Online ISSN 2690-1803

Aging Pathobiology and Therapeutics

Enhancing healthy aging with drugs that target insulin signaling

Guest Editor: Dr. Ian James Martins

SARICH NEUROSCIENCE RESEARCH INSTITUTE,
Perth, Western Australia

http://www.antpublisher.com/index.php/APT

Topic：Enhancing healthy aging with drugs that target insulin signaling

GUEST EDITOR

IAN JAMES MARTINS

Ph.D., D.Sc and Dr.Med, Doctrin de Science, Honoris Causa.

SARICH NEUROSCIENCE RESEARCH INSTITUTE, Perth, Western Australia.

Email: fellow.iasr@gmail.com

Website: https://uwa.academia.edu/IanMartins/CurriculumVitae

Topic：Enhancing healthy aging with drugs that target insulin signaling

EDITORIAL

Age-related diseases are becoming a major concern as the world's population grows older due to advances in technology, health, and nutrition. The process of aging is determined by various genetic and environmental factors with epigenetic alterations now considered as a major defect in insulin signaling and the global chronic disease epidemic. Nutritional interventions are now critical to reverse that aging process with the activation of anti-aging genes that prevent mitophagy and programmed cell death. The global diabetes epidemic is now closely linked to accelerated aging and nutritional interventions reverse the aging process and enhance insulin signaling that stabilizes the various organ diseases. The use of anti-aging drugs (metformin, acarbose, canagliflozin as well as others) has now become of importance to age-related diseases with the use of these drugs to reverse transcriptional dysregulation, subcellular changes, and membrane alterations in diabetes and Alzheimer's disease. Anti-aging drugs such as metformin and rapamycin are now important to activate anti-aging genes and reverse the aging process that is connected to insulin signaling, neurodegeneration, and global chronic disease. The use of drugs that target insulin signaling with nutritional interventions increases longevity by reducing oxidative stress and targeted insulin signaling with increased lifespan in animals and man.

Topic：Enhancing healthy aging with drugs that target insulin signaling

PARTICIPANTS

ELIZABETH RHEA AND WILLIAM ALLEN BANKS

VA Puget Sound Healthcare System, University of Washington, Seattle, WA, USA.

ADAM B SALMON

University of Texas Health Science Center at San Antonio, Barshop Institute for Longevity and Aging Studies, San Antonio, TX, USA.

ALBERT AUGUSTIN

Klinikum Karlsruhe, Augenklin, Moltkestr 90, D-76133 Karlsruhe, Germany.

NURTEN ARSLAN

Faculty of Health Sciences, Erzincan University, Erzincan, Turkey.

Topic：Enhancing healthy aging with drugs that target insulin signaling

CONTENTS

1 EVIDENCE FOR AN ALTERNATIVE INSULIN TRANSPORTER AT THE BLOODBRAIN BARRIER

William A Banks, Cassidy Noonan, Elizabeth M Rhea

Aging Pathobiol Ther, 2022, 4(4): 100-108. doi: 10.31491/APT.2022.12.100.

2 INFLUENCE OF METFORMIN ON AGE-RELATED MACULAR DEGENERATION

Albert J Augustin, Jenny Atorf

Aging Pathobiol Ther, 2022, 4(4): 119-128. doi: 10.31491/APT.2022.12.098.

Aging Pathobiology and Therapeutics 2022; 4(4): 100-108 ***100***
DOI: 10.31491/APT.2022.12.100

REVIEW

Evidence for an alternative insulin transporter at the blood-brain barrier

William A. Banks[a, b], Cassidy Noonan[c, d], Elizabeth M. Rhea[a, b, *]

[a] *Department of Medicine, Division of Gerontology and Geriatric Medicine, University of Washington, Seattle, WA 98195, USA.*

[b] *Geriatric Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, WA 98108, USA.*

[c] *Research and Development, Veterans Affairs Puget Sound Health Care System, Seattle, WA 98108, USA.*
[d] *University of Washington, Seattle, WA 98195, USA.*

This article belongs to the Special Issue: Enhancing healthy aging with drugs that target insulin signaling

Abstract

Accumulating evidence suggests there is an alternative insulin transporter besides the insulin receptor at the blood-brain barrier (BBB), responsible for shuttling insulin from the circulation into the brain. In this review, we summarize key features of the BBB and what makes it unique compared to other capillary beds; summarize what we know about insulin BBB transport; provide an extensive list of diseases, physiological states, and serum factors tested in modifying insulin BBB transport; and lastly, highlight potential alternative transport systems that may be involved in or have already been tested in mediating insulin BBB transport. Identifying the transport system for insulin at the BBB would aide in controlling central nervous system (CNS) insulin levels in multiple diseases and conditions including Alzheimer's disease (AD) and obesity, where availability of insulin to the CNS is limited.

Keywords: Insulin, transport, blood-brain barrier

Introduction

The ability of insulin to act within the brain has been known since the early 20th century [1, 2]. However, as blood substrate entry into the brain is regulated by the blood-brain barrier (BBB) interface, evidence of insulin crossing the brain barriers was identified decades later [3, 4]. It is now well recognized that the majority of insulin acting within the brain crosses the BBB via a saturable, receptor-mediated transport system that is affected by various physiological states [5, 6]. Once present within the brain, it is assumed insulin must navigate the brain parenchyma to reach various cell types to act as a ligand by binding its receptors, including the insulin receptor, insulin-like growth factor 1 receptor (IGF-1R), and hybrids of the two, activating intracellular signaling cascades. Insulin signaling within the central nervous system (CNS) is important not only for regulation of metabolism but also cognition. CNS insulin signaling can become dysfunctional with age and in neurodegenerative diseases such as Alzheimer's disease [7] and insulin BBB interactions are impaired [7-9]. BBB transport of insulin could be a regulator of CNS insulin signaling since it is one of the mediators of CNS insulin levels [7]. Additionally, insulin interactions with the BBB are impaired in obesity [10, 11]. Without sufficient ligand or receptor signaling, insulin functions within the CNS become impaired. Therefore, understanding more about the transport system and interactions at the BBB for insulin will aid in combating such deficiency in aging, Alzheimer's disease, and obesity.

Blood-brain barrier (BBB)

* Corresponding author: Elizabeth M. Rhea
Mailing address: 1660 S. Columbian Way, Seattle, WA 98108, USA.
Email: meredime@uw.edu
Received: 01 September 2022 / Revised: 04 November 2022
Accepted: 17 November 2022 / Published: 29 December 2022

Components of the BBB

Conceptually, the BBB can be thought of as those structures which inhibit or otherwise regulate the exchange of substances between the CNS and blood. These barriers include the vascular BBB, the choroid plexus, the tanycytic barrier located between the circumventricular organs and adjacent brain tissue, the meningeal barrier, and the barriers of the cranial nerves such as the blood-retinal barrier [12]. Likely, all these barriers participate in insulin/CNS interactions, but it is the vascular BBB that has been most studied in this regard.

The physical wall that forms the BBB is comprised of brain endothelial cells (BECs) and occurs in the arteriolar, capillary, and venule portions of the cerebral vasculature [13]. These cells are in constant communication with other cells of CNS, forming the neurovascular unit (NVU). The NVU includes microglia, pericytes, astrocytes, neurons, and mast cells, but it is the astrocytes and pericytes which have received the most attention in regard to their interactions with the BBB. Pericytes are anatomically connected by gap junctions with the BEC. Astrocytes form a sheath around the BBB capillaries and are separated from the abluminal surface of the BEC by the basement membranes. It is the pericytes and astrocytes which induce the BECs to express BBB characteristics, including the formation of tight junctions and the loss of fenestrae and micropinocytosis [14]. The cells of the NVU also modulate other BBB functions, such as cytokine secretions and transporter functions [14].

The BBB is also in communication with the circulating immune cells and, by way of secretions into blood, with the peripheral tissues. This communication can also affect various functions of the BBB. For example, lipopolysaccharide, a fragment of the cell wall of gram-negative bacteria that is a potent stimulator of the innate immune system, increases insulin transport across the BBB by inducing nitric oxide release from immune cells [15, 16].

Roles of the BBB

The most widely appreciated role of the BBB and for which it was named is that of limiting the unregulated leakage of substances from blood into the CNS. Unlike other capillary beds, that of the CNS has greatly reduced transcytosis, few fenestrations, and adjacent BEC cell membranes are cemented together by tight junctions [17]. Thus, paracellular (between cells) and transcellular (across a cell) leakage is essentially absent in the healthy BBB so that no ultrafiltrate is produced by the capillary bed of the CNS. This lack of an ultrafiltrate protects brain tissue from blood-borne substances, both endogenous and xenobiotic, which would be toxic to those tissues. The physical barrier is reinforced for some substances by the presence of brain-to-blood efflux systems which prevent circulating substances from entering or remaining in brain tissue. For example, the anti-helminthic ivermectin is prevented from entering the CNS by the brain-to-blood transporter p-glycoprotein (Pgp) [18]. In animals that do not express Pgp at their BBB, ivermectin is a potent neurotoxin [19].

The BBB can also be an enzymatic barrier, digesting substances such as the monoamines which could otherwise enter the brain from the circulation [20]. Insulin degrading enzyme (IDE) protein and mRNA is present in BECs, less than [21] and similar to levels present in neurons [22], respectively, regulating intracellular insulin levels.

The lack of an ultrafiltrate may protect the CNS from circulating toxins, but the ultrafiltrate is the major route by which most tissues receive their nourishment from the blood. Thus, the BBB has other mechanisms to provide the CNS with nutrients. The most prominent of these are the transport mechanisms. The BBB contains many transporters, and it is likely there are still more to be discovered. These transporters deliver to the brain the glucose, amino acids, free fatty acids, vitamins, and other nutrients needed by the brain. The transporters of the BBB also play a homeostatic role for the CNS by regulating electrolyte balance [23], bicarbonate levels [24], and as exemplified by Pgp eliminating from the CNS both endogenous and exogenous toxins [25]. The BBB also participates in brain-body communication by regulating the transport of various informational molecules, including insulin.

