*antioxidants*

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*Review*

# Metformin's Effects on Reactive Oxygen Species Production and Its Implications for Ocular Disease

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## Abstract

Mitochondrial dysfunction and metabolic stress are major contributors to the onset and progression of many ocular diseases, including cataract, glaucoma, diabetic retinopathy and age-related macular degeneration (AMD), which together account for approximately 65% of vision loss over the age of 50. These diseases can result from impaired antioxidant defences and excessive reactive oxygen species (ROS) production, resulting in oxidative stress. Interventions that can help to alleviate or prevent oxidative stress in the early stages of these diseases may hold potential as a therapeutic agent. Metformin, a first-line therapy for type 2 diabetes, has in recent years displayed pleiotropic effects in ocular tissues, influencing Complex I inhibition, modulation of AMP-activated protein kinase (AMPK) signalling, and upregulation of nuclear factor erythroid 2-related factor 2 (NRF2)-mediated antioxidant expression in both in vitro and in vivo models. In animal models, oral and topical modes of metformin administration have been shown to reach ocular tissues and reduce oxidative stress. However, a lack of human clinical evidence that is not confounded by diabetes is lacking and limits the case for metformin's therapeutic repurposing. This review examines the observed mechanisms through which metformin may exert beneficial effects on ocular disease outcomes, with an emphasis on its antioxidant capacity.

**Keywords:** oxidative stress; reactive oxygen species; antioxidant; metformin; age-related macular degeneration; glaucoma; diabetic retinopathy; cataract; ocular disease; AMPK

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## 1. Introduction

Metformin is a highly efficient biguanide antidiabetic agent utilised by over 150 million patients worldwide as a first-line treatment for type 2 diabetes [1,2]. Beyond metformin's mild inhibitory action on hepatic gluconeogenesis, metformin has shown promise for a variety of therapeutic benefits [3]. Beneficial anti-inflammatory [4], antioxidant [5,6], neuroprotective [7], antiangiogenic [8,9] and antifibrotic [10,11] effects have all been observed in prior research.

Metformin is a biguanide derived from galegine, an active chemical found in *Galega officinalis* (French lilac). Historically (Table 1), *Galega officinalis* was used as a treatment for relieving intense thirst and frequent urination in the 18th century, the symptoms that accompany the disease we now know as diabetes [12,13]. By the 19th century, the active ingredient responsible for *Galega officinalis* was determined to be galegine (isoamylene guanidine) [14]. The initial trials revealed that galegine inhibition of hepatic gluconeogenesis was too potent and carried a high risk of causing lactic acidosis as a side effect. Lactic acidosis caused by galegine or biguanide use is the result of potent inhibition of gluconeogenesis. The increased nicotinamide adenine dinucleotide reduced/oxidised (NADH/NAD+) ratio inhibits pyruvate dehydrogenase and leads to the accumulation of pyruvate, which metabolises into lactate [15]. The accumulation of lactate in the bloodstream drops blood pH to dangerous levels and results in a disruption of bodily functions. The mortality rate of lactic acidosis is approximately 50%, so even a small risk of lactic acidosis poses a great risk to patient safety [13,16]. Derivatives of galegine were synthesised throughout the 1920's to find a less toxic alternative with similar properties; this resulted in the first synthesis of metformin and other notable biguanides, such as phenformin and buformin [17].

Phenformin, buformin and metformin gained approval for clinical use in Europe throughout the 1950's. Among them, metformin was overshadowed in favour of other biguanides, such as phenformin and buformin, due to its comparative lack of potency, with a metformin dose ranging between 500–2000 mg [18]. After over a decade of clinical use, revelations about phenformin and buformin's high risk of lactic acidosis came to light, and their regulatory approval was removed in 1978 [12,14,19]. Studies have shown that metformin alone can have little to no risk of causing lactic acidosis, possibly due to its low potency. Oral doses of metformin are expelled unchanged via the kidneys, with an elimination half-life of approximately 5 h, and often require an existing renal impairment for metformin to accumulate to levels necessary to induce lactic acidosis [20,21]. Due to this, metformin's regulatory approval in Europe remained as other biguanides were removed, and it was eventually approved for use in the United States by 1995 [12,14]. Soon after, the World Health Organisation added metformin to its eighth report of essential medicines [2].

Throughout the 2000s–2010s, researchers began uncovering the mechanistic forces behind metformin's intracellular modes of action. The first canonical pathway demonstrated for metformin's inhibition of hepatic gluconeogenesis was through the mild inhibition of complex I (NADH:ubiquinone oxidoreductase) along the electron transport chain [22]. Further research by Zhou et al. [23] demonstrated that metformin suppressed lipogenic enzymes via activation of the AMP-activated protein kinase (AMPK). The researchers speculated that metformin could produce its pleiotropic benefits through AMPK-associated pathways, which was supported by mechanistic research that preceded it [24,25]. The revelation of metformin's antioxidant properties began with an observable reduction in intracellular reactive oxygen species (ROS), followed by findings that the antidiabetic drug can also decrease the activity of ROS-producing enzymes via AMPK-related pathways [26,27]. Observational studies during the 2010's consistently found that metformin produced significant beneficial outcomes in ocular disease progression of diabetic patients who have been treated with metformin compared to diabetic patients who had not [28–30]. Given metformin's ability to modulate multiple cellular pathways, there is a potential overlap between mechanisms underlying ocular disease pathogenesis and the pharmacological effects of metformin, which may provide opportunities for potential therapeutic benefit [31]. Within the past decade, researchers have begun to better understand the mechanisms through which metformin can benefit ocular disease outcomes.

Primarily, metformin is effective at modulating cellular pathways responsible for the generation and clearance of cellular ROS, such as the AMPK pathway, complex I elec-

tron leakage and nuclear factor erythroid 2-related factor 2 (NRF2)-mediated antioxidant expression [6]. This review seeks to evaluate metformin's relevance for ocular diseases, focusing on its influence on mitochondrial ROS production, stimulation of autophagy, stimulation of antioxidant responses and other pathways that may indicate its potential for ophthalmic repurposing.

**Table 1.** Milestones for the history of metformin and the discovery of antioxidative applications in the eye.

| Year | Milestone | Reference |
|---|---|---|
| ~1600–1772 | *Galega officinalis* was utilised as an herbal medicine for relief of diabetes symptoms. | [12] |
| 1918 | Watanabe demonstrated hypoglycaemia in animals after administration of guanidine. | [32] |
| 1922 | Werner and Bell first synthesised metformin (dimethylbiguanide). | [17] |
| 1929 | Slotta and Tschesche demonstrated the glucose lowering effect of metformin in animals. | [14] |
| 1957 | Phenformin was introduced to the United States market for the treatment of diabetes | [15] |
| 1957 | Sterne demonstrated the effectiveness of metformin in humans for the treatment of diabetes. | [33] |
| 1978 | FDA requested withdrawal of phenformin from the market due to concerns of lactic acidosis risk. | [19] |
| 1995 | Metformin was approved for use in the United States by the FDA. | [12] |
| 1997 | The World Health Organisation added metformin to their list of essential medicines. | [2] |
| 2000 | Pavlovic et al. found evidence that metformin increased activity of antioxidant enzymes in diabetic plasma samples. | [34] |
| 2000 | El-mir et al. established that metformin can indirectly inhibit complex I. | [22] |
| 2001 | Zhou et al. demonstrated the metformin dependent activation of AMP-activated protein kinase (AMPK). | [23] |
| 2002 | Hawley et al. presented evidence of AMPK activation independent of complex I inhibition. | [35] |
| 2003 | Bonnefont-Rousselot et al. found that metformin could reduce intracellular production of reactive oxygen species (ROS). | [26] |
| 2005 | Ouslimani et al. showed that metformin can modulate ROS generating enzymes such as nicotinamide adenine dinucleotide phosphate oxidase. | [27] |
| 2012 | Algire et al. attenuated endogenous production of ROS and ROS related DNA damage through metformin treatment. | [36] |
| 2013 | Martin-Montalvo et al. noted that metformin treatment delayed the onset of cataracts in a mouse model. | [37] |
| 2014 | Richards et al. determined metformin use to be associated with reduced risk of glaucoma development independent of glycaemic control. | [28] |
| 2017 | Kim et al. demonstrated metformin can decrease retinal pigment epithelial (RPE) programmed cell death by reducing nuclear factor kappa beta signalling. | [38] |
| 2018 | Xu et al. exhibited metformin's ability to cross the blood–brain barrier in vivo and prevent degeneration of RPE cells from oxidative stress. | [21] |
| 2018 | Li et al. observed a significant correlation between long term metformin use and a reduced severity of diabetic retinopathy in diabetic patients. | [39] |
| 2020 | Zhang et al. utilised metformin to prevent hydrogen peroxide-induced senescence of human lens epithelial cells. | [40] |
| 2020-present | More in-depth mechanistic research has been conducted and compiled to fill knowledge gaps in metformin's antioxidant pathways and the extent to which metformin can suppress the progression of ocular disease. | [5,6,41,42] |

