Influence of metformin on age-related macular degeneration

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Abstract

Metformin is the most commonly prescribed antihyperglycemic drug as first-line therapy in type II diabetic patients. In recent years, evidence is increasing that metformin has beneficial effects beyond its classical antihyperglycemic way of action. Those effects include anti-inflammation, anti-oxidation, anti-aging, anti-angiogenesis, anti-neoplasia, anti-apoptosis, and neuroprotection. The complex pathophysiology of age-related macular degeneration (AMD) includes age-related changes in the retinal pigment epithelium (RPE) and Bruch's membrane. An inflammatory and oxidative damage component has also been described. The dry form of late AMD is especially characterized by degeneration of the RPE, Bruch's membrane, the choriocapillaris and finally, loss of the photoreceptors (geographic atrophy), and the wet form of late AMD is characterized by pathological neovascularization. An increasing number of reports about the beneficial effects of metformin on AMD have been published in the last few years. Several effects of metformin could be linked to the AMPK pathway. A first prospective trial investigating the effect of metformin on dry AMD is ongoing with estimated results by the end of 2024. In this review, the current knowledge about the association between metformin and AMD is summarized.

Keywords: Metformin, age-related macular degeneration retina, insulin, diabetes, aging, drug therapy, AMPK pathway

Introduction

Metformin is one of the most commonly used oral antidiabetic drugs. Classically, it is used in non-insulin-dependent type 2 diabetic patients and most cases as the first oral antidiabetic medication. Metformin inhibits the formation of glucose in the liver and improves glucose turnover in the periphery (the muscles) of the body, thereby lowering the blood glucose level [1, 2].

There is increasing evidence that metformin may exert several beneficial effects beyond its original antidiabetic function [3-5]. In summary, *in vitro*, and *in vivo* investigations report anti-angiogenic, anti-inflammatory, anti-oxidative, anti-apoptotic, anti-aging, and neuroprotective effects of metformin [6, 7]. Most of these effects also play a crucial role in many retinal diseases such as diabetic retinopathy (DR), age-related macular degeneration (AMD), glaucoma, uveitis, or inherited retinal dystrophies as retinitis pigmentosa.

AMD is a vision-threatening disease of the elderly population worldwide with increasing prevalence. Wong *et al*. calculated an increase from 196 million affected people in 2020 to 288 million affected people in 2040 [8]. Together with diabetic retinopathy and glaucoma, AMD accounts for the majority of legal blindness cases in developed countries. In Germany, for example, it is estimated that AMD is responsible for up to 50% of legally blind people [9] .

AMD is a progressive, multi-factorial disease with a complex pathophysiology that is still not fully understood in all its details. The main risk factor is age. It is also known that a history of smoking, hyperlipidemia, ethnicity, and a certain genetic disposition as well as inflammatory processes play a role [10, 11]. Clinically, AMD is classified into different stages: an early, an intermediate, and two late stages: dry, non-neovascular, and wet, neovascular late-stage AMD (Figure 1) [12]. The early and intermediate stages are characterized by the size of the drusen deposits and by the presence or absence of pigmentary

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changes. The early and intermediate stages usually have no or only minimal symptoms [10]. Late, neovascular AMD, however, has more profound visual symptoms that can progress rapidly. The symptoms include distortion and/or large central scotoma or blind spot due to hemorrhage or fluid accumulation in the macular region. If left untreated, fibrosis and permanent vision loss are the consequences [10]. Late, non-neovascular AMD is characterized by progressive central vision loss due to degeneration

of the retinal pigment epithelium (RPE) and the photoreceptor cells, referred to as geographic atrophy (GA) [10, 11].

Approved treatment options are currently only available for late, neovascular AMD. The Standard of care is the intravitreal injection of anti-VEGF agents to block angiogenic factors that induce the formation of pathological neovascular vessels. Pathologic neovascularization leads to retinal damage by sub- and/or intraretinal fluid or blood accumulation [13]. Additionally, larger subretinal hemorrhages can be treated by surgical intervention to eliminate the subretinal blood mechanically [14]. The late, dry stage of AMD remains untreatable to date thus efforts are made to find a way to modify the disease. In the last years, an increasing number of scientific publications report on several potential associations of metformin with the course of the disease. This is true for both the development and the treatment of AMD. In this review, we summarize the current knowledge about these associations and the potential underlying (patho)physiological mechanisms.

Method

The systematic literature search was performed using the PubMed library. The search term "metformin age-related macular degeneration" revealed a total of 35 publications (the search was performed on July 20th, 2022). After the screening of titles and abstracts, 22 publications qualified as being suited for the topic of this review. Further database searches with adjusted search terms (metformin AMD, metformin macular degeneration pathways, *etc.*) did not reveal any further relevant articles.

Additional publications have been included for the introductory part as well as for the background part on the pathophysiology of AMD and the mode of action of metformin. These publications were identified by direct database search as well as by backward citation searching.