Types of transport systems at the BBB

Transport systems located in cell membranes can be categorized in various ways. Pharmacokinetically, transporters demonstrate saturation and biochemically, are typically transmembrane glycoproteins. Some BBB transporters, such as the glucose transporter 1 (GLUT-1) which transports glucose across the BBB, use facilitated diffusion [26, 27]. Facilitated diffusion systems are energy independent and transport substances bidirectionally from the side of higher concentration to the side of lower concentration. Active transporters require energy or an electrochemical gradient to function, can be unidirectional, and can transport substances against a concentration gradient. Facilitated diffusion systems can be channels or carriers (GLUT-1 is a carrier), but active transporters are carriers. Carriers typically open and close so that they can be open to one environment and closed to the other, whereas channels when active, are open simultaneously to the extracellular and intracellular environments. Carriers also tend to have highly selective binding sites so that they transport a specific ligand or class of ligands and follow Michaelis-Menten kinetics (GLUT-1 is specific for hexoses). Some channels can undergo conformational changes and so become inactive (closed to both environments), whereas pores are channels that are always open and active. Channels and carriers typically transport substances into or out of the cytoplasm, therefore, transcellular transport as occurs at the BBB would depend on a set of transporters located at both the luminal and abluminal cell membranes. These strict definitions from cellular biology [28, 29] are not always appropriately applied in the BBB literature. For example, the term “active” is often used to refer to any saturable transport system, whereas it should be reserved to refer only to energy-requiring carriers.

Receptor-mediated refers to a binding site for the ligand on the transporter and is a hallmark of carriers. Carrier-

mediated and receptor-mediated transport are not generally distinguishable terms. Receptor-mediated endocytosis refers to the internalization into the cell cytoplasm of the carrier protein with its ligand within a vesicle formed by the cell membrane. Those endosomes can be routed to various cellular structures, including back to the cell membrane. The term receptor-mediated endocytosis is often, but not always, used specifically to refer to endocytosis involving clathrin [30]. Clathrin-independent endocytic mechanisms include potocytosis (internalization of caveolae), adsorptive transcytosis, pinocytosis and phagocytosis.

Transcytosis occurs when the endosome moves from one membrane of a polarized cell to the other (*e.g.*, from apical to basal or luminal to abluminal). Transcytosis, therefore, requires that a cell have distinctive regions to its membrane as in the case of barrier cells. The BBB field tends to label any transport of a large molecule as receptor-mediated transcytosis, even when there is no evidence of involvement of vesicles or clathrin. The assumption is that vesicles are required to move larger substances, but enzymes and cytokines can be exported via carriers, as exemplified by interleukin 2 (IL-2) and Pgp [31]. Transport of insulin across the BBB is assumed to be clathrin-dependent but has not been directly tested *in vivo*. Insulin endocytosis is clathrin dependent for most cells [32], including peripheral endothelial cells [33] but caveolin-1 has also been shown to be involved in insulin uptake [34]. Insulin transcytosis across the retinal vascular endothelial cells is clathrin-dependent [35]. We recently showed insulin binding to isolated brain microvessels is clathrin-dependent [36]. We further went on to show transport across the BBB may be regionally mediated, involving caveolin-1 in the hypothalamus. This shows insulin transport across the various vascular beds can involve different processes.

Insulin BBB Transport

As established above, it is now well acknowledged that insulin can directly cross the BBB. This occurs in a saturable, specific, receptor-mediated process [37]. Saturability has been demonstrated by the nonlinear relation between CSF and blood levels of insulin [38-41], brain tissue and blood levels of insulin [41, 42], and by the inhibition of the rate of radioactive insulin transport across the BBB by unlabeled insulin [37, 43]. The transporter for insulin seems to be specific for it as no other ligands have to date been found, although substances have been found that modulate transporter activity (Table 1). The saturable, specific nature of the transport system which follows Michaelis-Menton kinetics suggests that it is receptor-mediated. The transport system is similar across species as human and rat insulin both cross the murine BBB [44], unlabeled human insulin is able to inhibit the blood to brain passage of radioactive rat insulin [37], and insulin BBB transport has been observed in dogs [38, 40, 41] and humans [45]. There is great variability in the insulin transport system throughout the brain as some regions have extremely fast transport [46]. Lastly, inactive insulin, either via freeze/thaw or heat-denatured methods, is unable to cross the BBB [10, 47]. Therefore, structural changes of insulin, such as deamidation, are likely necessary for recognition by the insulin transporter.

Over the years, insulin BBB transport has been investigated using various techniques. However, due to the small amounts of insulin transported across the BBB, there can be technical difficulties in some of the techniques. The ability to measure low amounts of immunoactive insulin present in brain compared to blood, and the need to inject high insulin concentrations of immunofluorescent tracer are some examples. Therefore, radioactivity is a great alternative to measuring pharmacokinetics of insulin transport. By investigating insulin BBB transport, independent researchers have identified this transport system is impacted by metabolic changes, during development and pregnancy, and even by exercise, Alzheimer's disease, and inflammation. There have also been factors and/or states that have had no effect on insulin transport. Most surprisingly, the loss or inhibition of the BEC insulin receptor had no effect on insulin transport [43, 48-50]. We have summarized this literature in Table 1 and refer readers to the specific references mentioned for each factor/disease state/intervention investigated.

The insulin receptor was long thought to serve as the protein responsible for insulin transport across the BBB, shuttling insulin from the circulation, across the BEC, and releasing it into the brain parenchyma. S961 is a potent, selective antagonist for the insulin receptor, but not IGF-1R or hybrid receptors [70], and has regularly been used to investigate the role of the insulin receptor in various processes, including transport. However, we recently showed that loss or inhibition of the insulin receptor in BECs in mice did not affect the transport rate of insulin across the BBB [43]. Since then, others have supported this finding, showing inhibition of the BEC insulin receptor did not impact insulin transport in an *in vitro* model [50] and in mice [48]. In an exciting new paper describing a novel, *in vivo* insulin PET tracer, co-administration of S961 also had no effect on brain insulin uptake in mice [49]. These data suggest there is another protein(s) responsible for transporting insulin across the BEC. However, to date, identification of this protein is unknown.

Alternatives for the insulin transporter

The involvement of another protein besides the insulin receptor for transporting insulin across the BBB makes evolutionary and physiological sense. Receptors and transporters are not static but modulated and regulated by a variety of factors. Separate receptor and transporter proteins would permit independent regulation of the effects of insulin on BEC functions and on brain activities. As the receptor is involved in many intracellular signaling cascades and acts as a tyrosine kinase, it further supports the primary role to be a signaling protein rather than a trans-

Table 1. Impact of disease, physiological states, and serum factors on insulin BBB transport.

Study	Disease/Intervention	Model	Model	Summary	Reference
Metabolic factors					
1	Diabetes- induced	streptozotocin (ip)	Mouse	↑	[51]
2	Diabetes- induced	alloxin (iv)	Mouse	↑	[51]
3	Hyperglycemia (non-diabetic)	D-glucose (ip)	Mouse	↔	[51]
4	Obesity	high-fat diet	Dog	↓	[11]
5	Obesity	retired breeders	Mouse	↓	[10]
6	Starvation in obesity	fasting (48 hr)	Mouse	↑	[10]
7	Triglycerides	cardiac perfusion	Mouse	↑	[10]
Developmental factors					
8	Newborn/Infancy	newborn, 3 wks	Rabbit	↑	[52]
9	Pregnancy	late pregnancy, BCSFB	Mouse	↓	[53]
10	Pregnancy	late pregnancy	Rat	↑	[54]
11	Age	C57B/6J (12, 24 mo)	Mouse	↓	[55]
12	Aging	SAMP8 (12 mo)	Mouse	↔	[8]
13	Alzheimer's	APP/PS1 (6 mo)	Mouse	↑	[56]
14	Alzheimer's	APP/PSN1 (6 mo)	Mouse	↑	[49]
15	Alzheimer's	moderate/severe AD, BCSFB	Human	↓	[57]
Physiological states					
16	Iron Deficiency	nutritional iron-deficiency	Rat	↑	[58]
17	Exercise	voluntary running wheel (24 hrs)	Mouse	↑	[59]
Insulin receptor loss					
18	Insulin receptor inhibition	S961	Mouse, BECs	↓	[47]
19	Insulin receptor loss/inhibition	EndoIRKO; S961	Mouse	↔	[43]
20	Insulin receptor inhibition	S961	BECs	↔	[50]
21	Insulin receptor inhibition	S961	Mouse	↔	[49]
Genetics					
22	Young ApoE mice	apoE3/apoE4, male/female	Mouse	↔	[60]
23	Aged ApoE mice	apoE3/apoE4, male/female, HFD	Mouse	↓	[61]
Factors/Drugs/Other					
24	IGFs	IGF-1, IGF-II (perfusion)	Mouse	↓	[62]
25	Leptin	iv, co-injection	Mouse	↔	[37]
26	Aluminum	ip	Rat	↑	[63]
27	Aluminum	ip	Mouse	↑	[37]
28	Pgp inhibitor	Verapamil iv, co-injection	Mouse	↔	[37]
29	Amino Acid	Tyrosine, iv, co-injection	Mouse	↔	[37]
30	Norepinephrine	iv, co-injeciton	Mouse	↔	[64]
31	Rapamycin	rapamycin (ip, 2 wks)	Mouse	↔	[65]
32	Rosiglitazone	iv, pre-treatment	Mouse	↔	[66]
33	CCK (Cholecystokinin)	ip, fasted (16 h)	Rat	↑	[67]
34	Acute estrogen	OVX female, male, ip (48 hr)	Rat	↔	[68]
35	Chronic estrogen	Male, ip (5 wks)	Rat	↔	[68]
36	Inflammation	LPS, ip (16, 24 h)	Mouse	↑	[16]
37	nNOS	3x ip LPS, inhibitor (4 h post)	Mouse	↓	[15]
38	iNOS, eNOS	3x ip LPS, inhibitor (4 h post)	Mouse	↑	[15]
39	Dexamethasone	oral (7 d)	Dog	↓	[69]

apoE: apolipoprotein E, BCSFB: blood-cerebrospinal fluid barrier, CCK: Cholecystokinin, EndoIRKO: endothelial insulin receptor knock-out, eNOS: endothelial nitric oxide synthase, HFD: high-fat diet, IGF: insulin-growth factor, iNOS: inducible nitric oxide synthase, ip: intraperitoneal, iv: intravenous, LPS: lipopolysaccharide, nNOS: neuronal nitric oxide synthase, OVX: ovariectomized, Pgp: p-glycoprotein.