## 2. Search Methodology

In this review, a targeted literature search was conducted using PubMed and Web of Science databases. Literature published between January 2016 and September 2026

was prioritised, although earlier studies were also included in instances where they provided historical context or foundational research. Search terms used (both individually and in combination) include: "Metformin", "Antioxidant", "Reactive Oxygen Species", "Cataract", "Glaucoma", "Age-Related Macular Degeneration", "Retina", "Lens", "Diabetic Retinopathy", and "Ocular Disease". Selection was based on relevance to the review, with an emphasis on studies that highlighted mechanisms and clinical evidence. Articles were excluded if they were not available in English or were not accessible through organisational avenues. Reference lists of retrieved articles were also manually searched for additional relevant studies.

## 3. Reactive Oxygen Species in the Eye

Reactive oxygen species (ROS) are produced in different tissues across the body and, in low levels, are even essential for physiological functions [43]. The maintenance of cellular signalling pathways often relies on small amounts of ROS for important cellular functions [44]. Under normal conditions, antioxidants manage the clearance of ROS, defending against accidental cellular damage [45]. Molecular antioxidants (e.g., ascorbate, glutathione, tocopherols) and enzymatic antioxidants (e.g., superoxide dismutase 2 [SOD2] and catalase) protect the cellular environment simultaneously and in different ways [46]. Age is a common risk factor for disease progression across the body; with the age-related deterioration of antioxidant defence mechanisms, even low levels of ROS production can result in the accumulation of ROS, resulting in cellular damage [44]. Ocular diseases such as cataract, glaucoma, diabetic retinopathy, and age-related macular degeneration (AMD) are among the leading causes of blindness. Combined, these diseases account for approximately 65% of blindness in people over the age of 50 [47]. The human eye is not resistant to the ageing process, and as control mechanisms fail, the assault from exogenous and endogenous oxidative stressors alike can lead to the deterioration of cellular function and the progression of these ocular diseases [48].

The eye is a highly specialised organ made up of many components that act to focus incoming light information and convert it into signals that our brains can interpret as sight [9,49]. Oxidative stress in ocular tissues can originate from multiple sources; however, as part of their basic function, the eyes are continuously exposed to light, including ultraviolet (UV) light. UV radiation has been linked to the generation of ROS through photo-induced free radical formation, which can subsequently lead to DNA damage and apoptosis [46]. While responsible for focusing light onto the retina to create a clear image, the cornea, aqueous humour and lens act as an antioxidant barrier, filtering UV light to protect the anterior segments of the eye from UV-induced oxidative damage [50,51]. The cornea and aqueous humour absorb UV-C and most wavelengths of UV-B by utilising ascorbate (vitamin C) molecules. Ascorbate acts as a natural UV light filter with an absorption maximum of 270 nm, and any longer wavelengths (UV-A and some UV-B) can pass through to the lens, which can absorb UV-A wavelengths up to 400 nm [45,46]. Though most UV wavelengths of light are absorbed by the cornea, aqueous humour and lens, small amounts of UV light are still able to reach the retina, where it may contribute to oxidative stress. UV radiation has been linked to the generation of mitochondrial DNA deletions through ROS-mediated mechanisms. As an example of UV-related DNA damage, the 4977-bp long deletion of mitochondrial DNA (mtDNA4977) is a "common" deletion often observed in skin cells after consistent sun exposure [52]. Notably, the incidence of mtDNA4977 has been demonstrated to increase mitochondrial ROS production through the deletion of genes responsible for transcription and complex formation along the electron transport chain [45,53]. This mtDNA4977 has also been observed in both the cornea and retina and has been attributed to mitochondrial dysfunction and accumulation of oxidative stress

in retinal pigment epithelium (RPE) cells [54,55]. The retina is an ocular tissue with a high energy demand for maintaining its basic function, which results in a large amount of metabolic output and a higher susceptibility to mitochondrial dysfunction and ROS overproduction [56]. ROS generation in RPE cells from UV radiation, exogenous oxidative agents and organelle dysfunction can result in unpredictable damage to DNA, lipids and proteins. The resulting dysfunction of cellular processes often leads to the initiation of inflammatory and apoptotic pathways or irreversible cell cycle arrest [18,48]. Mitochondrial production of ROS generally originates from leakage along the electron transport chain at Complex I (NADH dehydrogenase) and Complex III (ubisemiquinone-cytochrome b cycle) [57,58]. The generation of superoxide and other consequential ROS from the electron transport chain is often the result of an imbalance in electron transfer and ATP production, leading to electron uptake by free-floating oxygen to form superoxide [59]. Another source of intracellular ROS production is peroxisomes. The enzymes contained in the peroxisome facilitate the oxidation of fatty acids, and these reactions can produce hydrogen peroxide and superoxide as a by-product [45].

The deterioration of antioxidant systems, the high metabolic demand of ocular tissues, dysfunction of ROS-producing organelles and UV-radiation exposure can leave the eye highly susceptible to oxidative stress, leading to progression of ocular disease. Given the role of ROS in ocular tissue damage, there is a therapeutic need for an agent that attenuates ROS production and oxidative stress pathways.

## 4. Molecular Mechanisms Underlying Metformin's Antioxidant Effects

### *4.1. Organic Cation Transport Across Ocular Barriers*

Metformin acts as a cationic species at physiological pH, so it is unable to passively permeate through the blood–retinal barrier (BRB), blood–aqueous barrier (BAB), or the ocular surface. Due to this, the primary facilitators of metformin transport are organic cation transporters (OCTs) [42,60]. OCT1, encoded by the SLC22A1 gene, and OCT3, encoded by the SLC22A3 gene, play a major role in the intestinal uptake of metformin through oral ingestion [42]. These key metformin transporters have also been shown to be expressed in many different tissues in the eye, including the cornea, conjunctiva, ciliary body, lens and retina [5,61]. OCTs are saturable transporters, so retention of metformin in the tear film during application is a barrier to the therapeutic efficacy of topical application [31].