Pathophysiology of AMD

As mentioned above, the pathophysiology of AMD is complex and several risk factors are associated with this disease. As a neurosensory tissue, the retina, especially the photoreceptor cells, is metabolically highly active. This requires a constant balance between the breakdown of metabolic waste products and the supply of necessary nutrients, including oxygen. In the healthy retina, the RPE with its tight contact with the photoreceptor cells, Bruch's membrane, and the choroidal vasculature execute this important task [15]. The RPE cells form a single cell layer with neighboring cells being connected via tight junctions. This single-cell layer is supported by Bruch's membrane lying underneath. This complex forms the selective diffusion barrier known as the blood-retina-barrier (BRB) which precisely regulates the passage of ions, water, nutrients, proteins, and oxygen [16]. Any change to this sensitive interface, irrespective of its cause (*e.g.* age, disease,
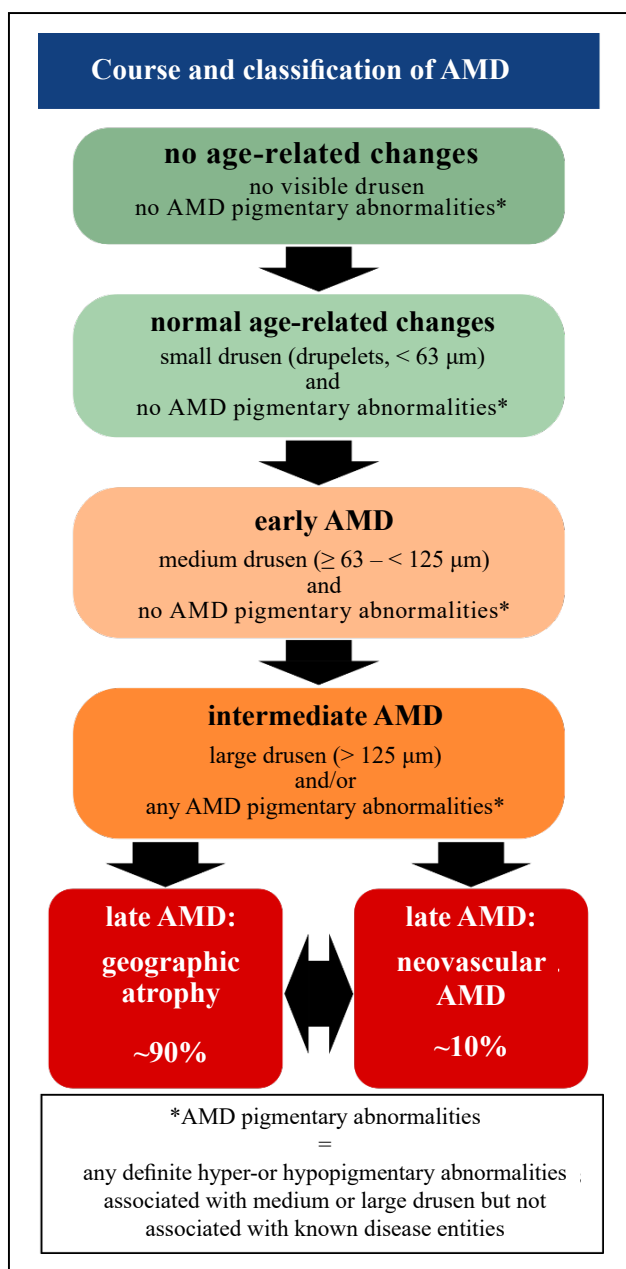

Figure 1. Classification and course of age-related macular degeneration (AMD). The earliest precursor signs of AMD are small drusen that are classified as normal age-related changes. Early AMD is characterized by the presence of medium drusen but the absence of AMD pigmentary changes which are defined as any hyper- or hypopigmentary abnormality. Intermediate AMD shows large drusen and/or the presence of any AMD pigmentary abnormalities. The late stages of AMD are its two distinct forms: neovascular AMD (wet AMD) and geographic atrophy (dry AMD) with the latter being the more common form. Both forms may merge into one another or be present simultaneously. (modified from Ferris *et al.* [12])

environmental factors), will affect the precise metabolic balance [11, 16, 17].

The three main risk factors for AMD are age, environmental risk factors, and genetic predisposition. Age itself influences the viability of both the RPE cells and Bruch's membrane [11]. Age has several negative effects on many intracellular structures of the RPE cells, finally leading to changes in RPE metabolism [11]. Similarly, Bruch's membrane suffers from age-related changes, such as thickening and other structural changes that change its permeability. Altogether, these age-related changes have the potential to negatively influence the integrity of the RPE/Bruch's membrane interface leading to the accumulation of debris that ultimately leads to the formation of drusen [11]. However, this assumption still has to be confirmed. According to Anderson *et al.* local inflammation as a response to debris accumulation plays a critical role in the formation of drusen [18]. Analyses of the composition of drusen have shown, that they are composed of lipids, polysaccharides, glycosaminoglycans, and proteins [18-20]. Additionally, many proteins showed oxidative modifications, supporting the hypothesis that oxidative stress is a further contributor to the pathophysiology of AMD [20].