porter. Recent proteomic studies of immortalized BECs support how critical the insulin receptor is in BECs, necessary for a multitude of functions, including regulation of a variety of BBB transporters, the transferrin receptor, and the tight junction protein claudin-5 [71]. A separate transporter would allow for insulin transport across the BEC, while also allowing critical intracellular signaling events via the receptor. Endothelial cell intracellular insulin signaling is a critical metabolic event. There is evidence in other receptor/transport systems supporting different proteins to accomplish these two independent events, as described next. We also discuss other alternatives for the insulin transporter that have been hypothesized.

Evidence for alternative transporters to canonical receptors at the BBB

Insulin binding to the luminal surface of BECs has two fates. It may activate the intracellular machinery that affects cellular functions (here termed the signaling receptor) or it may be transported across the BBB (here termed the transporter binding site). Binding to either the signaling receptor or the transporter binding site results in insulin endocytosis and exocytosis. In the case of signaling receptor binding, the exocytosis is at the luminal membrane of the BEC and in the case of transporter binding site, the exocytosis is to the abluminal membrane of the BEC. The question arises as to whether the protein forming the signaling receptor is the same protein as the transporter binding site. As we have previously reviewed [72], it seems that the usual situation is that the signaling receptor protein and the BBB transporter binding site are usually different proteins, as exemplified by prolactin [73], epidermal growth factor, Tyr-MIF-1, the enkephalins, pituitary adenylate cyclase activating polypeptide, and thyroid hormones. Our data argues that a similar dichotomy exists for insulin. We found that the insulin antagonist S961 binds avidly to the BEC, but is not transported across the BBB [43]. This means that the transporter binding site differs sufficiently from the signaling receptor as to not recognize S961 as a ligand. We also found that S961 largely blocked the ability of radioactive insulin to bind to BECs, but not its ability to cross the BBB. Finally, mice with loss of the signaling receptor in BECs demonstrated poor binding to BECs, but unimpaired transport activity. These studies are consistent with the insulin signaling receptor and the transporter binding site being different proteins.

Insulin-like growth factor-1 receptor (IGF-1R)

Could IGF-1R be insulin's transporter binding site? Insulin and IGF-1 each bind to the other's receptors, although much less avidly. IGF-1R is expressed at the BBB and choroid plexus [74]. IGF-1 crosses the BBB and inhibits the transport of radioactive insulin transport across the BBB, just as insulin inhibits the transport of radioactive IGF-1 [62, 75]. Both insulin and IGF-1 transport are reduced in obese animals and affected by triglycerides [10, 76]. However, cross inhibition studies suggest that there is a separate insulin-favoring transporter and an IGF-1-favoring transporter [62, 77]. Regulation of the two transporters also differ, as triglycerides increase insulin transport across the BBB but inhibit transport of IGF-1 [10, 76]. Furthermore, evidence suggests that IGF-1R does not transport IGF-1 across the BBB [78], but that low-density lipoprotein receptor-related protein (LRP)-1 is involved at the vascular BBB and LRP-2 at the choroid plexus [76, 79]. Therefore, IGF-1R is not a candidate for being the BBB insulin transport protein.

Low-density lipoprotein receptor-related proteins (LRP)

The LRP family of proteins are structurally similar but participate in a wide range of physiological processes including lipid metabolism, neurodevelopment, and transport of nutrients [80]. Megalin, also known as LRP-2, is the largest sized protein in the family and can bind a wide variety of ligands. While it can play a role in reabsorption of various molecules in the proximal renal tubule, including insulin [81], it can also act as a cell signaling transducer within the CNS [80, 82]. LRP-8, also referred to as apolipoprotein E receptor 2 (apoER2), has been recognized as a signal transducer critical in brain development [83]. Both of these have been suggested to play a role in insulin transport in peripheral systems.

LRP-2/Megalin can regulate insulin transport in kidney proximal tubule cells [81] and can take up other hormones as well, including leptin [84] and IGF-1 [85]. Receptor-associated protein (RAP) is a 39 kDa protein that is a natural inhibitor of ligand binding to LRP-2. We used this non-specific inhibitor of LRP-2, RAP, and reported insulin BBB transport was unchanged [43]. However, Orlando et al has also reported that RAP does not affect insulin binding to proximal tubule cells, compared to excess, unlabeled insulin [81]. Therefore, a more specific inhibitor of LRP-2 would aid in fully identifying a role for LRP-2 in insulin BBB transport. Further evidence suggests leptin is also not transported across the BBB via LRP-2 [86], despite its role in transport at the choroid plexus [87]. IGF-1 is also transported across the choroid plexus by LRP-2 [85].

LRP-8/ApoER2 is not only a receptor for apoE but also acts as the primary receptor for the critical brain development protein Reelin [83]. ApoER2 is involved in long-term potentiation, learning, and memory. In the last few years, due to the AD risk gene allele, ApoE4, the role for ApoER2 in AD has begun to be explored. Post-translational proteolytic cleavage of ApoER2 [88] and pre-translational splicing [89] is dysfunctional in AD. Additionally, the risk allele ApoE4 impairs the trafficking of the insulin receptor, resulting in decreased insulin signaling [90]. How ApoER2 may fit into this pathway remains to be determined.

Amino Acid Transporter Involvement

Amino acids are transported across the BBB involving both facilitative systems and active transporters. Some of these transporters are selective for a single substrate or group of substrates while others are non-selective [91]. Recently, it was identified in a high-throughput screen that

the amino acid transporter, SLC7A1, also known as CAT-1, could regulate leptin transport across an iPSC- derived BEC model [92]. This raises as a possibility that the same, or another amino acid transporter, could regulate insulin transport across the BBB. Transport of amino acids could modify transporter expression, activity, and cellular distribution. Additionally, it is possible that the amino acid itself could aid as a co-factor for the insulin transporter. While insulin is known to impact amino acid transport, either directly or indirectly [5], the converse is less well established. The amino acid-derived hormone norepinephrine did not affect insulin BBB transport contrary to a 2-3 fold increase of leptin BBB transport [64]. In an *in vitro* co-culture model of astrocytes and brain endothelial cells, L-glutamate enhances insulin transcytosis [47]. L-arginine, in the presence of LPS, also enhances insulin BBB transport [15]. L-arginine is a nitric oxide precursor and nitric oxide has been shown to regulate insulin BBB transport, as discussed next.

Involvement of Nitric Oxide Synthase (NOS)

Nitric oxide is a common secondary messenger that helps orchestrate multiple signaling pathways. Synthesis of nitric oxide from L-arginine is primarily converted by NOS, present in multiple different cell types. One of the more common roles is to act as a vasodilator, relaxing the smooth muscle cells around the blood vessels. In the brain, there are three main NOS enzymes: endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS). NOS and nitric oxide have an important role at the BBB, regulating its structure and function. Under inflammatory-stimulated conditions *in vivo*, NOS inhibitors enhance insulin BBB transport, specifically nitric oxide coming from nNOS [15]. In an *in vitro* co-culture model, astrocytic inhibition of NOS decreases insulin transcytosis [47]. This suggests the source of the nitric oxide stimulation can regulate insulin transport. The transport system is also suspected to involve calcium signaling as pre-treating astrocytes with a calcium donor enhanced insulin transport across a BEC model [47]. As these second messengers are complex, further investigation on the interaction and the NVU cell types involved in regulating insulin BBB transport are warranted.

Conclusions

We have presented evidence that the insulin transport system at the BBB involves a protein other than the insulin receptor. Identification of this transport system will be critical in treating diseases with deficient CNS insulin signaling, such as Alzheimer's disease or dysregulated metabolism, as insulin availability could be a contributing factor to such a deficiency. While there are ways to deliver exogenous insulin to the CNS, such as via intranasal insulin [93], that have proven to be beneficial, preventing and/or restoring the endogenous insulin BBB transport system would likely be more effective and potentially even prevent a deficiency in the first place. Insulin clearly has multiple impacts not only within the CNS but also in regulating BBB function, that any slight modification of this signaling has downstream detrimental effects. Whether this transport system is unique to the BBB or is similar to other peripheral endothelial beds remains to be determined. Leveraging multiple genetic data sets could hopefully shed light on potential targets for the transport system, but proteomic data will also be necessary. In a recent proteomics study, protein levels of the insulin receptor were detected at similar levels between rat microvessels isolated from various regions including white matter, cortical grey matter, and spinal cord [94]. While protein expression level does not necessarily translate to activity of a transporter, equivalent expression of the insulin receptor across brain regions does not support the high variability of insulin transport rate across brain regions. Additionally, if the transport system involves co-factors, the identification of the transporter could prove to be even more difficult. It is likely the abundance of the transporter(s) within BECs is low, given the limited entry of insulin into the CNS, which will further add to the difficulty. Despite these difficulties, recent technological advances in microvessel isolation, omics-based discovery approaches, and cell culture screening tools will help elucidate the transport system for insulin.