The importance of OCTs in facilitating the transport of metformin past ocular barriers is evident from the work of Nirmal et al. [61], who demonstrated metformin's capability of trans-corneal penetration via OCTs in vivo. Through the periodic collection of aqueous humour samples from the eyes of rabbits, they determined that topically applied metformin solution (7.85 mM) reached a maximal concentration (0.108 nmol/mL) after only 30 min. They verified the involvement of OCTs in this experiment through pretreatment with known cationic blockers of OCTs (quinidine and atropine), which competitively reduced the trans-corneal passage of metformin [61]. Due to the eye's natural barriers, penetration of topical metformin into the deeper posterior segments of the eye at therapeutic levels would be inefficient, if not unachievable. Nirmal et al. [62] also demonstrated the use of OCTs to transport organic cations across the BRB and the BAB. Although this study utilised Tetraethylammonium (TEA) rather than metformin, TEA is an organic cation and a well-characterised OCT substrate that has previously been shown to exhibit comparable OCTs-mediated uptake to metformin [61]. OCTs are an established transporter for metformin uptake in the intestines and are also expressed within ocular tissues. The tissue-specific pharmacokinetics of metformin in the eye following oral or topical administration are still largely under-investigated. In particular, the specific saturability of ocular OCTs and how effective each administration route is at delivering therapeutically relevant concentrations

to the anterior and posterior segments of the eye are still unclear. The safe and effective dosages of topically applied metformin in humans also remain to be determined, all of which requires further research.

### *4.2. Complex I Inhibition and Modulation of ROS Output*

A popular founding theory proposed to explain metformin's anti-hyperglycaemic action was the indirect and transient inhibition of complex I (Figure 1). This mechanism was first proposed by El-Mir et al. [22] and was observed through the incubation of hepatocytes and isolated mitochondria. As metformin has a positive charge at physiological pH, it has an affinity to accumulate in the mitochondrial matrix as a response to the mitochondria's changes in membrane potential [63,64]. Complex I is the first step along the electron transport chain and is responsible for oxidising NADH to NAD+, using the two electrons gained to reduce ubiquinone to move electrons along the chain. This supplies complexes III and IV with electrons to reduce $O_2$ to water. The energy released from the NADH:ubiquinone redox reaction drives the proton motive force across the inner membrane, powering the synthesis of ATP [63]. Through inhibition of complex I, the proton gradient along the inner membrane of the mitochondria is reduced, leading to a reduction in ATP synthesis, thus increasing the AMP/ATP ratio [63]. It has been additionally theorised that metformin can reduce oxidative stress through complex I by reducing the incidence of superoxide generation driven by the reverse electron transport (RET) process. RET may occur in dysfunctional mitochondria where the regular flow of the electron transport chain is disrupted. An increase in the proton motive force and an overly reduced coenzyme Q pool can drive electrons against the redox potential gradient back to complex I [18]. During RET, NAD+ is reduced back into NADH at complex I and, as a consequence of this process, superoxide is produced from free-floating oxygen at the flavin monohydrate site of complex I [59,65]. By mildly inhibiting complex I, metformin could theoretically lower the proton motive force, limiting RET-associated superoxide production driven by dysfunctional mitochondria [42].

Although complex I inhibition is often cited as metformin's primary mechanism of action, a common criticism of its therapeutic relevance is that the concentration required to observe this mechanism in vitro (>1 mM) typically exceeds the therapeutic levels achievable systemically after an oral dose [66,67]. Ravera et al. [68] were able to measure metformin's inhibition of complex I in bovine rod outer segments by observing the effect on aerobic respiration. They observed the inhibition of complex I and a reduction in reactive oxygen intermediates at metformin levels above therapeutic concentrations (>1.5 mM). Conversely, an in vivo study conducted by Xu et al. [21] measured complex I activity in the retina of mice to have decreased 2 h after 300–500 mg/kg of metformin had been administered subcutaneously (an equivalent dose to oral metformin in humans). To this day, the relevance of complex I inhibition as a realistic therapeutic target of metformin remains contentious and requires more in vivo research. However, metformin has also displayed antioxidant effects through other avenues within a more therapeutically achievable range.

### *4.3. AMPK Activation*

AMPK is the energy sensor for the cell; it plays a crucial role in adapting the cell to low energy conditions. Cellular processes including cell growth, proliferation, apoptosis and autophagy are regulated by AMPK to conserve energy and cellular materials [69,70]. AMPK consists of a catalytic α-subunit and two regulatory subunits labelled β- and γ-. AMP allosterically binds to the γ-subunit of AMPK, promoting the phosphorylation of Thr172 within the α-subunit by Liver kinase B1 (LKB1), causing an upstream kinase of AMPK [13,63]. Inversely, ATP can also bind to the γ-subunit, inhibiting its activation [71]. Through this pathway, metformin can indirectly influence the activation of AMPK through

inhibition of complex I, hindering ATP synthesis and increasing the availability of AMP. However, as stated earlier, the complex I-dependent pathway of AMPK activation has since been complicated by questions of therapeutic relevance. Through knockout trials on animal models, Zhang et al. [72] determined that metformin can activate AMPK independently of its complex I inhibition by acting on the lysosomal membrane-bound v-ATPase-regulator complex (Figure 1). This conformational change allows for the anchoring of the scaffolding protein Axin, which translocates to the lysosome and promotes LKB1 phosphorylation of AMPK through the formation of a v-ATPase-regulator-AXIN/LKB1-AMPK complex [72]. Ma et al. [41] later determined this method of AMPK activation to be the result of metformin directly binding to Presenilin Enhancer 2 (PEN2) along the lysosomal membrane, and once bound, PEN2 forms a complex with the ATP6AP1 subunit of v-ATPase [41], finally leading to metformin activation of AMPK independent of the complex I inhibition pathway.

