

# Newly-found functions of metformin for the prevention and treatment of age-related macular degeneration

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## Abstract

• Metformin (MET), a first-line oral agent used to treat diabetes, exerts its function mainly by activating adenosine monophosphate-activated protein. The accumulation of oxidized phospholipids in the outer layer of the retina plays a key role in retinal pigment epithelium (RPE) cells death and the formation of choroidal neovascularization (CNV), which mean the development of age-related macular degeneration (AMD). Recent studies have shown that MET can regulate lipid metabolism, inhibit inflammation, and prohibit retinal cell death and CNV formation due to various pathological factors. Here, newly discovered functions of MET that may be used for the prevention and treatment of AMD were reviewed.

• **KEYWORDS:** metformin; age-related macular degeneration; liver X receptor

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## INTRODUCTION

Metformin (MET) was first used to treat diabetes in 1957 and is currently one of the most widely used oral glucose-lowering drugs. Recent studies showed that MET can also decrease the risk of developing cardiovascular disease, exert antioxidant, anti-senescence, weight loss, nephroprotective and antineoplastic effects<sup>[1-4]</sup>. In the field of ophthalmology, it has been demonstrated that MET can reduce retinal cell death due to various pathological factors, alleviate

diabetic retinopathy, and inhibit corneal and intraocular neovascularization<sup>[5-15]</sup>. The latest clinical studies revealed that MET can reduce the occurrence or delay the progression of age-related macular degeneration (AMD)<sup>[16-19]</sup>. However, the mechanisms of MET in this process remain unclear. This paper provides a literature review on newly discovered functions of MET that may be used for the prevention and treatment of AMD and the mechanisms involved.

## ROLE OF OXIDIZED PHOSPHOLIPIDS IN PATHOGENESIS OF AMD

AMD is the primary cause of irreversible blindness in elderly people. There are an estimated 196 million patients with AMD globally in 2020<sup>[20]</sup>. AMD is classified as the early, moderate and late stages. The late stage of AMD is classified as dry (atrophic) or wet (neovascular) types according to pathological changes. The former occurs mainly because of the death of retinal pigment epithelium (RPE) cells, resulting in the degeneration of neuroretina, whereas the latter is due to choroidal neovascularization (CNV), which further causes bleeding and exudation.

Currently, AMD is thought to result from a combination of genetic, age-related, environmental, dietary, and other factors, among which age plays a crucial role<sup>[20-21]</sup>. With aging, the phagocytosis of RPE cells is reduced and detached outer segments of photoreceptor cells accumulate in RPE cells and beneath the RPE layer. This results in thickening of the Bruch's membrane and drusen formation<sup>[22]</sup>, *i.e.*, early AMD. Drusen contains abundant apolipoproteins, unsaturated fatty acid phospholipids, and cholesterol. Phospholipids are susceptible to oxidation and tend to form oxidized phospholipids (OxPLs). OxPLs mainly exists as oxidized low-density lipoprotein (OxLDL) *in vivo*. OxLDL enters RPE cells through CD36 receptors and further activates nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3)<sup>[23-24]</sup>. The activated NLRP3 inflammasome can induce the secretion of interleukin-1 $\beta$  (IL-1 $\beta$ ) and IL-18, resulting in RPE cell pyroptosis. Secreted IL-1 $\beta$  can activate the inflammasome to further aggravate inflammation. Additionally, the activated NLRP3 inflammasome can promote CD36 expression and increase OxLDL uptake. These actions reduce ATP-binding cassette transporter A1 (ABCA1)-mediated cholesterol efflux,

further exacerbating intracellular cholesterol overload<sup>[23,25-26]</sup>. Given its strong oxidative and pro-inflammatory properties, OxPLs is considered as a key factor in AMD (Figure 1).

### CURRENT STATUS OF AMD TREATMENT

Pathogenesis of AMD is not completely understood and its treatment remains unsatisfactory. Currently, treatments mainly target CNV in wet AMD, and available treatments include laser photocoagulation, photodynamic therapy, transpupillary thermotherapy, vitreous surgery for the excision of submacular neovascular membranes, as well as intravitreal injection of glucocorticoid and anti-vascular endothelial growth factor (VEGF) agents. Among these methods, intraocular injection of anti-VEGF agents is currently a mainstay treatment and has shown positive effects. However, the recurrence of CNV, high cost, injection-related complications, RPE cell death due to VEGF inhibition, vision loss, etc., should not be ignored<sup>[27]</sup>. In addition, no effective treatment is available for dry AMD, which accounts for more than 85% of AMD cases. The results of the AREDS study showed that antioxidant treatment can only limit disease progression<sup>[28]</sup>. Stem cell and complement-related treatments are still being researched<sup>[27,29]</sup>.

