [Box 1](#).)

With diabetes, mtDNA damage includes an increase in oxidatively modified guanine bases (8-OHdG) and sequence variants in retinal microvessels and endothelial cells in culture [44,45]. Although the entire mitochondrial genome is damaged, the noncoding D-loop is the most extensively damaged region [15]. The effect of this damage is further amplified by impaired machinery responsible for repairing the damaged mtDNA. Mitochondrial reductions in both the base excision repair enzyme 8-oxoguanine DNA glycosylase (Ogg1) and the mismatch repair enzyme MutLhomolog 1 (Mlh1) have been reported in endothelial cells and microvessels [44–46]. As a consequence of the increased mtDNA damage and suboptimal DNA repair systems, the transcription of mtDNA-encoded genes is decreased, further compromising the already damaged ETC system [44,45]. This mitochondrial damage and decreased transcription induces a vicious cycle of free radical production that eventually leads to the capillary cell death observed in DR.

With AMD, there are numerous reports of elevated mtDNA damage in the RPE from human donor tissue as well as in primary RPE cultures. In the RPE from donors with AMD, mtDNA damage increased by ~350% compared with RPE from age-matched controls [39,47]. This damage was localized to specific regions of the genome, including the regulatory D-loop and in coding regions for subunits of complex I, complex IV, and complex V [47]. Consistent with findings in donor tissue, an analysis of primary cultures of RPE cells reported increased mtDNA damage and a fivefold increase in somatic mutations in RPE cultures from AMD donors compared with age-matched controls [40].

Comparison of the type and extent of mtDNA damage in each disease is limited due to the different assays used. However, both find damage localized to the D-loop, the regulatory region of mtDNA. This damage would lead to decreased replication and transcription, thereby affecting the production of new mitochondria and nascent mitochondrion-encoded proteins, hence negatively impacting mitochondrial homeostasis.

Current Treatments and Novel Strategies

Standard of Care

The complex etiology of DR has allowed only limited therapeutic advances. Therefore, tight glycemic control and laser photocoagulation remain the conventional management options for controlling its progression. Insulin not only helps to regulate blood glucose levels but may have a positive effect on mitochondrial function since it has been shown to maintain cardiac mitochondrial homeostasis in streptozotocin-induced diabetic rats [48]. Maintenance of glycemic control for long durations becomes challenging for some diabetic patients and laser photocoagulation has the possibility of unwanted damage to the retina and loss of central vision. Although intravitreal anti-VEGF administration is routinely used for DME, its use for nonproliferative DR still requires further investigation [49]. The limitation of these treatments is that they treat disease symptoms only rather than targeting the pathological mechanism. Thus, there is an urgent need to identify novel therapies targeting the mechanism that could be used to treat patients prior to the development of DR.

Currently, the only treatment available for dry AMD is a supplement of antioxidants plus zinc, identified by the Age-Related Eye Disease Study (AREDS), which slowed AMD progression [50]. However, only ~20% of patients with intermediate AMD had a positive response to the AREDS formulation, so additional treatments that affect a larger patient population are needed. Several clinical trials have tested new drugs that target specific pathways or mechanisms linked to AMD, including complement, inflammation, neuroprotection, and oxidative stress [51]. For example, drugs targeting different points in the three pathways (classical, lectin, and alternative) that constitute the complement cascade capitalize on blocking the formation of the membrane attack complex, which can cause cell lysis and release cytokines that trigger the recruitment of inflammatory cells. One of the early successes in inhibiting the complement system in the retina was lampalizumab, an antibody that inhibits

complement factor D, the final step in the alternative pathway. The outcome of the Phase II multicenter, randomized sham-controlled trial (NCT01602120) noted a significant 20% reduction in the mean growth of geographic atrophy lesions, which are retinal areas where the RPE has died. The publication of these encouraging results [52] prompted the initiation of two identically designed Phase III trials (NCT02247531ⁱⁱ, NCT02247479ⁱⁱⁱ) conducted at 275 sites in 23 countries. The outcome of the double-masked, sham-controlled trials with 1733 participants found no significant benefit with lampalizumab versus sham controls over 48 weeks of treatment [53]. These trials were the most comprehensive studies of geographic atrophy to date and while their results did not support lampalizumab's efficacy, they did provide a wealth of information about the natural history of AMD in a diverse population with geographic atrophy.

While several additional drugs targeting specific molecules in the complement cascade are currently in clinical trials (see [51] for a summary), two ongoing Phase III clinical trials (NCT03525613^{iv} and NCT03525600^v) are worth noting. These trials are testing the efficacy and safety of intravitreal injections of the drug APL-2, a cyclic peptide that inhibits C3. This protein is located downstream of complement factor D at the convergence of the three pathways in the complement system. As APL-2 affects C3, which is involved in all complement pathways, it may be more effective than lampalizumab, which inhibits one arm of the complement cascade. The multicenter Phase III trials are randomized, double-masked, sham-controlled studies of subjects with advanced dry AMD that will use growth of geographic atrophy lesions as the primary outcome. These Phase III trials are still recruiting to meet their target of 600 subjects, with an expected completion date of November 2022.