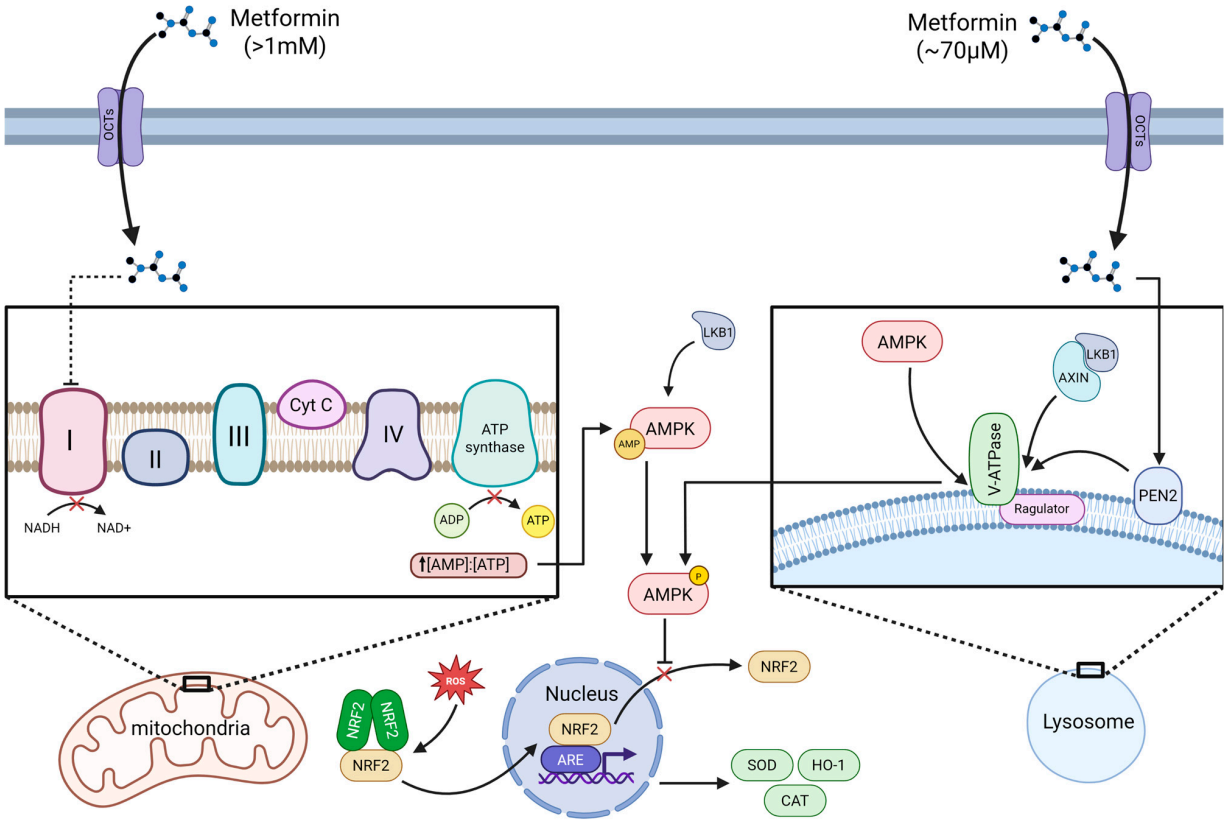


**Figure 1.** Visualisation of pathways for metformin-induced AMPK activation. Metformin enters the cell via organic cation transporters (OCTs). At higher dosages (>1 mM), metformin transiently inhibits complex I activity, leading to an increase in available AMP and a decrease in ATP. AMP binds to AMPK, priming it for activation via Liver kinase B1 (LKB1) phosphorylation. At lower dosages (~70 μM), metformin binds to Presenilin Enhancer 2 (PEN2), leading to the formation of a v-ATPase-regulator-AXIN/LKB1-AMPK mega complex and the phosphorylation of AMPK. Phosphorylated AMPK acts on NRF2 expression by inhibiting its export from the nucleus, leading to prolonged antioxidant transcription. Created in BioRender. Suphioglu, C. (2026) https://BioRender.com/ezbnwsq.

At clinically relevant concentrations in the liver (~70 μM), AMPK has been found to be activated in hepatocytes without the inhibition of complex I and the subsequent increase in the AMP:ATP ratio [35,72]. Available systemic plasma concentrations after hepatic uptake reduce to 10–40 μM in humans, which falls well below the concentrations often used to observe complex I inhibition [73]. Studies measuring ocular tissue metformin accumulation with a correlation of AMPK pathway activation remain limited. A study by Xu et al. [21]

demonstrated that an average of ~100 ng had accumulated in the retinas of mice that had been subcutaneously injected with 300–500 mg/kg of metformin. They identified that at this level of retinal accumulation, metformin increased retinal expression of AMPK [21]. With the activation of AMPK, metformin can influence a variety of signalling pathways that can contribute to reduced oxidative stress.

The nuclear factor kappa B (NF-κB) mediated signalling pathway is a major regulator of immune response and a source of oxidative stress. NF-κB can be inhibited by AMPK via several downstream targets of AMPK activation. Deacetylation of the p65 protein of NF-κB by silent information regulator type 1 (SIRT1), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) binding to p65, p53 inhibition of glycolysis, and Forkhead box O 1 (FOXO1)-mediated inhibition are all downstream targets of AMPK activation that can inhibit NF-κB activity [24]. Through each of these avenues, AMPK can attenuate immune response and associated oxidative stress.

PGC-1α, in addition to inhibiting NF-κB, also stimulates the production of new mitochondria via the activation of mitochondrial biogenesis [74]. Additionally, autophagy/mitophagy can occur through the AMPK-dependent phosphorylation of Unc-51-like autophagy-activating kinase 1 (ULK1) and inhibition of the mechanistic target of rapamycin (mTOR) [25]. In the event of mitochondrial dysfunction due to oxidative damage, the combination of these downstream targets of AMPK can influence the creation of new mitochondria and recycle dysfunctional ROS-producing mitochondria. AMPK can additionally prolong the production of antioxidants in the presence of oxidative stress by acting on the NRF2 pathway.

### *4.4. NRF2 Nuclear Translocation and Upregulation of Antioxidant Response Gene*

NRF2 is a major regulator of oxidative stress response and a primary source of metformin-induced expression of antioxidant enzymes (e.g., SOD2, Glutathione-peroxidase, catalase, Heme oxygenase-1, and nicotinamide adenine dinucleotide phosphate [NADPH] quinone oxidoreductase 1) [5,75,76]. Under regular conditions, NRF2 is kept sequestered in the cytoplasm in an inactive state by being bound to Kelch-like ECH-associated protein 1 (Keap1) [75]. Keap1 is a substrate adaptor protein of Cullin 3 and facilitates the attachment of ubiquitin to NRF2, ensuring its proteasomal degradation [75]. This is a rapid process and ensures a consistent turnover of NRF2 production (5–7 min half-life) to maintain a baseline concentration [43]. Keap1 acts as a sensor for oxidative stress, and in a heavily oxidative environment, cysteine residues become oxidised, leading to a conformational change in Keap1 and the release of NRF2. Once free of Keap1, NRF2 accumulates in the cytoplasm until it is phosphorylated at the Ser40 residue by protein kinase Cδ, which promotes its translocation to the nucleus [77]. Upon arriving in the nucleus, NRF2 binds to the antioxidant response element (ARE) and begins the expression of NRF2-targeted antioxidant genes [43]. The continuation of the NRF2 transcriptional pathway can be promoted via activation of the AMPK (Figure 1). AMPK-dependent phosphorylation of NRF2 at the Ser558 residue, a canonical nuclear export signal, can lead to the retention and accumulation of NRF2 in the nucleus [77,78]. Further to this effect, AMPK can also inhibit glycogen synthase kinase 3 beta (GSK3β), which is responsible for the downstream nuclear export of NRF2 and resumption of Keap1-facilitated degradation [77]. Through AMPK-mediated phosphorylation of Ser558 on NRF2 alongside the inhibition of GSK3β, metformin can extend the length of ARE antioxidant gene expression in response to cellular oxidative stress. Through these, metformin has demonstrated that it has an adaptable catalogue of pathways at its disposal, in which it can reduce consequential cellular responses in the presence of exogenous and endogenous oxidative stress factors. This can allow metformin to be a versatile treatment across multiple categories of ocular disease.

## 5. Disease-Specific Evidence

### *5.1. Cataract*

Cataracts are characterised by the gradual opacification of the lens, altering its light refractive properties and leading to progressively cloudy vision. The transparency and refractive properties of the lens depend on the specific arrangement of lens fibres [79]. Protein aggregation and fibre alterations caused by oxidative damage are the main drivers of opacification of the crystalline lens [76,79]. Ageing is the predominant risk factor for development of cataracts; the deterioration of antioxidant systems and induction of cellular senescence can leave lens cells vulnerable to external stressors such as UV radiation [76]. The accumulation of UV-related oxidative damage, alongside age-deteriorated antioxidant capacity and other factors, can lead to the aggregation of lens proteins, which can reduce the transparency of the lens [80]. Crystallin proteins play a key role in the maintenance of lens structure; they act as chaperones and maintain lens transparency by preventing protein misfolding and aggregation [79]. Senescence of lens cells via oxidative stress has been associated with the modification of crystallin proteins, leading to a reduction in their functional capacity and even the formation of light-scattering crystallin aggregates [79–81]. Spartalis et al. [82] (Table 2) retrieved lens specimens from type 2 diabetic patients treated with oral metformin that had undergone cataract surgery. Through analysis of metformin concentrations, they were able to confirm the presence of metformin within the lens cells at similar concentrations (~0.75 pg/µL) between low-dose (≤1000 mg/day) and high-dose (>1000 mg/day) groups. Through the cultivation of healthy human lens epithelial cells, they were able to confirm a significant reduction in cellular proliferation without inducing arrested growth at the concentration observed in the human lens samples [82]. This observed effect is consistent with the AMPK-dependent inhibition of growth pathways. Further to this, Chen et al. [81] (Table 2) demonstrated that metformin can discourage the senescence of lens cells in vivo via its influence on the AMPK pathway. They determined that AMPK-dependent pathways were inactive in senescent lens cells. Metformin was able to stimulate AMPK activation (Figure 2) and restore the autophagic flux via inhibition of mTOR and activation of SIRT1. SIRT1 deacetylates FOXO1, which in turn promotes autophagy [81,83]. These findings highlight metformin's potential to delay the onset of cataract formation through stimulation of the body's natural repair mechanisms.