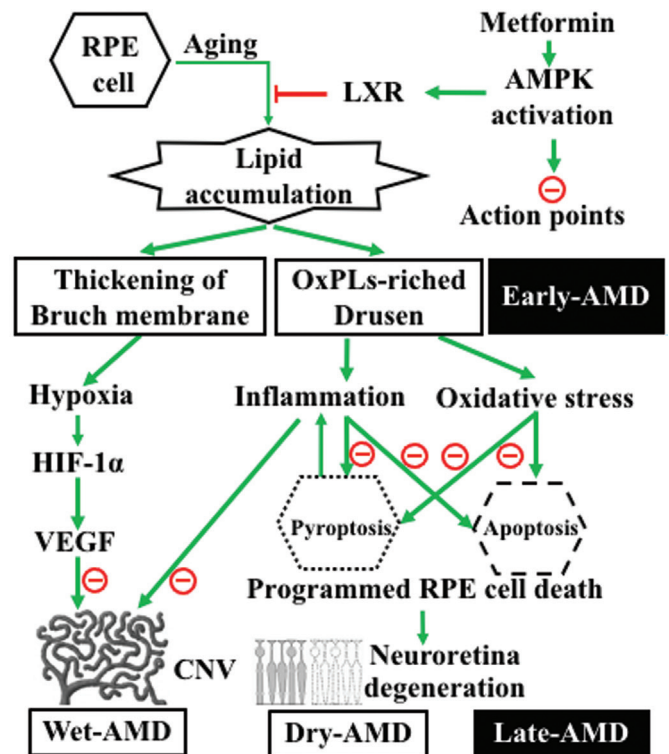
### MAIN FUNCTIONS AND MECHANISM OF MET

MET was isolated from the extract of goat's rue (*Galega officinalis*) and was first used to treat diabetes 64 years ago. In addition to its glucose-lowering effects, an increasing number of studies have shown that MET can regulate lipid metabolism, reduce inflammatory and oxidative damage, and inhibit neovascularization<sup>[3]</sup>. Oxidative stress and inflammation induced by OxPLs are thought to play an important role in the pathogenesis of AMD, hyperglycemia is also considered as a risk factor for AMD. Therefore, it is reasonable to believe that MET may be used for the prevention and treatment of AMD through above effects.

About the mechanism of MET, it is thought that the glucose-lowering effect occur through inhibition of NADH: ubiquinone oxidoreductase (mitochondrial electron transport chain "complex I") in the inner mitochondrial membrane to decrease ATP yield and increase the AMP/ATP ratio<sup>[30]</sup>. This metabolic transformation results in adenosine monophosphate-activated protein kinase (AMPK) activation, which is a key molecule in regulating energy metabolism and plays an important role in diabetes and other metabolic disorders. After activated, AMPK can shut down many downstream synthetic pathways that consume ATP and activate ATP degradation pathways to restore physiological energy equilibrium<sup>[31]</sup>.

### CRUCIAL ACTIONS OF MET FOR CONTROLLING AMD

Recently, retrospective studies<sup>[16-19]</sup> showed that MET decreased the incidence of AMD in a time- and dose-dependent manner. To exclude the effects of glucose-lowering on AMD



**Figure 1** The role of OxPLs in pathogenesis of AMD and the main functions of MET to prevent and treat AMD Lipid accumulation resulted from aging of RPE on one hand leads to the thickening of Bruch's membrane, which further leads to choroidal hypoxia, the expression of HIF-1 and VEGF, and ultimately the formation of CNV; on the other hand, it leads to the formation of drusen, which is the hallmark of early AMD. OxPLs in drusen induce inflammation and oxidative stress, which further cause programmed cell death, such as pyroptosis and apoptosis of RPE cells. Pyroptotic cell in turn aggravates inflammation by releasing pro-inflammation factors, and further promotes CNV formation. The death of RPE cells leads to the degeneration of the neuroretina. CNV formation and retinal degeneration mark the late stage of AMD. MET can activate AMPK. AMPK activation can reduce lipid accumulation through AMPK/LXR pathway, suppress inflammation, prohibit retinal cells from death, and inhibit CNV formation, thus to prevent and treat AMD. OxPLs: Oxidized phospholipids; RPE: Retinal pigment epithelium; AMPK: Adenosine monophosphate-activated protein kinase; LXR: Liver X receptor; HIF-1: Hypoxia inducible factor-1; VEGF: Vascular endothelial growth factor; AMD: Age-related macular degeneration; CNV: Choroidal neovascularization.