Declarations

Authors' contributions: EMR designed the outline of the review. All authors contributed to the writing of the manuscript and reviewed the final version.

Financial support and sponsorship: This research was funded by The Brown Foundation (EMR) and the National Institute of Health (EMR: P30 DK017047-44 and P30 AG066509; WAB: RF1AG059088).

Conflicts of interest: The authors declare there are no conflicts of interest.

References

1. Supniewski JV, Ishikawa Y, & Geiling EMK. The effect of insulin injected into the cerebrospinal fluid. *Journal of Biological Chemistry*, 1927, 74(2): 241-246.
2. Kasahara M, & Uetani E. The effect of insulin upon the reducing substance in the cerebrospinal fluid of normal rabbits. *Journal of Biological Chemistry*, 1924, 59(2): 433-436.
3. Haugaard N, Vaughan M, Haugaard ES, & Stadie WC. Studies of radioactive injected labeled insulin. *J Biol Chem*, 1954, 208(2): 549-563.
4. Rhea EM, & Banks WA. A historical perspective on the interactions of insulin at the blood-brain barrier. *J Neuroendocrinol*, 2021, 33(4): e12929. [Crossref]
5. Banks WA, Owen JB, & Erickson MA. Insulin in the brain: there and back again. *Pharmacol Ther*, 2012, 136(1): 82-93. [Crossref]

6. Rhea EM, & Banks WA. Role of the Blood-Brain Barrier in Central Nervous System Insulin Resistance. *Frontiers in Neuroscience*, 2019, 13(521). [Crossref]
7. Rhea EM, Banks WA, & Raber J. Insulin Resistance in Peripheral Tissues and the Brain: A Tale of Two Sites. *Biomedicines*, 2022, 10(7). [Crossref]
8. Banks WA, Farr SA, & Morley JE. Permeability of the blood-brain barrier to albumin and insulin in the young and aged SAMP8 mouse. *J Gerontol A Biol Sci Med Sci*, 2000, 55(12): B601-606.
9. Sartorius T, Peter A, Heni M, Maetzler W, Fritsche A, Haring HU, *et al*. The brain response to peripheral insulin declines with age: a contribution of the blood-brain barrier? *PLoS One*, 2015, 10(5): e0126804. [Crossref]
10. Urayama A, & Banks WA. Starvation and triglycerides reverse the obesity-induced impairment of insulin transport at the blood-brain barrier. *Endocrinology*, 2008, 149(7): 3592-3597. [Crossref]
11. Kaiyala KJ, Prigeon RL, Kahn SE, Woods SC, & Schwartz MW. Obesity induced by a high-fat diet is associated with reduced brain insulin transport in dogs. *Diabetes*, 2000, 49(9): 1525-1533.
12. Neuwelt E, Abbott NJ, Abrey L, Banks WA, Blakley B, Davis T, *et al*. Strategies to advance translational research into brain barriers. *Lancet Neurology*, 2008, 7: 84-96.
13. Sweeney MD, Zhao Z, Montagne A, Nelson AR, & Zlokovic BV. Blood-Brain Barrier: From Physiology to Disease and Back. *Physiological reviews*, 2019, 99(1): 21-78. [Crossref]
14. Vangilder RL, Rosen CL, Barr TL, & Huber JD. Targeting the neurovascular unit for treatment of neurological disorders. *Pharmacol Ther*, 2011, 130: 239-247.
15. Banks WA, Dohgu S, Lynch JL, Fleegal-DeMotta MA, Erickson MA, Nakaoke R, *et al*. Nitric oxide isoenzymes regulate lipopolysaccharide-enhanced insulin transport across the blood-brain barrier. *Endocrinology*, 2008, 149(4): 1514-1523. [Crossref]
16. Xaio H, Banks WA, Niehoff ML, & Morley JE. Effect of LPS on the permeability of the blood-brain barrier to insulin. *Brain Res*, 2001, 896(1-2): 36-42.
17. Reese TS, & Karnovsky MJ. Fine structural localization of a blood-brain barrier to endogenous peroxidase. *J Cell Biol*, 1967, 34: 207-217.
18. Kiki-Mvouaka S, Menez C, Borin C, Lyazrhi F, Foucaud-Vignault M, Dupuy J, *et al*. Role of P-glycoprotein in the disposition of macrocyclic lactones: A comparison between ivermectin, eprinomectin, and moxidectin in mice. *Drug Metab Dispos*, 2010, 38(4): 573-580. [Crossref]
19. Mealey KL, Bentjen SA, Gay JM, & Cantor GH. Ivermectin sensitivity is associated with a deletion mutation of the mdr1 gene. *Pharmacogenetics*, 2001, 11: 727-733.
20. Hardebo JE, & Owman C: Enzymatic barrier mechanisms for neurotransmitter monoamines and their precursors at the blood-brain barrier. In: *Pathophysiology of the Blood-Brain Barrier*. edn. Edited by Johansson BB, Owman C, Widner H. Amsterdam: Elsevier; 1990: 41-55.
21. Bernstein HG, Lendeckel U, Bukowska A, Ansorge S, Ernst T, Stauch R, *et al*. Regional and cellular distribution patterns of insulin-degrading enzyme in the adult human brain and pituitary. *J Chem Neuroanat*, 2008, 35(2): 216-224. [Crossref]
22. Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, *et al*. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J Neurosci*, 2014, 34(36): 11929-11947. [Crossref]
23. Lee G, Dallas S, Hong M, & Bendayan R. Drug transporters in the central nervous system: brain barriers and brain parenchyma considerations. *Pharmacol Rev*, 2001, 53(4): 569-596.
24. Hawkins RA. The blood-brain barrier and glutamate. *Am J Clin Nutr*, 2009, 90(3): 867S-874S. [Crossref]
25. de Boer AG, van der Sandt IC, & Gaillard PJ. The role of drug transporters at the blood-brain barrier. *Annu Rev Pharmacol Toxicol*, 2003, 43: 629-656. [Crossref]
26. Sanchez-Covarrubias L, Slosky LM, Thompson BJ, Davis TP, & Ronaldson PT. Transporters at CNS barrier sites: obstacles or opportunities for drug delivery? *Curr Pharm Des*, 2014, 20(10): 1422-1449. [Crossref]
27. Tsuji A, & Tamai II. Carrier-mediated or specialized transport of drugs across the blood-brain barrier. *Adv Drug Deliv Rev*, 1999, 36(2-3): 277-290. [Crossref]
28. Stein WD: Transport and diffusion across cell membranes. London: Academic Press, *Inc*; 1986.
29. Yeagle P: Transport. In: *The Membranes of Cells*. edn. Orlando: Academic Press, Inc.; 1987: 191-215.
30. Toth AE, Holst MR, & Nielsen MS. Vesicular Transport Machinery in Brain Endothelial Cells: What We Know and What We Do not. *Curr Pharm Des*, 2020, 26(13): 1405-1416. [Crossref]
31. Drach J, Gsur A, Hamilton G, Zhao S, Angerler J, Fiegl M, *et al*. Involvement of P-glycoprotein in the transmembrane transport of interleukin-2 (IL-2), IL-4, and interferon-gamma in normal human T lymphocytes. *Blood*, 1996, 88: 1747-1754.
32. Fan JY, Carpentier JL, Gorden P, Van Obberghen E, Blackett NM, Grunfeld C, *et al*. Receptor-mediated endocytosis of insulin: role of microvilli, coated pits, and coated vesicles. *Proc Natl Acad Sci U S A*, 1982, 79(24): 7788-7791. [Crossref]
33. Azizi PM, Zyla RE, Guan S, Wang C, Liu J, Bolz SS, *et al*. Clathrin-dependent entry and vesicle-mediated exocytosis define insulin transcytosis across microvascular endothelial cells. *Mol Biol Cell*, 2015, 26(4): 740-750. [Crossref]
34. Wang H, Wang AX, & Barrett EJ. Caveolin-1 is required for vascular endothelial insulin uptake. *Am J Physiol Endocrinol Metab*, 2011, 300(1): E134-144. [Crossref]
35. Stitt AW, Anderson HR, Gardiner TA, Bailie JR, & Archer DB. Receptor-mediated endocytosis and intracellular trafficking of insulin and low-density lipoprotein by retinal vascular endothelial cells. *Invest Ophthalmol Vis Sci*, 1994, 35(9): 3384-3392.
36. Pemberton SG, DC; Schwartz, MW; Banks, WA; Rhea, EM. Endocytosis of insulin at the blood-brain barrier. *Frontiers in Drug Delivery*, 2022, [Crossref]
37. Banks WA, Jaspan JB, Huang W, & Kastin AJ. Transport of insulin across the blood-brain barrier: saturability at euglycemic doses of insulin. *Peptides*, 1997, 18(9): 1423-1429.