**Table 2.** Evidence hierarchy table of metformin's effects on ocular diseases.

| Study | Design/Model | Findings | References |
|---|---|---|---|
| Cataract | | | |
| In vitro | Senescence of human HLE-B3 cells was induced via hydrogen peroxide treatment. | Metformin (2 mM) activated AMPK in senescent cells, stimulating autophagy and downregulating mTOR expression | [80] |
| | Human HLE-B3 cells were pre-treated with metformin 24 h prior to oxidative injury induced via TBHP treatment. | Metformin (0.5 mM) pre-treatment lowered ROS, lipid-peroxidation, and apoptosis. Protection was SIRT1/FOXO1-autophagy dependent | [84] |
| In vivo | Mice were allowed to age naturally in a controlled environment. At 10 months old the diet of the treatment group mice was supplemented with metformin. Both groups were euthanized at 20 months old and lenses were extracted. | The incidence of cataracts was significantly reduced in the metformin (0.1%) treated group (19%) compared to the control (95.23%). Activation of AMPK increased in metformin group compared to the control. | [81] |
| | Human lens capsule specimens were collected from patients undergoing cataract surgery. Patient groups were stratified by low-dose (≤1000 mg/day, n = 10) and high-dose (>1000 mg/day, n = 10) | Metformin was present in the lens samples of both low dose (0.77 pg/µL) and high dose (0.73 pg/µL). | [82] |
| Observational | Three independent cohorts from the prospective, population-based Rotterdam Study (n = 11,260) | Untreated type 2 diabetes was associated with higher cataract risk, but no association between metformin use and cataract formation. | [85] |

**Table 2.** *Cont.*

| Study | Design/Model | Findings | References |
|---|---|---|---|
| RCT | None identified | | |
| | Glaucoma | | |
| In vitro | Human TM cells were exposed to dexamethasone and TBHP separately and then metformin. | Low dose metformin (1 mM) reduced the intracellular ROS induced by both dexamethasone and TBHP. | [86] |
| In vivo | Dexamethasone-induced ocular hypertension was established in mice. Mice were then treated with topical metformin. | Metformin (0.3%) attenuated the fibrosis in the TM cells of the mice, lowering IOP compared to the control. | [86] |
| | Sprague–Dawley rats underwent glaucoma filtrating surgery. Metformin was administered topically four times a day post operation. | Metformin eyedrops (7.85 mM) resulted in reduced collagen deposition and reduced static markers of fibrosis. | [11] |
| | Mouse retinal I/R model was achieved through artificially elevating IOP, metformin was administered once a day via intraperitoneal injection | RGC density was preserved at 10 mg/kg of metformin but not at 50 mg/kg. Metformin also raised AMPK phosphorylation and mitophagy markers. | [7] |
| | Mouse retinal I/R model was achieved through artificially elevating IOP, metformin was administered the day prior to and the day of I/R injury. | Intravitreal injections of metformin (25 µg) protected RGC's from I/R injury though AMPK-driven mitochondrial fusion. Metformin protection of RGC's cell viability mimicked that of mitochondrial ROS scavenging antioxidant mito-TEMPO. | [87] |
| Observational | Three independent cohorts from the prospective, population-based Rotterdam Study (n = 11,260) | Untreated type 2 diabetes was associated with higher glaucoma risk. Metformin was found to be associated with a lower risk of glaucoma disease progression compared to control and other diabetes medication (insulin and sulfonylurea) | [85] |
| RCT | None identified | | |
| | Diabetic retinopathy | | |
| In vitro | Using metformin, a human RPE cell line was pre-treated, co-treated, and post alongside methylglyoxal | Metformin (2 mM, 6 mM, and 20 mM) was capable of protecting the cells from methylglyoxal-induced mitochondrial ROS at all treatment points. | [88] |
| In vivo | Mice received intravitreal injections of methylglyoxal and metformin | Metformin (5 µg, 7 mM) reversed methylglyoxal-induced cotton-wool spots, macular oedema and retinal damage. | [88] |
| | A type 2 diabetic rat model was induced by high-fat diet and streptozotocin injection, diabetic retinopathy development was confirmed prior to metformin treatment. | Oral metformin monotherapy (300 mg/kg) reduced retinal apoptosis and ROS expression | [89] |
| Observational | Taiwan patients ≥ 20 years diagnosed with type 2 diabetes and prescribed long-term Metformin (n = 10,044) | Metformin was associated with lower NDPR risk (aHR = 0.76) and lower PDR (aHR = 0.29) compared to the control in an inverse dose response manner. | [90] |
| RCT | None identified | | |
| | Age-related macular degeneration | | |
| In vitro | Human retinal epithelial cells (D407) were treated with hydrogen peroxide to induce cytotoxicity | Metformin (1 mM) attenuated $H_2O_2$-induced viability loss, apoptosis, ROS elevation and mitochondrial-potential collapse through AMPK-dependent autophagy. | [91] |
| In vivo | Mice injected with metformin subcutaneously were exposed to bright light and sodium iodate treatment to induce RPE damage. *Pde6b*$^{rd10}$ mice were used to test metformin's effects on inherited aggressive rod degeneration. | Subcutaneous metformin injections (300–500 mg/kg) once-daily activated retinal AMPK, protected photoreceptors and RPE from degeneration due to inherited mutations or oxidative stress. | [21] |
| Observational | A Case-control study of patients with and without AMD matched with controls without a type 2 diabetes diagnosis (n = 464,021) | Metformin was associated with reduced odds of any AMD in people without diabetes. | [92] |
| RCT | Randomised phase II clinical trial, patients presenting for late-stage atrophic AMD (n = 66) were given oral metformin 2000 mg daily for 18 months | Mean atrophy area enlargement rate of metformin group (0.35 ± 0.04 mm/year) was not statistically different from the control (0.42 ± 0.04 mm/year) | [93] |