occurrence, the authors simultaneously studied the effects of another diabetes drug on AMD occurrence. The results revealed no significant relationship between this drug and AMD. Currently, it is thought that the mechanisms involved are as follows:

**MET Can Reduce Drusen Formation Through AMPK/LXR Pathway** Liver X receptor (LXR) is a ligand-activated nuclear transcription factor, its activation can induce the

expression of lipid metabolism regulating genes and reduce cholesterol accumulation<sup>[32]</sup>. Studies in macrophage showed that MET depends on the AMPK/LXR pathway to increase the expression of inducible degrader of the LDL receptor, increase LDL receptor degradation, and reduce OxLDL uptake. Additionally, MET increases ABCA1/G1 expression to promote cholesterol efflux, thus to reduce intracellular cholesterol accumulation and drusen formation<sup>[33-37]</sup>.

**MET Can Inhibit Inflammation** Macrophages are the most important inflammatory cells. Studies have shown that MET can activate AMPK to inhibit lipopolysaccharide (LPS)-induced synthesis of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), monocyte chemoattractant protein-1 (MCP-1), and reactive oxygen species (ROS)<sup>[38-39]</sup>. Kelly *et al*<sup>[40]</sup> found that MET can inhibit LPS-induced pro-IL-1 $\beta$  expression in a dose-dependent manner while simultaneously increasing the expression of anti-inflammatory IL-10 in macrophages.

In addition to its effects on macrophages, addition of MET to the culture medium of human umbilical vein endothelial cells (HUVEs) *in vitro* significantly inhibited TNF- $\alpha$  induced expression of vascular cell-adhesion molecule-1 (VCAM-1), intercellular cell-adhesion molecule-1 (ICAM-1), E-selectin, and MCP-1. AMPK played a central role in this process<sup>[41-42]</sup>. *In vivo* studies showed that treatment with MET in patients with type 2 diabetes for 3mo significantly reduced the levels of vascular endothelium-related factors, such as tissue plasminogen activator, VCAM-1, and ICAM-1, and improved vascular function<sup>[43]</sup>. In a model of ischemia reperfusion injury, MET decreased the expression of IL-1 $\beta$ , TNF- $\alpha$ , toll-like receptor 4 (TLR4), and chemokine C-C motif receptor 2 (CCR2), and reduced infiltration by monocytes and macrophages, thereby reducing inflammatory damage to the liver<sup>[44]</sup>. In addition, MET promotes a shift from the pro-inflammatory M1 phenotype to an anti-inflammatory M2 phenotype in macrophages to exert its anti-inflammatory effects<sup>[45]</sup>.

In experimental uveitis, the levels of IL-1 $\beta$ , TNF- $\alpha$ , MCP-1, IL-6, and IL-18 are increased in the aqueous humor and the outer segments of photoreceptor cells shrink. MET and AMPK agonists can activate AMPK, thereby inhibiting activation of cyclooxygenase 2, inducible nitric oxide synthase, and NF- $\kappa$ B to inhibit these changes<sup>[46-47]</sup>.

**MET Can Prohibit RPE and Photoreceptor from Death** RPE and photoreceptor death are main pathological changes of late stage AMD. Cell death can be classified as programmed cell death (apoptosis, autophagy, pyroptosis, necroptosis, ferroptosis *etc.*) and necrosis. Studies showed that pyroptosis and apoptosis are the main forms of cell death induced by OxPLs<sup>[48]</sup>.

MET can maintain intracellular lipid metabolic equilibrium through AMPK/LXR pathway, prevent NLRP3 protein

expression and inflammasome activation, and inhibit the release of inflammatory factors, thereby reducing cell death<sup>[49-51]</sup>. In addition, cardiac studies showed that MET can reduce sodium arsenite-induced secretion of IL-5, TNF- $\alpha$ , IL-1 $\beta$ , caspase-3 activation, and cardiomyocyte apoptosis<sup>[52]</sup>. Endothelial cell studies showed that MET can prevent high glucose-induced increased mitochondrial permeability and cytochrome C (Cyt-C) release to prevent endothelial cell apoptosis<sup>[53]</sup>.