The most important environmental risk factors are smoking and diet. Smoking increases the risk to develop AMD by two- to three-fold. Moreover, there is evidence that there is a dose-dependent association as well as a reversibility in the case of quitting smoking [21]. Regarding an individual's diet, healthy forms, *e.g.* the Mediterranean diet, is associated with reduced risk due to the high content of antioxidants and vitamins [22, 23]. In contrast, high-fat or high glucose/fructose diets represent a significant risk factor for AMD. Both direct influences of the nutritional components, as well as more indirect influences like dysbiosis of the gut microbiota, are thought to be associated with AMD formation. The latter is thought to result in systemic low-grade inflammation [24].

To date, the largest study of the genome-wide association of AMD revealed 52 gene variants across 34 loci [25]. 45 out of 52 were classified as common variants and the remaining 7 as rare variants. Furthermore, the analyses showed that the genetic risk is shared between the neovascular and the non-neovascular form of AMD except for one genetic variant that seems to be exclusive for neovascular AMD [25]. Further enrichment analyses narrowed down the following molecular mechanisms that could be affected by the identified gene variants: lipid metabolism, extracellular matrix organization, and assembly as well as the complement pathway [25]. The possible role of the complement system in the pathophysiology of AMD was recently reviewed by Armento *et al.* [11]. In summary, increasing evidence supports the involvement of the activation of the alternative pathway of the complement system, both in a local fashion as well as on a systemic basis.

It is hypothesized that the crucial anatomic site where AMD pathophysiology begins is the complex of RPE cells, Bruch's membrane, and the choroid. In the healthy retina, this complex does not only mediate the precisely regulated exchange of nutrients and metabolic waste products, but it also inhibits the activation of the alternative pathway of the complement system. As soon as the AMD pathophysiology has been triggered through one or more of its risk factors, the normal function of the complex is unbalanced. Consequently, both the integrity of RPE cells and Bruch's membrane become more and more impaired. This leads to a cascade of events that disturb retinal homeostasis: accumulation of metabolic end products, oxidative stress, and activation of the complement system thereby inducing local inflammation and cell senescence [11, 26, 27]. The exact temporal relationships between the degeneration of RPE and photoreceptor cells and changes in Bruch's membrane and the choriocapillaris are not yet clear. The first changes may occur in Bruch's membrane or the choriocapillaris, leading to RPE and photoreceptor degeneration. Alternatively, changes in the RPE and photoreceptors could drive the changes in Bruch's membrane and choriocapillaris.

Metformin

Metformin is a synthetic derivative of the naturally occurring galegine from the plant *Galega officinalis* [1]. Chemically, metformin is a biguanide consisting of two coupled guanidine molecules with some additional substitutes. As a derivative of a naturally occurring molecule, metformin has not been designed to target specific pathways, nor did it go through the regulatory process of preclinical and clinical trials which are mandatory today. After its safety and efficacy had been established, metformin has been used as a glucose-lowering agent since the 1950s [1]. FDA approval followed in 1994 and since the UK Prospective Diabetes Study in 1997 (UKPDS) had demonstrated the beneficial effects of metformin, it has been recommended as first-line treatment for type 2 diabetic patients [2].

Metformin mechanisms of action

The classical antihyperglycemic function of metformin takes place at multiple sites of action in the body and through multiple molecular mechanisms that have been described in detail elsewhere [1, 2]. Briefly, its blood glucose-lowering ability is a combination of effects that metformin exerts in the liver, the gastrointestinal tract, and the muscles.

In the liver, gluconeogenesis is downregulated through both AMPK-dependent and -independent signaling pathways. The AMPK pathway is the cellular energy sensor and regulator of the cell's energy homeostasis. If the ratio of AMP: to ATP increases, the AMPK pathway induces a switch from ATP-consuming pathways to ATP-generating pathways. This includes the downregulation of gluconeogenesis and hence, a reduction of glucose levels [1, 2].

In the gastrointestinal tract, metformin is thought to increase glucose uptake and metabolism by colonic enterocytes [1]. Moreover, increased glucagon-like peptide-1 receptor (GLP-1 receptor) secretion has been reported in response to metformin. Activation of the GLP-1 receptor results in increased insulin release [2]. Finally, metformin

seems to be related to shifts in the composition of the gut microbiome, but it remains unclear if and how changes in the gut microbiome lead to glucose-lowering effects [2]. It is postulated that a healthier gut microbiome suppresses postprandial hyperglycemia and that levels of inflammatory cytokines are reduced [1].