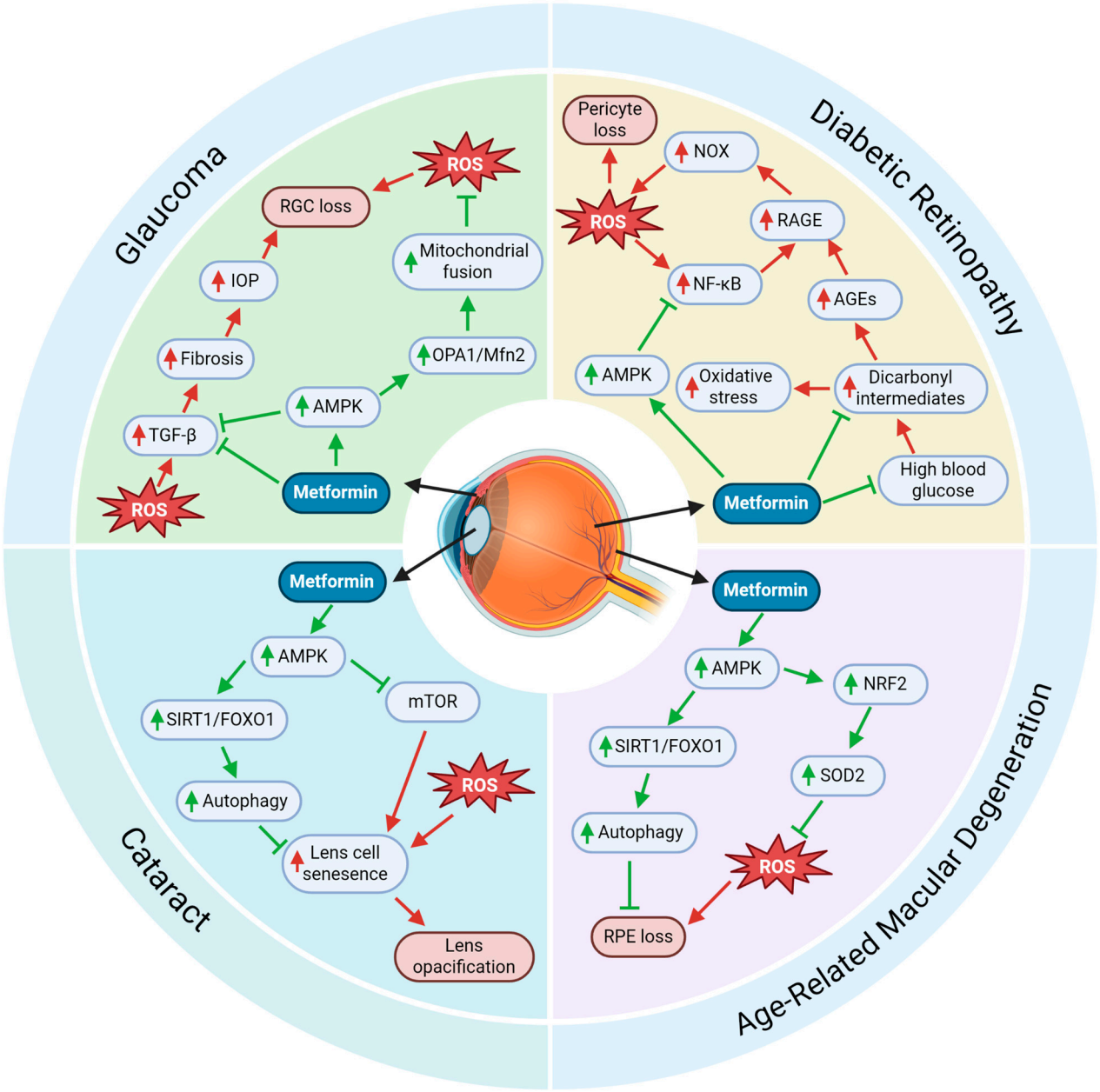


**Figure 2.** Visualisation of ROS in ocular disease and observed pathways in which metformin prevents/alleviates oxidative stress and the detrimental progression of cataract, glaucoma, diabetic retinopathy and age-related macular degeneration. Arrows in red indicate the disease progression pathway; arrows in green represent the metformin pathway; flat-headed arrows signify the point at which metformin interrupts the disease progression pathway. Created in BioRender. Suphioglu, C. (2026) https://BioRender.com/0ia3dya.

### *5.2. Glaucoma*

Glaucoma is a class of optic neuropathy that is characterised by progressive and irreversible damage to the optic nerve head and, consequently, the visual field. The condition is one of the most common causes of irreversible blindness in the world and affects more than 70 million patients worldwide, with the most common form being primary open-angle glaucoma, representing 74% of all cases [94]. A major risk factor of glaucoma is high intraocular pressure (IOP) resulting from impaired aqueous humour drainage [94]. The increased IOP places strain on retinal ganglion cells (RGC), resulting in ganglion cell degradation and mechanical distortion, which leads to the characteristic visual field loss of

glaucoma [10]. Located at the iridocorneal angle, the trabecular meshwork (TM) is the primary outlet for flow of aqueous humour out of the anterior chamber [95]. Oxidative stress can lead to major damage in TM cells, leading to DNA damage, morphological changes and extracellular matrix (ECM) accumulations that can reduce the permeability of TM cells [45]. In patients with glaucoma, mitochondrial DNA damage and oxidative stress in the TM can be observed to be significantly higher than in healthy patients [45]. The aqueous humour consistently generates ROS due to exposure to UV light and byproducts of cellular metabolism [96]. Because the aqueous humour is in constant contact with the TM as it is drained, the TM is being consistently assaulted by exogenous ROS. Oxidative stress can impair normal tissue repair, leading to the recruitment of pro-inflammatory cytokines and growth factors, such as transforming growth factor-beta (TGF-β), which have been shown to be responsible for the proliferation of fibroblast activity [10]. Metformin (2 mM) has been shown to be capable of inhibiting TGF-β activity in human conjunctival cells in vitro, reducing cell proliferation and fibrosis [11]. These results were reflected in a subsequent rat glaucoma filtration surgery model where rats treated with metformin eye drops (7.85 mM/drop) demonstrated a reduction in fibrosis under histological examination [11]. The pro-fibrotic ECM remodelling in glaucoma results in increased tissue stiffness, increasing the resistance of aqueous humour outflow, which increases the IOP within the anterior chamber [10]. A study by Ren et al. [94] demonstrated a metformin-induced reduction, both in vitro and in vivo, of fibrotic development via AMPK-mediated promotion of autophagy, as well as inhibition of TGF-β (Figure 2), alleviating fibrosis development and clearing excessive ECM, preventing the accumulation of IOP that results in strain on RGCs in glaucoma patients [94]. Additionally, there is evidence that metformin can directly bind to TGF-β, antagonising its signalling independent of activation of AMPK [97].

In addition to IOP elevation, glaucoma is characterised by the progressive degeneration of RGCs. Mitochondrial dysfunction and oxidative stress are among the key pathological mechanisms associated with the loss of RGCs in cases of glaucoma [98]. A study conducted by Zhang et al. [87] found that following ischemia/reperfusion (I/R) injury, RGC mitochondrial fission was heavily favoured, leading to increased ROS production and subsequent neuronal cell death [87]. Through AMPK-dependent upregulation of mitofusin-2 (Mfn2) and Optic Atrophy 1 (OPA1) (Figure 2), metformin favoured mitochondrial fusion, leading to a reduction in ROS production following an I/R event [87]. Supporting this, Satriano et al. [7] demonstrated the effectiveness of metformin's neuroprotective effect by running an eleven-day low-dose (10 mg/kg) metformin treatment on a mouse model. After four days of metformin treatment, they induced a retinal I/R event in the mice and resumed metformin treatment for an additional seven days. At the end of the treatment period, they found that mice treated with metformin significantly preserved RGC density following the ischemic event [7]. Consequently, what these papers have demonstrated through in vivo animal models is the neuroprotective effects of metformin in RGCs against the underlying mechanisms of oxidative stress.