Studies have shown that MET can activate the AMPK and PI3K/Akt/mTOR/S6K pathways, increase superoxide dismutase (SOD) and glutathione levels, induce nuclear factor erythroid-2-related factor 2 (Nrf2) aggregation, and increase mitochondrial energy reserves to reduce retinal ganglion cell and RPE cell death in many oxidative stress and inflammation models<sup>[6,8,47,54-63]</sup>. Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 $\alpha$ ) regulates mitochondrial biosynthesis. AMPK activation can regulate mitochondrial function through PGC-1 $\alpha$  and alleviate senescence and injury in RPE cells caused by hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), TNF- $\alpha$ , and ultraviolet light<sup>[56-57,64]</sup>, and photoreceptor cell death<sup>[65]</sup>. Other studies showed that the AMPK-mTOR pathway can induce autophagy in RPE cells which plays an important role in self-clearance and the maintenance of normal cellular function. Therefore, this pathway is also regarded as useful for AMD prevention and treatment<sup>[66-67]</sup>. In summary, MET can activate AMPK to reduce RPE and photoreceptor cell death in the prevention and treatment AMD.

**MET Can Inhibit CNV Formation** The relationship between inflammation and neovascularization has been demonstrated<sup>[68-69]</sup>. OxLDL can induce cell pyroptosis and releasing of IL-1 $\beta$ , which promotes the expression of hypoxia-inducible factor-1 alpha (HIF-1 $\alpha$ ) and VEGF. IL-1 $\beta$  can also stimulate mast cells to secrete IL-8, promoting the survival and proliferation of vascular endothelial cells and expression of matrix metalloproteinase (MMP)-2 and MMP-9, thereby promoting neovascularization<sup>[69-72]</sup>. In addition, the disrupted outer retinal barrier facilitates the spread of inflammatory factors and growth factors as well as the subsequent growth of CNV into the retina. In addition to increasing the release of inflammatory factors, OxLDL can also promote CNV by increasing VEGF, VEGF receptor 2 (VEGFR2), and transforming growth factor beta (TGF- $\beta$ ) expression of vascular endothelial cells<sup>[73-74]</sup>.

As MET can inhibit inflammation, it can reduce inflammation-related neovascularization<sup>[75]</sup>. In addition, experiments showed that AMPK activation can decrease MMP-2 and MMP-9 expression in endothelial cells and endothelial progenitor cells, and inhibit cell proliferation, migration, and tube formation<sup>[76-77]</sup>. Han *et al*<sup>[78]</sup> showed that MET can inhibit TNF-induced expression of ICAM-1 and MCP-1, as well as inhibit the proliferation,



migration, and tube formation of endothelial cells, thereby preventing retinal neovascularization. MET can also reduce intracellular cholesterol in vascular endothelial cells, thus to disrupt the structure of lipid rafts, inhibit VEGFR2 dimerization and phosphorylation, and block VEGF-induced neovascularization<sup>[79]</sup>. Further analysis showed that MET can induce VEGF-A mRNA splicing to form VEGF120, thereby reducing VEGFR2 activation<sup>[5,80]</sup>. Studies of oxygen-induced retinopathy (OIR) and very low-density lipoprotein receptor (VLDL-R) knockout animal models also showed that MET can decrease vascular inflammation and inhibit retinal neovascularization<sup>[5,78]</sup>. In the laser-induced mouse CNV model, intraperitoneal injection of MET could inhibit CNV formation<sup>[9]</sup>. Clinical studies also showed that long-term MET treatment decreased the levels of VEGF and plasminogen activator inhibitor (PAI-1) in patients with type 2, thereby alleviating the severity of diabetic retinopathy and reducing the incidence of AMD<sup>[81]</sup>.

## CONCLUSION

In summary, oxidative stress and inflammation induced by OxPLs, a main component of drusen, can cause RPE cell death and CNV formation, which are the main pathological changes in late-stage AMD. MET was recently found to have the functions to regulate lipid metabolism, inhibit inflammation, prohibit retinal cells from death, and inhibit CNV formation (Figure 1). This makes MET a good candidate drug to prevent and treat AMD. Further in-depth investigation of the mechanism by which MET affects AMD and multicenter clinical studies are needed to validate the efficacy of MET and the way of its administration in AMD.

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