38. Woods SC, & Porte D, Jr. Relationship between plasma and cerebrospinal fluid insulin levels of dogs. *Am J Physiol*, 1977, 233(4): E331-334.
39. Greco AV, Ghirlanda G, Fedeli G, & Gambassi G. Insulin in the cerebro spinal fluid of man. *Eur Neurol*, 1970, 3(5): 303-307. [Crossref]
40. Margolis RU, & Altszuler N. Insulin in the cerebrospinal fluid. *Nature*, 1967, 215(5108): 1375-1376.
41. Baura GD, Foster DM, Porte D, Jr., Kahn SE, Bergman RN, Cobelli C, *et al*. Saturable transport of insulin from plasma into the central nervous system of dogs in vivo. A mechanism for regulated insulin delivery to the brain. *J Clin Invest, 1993*, 92(4): 1824-1830. [Crossref]
42. Stanley M, Macauley SL, Caesar EE, Koscal LJ, Moritz W, Robinson GO, *et al*. The Effects of Peripheral and Central High Insulin on Brain Insulin Signaling and Amyloid-beta in Young and Old APP/PS1 Mice. *J Neurosci*, 2016, 36(46): 11704-11715. [Crossref]
43. Rhea EM, Rask-Madsen C, & Banks WA. Insulin transport across the blood-brain barrier can occur independently of the insulin receptor. *J Physiol*, 2018, 596(19): 4753-4765. [Crossref]
44. Banks WA, Jaspan JB, & Kastin AJ. Selective, physiological transport of insulin across the blood-brain barrier: novel demonstration by species-specific radioimmunoassays. *Peptides*, 1997, 18(8): 1257-1262.
45. Heni M, Schopfer P, Peter A, Sartorius T, Fritsche A, Synofzik M, *et al*. Evidence for altered transport of insulin across the blood-brain barrier in insulin-resistant humans. *Acta Diabetol*, 2014, 51(4): 679-681. [Crossref]
46. Banks WA, Kastin AJ, & Pan W. Uptake and degradation of blood-borne insulin by the olfactory bulb. *Peptides*, 1999, 20(3): 373-378.
47. Gray SM, Aylor KW, & Barrett EJ. Unravelling the regulation of insulin transport across the brain endothelial cell. *Diabetologia*, 2017, 60(8): 1512-1521. [Crossref]
48. Leclerc M, Bourassa P, Tremblay C, Caron V, Sugere C, Emond V, *et al*. Cerebrovascular insulin receptors are defective in Alzheimer's disease. *Brain*, 2022, [Crossref]
49. Taubel JC, Nelson NR, Bansal A, Curran GL, Wang L, Wang Z, *et al*. Design, Synthesis, and Preliminary Evaluation of [(68)Ga]Ga-NOTA-Insulin as a PET Probe in an Alzheimer's Disease Mouse Model. *Bioconjug Chem*, 2022, 33(5): 892-906. [Crossref]
50. Hersom M, Helms HC, Schmalz C, Pedersen TA, Buckley ST, & Brodin B. The insulin receptor is expressed and functional in cultured blood-brain barrier endothelial cells but does not mediate insulin entry from blood to brain. *Am J Physiol Endocrinol Metab*, 2018, 315(4): E531-E542. [Crossref]
51. Banks WA, Jaspan JB, & Kastin AJ. Effect of diabetes mellitus on the permeability of the blood-brain barrier to insulin. *Peptides*, 1997, 18(10): 1577-1584.
52. Frank HJ, Jankovic-Vokes T, Pardridge WM, & Morris WL. Enhanced insulin binding to blood-brain barrier in vivo and to brain microvessels in vitro in newborn rabbits. *Diabetes*, 1985, 34(8): 728-733.
53. Azar AS, & Brooks VL. Impaired baroreflex gain during pregnancy in conscious rats: role of brain insulin. *Hypertension*, 2011, 57(2): 283-288. [Crossref]
54. Shi Z, Hansen KM, Bullock KM, Morofuji Y, Banks WA, & Brooks VL. Resistance to the sympathoexcitatory effects of insulin and leptin in late pregnant rats. *J Physiol*, 2019, 597(15): 4087-4100. [Crossref]
55. Zhou AL, Sharda N, Sarma VV, Ahlschwede KM, Curran GL, Tang X, *et al*. Age-Dependent Changes in the Plasma and Brain Pharmacokinetics of Amyloid-beta Peptides and Insulin. *J Alzheimers Dis*, 2022, 85(3): 1031-1044. [Crossref]
56. Poduslo JF, Curran GL, Wengenack TM, Malester B, & Duff K. Permeability of proteins at the blood-brain barrier in the normal adult mouse and double transgenic mouse model of Alzheimer's disease. *Neurobiol Dis*, 2001, 8(4): 555-567. [Crossref]
57. Craft S, Peskind E, Schwartz MW, Schellenberg GD, Raskind M, & Porte D, Jr. Cerebrospinal fluid and plasma insulin levels in Alzheimer's disease: relationship to severity of dementia and apolipoprotein E genotype. *Neurology*, 1998, 50(1): 164-168.
58. Ben-Shachar D, Yehuda S, Finberg JP, Spanier I, & Youdim MB. Selective alteration in blood-brain barrier and insulin transport in iron-deficient rats. *J Neurochem*, 1988, 50(5): 1434-1437. [Crossref]
59. Brown C, Pemberton S, Babin A, Abdulhameed N, Noonan C, Brown MB, *et al*. Insulin blood-brain barrier transport and interactions are greater following exercise in mice. *J Appl Physiol* (1985), 2022. [Crossref]
60. Rhea EM, Torres ERS, Raber J, & Banks WA. Insulin BBB pharmacokinetics in young apoE male and female transgenic mice. *PLoS One*, 2020, 15(1): e0228455. [Crossref]
61. Rhea EM, Hansen K, Pemberton S, Torres ERS, Holden S, Raber J, *et al*. Effects of apolipoprotein E isoform, sex, and diet on insulin BBB pharmacokinetics in mice. *Sci Rep*, 2021, 11(1): 18636. [Crossref]
62. Reinhardt RR, & Bondy CA. Insulin-like growth factors cross the blood-brain barrier. *Endocrinology*, 1994, 135(5): 1753-1761. [Crossref]
63. Banks WA, & Kastin AJ. Peptides and the blood-brain barrier: lipophilicity as a predictor of permeability. *Brain Res Bull*, 1985, 15(3): 287-292. [Crossref]
64. Banks WA. Enhanced leptin transport across the blood-brain barrier by alpha 1-adrenergic agents. *Brain Res*, 2001, 899(1-2): 209-217.
65. Nguyen S, Banks WA, & Rhea EM. Effects of Rapamycin on Insulin Brain Endothelial Cell Binding and Blood-Brain Barrier Transport. *Med Sci (Basel)*, 2021, 9(3). [Crossref]
66. Galindo DC, Banks WA, & Rhea EM. The impact of acute rosiglitazone on insulin pharmacokinetics at the blood-brain barrier. *Endocrinol Diabetes Metab*, 2020, 3(3): e00149. [Crossref]
67. May AA, Liu M, Woods SC, & Begg DP. CCK increases the transport of insulin into the brain. *Physiol Behav*, 2016, 165: 392-397. [Crossref]
68. May AA, Bedel ND, Shen L, Woods SC, & Liu M. Estrogen and insulin transport through the blood-brain barrier. *Physiol Behav*, 2016, 163: 312-321. [Crossref]
69. Baura GD, Foster DM, Kaiyala K, Porte D, Jr., Kahn SE, & Schwartz MW. Insulin transport from plasma into the

central nervous system is inhibited by dexamethasone in dogs. *Diabetes*, 1996, 45(1): 86-90. [Crossref]