In skeletal muscles, metformin has been reported to increase insulin-stimulated uptake of glucose. Newer investigations, however, indicate that this effect is more secondary by the metformin-induced overall improvement of glycemic control and reversal of glucose toxicity [2].

Metformin and AMD

As described above, the pathophysiology of AMD primarily affects the interface of photoreceptors, RPE cells, the choroid, and choriocapillaris. The association of metformin with AMD has been investigated in some preclinical trials, some retrospective trials, and some systematic reviews and meta-analyses based on the reported mechanisms of action of metformin. Before reporting the results of these trials, we will summarize the proposed mechanisms of action of metformin, that could play a role in its influence on the AMD pathophysiology.

Proposed mechanisms of action

The exact mechanisms of the multiple effects of metformin are still under investigation. However, some possible signaling pathways and/or modes of action have already been identified.

The AMPK pathway appears to play a central role in the action of metformin (Figure 2). The AMPK pathway is a central regulator of cellular metabolism [28]. AMPK becomes activated when the level of ATP decreases indicating high metabolic activity. Via direct phosphorylation of several proteins, AMPK downregulates energy-consuming pathways and promotes the activation of energy-producing pathways to restore the energy homeostasis of the cell [28]. In this way, the AMPK pathway plays a major role in the regulation of glucose metabolism, lipid metabolism, cell growth, and autophagy. As described earlier, AMD pathophysiology relies on the integrity of the RPE cells, which are the critical interface between photoreceptor cells and the choroid. Dysregulation of RPE metabolic pathways, especially of the AMPK/SIRT1/PGC-1 and of the mTOR pathway is strongly associated with AMD pathophysiolog [29]. Metformin directly influences the mitochondrial respiratory chain thereby inducing the AMP-mediated activation of AMPK, the initial step of the AMPK pathway [2, 7]. Downstream signaling within the AMPK pathway is complex. This could explain why the beneficial functions of metformin are as diverse as anti-inflammatory, anti-oxidative, anti-angiogenic, and anti-apoptotic [30]. The second mode of action is the ability of metformin to reduce chronic inflammation by improving the metabolic state. Additionally, several direct anti-inflammatory effects have been described, although not directly in the context of AMD but as a general effect of metformin [31]. This includes decreasing reactive oxygen species and lowering levels of inflammatory cytokines [31]. Interestingly, in the context of the acute respiratory distressed syndrome (ARDS), a common inflammatory condition in severe COVID-19, metformin has been shown to inhibit the activation of the NLRP3 inflammasome thereby ameliorating the course of this life-threatening complication [32]. This is in line with the finding that fluoxetine, a direct inhibitor of NLRP3, is associated with a reduced risk to develop AMD. Possibly, metformin is likewise able to prevent NLRP3 inflammasome activation in RPE cells to prevent their degeneration [33].

Effects of metformin in preclinical trials

The group of Ying *et al*. investigated the effects of metformin in a mouse model of laser-induced CNV as well as in the human umbilical vein endothelial cell (HUVEC) line [34]. Mice treated with metformin had significantly smaller CNV lesions with reduced vascular density than the control group. Their experiments with HUVEC cells showed that activin receptor-like kinase 1 (ALK1), a receptor that is essential for vascular development, remodeling, and pathological angiogenesis, is inhibited by AMP-activated protein kinase (AMPK) and that metformin is a potent activator of AMPK [34].

The group of Han *et al*. elucidated the anti-angiogenic and anti-inflammatory effects of metformin in a set of *in-vitro* and *in-vivo* experiments [35]. They found that metformin had significant anti-angiogenic effects by inhibiting proliferation, migration, and tube formation of human retinal vascular endothelial cells. In addition, metformin had potent anti-inflammatory effects by suppressing several inflammatory cytokines through both AMPK-dependent and AMPK-independent pathways [35]. The authors did not specify which AMPK-independent pathways are involved in the mode of action of metformin. However, their experiments showed that suppression of NFB and interleukin-8 by metformin was independent of the AMPK pathway.

Qu *et al*. examined the effect of metformin on the human retinal pigment epithelium cell line ARPE-19. Cells were put under oxidative stress via glyoxal-induced cytotoxicity [36]. Metformin was able to protect ARPE-19 cells by inhibiting cell death, reducing intracellular reactive oxygen species (ROS) production, decreasing the apoptosis rate, and increasing intracellular nitric oxide (NO) levels, an important molecule for maintaining retinal homeostasis [36]. A subset of experiments confirmed that metformin influences antioxidant and autophagy pathways to exert its function [36]. Similar experiments have been performed by Zhao *et al*. using two different human pigment epithelium cell lines [37]. Their experiments showed that H_2O_2-induced oxidative damage was attenuated by metformin. Metformin stimulated autophagy via the AMPK pathway [37].