### *5.3. Diabetic Retinopathy*

Diabetic retinopathy is a detrimental microvascular complication that results in the visual impairment of approximately 30–40% of adult diabetic patients [99]. Non-proliferative diabetic retinopathy (NPDR) refers to the early stages of diabetic retinopathy in which microaneurysms can form and vascular permeability increases, compromising the integrity of the BRB [99,100]. These vascular complications can result in ischemia, leading to the release of vascular endothelial growth factor (VEGF), which can induce neovascularisation of the compromised vessels [101]. This can contribute to the progression of the disease to proliferative diabetic retinopathy (PDR), which can lead to haemorrhage and retinal

detachment, greatly increasing the risk of vision loss [100]. A large contributor to oxidative stress along the retinal capillaries is advanced glycation end-products (AGEs) [102]. AGEs are a collection of irreversible end-products that are majorly implicated in the development of diabetic retinopathy; they are formed through non-enzymatic multi-step processes commonly referred to as a Maillard reaction [88,99]. Reducing sugars (glucose) react with a free amino group (typically from lysine or arginine) in the bloodstream to form a Schiff base. The Schiff base undergoes an Amadori rearrangement of its molecular structure to become more stable, resulting in an Amadori product [102,103]. The Amadori product then undergoes a prolonged series of reactions and rearrangements through dicarbonyl reactive intermediates, eventually resulting in stable and irreversible AGEs [104].

One of the largest contributors to the accelerated formation of AGEs in the bloodstream is the accumulation of glucose as a result of diabetic hyperglycaemia [102]. Through metformin's mild inhibition of hepatic gluconeogenesis (Figure 2), the amount of available glucose in the bloodstream is reduced, thereby indirectly reducing the frequency of the glycation process [99,105]. Metformin has also been shown to be able to directly bind with reactive α-dicarbonyl compounds in the bloodstream, more directly reducing oxidative stress in plasma and directly discouraging the final steps of AGEs formation through the elimination of intermediates [102,105]. The accumulation of AGEs in the bloodstream can reach retinal capillaries and can initiate the NPDR stage. Along the outside of retinal capillary endothelial cells, there are mural cells called pericytes that contribute to contractile function and microvascular integrity of retinal capillaries [106]. During NDRP, pericytes are lost via apoptosis, resulting in increased capillary permeability and formation of microaneurysms [100,107]. The binding of AGEs to their receptor (RAGE) in retinal pericytes promotes the production of ROS, eliciting apoptotic cell death, and initiating the early stage of diabetic retinopathy [100].

The AGE-RAGE interaction stimulates excessive p47phox- and Rac1-dependent NADPH oxidase (NOX) activation. NOX produces superoxide molecules by catalysing NADPH oxidation to NADP+ [100,106]. The resulting oxidative stress leads to the upregulation of NF-κB, leading to greater expression of RAGE and pro-inflammatory cytokines, creating a self-feeding cycle that increases the generation of oxidative stress in pericytes and endothelial cells (Figure 2) [100,105]. Metformin can suppress RAGE expression via AMPK-mediated suppression of NF-κB signalling, suppressing the uncontrolled cascading expression of RAGE that leads to excessive oxidative stress [100].

The vessel ischemia resulting from the microaneurysms formed from pericyte loss triggers the expression of VEGF, leading into the second stage of diabetic retinopathy (proliferative). Pericytes discourage angiogenesis through the suppression of VEGF signalling [106]. Pericyte loss via apoptosis removes this limiter and allows for neovascularisation of retinal capillaries. The newly formed vessels are fragile and can easily haemorrhage, leading to the accumulation of blood in the vitreous cavity and the eventual vision loss of the patient [107]. Metformin has been shown to attenuate VEGF receptor 2 (VEGFR2) proteins in the retinas of mice with oxygen-induced retinopathy [108]. Through a reduction in VEGFR2 availability, metformin demonstrated a capacity to reduce the VEGF-dependent formation of new vessels, hindering the progression of NPDR to PDR and reducing the risk of detrimental outcomes. As diabetic retinopathy is a complication of diabetes, the indirect benefits of glycaemic control and the direct antioxidant mechanisms are difficult to separate. Mechanistic studies nonetheless indicate that through lowering the availability of glucose in the bloodstream, directly binding to dicarbonyl intermediates, and disrupting the AGE-RAGE expression cascade, metformin may discourage oxidative stress and diabetic retinopathy progression at multiple stages.

### *5.4. Age-Related Macular Degeneration*

Age-related macular degeneration (AMD) is the leading cause of blindness in those over the age of 50 and affects more than 30 million people worldwide [21,91]. As the name suggests, age is a predominant risk factor in the development and progression of AMD. Endogenous factors (genetic predisposition and cardiovascular malfunction) as well as exogenous oxidative stressors (smoking and poor dietary intake) are also major contributors to AMD risk in addition to age [49]. As per the Beckman classification system, AMD is classified into three main stages: early AMD, intermediate AMD and late AMD [109]. Early/intermediate AMD is characterised by the formation of extracellular deposits of lipids, minerals, and proteins called drusen. Early AMD is characterised by small to medium drusen formations (>63 µm and ≤125 µm) and the progression to intermediate is characterised by the formation of at least one large drusen (>125 µm) and/or the presence of pigmentary abnormalities [110]. The vision loss associated with AMD is related to the further progression into the late stage neovascular AMD or atrophic AMD [109,111]. Neovascular AMD is characterised by the formation of abnormal blood vessels in the macula in response to the abnormal expression of VEGF; the stimulation of new vessel growth and permeability can lead to fluid leakage, causing haemorrhaging and fibrosis [110]. Neovascular AMD is less common than non-neovascular forms of AMD; however, it is disproportionately responsible for approximately 75% of severe vision loss in AMD patients [112]. While neovascular AMD can result in more detrimental patient outcomes, the intravitreal injection of VEGF inhibitors is an existing and effective treatment, reducing induced blindness by approximately half [113]. Atrophic AMD, often referred to as geographic atrophy, is characterised by areas of RPE and photoreceptor atrophy [57,91]. As the retina is one of the highest oxygen-consuming tissues in the body, the age-related reduction in antioxidant defences in RPE cells and the accumulation of oxidative stress can damage RPE tight junctions and disrupt the BRB [56]. As RPE cells are responsible for supplying nutrients to the photoreceptors, a secondary effect of impaired RPE function results in the secondary deterioration of photoreceptors, leading to vision degradation [91,110].

Using human AMD donor RPE cells in vitro, Golestaneh et al. [114] found that AMD patient RPE cells were unable to respond to an increase in oxidative stress due to a reduction in antioxidant function. AMD patient cells were unable to increase the expression of mitochondrial SOD2 compared to the control under induced stress conditions [114]. The inability to increase SOD2 expression was correlated with oxidative-induced RPE cell death, likely due to the diminished clearance of ROS. Through the AMPK/SIRT1/FOXO1 pathway described in the prevention of lens cell senescence, AMPK can prevent the deterioration of RPE cells via the activation of autophagic pathways [115]. Additionally, through the NRF2 pathway (Figure 2), metformin promotes the transcription of SOD2, alleviating mitochondrial oxidative stress in RPE cells [75].

To the best of our knowledge, one randomised controlled trial (RCT) of non-diabetic patients has been conducted. Shen et al. [93], through their RCT, were able to establish an acceptable safety profile for the oral use of twice daily 1000 mg of metformin in non-diabetic patients. However, they found no significant benefit of metformin in the management or treatment of late atrophic AMD. While their findings indicated a lack of efficacy in the progression of patients already diagnosed with late atrophic AMD, their restricted sample size (n = 66) may have hindered their statistical power. Additionally, their study did not test the progression of early/intermediate AMD to late atrophic AMD, as the eligibility criteria for participant involvement required observable atrophy (1.25–17.5 $mm^2$) [93].

The chronic nature of neovascular AMD is related to the irregularly high expression of VEGF, which promotes the creation of new vessels, increasing permeability and leading to fluid leakage, haemorrhages and fibrosis [110]. Early/intermediate AMD can progress into

late AMD through neovascularisation [57]. Similarly to diabetic retinopathy, metformin can reduce the formation and permeability of new vessels by discouraging VEGF-VEGFR2 binding [108]. However, the intravitreal injection of anti-VEGF agents is an effective first-line treatment for the progression of neovascular AMD [111]. As the potential preventative benefits of metformin for progression of neovascular AMD are a secondary function, it would be unlikely to be utilised as a primary treatment.