There are a number of challenges associated with conducting AMD clinical trials that are likely to contribute to the limited success observed to date. For example, due to slow disease progression, significant changes in outcomes (i.e., size of atrophic lesions) require lengthy clinical trials that are cost-prohibitive. Additionally, most trials randomly assign patients to treatment or control groups without consideration of their genetic background or environmental factors that could influence the results. Due to constraints placed by the FDA, most clinical trials are treating patients with late-stage disease when the ability to 'rescue' the retina is very limited. The mode of drug delivery (oral, intravitreal, subcutaneous, or intravenous injections) could also influence the outcome due to challenges associated with the blood-retina barrier and in maintaining an optimal dose that targets the correct cell. It is also important to note that most clinical trials may not be targeting the correct molecular pathway. Therefore, a greater understanding of disease mechanism is required to develop better therapies that target the primary defect in the retina.

Emerging Strategies That Target Mitochondria

Based on the central role of mitochondria in DR and in AMD, strategies targeting mitochondrial homeostasis have great potential. For example, the tetrapeptide SS-31, which targets mitochondrial cardiolipin, was effective in reversing visual decline in a streptozotocin-induced mouse model of diabetes [54]. Cardiolipin is a lipid found in the inner mitochondrial membrane (IMM) and is crucial for maintaining OxPhos function [55]. Since this drug promotes proper mitochondrial function, SS-31 has also been used to treat diseases involving mitochondrial pathology [56]. SS-31 (commercial name Elamipretide) is currently in a Phase II randomized, double-masked, placebo-controlled clinical trial to evaluate its safety, efficacy, and pharmacokinetics in subjects with dry AMD (NCT03891875^{vi}). This clinical trial is still recruiting subjects, with a target of 180 patients, and will use best-corrected visual acuity under low-light conditions as the primary outcome.

In addition to chemical modulators of mitochondria, far-red to near-IR light (590–850 nm), referred to as photobiomodulation, has also demonstrated beneficial effects in multiple retinal degenerative models and patients with DR or AMD [11,57]. Photobiomodulation targets mitochondrial cytochrome oxidase C, a protein that modulates the transfer of electrons between ETC complexes, increasing the mitochondrial membrane potential and ATP synthesis [57]. In streptozotocin-diabetic mice, treatment with 670-nm light inhibited the diabetes-induced leakage of retinal capillaries and reduction in vision [58]. In aged mice and an AMD mouse model with a genetic disruption of complement factor

H, photobiomodulation significantly increased retinal mitochondrial function and reduced signs of inflammation [59,60]. These studies provide insight into the molecular basis for the beneficial effects of photobiomodulation.

Clinical studies have also reported positive results after photobiomodulation for patients with DR. In a nonrandomized, consecutive case study of four type 2 diabetic patients with DME, photobiomodulation treatment caused a significant reduction in macular thickness [61]. A pilot study evaluating photobiomodulation therapy for DME, initiated in 2019, will evaluate macular thickness, mean change in retinal volume, and visual acuity from baseline to 4 months of treatment (NCT03866473^{vii}). This randomized, sham-controlled, quadruple-masked trial anticipates 134 participants.

Multiple clinical studies treating AMD patients with photobiomodulation have also produced promising results. In a prospective intervention study with 21 dry AMD patients, photobiomodulation significantly improved vision (best-corrected visual acuity and contrast sensitivity) and reduced drusen volume [62]. Based on these positive outcomes, a pilot study with 30 participants was conducted and, importantly, this study design included double-masked, randomized patient assignment to either treatment or sham-control groups (NCT02725762^{viii}). Outcomes were visual acuity, contrast sensitivity, and retina structural analysis measured by optical coherence tomography. Published results reported a statistically significant improvement in visual acuity and contrast sensitivity and a reduction in drusen volume [63]. As a follow up from this first study, a second multicenter clinical trial based in Europe is currently enrolling dry AMD patients (NCT03878420^{ix}). This double-masked, randomized, sham-controlled study has an anticipated completion date of December 2020.

The antidiabetic drug metformin has also demonstrated promise in improving mitochondrial function. Systemic treatment of mice with metformin was effective in increasing mitochondrial biogenesis and improving mitochondrial health [64]. The mechanism may be due to metformin's activation of AMP-activating protein kinase (AMPK), which in turn activates **peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1 α)**, the regulator of mitochondrial biogenesis. The link between improved mitochondrial health and reduced risk for AMD was recently reported in a retrospective, case-controlled study that used univariate conditional multivariate logistic regression to examine the association between metformin use and AMD diagnosis in over 7000 patients. The significant odds ratio of 0.58 shows that diabetic patients taking metformin had significantly decreased risk of developing AMD [65].