The *in-vivo* experiments performed by Xu *et al*. using different mouse models for retinal and photoreceptor degeneration corroborate the results of the above-described *in-vitro* experiments [38]. Xu *et al*. used the albino BALB/cJ mouse strain to analyze whether metformin can protect against light-induced photoreceptor loss. If mice were pretreated with metformin at least 4 days before light

damage was induced via 4h exposure to 4.000 lx bright white fluorescent light, photoreceptor loss was prevented. In a subset of experiments, the group used knockout mice for the AMPK1- and AMPK2-subunit, and showed, that presence of the 2-subunit was crucial for the protective effect of metformin. As the protection by metformin was the same between systemic and local (intravitreal) injections, the authors followed that metformin's protection is based on local influences. Xu *et al.* used a second mouse model, the Rd10 model for inherited retinal degeneration to analyze the protective effect of metformin. Starting on postnatal day 16, Rd10 mice aggressively lose their rod photoreceptors followed by cone photoreceptor loss. Treatment with metformin starting on postnatal day 13 delayed the loss of both photoreceptor types. Via mitochondrial protein expression experiments, Xu *et al.* could associate metformin's protection with increased metabolic activity. In the third set of experiments, the group injected sodium-
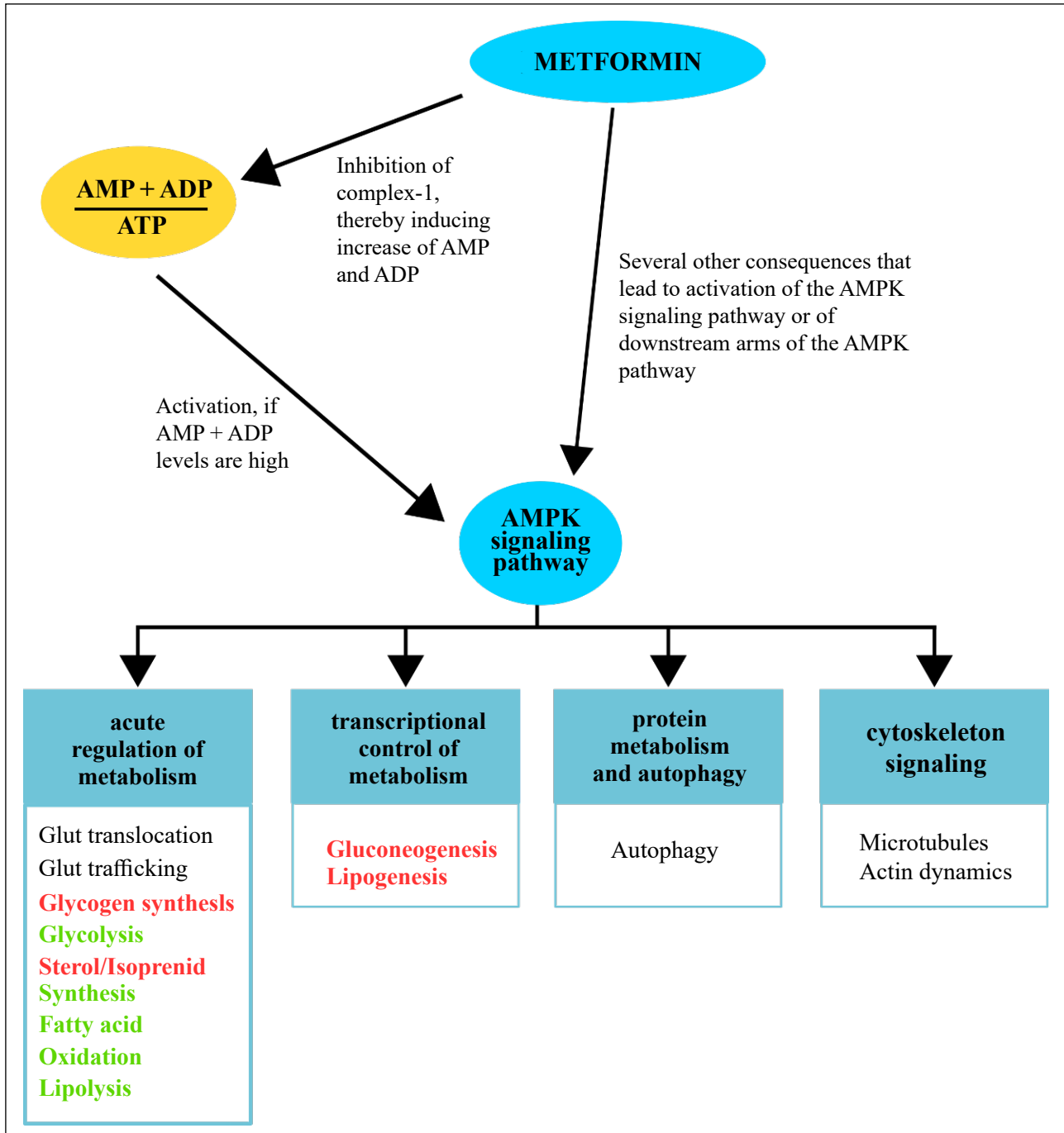