70. Schaffer L, Brand CL, Hansen BF, Ribel U, Shaw AC, Slaaby R, *et al*. A novel high-affinity peptide antagonist to the insulin receptor. *Biochem Biophys Res Commun*, 2008, 376(2): 380-383. [Crossref]
71. Nagano H, Ito S, Masuda T, & Ohtsuki S. Effect of Insulin Receptor-Knockdown on the Expression Levels of Blood-Brain Barrier Functional Proteins in Human Brain Microvascular Endothelial Cells. *Pharm Res*, 2022, 39(7): 1561-1574. [Crossref]
72. Banks WA. The source of cerebral insulin. *European Journal of Pharmacology*, 2004, 490: 5-12.
73. Brown RS, Wyatt AK, Herbison RE, Knowles PJ, Ladyman SR, Binart N, *et al*. Prolactin transport into mouse brain is independent of prolactin receptor. *FASEB J*, 2016, 30(2): 1002-1010. [Crossref]
74. Rosenfeld RG, Pham H, Keller BT, Borchardt RT, & Pardridge WM. Demonstration and structural comparison of receptors for insulin-like growth factor-I and -II (IGF-I and -II) in brain and blood-brain barrier. *Biochem Biophys Res Commun*, 1987, 149(1): 159-166. [Crossref]
75. Pan W, & Kastin AJ. Interactions of IGF-1 with the blood-brain barrier in vivo and in situ. *Neuroendocrinology*, 2000, 72(3): 171-178. [Crossref]
76. Dietrich MO, Muller A, Bolos M, Carro E, Perry ML, Portela LV, *et al*. Western style diet impairs entrance of blood-borne insulin-like growth factor-1 into the brain. *Neuromolecular Med*, 2007, 9(4): 324-330. [Crossref]
77. Yu Y, Kastin AJ, & Pan W. Reciprocal interactions of insulin and insulin-like growth factor I in receptor-mediated transport across the blood-brain barrier. *Endocrinology*, 2006, 147(6): 2611-2615. [Crossref]
78. Pulford BE, & Ishii DN. Uptake of circulating insulin-like growth factors (IGFs) into cerebrospinal fluid appears to be independent of the IGF receptors as well as IGF-binding proteins. *Endocrinology*, 2001, 142(1): 213-220. [Crossref]
79. Nishijima T, Piriz J, Duflot S, Fernandez AM, Gaitan G, Gomez-Pinedo U, *et al*. Neuronal activity drives localized blood-brain-barrier transport of serum insulin-like growth factor-I into the CNS. *Neuron*, 2010, 67(5): 834-846. [Crossref]
80. May P, Woldt E, Matz RL, & Boucher P. The LDL receptor-related protein (LRP) family: an old family of proteins with new physiological functions. *Ann Med*, 2007, 39(3): 219-228. [Crossref]
81. Orlando RA, Rader K, Authier F, Yamazaki H, Posner BI, Bergeron JJ, *et al*. Megalin is an endocytic receptor for insulin. *J Am Soc Nephrol*, 1998, 9(10): 1759-1766.
82. Willnow TE, Hilpert J, Armstrong SA, Rohlmann A, Hammer RE, Burns DK, *et al*. Defective forebrain development in mice lacking gp330/megalin. *Proc Natl Acad Sci U S A*, 1996, 93(16): 8460-8464. [Crossref]
83. D'Arcangelo G. Apoer2: a reelin receptor to remember. *Neuron*, 2005, 47(4): 471-473. [Crossref]
84. Hama H, Saito A, Takeda T, Tanuma A, Xie Y, Sato K, *et al*. Evidence indicating that renal tubular metabolism of leptin is mediated by megalin but not by the leptin receptors. *Endocrinology*, 2004, 145(8): 3935-3940. [Crossref]
85. Carro E, Spuch C, Trejo JL, Antequera D, & Torres-Aleman I. Choroid plexus megalin is involved in neuroprotection by serum insulin-like growth factor I. *J Neurosci*, 2005, 25(47): 10884-10893. [Crossref]
86. Sandin ES, Folberth J, Muller-Fielitz H, Pietrzik CU, Herold E, Willnow TE, *et al*. Is LRP2 Involved in Leptin Transport over the Blood-Brain Barrier and Development of Obesity? *Int J Mol Sci*, 2021, 22(9). [Crossref]
87. Dietrich MO, Spuch C, Antequera D, Rodal I, de Yebenes JG, Molina JA, *et al*. Megalin mediates the transport of leptin across the blood-CSF barrier. *Neurobiol Aging*, 2008, 29(6): 902-912. [Crossref]
88. Mata-Balaguer T, Cuchillo-Ibanez I, Calero M, Ferrer I, & Saez-Valero J. Decreased generation of C-terminal fragments of ApoER2 and increased reelin expression in Alzheimer's disease. *FASEB J*, 2018, 32(7): 3536-3546. [Crossref]
89. Hinrich AJ, Jodelka FM, Chang JL, Brutman D, Bruno AM, Briggs CA, *et al*. Therapeutic correction of ApoER2 splicing in Alzheimer's disease mice using antisense oligonucleotides. *EMBO Mol Med*, 2016, 8(4): 328-345. [Crossref]
90. Zhao N, Liu CC, Van Ingelgom AJ, Martens YA, Linares C, Knight JA, *et al*. Apolipoprotein E4 Impairs Neuronal Insulin Signaling by Trapping Insulin Receptor in the Endosomes. *Neuron*, 2017, 96(1): 115-+. [Crossref]
91. Zaragoza R. Transport of Amino Acids Across the Blood-Brain Barrier. *Front Physiol*, 2020, 11: 973. [Crossref]
92. Shi Y, Kim H, Hamann CA, Rhea EM, Brunger JM, & Lippmann ES. Nuclear receptor ligand screening in an iPSC-derived in vitro blood-brain barrier model identifies new contributors to leptin transport. *Fluids Barriers CNS*, 2022, 19(1): 77. [Crossref]
93. Rhea EM, Salameh TS, & Banks WA. Routes for the delivery of insulin to the central nervous system: A comparative review. *Exp Neurol*, 2019, 313: 10-15. [Crossref]
94. Uchida Y, Yagi Y, Takao M, Tano M, Umetsu M, Hirano S, *et al*. Comparison of Absolute Protein Abundances of Transporters and Receptors among Blood-Brain Barriers at Different Cerebral Regions and the Blood-Spinal Cord Barrier in Humans and Rats. *Mol Pharm*, 2020, 17(6): 2006-2020. [Crossref]

Cite this article as: Banks WA, Noonan C, & Rhea EM. Evidence for an alternative insulin transporter at the blood-brain barrier. *Aging Pathobiol Ther*, 2022, 4(4): 100-108. doi: 10.31491/APT.2022.12.100

Aging Pathobiology and Therapeutics 2022; 4(4): 119-128 ***119***
DOI: 10.31491/APT.2022.12.098

Influence of metformin on age-related macular degeneration

Albert J. Augustin[a,*], Jenny Atorf[a]

[a] *Department of Ophthalmology, Staedtisches Klinikum Karlsruhe, Moltkestr. 90, 76133 Karlsruhe, Germany.*

This article belongs to the Special Issue: Enhancing healthy aging with drugs that target insulin signaling

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

* Corresponding author: Albert J. Augustin
Mailing address: Department of Ophthalmology, Staedtisches Klinikum Karlsruhe, Moltkestr. 90, 76133 Karlsruhe, Germany.
Email: albertjaugustin@googlemail.com
Received: 26 August 2022 / Revised: 19 September 2022
Accepted: 21 November 2022 / Published: 29 December 2022

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].

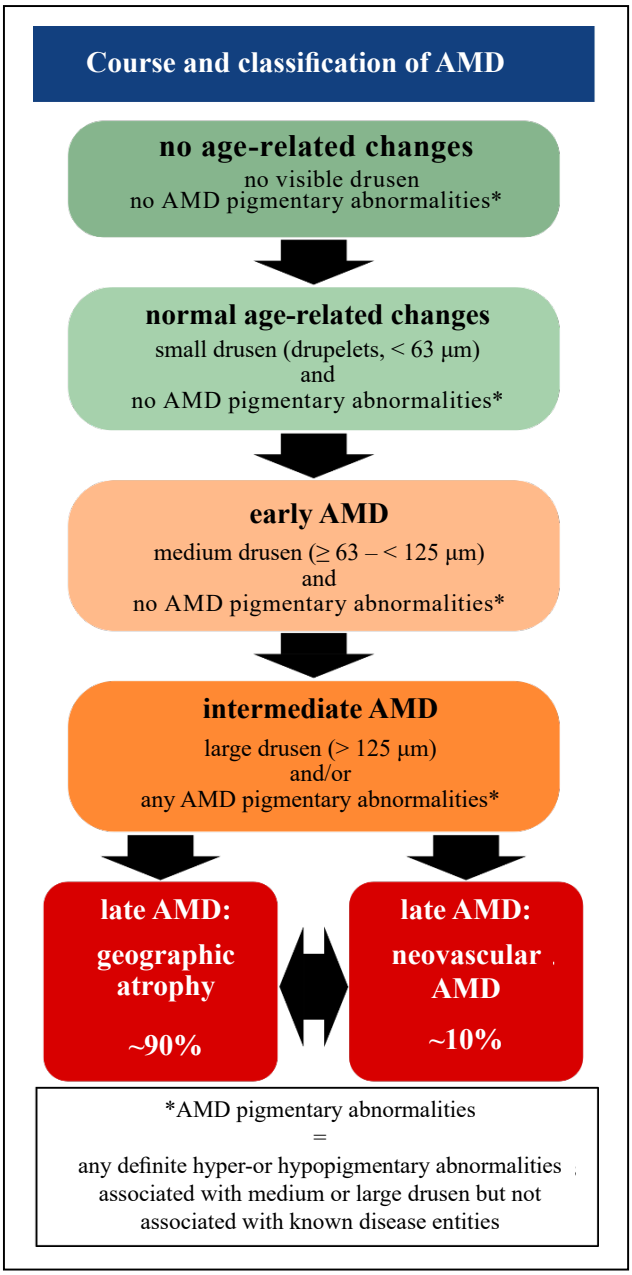

Figure 1. Classification and course of age-related macular degeneration (AMD). The earliest precursor signs of AMD are small drusen that are classified as normal age-related changes. Early AMD is characterized by the presence of medium drusen but the absence of AMD pigmentary changes which are defined 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])