## 6. Therapeutic Implications for Ocular Disease and Gaps in Current Knowledge

Metformin is typically prescribed as an oral medication, ranging from a dosage of 500 mg to 2000 mg and is not commercially available in a topical eyedrop formulation since ocular treatment is still a novel endeavour [13]. As mentioned previously, the eye is a specialised organ with multiple static barriers of entry, including the BRB, BAB, and the cornea/sclera. As demonstrated by Xu et al. [21] (Table 2), mice injected with metformin subcutaneously (300–500 mg/kg, the equivalent of 1500–2500 mg dose in humans) resulted in the accumulation of metformin in retinal pigment epithelial cells (~100 ng/retina), indicating that metformin may be capable of crossing the BRB. While no studies to date have directly investigated metformin's ability to cross the BAB, Pietrowska et al. [116] reported evidence of metformin in the aqueous humour samples of diabetic patients who had undergone cataract surgery. Similarly, Spartalis et al. [82] found metformin in patient lens samples. Although more direct evidence is needed, these studies suggest the possibility that metformin from blood plasma can cross the BRB and BAB.

As metformin cannot passively diffuse across the ocular surface and instead relies on saturable transporters such as OCTs, retention along the ocular surface is an issue. More recent efforts have been made to optimise metformin for ophthalmic application, utilising formulations such as in situ gels and microparticles to increase retention and reduce clearance from lacrimal drainage systems [9,31]. Through loading metformin into silk fibroin microparticles, Polat et al. [9] were able to increase the retention time of metformin on the ocular surface and increase uptake. Through a rat corneal burn model, they demonstrated that a solution containing just metformin improved corneal neovascularisation compared to the control. However, metformin-loaded silk fibroin microparticles were able to exert a significant reduction in corneal neovascularisation comparable to dexamethasone at a third of the application frequency [9]. While there are studies demonstrating the viability of metformin uptake on the ocular surface, there has been little mechanistic demonstration of metformin's antioxidant effects on ocular surface diseases; however, a study by Rescher et al. [117] demonstrated an increase in lipid production of meibomian gland epithelial cells in vitro in response to metformin treatment. This finding can potentially have positive implications for treatment of dry eye, as impaired lipid production can lead to excessive tear evaporation. However, it should be noted that metformin was found to induce cell death in a dose- and duration-dependent manner (5–10 mM, 48 h), indicating a potential upper limit to metformin's effectiveness in a highly concentrated topical solution [117]. Additionally, Tsoi et al. [118] evaluated an improvement in the corneal nerve fibre density in diabetic patients treated with oral metformin by utilising in vivo corneal confocal microscopy. This tool could be similarly employed to monitor corneal response to topical metformin treatment alongside tear film metrics and biomarker readings.

Other administration methods, such as the intravitreal injection of metformin (5–25 μg) into the eyes of mice, have demonstrated positive outcomes for RPE cell viability [87,88].

Despite decades of clinical use, the true extent of metformin's interactions and benefits is still not fully understood. Metformin's influence can be demonstrated in vitro and in vivo through both direct and indirect modulation of key pathways such as AMPK/mTOR,

autophagy, AGEs formation, NRF2 antioxidant cascades, VEGF angiogenesis, and NF-κB inflammatory response. However, our understanding of these pathways remains incomplete, as some mechanistic links are still yet to be discovered [6].

To date, most human studies investigating metformin's effects on ocular diseases are observational from predominantly diabetic populations. Their outcomes can only demonstrate associations and are often unable to control for confounding factors and bias. Type 2 diabetes is heavily associated with the progression of ocular diseases such as cataracts, glaucoma, and diabetic retinopathy, leaving findings vulnerable to confounding due to patient compliance, diabetes severity, and concomitant medications [85]. Claims that metformin prevents ocular disease therefore remain theoretical, based on in vitro, in vivo, and observational evidence. Metformin is a promising candidate for therapeutic repurposing; however, establishing metformin as a viable ocular therapy would require more human data, particularly from RCTs.

## 7. Conclusions

Metformin influences several mechanistic pathways associated with oxidative stress and cellular homeostasis. These mechanisms provide a plausible rationale for repurposing metformin for use in several ocular diseases. AMD, glaucoma, diabetic retinopathy and cataract together represent a large share of vision loss worldwide. Mechanistically, through both complex I-dependent and -independent activation of AMPK, metformin has been shown to exert pleiotropic effects within many different ocular tissues. Through antioxidant activity combined with modulation of inflammatory, angiogenic and fibrotic pathways, metformin has demonstrated a multifaceted mechanistic approach to the treatment of ocular disease beyond its influence on hepatic gluconeogenesis. However, this evidence is primarily preclinical, and it remains uncertain whether these effects can occur at clinically achievable ocular concentrations or translate into meaningful clinical benefits in humans. It is recommended that future research should therefore focus on characterisation of metformin's pharmacokinetics in ocular tissues after systemic and topical administration, compare disease outcomes and administration routes in animal models and early human studies, and conduct RCTs to determine the efficacy of metformin in both diabetic and non-diabetic patients at risk of disease onset.

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## Abbreviations

The following abbreviations are used in this manuscript:

| | |
|---|---|
| AGEs | Advanced glycation end-products |
| aHR | Adjusted hazard ratio |
| ARE | Antioxidant response element |

| | |
|---|---|
| RAGE | AGEs receptor |
| AMD | Age-related macular degeneration |
| AMP | Adenosine monophosphate |
| AMPK | AMP-activated protein kinase |
| ATP | Adenosine triphosphate |
| BAB | Blood–aqueous barrier |
| BRB | Blood–retinal barrier |
| DNA | Deoxyribonucleic acid |
| ECM | Extracellular matrix |
| FOXO1 | Forkhead box O 1 |
| GSK3β | glycogen synthase kinase 3 beta |
| I/R | Ischemia/reperfusion |
| Keap1 | Kelch-like ECH-associated protein 1 |
| LKB1 | Liver kinase B1 |
| mtDNA4977 | 4977-bp long deletion of mitochondrial DNA |
| mTOR | Mechanistic target of rapamycin |
| NADH/NAD+ | Nicotinamide adenine dinucleotide (reduced/oxidised) |
| NADPH | Nicotinamide adenine dinucleotide phosphate |
| NF-κB | Nuclear factor kappa B |
| NOX | NADPH oxidase |
| NPDR | Non-proliferative diabetic retinopathy |
| NRF2 | Nuclear factor erythroid 2-related factor 2 |
| OCT | Organic cation transporter |
| PDR | Proliferative diabetic retinopathy |
| PEN2 | Presenilin enhancer 2 |
| PGC-1α | Peroxisome proliferator-activated receptor gamma coactivator 1-alpha |
| RCT | Randomised controlled trial |
| RET | Reverse electron transport |
| RGC | Retinal ganglion cells |
| RPE | Retinal pigment epithelium |
| SIRT1 | Silent information regulator type 1 |
| SOD2 | Superoxide dismutase2 |
| TEA | Tetraethylammonium |
| TGF-β | Transforming growth factor-beta |
| TM | Trabecular meshwork |
| ROS | Reactive oxygen species |
| ULK1 | Unc-51-like autophagy-activating kinase 1 |
| UV | Ultraviolet |
| VEGF | Vascular endothelial growth factor |
| VEGFR2 | VEGF receptor 2 |

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