Figure 2. Influence of metformin on the AMPK signaling pathway and consequences of AMPK activation. Without metformin, the AMPK signaling pathway is activated when the cellular levels of AMP and ADP increase. Activation of the pathway leads to a switch from energy-consuming metabolism to energy-providing metabolism. Metformin has been shown to exert parts of its function through the activation of the AMPK pathway. A confirmed mechanism is that metformin can inhibit complex1 of the respiratory chain, thereby inducing the accumulation of AMP and ADP. Furthermore, several other, more direct influences of metformin on downstream components of the AMPK pathway have been reported. AMP = adenosine-monophosphate, ATP = adenosine-triphosphate, ADP = adenosine-diphosphate, AMPK = adenosine-monophosphate dependent kinase, Glut = glucose transporter. Bold red font: inhibition, bold green font: promotion.

iodate into BALB/cJ mice to induce acute oxidative stress to the RPE and the photoreceptors. This oxidative stress mimics the early oxidative stress factor of early AMD. If mice were pretreated with metformin, either 30 or 35 mg/kg the RPE and photoreceptors were resistant to the damage in a dose-dependent manner: ~50% and ~90% of cells were protected [38]. None of these mouse models is a perfect model for AMD and such a model does not exist. But each experiment gives insights into relevant aspects of the AMD pathophysiology and potential mechanisms that could be impacted by metformin.

Effects of metformin use on AMD in retrospective clinical trials

Eight retrospective studies have analyzed the association of metformin use with AMD, of which one was a cross-sectional study [39], four were cohort studies [40-43], two were case-control studies [44, 45], and one was a nested case-control study [46]. Five studies exclusively determined the association between metformin use and the risk of developing AMD in diabetic patients [39-43], whereas three studies included broader patient groups according to their cohort definition [44-46]. One study examined the association of metformin use with dry AMD only [43], while the remaining studies considered all forms of AMD or did not further specify. All studies took into account possible confounders like age, sex, ethnicity, smoking status, insurance status, other oral (antidiabetic) medications, insulin use, cardiovascular disease, hypertension, hyperlipidemia, obesity, BMI, HbA1c, kidney disease, or Charlson comorbidity index as far as data were available. Stewart *et al.* performed a cross-sectional study using the electronic medical record database of the University of California, San Francisco [39]. They included 3,120 diabetic patients who had documented ophthalmologic examinations and a documented metformin use before or at their first documented ophthalmologic exam. The outcome of interest was a diagnosis of either non-neovascular or neovascular AMD at the first ophthalmologic exam. Using propensity score-weighted logistic regression models, Stewart *et al.* found that metformin use was significantly associated with a reduced odd ratio (OR) to develop AMD (OR 0.70, 95% confidence interval, *p*-value 0.003). The association was even stronger when analyzing non-neovascular AMD alone (OR 0.59, 95% CI, *p*-value < 0.001). All other antidiabetic drugs studied showed no association. Limitations of this study are the retrospective nature, the relatively small sample size, the exclusion of drusen as an early stage of AMD, because authors found the diagnosis of drusen to be unreliable, and missing information about the duration of metformin use [39].

Chen *et al.* investigated the association between metformin use and the risk of AMD in a cohort study with type 2 diabetic patients [40]. They included 68,205 patients who had a diagnosis of type 2 diabetes mellitus during the study period. Patients were followed up to identify the onset of AMD (unspecified, non-exudative, or exudative). The main independent variable was the use of metformin, which was true for 66.7% of the identified patients. Ad-justed hazard ratios (HRs) were obtained via multivariate Cox regression analyses. Patients taking metformin had a significantly lower HR to develop AMD than metformin non-users (HR 0.54, 95% Ci, *p*-value < 0.0001). Chen *et al.* also calculated HRs for the duration and cumulative dose of metformin and their association with the development of AMD and found that both significantly lowered AMD risk. Limitations of this study are the retrospective nature, and some missing details in the database like smoking status, diet, and laboratory values [40].

Another cohort study was performed by Jiang *et al.* [41]. The group reviewed medical records of the ophthalmology department of the China-Japan Friendship Hospital in Bejing, China. 324 patients with a diagnosis of diabetes mellitus type 2 for at least 10 years, were identified and followed-up over 5 years. Patients were excluded if they had a diagnosis of AMD before the diagnosis of diabetes. AMD was graded into early and late-stage AMD. Metformin users and non-users were compared using the X^2 test and multivariate logistic regression models were used to characterize the influence of confounders. AMD occurrence in the metformin group was 15.8% and 45.2% in the metformin non-users (*p* < 0.0001), thus patients taking metformin had significantly less risk to develop any AMD. Subgroup analysis revealed, that metformin use only influenced the development of early AMD and not late AMD. Further analysis showed that both duration of metformin use and cumulative metformin dose was associated with significantly lower risks to develop any early but not late AMD. The retrospective design, the small sample size, and the missing data on some important confounders are the limitations of this study.