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,

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-

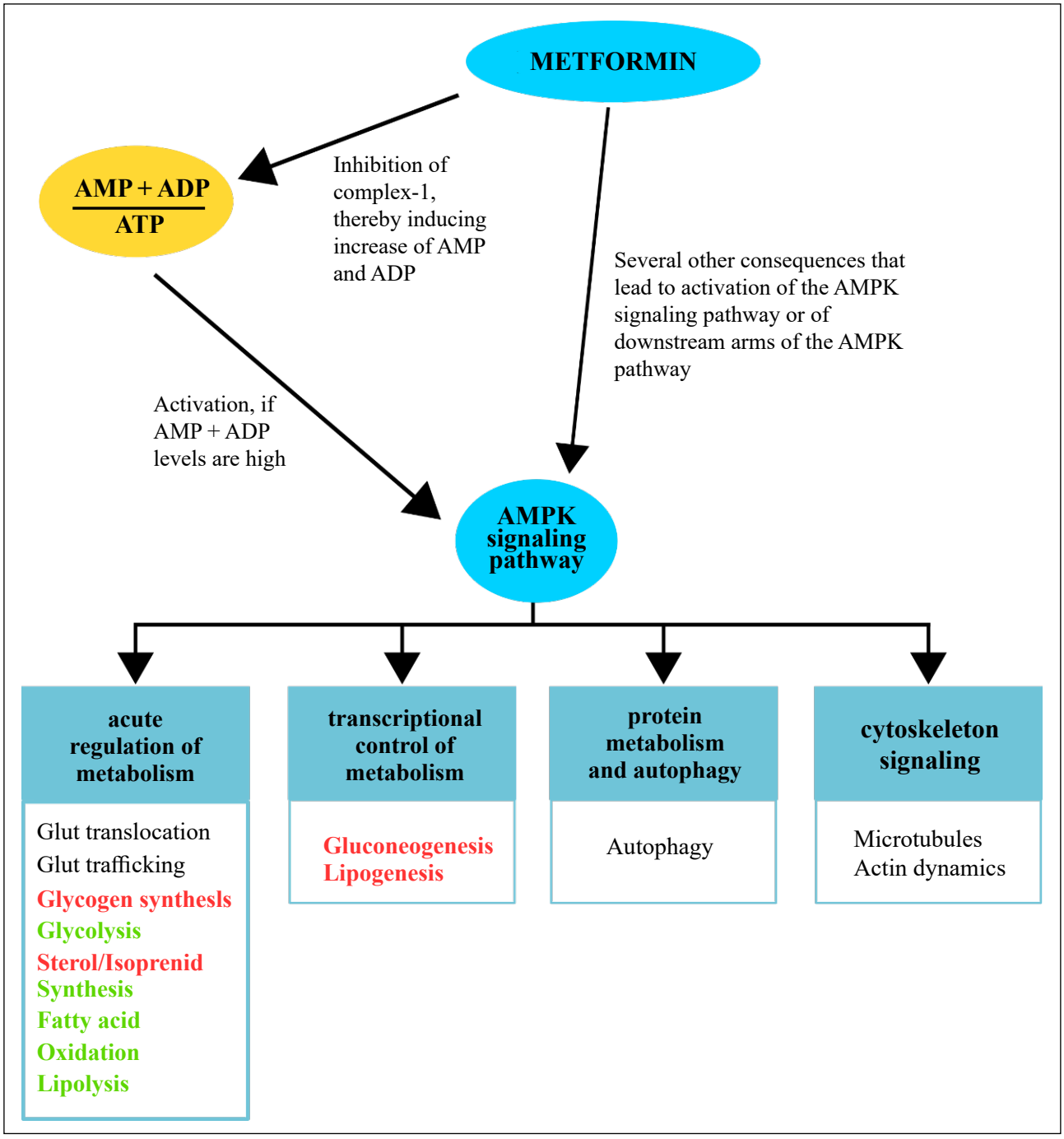

Figure 2. Influence of metformin on the AMPK signaling pathway and consequences of AMPK activation. Without metformin, the AMPK signaling pathway is activated when the cellular levels of AMP and ADP increase. Activation of the pathway leads to a switch from energy-consuming metabolism to energy-providing 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. Adjusted 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

Authors' contributions: The authors contributed equally to the article.

Conflicts of interest: I declare that I have no conflicts of interest.

Ethical approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

References

1. Rena G, Hardie DG, & Pearson ER. The mechanisms of action of metformin. *Diabetologia*, 2017, 60(9): 1577-1585. [Crossref]
2. LaMoia TE, & Shulman GI. Cellular and Molecular Mechanisms of Metformin Action. *Endocr Rev*, 2021, 42(1): 77-96. [Crossref]
3. Romdhoniyyah DF, Harding SP, Cheyne CP, & Beare NAV. Metformin, A Potential Role in Age-Related Macular Degeneration: A Systematic Review and Meta-Analysis. *Ophthalmol Ther*, 2021, 10(2): 245-260. [Crossref]
4. Campbell JM, Bellman SM, Stephenson MD, & Lisy K. Metformin reduces all-cause mortality and diseases of ageing independent of its effect on diabetes control: A systematic review and meta-analysis. *Ageing Res Rev*, 2017, 40: 31-44. [Crossref]
5. Saisho Y. Metformin and Inflammation: Its Potential Beyond Glucose-lowering Effect. *Endocr Metab Immune Disord Drug Targets*, 2015, 15(3): 196-205. [Crossref]
6. Amin SV, Khanna S, Parvar SP, Shaw LT, Dao D, Hariprasad SM, *et al*. Metformin and retinal diseases in preclinical and clinical studies: Insights and review of literature. *Exp Biol Med (Maywood)*, 2022, 247(4): 317-329. [Crossref]
7. Soukas AA, Hao H, & Wu L. Metformin as Anti-Aging Therapy: Is It for Everyone? *Trends Endocrinol Metab*, 2019, 30(10): 745-755. [Crossref]
8. Wong WL, Su X, Li X, Cheung CM, Klein R, Cheng CY, *et al*. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *Lancet Glob Health*, 2014, 2(2): e106-116. [Crossref]
9. Stahl A. The Diagnosis and Treatment of Age-Related Macular Degeneration. *Dtsch Arztebl Int*, 2020, 117(29-30): 513-520. [Crossref]
10. Thomas CJ, Mirza RG, & Gill MK. Age-Related Macular Degeneration. *Med Clin North Am*, 2021, 105(3): 473-491. [Crossref]
11. Armento A, Ueffing M, & Clark SJ. The complement system in age-related macular degeneration. *Cell Mol Life Sci*, 2021, 78(10): 4487-4505. [Crossref]

12. Ferris FL, 3rd, Wilkinson CP, Bird A, Chakravarthy U, Chew E, Csaky K, *et al*. Clinical classification of age-related macular degeneration. *Ophthalmology*, 2013, 120(4): 844-851. [Crossref]
13. Schmidt-Erfurth U, Chong V, Loewenstein A, Larsen M, Souied E, Schlingemann R, *et al*. Guidelines for the management of neovascular age-related macular degeneration by the European Society of Retina Specialists (EURETINA). *Br J Ophthalmol*, 2014, 98(9): 1144-1167. [Crossref]
14. Augustin AJ, Atorf J. Chirurgie bei Makulablutung: Wann und unter welchen Umständen? *Ophthalmo-Chirurgie*. 2022, 34(1): 27–31. German.
15. Hurley JB. Retina Metabolism and Metabolism in the Pigmented Epithelium: A Busy Intersection. *Annu Rev Vis Sci*, 2021, 7: 665-692. [Crossref]
16. Cunha-Vaz J, Bernardes R, & Lobo C. Blood-retinal barrier. *Eur J Ophthalmol*, 2011, 21 Suppl 6: S3-9. [Crossref]
17. Gu X, Neric NJ, Crabb JS, Crabb JW, Bhattacharya SK, Rayborn ME, *et al*. Age-related changes in the retinal pigment epithelium (RPE). *PLoS One*, 2012, 7(6): e38673. [Crossref]
18. Anderson DH, Mullins RF, Hageman GS, & Johnson LV. A role for local inflammation in the formation of drusen in the aging eye. *Am J Ophthalmol*, 2002, 134(3): 411-431. [Crossref]
19. Wang L, Clark ME, Crossman DK, Kojima K, Messinger JD, Mobley JA, *et al*. Abundant lipid and protein components of drusen. *PLoS One*, 2010, 5(4): e10329. [Crossref]
20. Crabb JW, Miyagi M, Gu X, Shadrach K, West KA, Sakaguchi H, *et al*. Drusen proteome analysis: an approach to the etiology of age-related macular degeneration. *Proc Natl Acad Sci U S A*, 2002, 99(23): 14682-14687. [Crossref]
21. Thornton J, Edwards R, Mitchell P, Harrison RA, Buchan I, & Kelly SP. Smoking and age-related macular degeneration: a review of association. *Eye (Lond)*, 2005, 19(9): 935-944. [Crossref]
22. Raimundo M, Mira F, Cachulo MDL, Barreto P, Ribeiro L, Farinha C, *et al*. Adherence to a Mediterranean diet, lifestyle and age-related macular degeneration: the Coimbra Eye Study - report 3. *Acta Ophthalmol*, 2018, 96(8): e926-e932. [Crossref]
23. Merle BMJ, Colijn JM, Cougnard-Grégoire A, de Koning-Backus APM, Delyfer MN, Kiefte-de Jong JC, *et al*. Mediterranean Diet and Incidence of Advanced Age-Related Macular Degeneration: The EYE-RISK Consortium. *Ophthalmology*, 2019, 126(3): 381-390. [Crossref]
24. Rinninella E, Mele MC, Merendino N, Cintoni M, Anselmi G, Caporossi A, *et al*. The Role of Diet, Micronutrients and the Gut Microbiota in Age-Related Macular Degeneration: New Perspectives from the Gut⁻Retina Axis. *Nutrients*, 2018, 10(11). [Crossref]
25. Fritsche LG, Igl W, Bailey JN, Grassmann F, Sengupta S, Bragg-Gresham JL, *et al*. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. *Nat Genet*, 2016, 48(2): 134-143. [Crossref]
26. Romero-Vazquez S, Llorens V, Soler-Boronat A, Figueras-Roca M, Adan A, & Molins B. Interlink between Inflammation and Oxidative Stress in Age-Related Macular Degeneration: Role of Complement Factor H. *Biomedicines*, 2021, 9(7). [Crossref]
27. Nita M, & Grzybowski A. Interplay between reactive oxygen species and autophagy in the course of age-related macular degeneration. *Excli j*, 2020, 19: 1353-1371. [Crossref]
28. Mihaylova MM, & Shaw RJ. The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. *Nat Cell Biol*, 2011, 13(9): 1016-1023. [Crossref]
29. Zhang M, Jiang N, Chu Y, Postnikova O, Varghese R, Horvath A, *et al*. Dysregulated metabolic pathways in age-related macular degeneration. *Sci Rep*, 2020, 10(1): 2464. [Crossref]
30. Dang KR, Wu T, Hui YN, & Du HJ. Newly-found functions of metformin for the prevention and treatment of age-related macular degeneration. I*nt J Ophthalmol*, 2021, 14(8): 1274-1280. [Crossref]
31. Bharath LP, & Nikolajczyk BS. The intersection of metformin and inflammation. *Am J Physiol Cell Physiol*, 2021, 320(5): C873-c879. [Crossref]
32. Xian H, Liu Y, Rundberg Nilsson A, Gatchalian R, Crother TR, Tourtellotte WG, *et al*. Metformin inhibition of mitochondrial ATP and DNA synthesis abrogates NLRP3 inflammasome activation and pulmonary inflammation. *Immunity*, 2021, 54(7): 1463-1477.e1411. [Crossref]
33. Ambati M, Apicella I, Wang SB, Narendran S, Leung H, Pereira F, *et al*. Identification of fluoxetine as a direct NLRP3 inhibitor to treat atrophic macular degeneration. *Proc Natl Acad Sci U S A*, 2021, 118(41). [Crossref]
34. Ying Y, Ueta T, Jiang S, Lin H, Wang Y, Vavvas D, *et al*. Metformin inhibits ALK1-mediated angiogenesis via activation of AMPK. *Oncotarget*, 2017, 8(20): 32794-32806. [Crossref]
35. Han J, Li Y, Liu X, Zhou T, Sun H, Edwards P, *et al*. Metformin suppresses retinal angiogenesis and inflammation in vitro and in vivo. *PLoS One*, 2018, 13(3): e0193031. [Crossref]
36. Qu S, Zhang C, Liu D, Wu J, Tian H, Lu L, *et al*. Metformin Protects ARPE-19 Cells from Glyoxal-Induced Oxidative Stress. *Oxid Med Cell Longev*, 2020, 2020: 1740943. [Crossref]
37. Zhao X, Liu L, Jiang Y, Silva M, Zhen X, & Zheng W. Protective Effect of Metformin against Hydrogen Peroxide-Induced Oxidative Damage in Human Retinal Pigment Epithelial (RPE) Cells by Enhancing Autophagy through Activation of AMPK Pathway. *Oxid Med Cell Longev*, 2020, 2020: 2524174. [Crossref]
38. Xu L, Kong L, Wang J, & Ash JD. Stimulation of AMPK prevents degeneration of photoreceptors and the retinal pigment epithelium. *Proc Natl Acad Sci U S A*, 2018, 115(41): 10475-10480. [Crossref]
39. Stewart JM, Lamy R, Wu F, & Keenan JD. Relationship between Oral Metformin Use and Age-Related Macular Degeneration. *Ophthalmol Retina*, 2020, 4(11): 1118-1119. [Crossref]
40. Chen YY, Shen YC, Lai YJ, Wang CY, Lin KH, Feng SC, *et al*. Association between Metformin and a Lower Risk of