These early studies and clinical trials show promising effects by targeting the mitochondria. SS-31 and photobiomodulation interact with the ETC and improve bioenergetics. Metformin has many targets and therefore its beneficial effect on bioenergetics is less clearly defined. As early intervention strategies, these treatments should have minimal side effects and demonstrate long-term benefits. Important optimization must be conducted once their efficacy is established, including timing, dose, and method of therapeutic delivery.

Concluding Remarks

Mitochondrial dysfunction drives disease pathology in two distinct retinal diseases affecting unique cell types (Figure 2). While recent advances highlight the importance of mitochondrial homeostasis in disease mechanism, several outstanding questions and key issues remain to be investigated (see Outstanding Questions). For example, although mitochondrial damage is observed in specific cells (e.g., vasculature in DR, RPE in AMD), the entire neural retina is ultimately affected as the disease progresses. It is unclear how metabolic defects in a single cell can affect the health of adjacent cells. One of the challenges is the complexity of the retina, which comprises eight distinct cell types, including neurons (PR, horizontal, bipolar, ganglion), glia (Müller, microglia, astrocytes), and RPE. An additional layer of complexity comes from region-specific differences in the distribution and multiple subpopulations of these cell types. For example, the macula has a high density of cone PRs, with the rest of the retina containing primarily rod PRs. To thoroughly understand effects of the metabolic defect on

Clinician's Corner

Diabetes is a chronic disease that can lead to diabetic retinopathy (DR), the leading cause of blindness in working-age adults. Its effective management depends heavily on early diagnosis and the maintenance of tight glycemic control, which becomes difficult or impossible for many patients. Current options to prevent progression of DR remain focal laser and panretinal laser photocoagulation, both carrying risks of visual field deficits and exudative retinal detachments.

Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly, manifesting as loss of central vision and blurring of horizontal and vertical lines due to cell death in the macula.

AMD includes two forms of the disease, 'wet' and 'dry'. While wet AMD is treated with ocular injections of anti-VEGF, there are no effective treatments for dry AMD. As both DR and AMD develop due to mitochondrial damage and dysfunction, new therapies that target the mitochondria may be a viable option.

each cell type in the retina, single-cell analysis is required to grant insight into how specific retinal populations are affected.

Fortunately, with technical advances our understanding of the molecular mechanisms responsible for imbalanced mitochondrial homeostasis in DR and AMD is improving. Major effort needs to be placed in the development of therapies that target the mitochondria and are administered early in disease, so that irreversible cellular damage can be prevented. Currently, new mitochondria-targeted molecules are being generated and advanced strategies for sustained drug delivery to the back of the eye are now becoming a reality. Thus, the future for preventing vision loss in patients with DR or AMD looks optimistic.

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Resources

- ⁱ<https://clinicaltrials.gov/ct2/show/NCT01602120>
- ⁱⁱ<https://clinicaltrials.gov/ct2/show/NCT02247531>
- ⁱⁱⁱ<https://clinicaltrials.gov/ct2/show/NCT02247479>
- ^{iv}<https://clinicaltrials.gov/ct2/show/NCT03525613>
- ^v<https://clinicaltrials.gov/ct2/show/NCT03525600>
- ^{vi}<https://clinicaltrials.gov/ct2/show/NCT03891875>
- ^{vii}<https://clinicaltrials.gov/ct2/show/NCT03866473>
- ^{viii}<https://clinicaltrials.gov/ct2/show/NCT02725762>
- ^{ix}<https://clinicaltrials.gov/ct2/show/NCT03878420>

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Outstanding Questions

Why is retinal microvascular mitochondrial damage not an early event in the pathogenesis of DR? In the initial stages of DR, mitophagy may efficiently remove the damaged mitochondria. However, with time the mitophagy machinery becomes exhausted and damaged mitochondria accumulate. A systematic temporal relationship between mitochondrial mitophagy, biogenesis, mtDNA damage, and the DNA repair machinery could help to unravel this mystery.

In DR, what cell types exhibit mitochondrial damage and when does it occur? We need new models and techniques to decipher cell-specific changes with mitochondrial damage and dysfunction. A better understanding of the epigenetic regulation of mitochondrial homeostasis would shine light on this question.

Why does preferential death of the macular RPE and PRs occur when both clinical and biochemical evidence shows that the detrimental effect of AMD impacts the entire retina? A potential mechanism involves the higher bioenergetic demand of the macula and metabolic uncoupling of the retinal ecosystem with AMD.

In AMD, why is mitochondrial damage limited to the RPE? The answer may involve cell-specific differences in metabolism. In the healthy retina, the RPE relies almost exclusively on mitochondria to generate energy, whereas the PRs depend on glycolysis.

What drugs will provide the greatest protection of mitochondrial function? It is important that new and current drugs are investigated for their effect on mitochondrial homeostasis and function in both DR and AMD. It is important to test these drugs in the most appropriate model system and cell type.

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