Gokhale *et al.* performed a further cohort study investigating the influence of metformin on the risk of AMD in patients with type 2 diabetes [42]. The group identified 173,689 patients with newly diagnosed type 2 diabetes from the United Kingdom IQVIA Medical Research Data. Patients were excluded if they had an AMD diagnosis before diabetes diagnosis and if they had no prescription for antidiabetic medication. 89% of the identified patients had a prescription for metformin alone or in combination with other antidiabetic drugs. The control group had any medication except metformin. The outcome of interest was a diagnosis of AMD during the study period. HRs were defined in a time-dependent manner using extended Cox proportional hazard models. For the time-dependent analysis, the follow-up intervals were set to 3 months. AMD occurred in 3,111 (1.8%) of the patients. Gokhale *et al.* did not find an association between metformin and the development of AMD. This finding was independent of the use of other antidiabetic drugs as well as from the duration of diabetes and the duration of metformin use. Limitations of this study include the retrospective design, and the missing differentiation between AMD stages (early, late) that could for example mask findings if metformin was only protective for certain AMD stages [42].

The group of Eton *et al.* investigated the association of metformin and dry AMD only [43]. In their cohort study, they included patients with a diagnosis of diabetes mel-

litus and sufficient follow-up visits. Furthermore, Eton *et al.* distinguished between the current and historical use of metformin. Current metformin use was defined as metformin use during the study period, historical metformin use was based on any metformin use before the patients' enrollment date (defined as aged 55 years or more, a diagnosis of diabetes mellitus and at least two years of follow-up data). Current metformin use was associated with a small, but significantly increased HR to develop dry AMD (HR 1.08; 95% CI, *p* < 0.0001). Historical metformin use, however, showed a protective effect (HR 0.95; 95% CI, *p* = 0.002). The analysis of the cumulative dose of metformin revealed slightly decreased HRs for cumulative doses below 720,000 mg, but slightly increased HRs for cumulative doses above 720,000 mg. Overall, the study by Eton *et al.* showed conflicting results for the effect of metformin on the development of dry AMD. Study limitations include the retrospective design, potentially the restriction to dry AMD only, and a probable observation bias [43].

Lee *et al.* used a different study design, a nested case-control study, and they also had a broader definition for the study eligibility as they not only included patients with a diagnosis of diabetes mellitus type 1 and 2 but also patients with a diagnosis of cardiovascular disease [46]. Above that, they were not only interested in the effect of metformin, but also the effects of statins, angiotensin-converting enzyme (ACE)-inhibitors, and angiotensin II receptor blockers on AMD. During the study, 2,330 patients developed AMD. For each case, 10 controls were matched by sex, age, and cohort entry date, leading to a control group of 23,278 patients. Study outcomes were, that none of the investigated drugs had a protective effect on the development of AMD. These findings were independent of the duration of drug use. The nested case-control design overcomes some of the disadvantages of traditional case-control studies, such as the reduction of selection bias. The retrospective design and its disadvantages remain, and sample sizes were relatively small.

Two case-control studies investigated the effect of metformin on AMD independent of a diagnosis of diabetes [44, 45]. However, both studies examined diabetic patients separately as subgroups of the initial total study cohort. Cases were defined as patients who had a diagnosis of AMD during the study period. Brown *et al.* included patients with all types of AMD (non-exudative, exudative, or unspecified), controls had no AMD and were propensity score matched using age, Charlson comorbidity index (CCI), hypertension, and anemia as matching variables [45]. They found that metformin was associated with statistically significant decreased odds of developing AMD (OR 0.58; 95% CI, *p* = 0.0005). Other diabetic and non-diabetic medications showed no association with AMD. The subgroup analysis of diabetic patients taking metformin versus non-metformin users showed that metformin was significantly associated with decreased odds of developing AMD in univariate and multivariate logistic regression (OR 0.68; 95% Ci, *p* = 0.002 and OR 0.7; 95% CI, *p* = 0.043). Blitzer *et al.* defined their study cohort as patients with newly diagnosed AMD during the study

period and powered their study to detect ORs of 0.95 with 90% power in a subgroup of diabetic patients [44]. Controls were selected 1:1 and matched based on age, anemia, hypertension, region, and CCI score. The effects of diabetes were tested after control matching. Metformin use was similar in the case and control groups (12.8% and 13.0%). Use of any metformin was significantly associated with decreased odds of developing AMD (OR 0.94; 95% CI, *p* < 0.001). In addition, it was found that low to moderate total metformin doses had a dose-dependent effect, while there was no association between high metformin (> 1080 g cumulative dose) doses and AMD. The subgroup analyses of diabetic patients showed similar results. Metformin use significantly decreased the odds of developing AMD (OR 0.95; 95% CI, *p* < 0.001), and again a dose-dependent effect for low to medium cumulative metformin doses was found.

In summary, five out of eight retrospective studies found associations of metformin with decreased odds of developing AMD [39-41, 44, 45], one study found conflicting associations [43], and two studies report no association of metformin use with the development of AMD [42, 46]. Three studies found positive associations with either the duration of metformin use or dose-dependent effects [40, 41, 44], while one study did not detect an association with longer metformin use [46]. In addition, a meta-analysis by Romdhoniyyah *et al.* over five of the above-reported retrospective trials did not find a significant association between metformin use and the risk to develop AMD [3]. In contrast, a very recently published meta-analysis by Mauschitz *et al.* over 14 European population- or hospital-based studies found a lower AMD prevalence in patients taking lipid-lowering and/or antidiabetic drugs (including metformin) (OR 0.78; 95% CI, *p* = 0.002) [47]. However, no association was found for late AMD stages (OR 1.12, 95% CI, *p* = 0.37).

The main limitation of all retrospective studies is that they can only detect associations but cannot determine causal relationships. The latter is only possible in the context of prospective trials. In addition, retrospective trials are prone to other limitations such as selection bias, recall bias, loss to follow-up, and confounding factors [48]. Nevertheless, the majority of the described retrospective analyses found that metformin was associated with decreased odds to develop AMD. Selection bias is especially small for cohort studies like those of Chen *et al.*, Jiang *et al.*, Gokhale *et al.*, and Eton *et al.* [40-43, 48]. All eight studies considered confounding factors and comorbidities in their analyses. The limitation of loss to follow-up was reduced by adjusting the eligibility criteria and only patients for whom sufficient follow-up visits were available were allowed to enter the study cohorts. As metformin is predominantly described to type 2 diabetic patients, five studies exclusively investigated the effect of metformin on AMD in diabetic patients. Three studies included broader patient groups. Two of them found that metformin decreased the odds of developing AMD independently from a diagnosis of diabetes. This suggests that diabetes itself probably has little influence on the development of AMD.

Prospective clinical trials

There is one ongoing prospective, phase II clinical trial that is investigating the ability of metformin to decrease the progression of geographic atrophy (GA) in non-diabetic patients with AMD [49]. A planned population of 186 subjects will be stratified 1:1 into a treatment and an observation group. The treatment group will be assigned to oral metformin for 18 months. At an additional follow-up visit at month 24, the progression of geographic atrophy will be measured and compared between groups. The primary outcome measures are the change in the area of GA or drusen growth. Secondary outcome measures include best-corrected and low-luminance visual acuity, ocular and systemic safety of metformin use, and score changes of the National Eye Institute Visual Function Questionnaire. Subjects with type 1 or 2 diabetes are excluded from the study as well as subjects that are already taking metformin for other reasons. Study completion is expected by the end of 2024.

Limitations of metformin use

Beyond all the reported beneficial properties of metformin, there are also some disadvantages associated with the use of metformin that should be taken into account before using metformin as a "cure it medication". Reported disadvantages include vitamin B12 deficiency, increased risk of lactic acidosis, and alteration of 745 proteins with uncertain consequences [7]. In addition, metformin is known to have various gastrointestinal side effects.

Furthermore, a study by Ebeling *et al*. that analyzed the influence of metformin on individual patient-derived RPE cell lines indicated that the effect of metformin was not uniform across all patients. The group suggests that patient-specific responsiveness to metformin should be taken into account before prescription and that approaches toward personalized medicine are necessary [50].

Conclusion

Evidence is increasing that metformin, the most commonly prescribed oral antihyperglycemic drug, influences a variety of physiological functions besides its classical glucose-lowering effect. Essentially, this includes anti-inflammatory, anti-angiogenic, anti-oxidative, anti-apoptotic, neuroprotective, and anti-aging effects.

The ongoing prospective trial about the effect of metformin on the progression of geographic atrophy could deliver the first results for this subgroup of late-stage AMD patients. In the future, more prospective trials are needed to confirm in more detail how the beneficial effects of metformin influence the pathophysiology of AMD and if metformin qualifies as a treatment option in patients with a diagnosis of AMD. Additionally, prospective trials should not only concentrate on late-stage dry AMD but consider all AMD stages. Jiang *et al*. [41] found for example, that especially the early stage of AMD was associated with a beneficial effect of metformin. Finally, prospective trials should consider patients with and without a diagnosis of diabetes to rule out possible confounding effects of the diabetic disease.

Declarations

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