Age-Related Macular Degeneration in Patients with Type 2 Diabetes. *J Ophthalmol*, 2019, 2019: 1649156. [Crossref]

41. Jiang J, Chen Y, Zhang H, Yuan W, Zhao T, Wang N, *et al*. Association between metformin use and the risk of age-related macular degeneration in patients with type 2 diabetes: a retrospective study. *BMJ Open*, 2022, 12(4): e054420. [Crossref]
42. Gokhale KM, Adderley NJ, Subramanian A, Lee WH, Han D, Coker J, *et al*. Metformin and risk of age-related macular degeneration in individuals with type 2 diabetes: a retrospective cohort study. *Br J Ophthalmol*, 2022. [Crossref]
43. Eton EA, Wubben TJ, Besirli CG, Hua P, McGeehan B, & VanderBeek BL. Association of metformin and development of dry age-related macular degeneration in a U.S. insurance claims database. *Eur J Ophthalmol*, 2022, 32(1): 417-423. [Crossref]
44. Blitzer AL, Ham SA, Colby KA, & Skondra D. Association of Metformin Use With Age-Related Macular Degeneration: A Case-Control Study. *JAMA Ophthalmol*, 2021, 139(3): 302-309. [Crossref]
45. Brown EE, Ball JD, Chen Z, Khurshid GS, Prosperi M, & Ash JD. The Common Antidiabetic Drug Metformin Reduces Odds of Developing Age-Related Macular Degeneration. *Invest Ophthalmol Vis Sci*, 2019, 60(5): 1470-1477. [Crossref]
46. Lee H, Jeon HL, Park SJ, & Shin JY. Effect of Statins, Metformin, Angiotensin-Converting Enzyme Inhibitors, and Angiotensin II Receptor Blockers on Age-Related Macular Degeneration. *Yonsei Med J*, 2019, 60(7): 679-686. [Crossref]
47. Mauschitz MM, Verzijden T, Schuster AK, Elbaz H, Pfeiffer N, Khawaja A, *et al*. Association of lipid-lowering drugs and antidiabetic drugs with age-related macular degeneration: a meta-analysis in Europeans. *Br J Ophthalmol*, 2022. [Crossref]
48. Röhrig B, du Prel JB, Wachtlin D, & Blettner M. Types of study in medical research: part 3 of a series on evaluation of scientific publications. *Dtsch Arztebl Int*, 2009, 106(15): 262-268. [Crossref]
49. University of California, San Francisco. Metformin for the Minimization of Geographic Atrophy Progression in Patients With AMD (METforMIN). 2022 [cited 2022 Aug 12]. Available from: https://clinicaltrials.gov/ct2/show/NCT02684578?cond=metformin+macular+degeneration&draw=2&rank=1
50. Ebeling MC, Geng Z, Stahl MR, Kapphahn RJ, Roehrich H, Montezuma SR, *et al*. Testing Mitochondrial-Targeted Drugs in iPSC-RPE from Patients with Age-Related Macular Degeneration. *Pharmaceuticals (Basel)*, 2022, 15(1). [Crossref]

Cite this article as: Augustin AJ, Atorf J. Influence of metformin on age-related macular degeneration. *Aging Pathobiol Ther*, 2022, 4(4): 119-128. doi: 10.31491/APT.2022.12.098

Aging Pathobiology and Therapeutics

ABOUT

AIMS AND SCOPE

Aging Pathobiology and Therapeutics (APT) is an open access, peer-reviewed, interdisciplinary journal available online, committed to disseminating information on the pathological progression and intervention of aging and age-related disease phenotypes in mammalian species, providing an opportunity to communicate pathology data as a primary scientific focus of aging. Scholars from the disciplines of geriatrics, gerontology, physiology, pathology, pharmacology, toxicology, biology, neuropsychology, psychology, nursing, epidemiology, and genetics are encouraged to contribute articles to the journal. Article types considered include research articles, reviews, editorials, case reports, opinions, letters, abstracts and drug therapeutic brief relating to research on Aging.

The Editorial Board of the Journal comprises a dedicated team of local and international experts in the field of geriatrics, gerontology, physiology, pathology, pharmacology, biology, neuropsychology, psychology, nursing, epidemiology, and genetics. All members of the Editorial Board actively guide and set the direction of the journal. With the aim of promoting effective and accurate scientific information, an expert panel of referees constitutes the backbone of the peer-review process in evaluating the quality and content of manuscripts submitted for publication.

INFORMATION FOR AUTHORS

APT follows the Committee on Publication Ethics (COPE)'s guidelines and the ICMJE recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journal.

Manuscripts submitted must be the original work of the author(s) and must not be published previously or under consideration for publication elsewhere.

JOURNAL INFORMATION

- Official Publication: Geropathology Research Network, Geriatric Department of Guangzhou First People's Hosipital (CN)

- Funded: APT is funded by ANT Publishing Corporation
- Ownership: APT is owned by Geropathology Research Network and published by ANT Publishing Corporation

JOURNAL STATISTICS

- Launch date: 11 November 2019
- Publication model: Open access
- ISSN: 2690-1803 (Online)
- Digital archive: Portico
- Frequency: Quarterly published
- DOI: 10.31491/APT

SUBMISSION TURNAROUND TIME

- Initial review: 3 days
- External peer review: 2–3 weeks
- Publication ahead of print: within 2 weeks after being accepted
- Formal publication: within 1-2 months after being accepted

INDEXING/ABSTRACTING

- Scopus
- Chemical Abstracts Service
- CNKI Scholar
- Google Scholar
- ICI World of Journals
- Portico
- Research Gate
- Scilit

- Semantic Scholar
- Sherpa/Romeo
- ScienceOpen
- WorldCat

COPYRIGHT AND LICENSE TO PUBLISH

EDITORIAL OFFICE

- Email: apt@antpublisher.com
- US Office: 2879 Tynewick Dr, Roswell, Georgia, 30075, USA
- China Office: Room 1006, Wuxing Building, No. 280-1 Tongyang Road, Liangxi District, Wuxi, China

ANT Publishing

ABOUT

Headquartered in Georgia, USA, ANT Publishing Corporation is a scholarly open access and peer-reviewed publisher specializing in the publication of biomedical journals in print and online across the broad spectrum of science, technology and medicine. ANT possesses standard publication ethics, a rigorous peer review process, and rapid publication, aiming to provide a significant contribution to the academic exchange. All journals published by ANT currently working towards being indexed in PubMed, Science Citation Index Expanded (SCIE), BIOSIS, Scopus. We strive to provide an easily and freely accessible platform to researchers and practitioners in support of their novel and valuable ideas. Currently, we are concentrating on building the following journals.

AGING PATHOBIOLOGY AND THERAPEUTICS

URO-TECHNOLOGY JOURNAL

ANIMAL HEALTH AND THERAPY REPORTS

All ANT journals are international, peer-reviewed Open Access journals. In accordance with our Open Access policy (under the Creative Commons Attribution (CC BY 4.0) License), all papers, including supplements, published in ANT journals are immediately and permanently available online, and free to access, distribute, and reuse provided that the original work is correctly cited. For more details, please see our Open Access Polices.

All ANT journals maintain a rigorous, thorough, unbiased peer review. Besides, quality control is imbedded in each step of the editorial process, and in each internal and external editors' responsibilities. In this quality control system, all editors abide by the procedures, standards and ethical polices outlined by the Committee on Publication Ethics (COPE) and International Committee of Medical Journal Editors (ICMJE), the Open Access Scholarly Publishers Association (OASPA), the World Association of Medical Editors (WAME) and Directory of Open Access Journals (DOAJ). More details regarding ANT peer review and quality control system are introduced in Editorial Process.

CONTACT US

- Website: http://www.antpublisher.com
- Tel: +1(513)291-3698
- US Office: 2879 Tynewick Dr, Roswell, Georgia, 30075, USA
- China Office: Room 1006, Wuxing Building, No. 280-1 Tongyang Road, Liangxi District, Wuxi, China
- General editorial enquiries: editorialoffice@antpublisher.com
- Cooperation enquiries: support@antpublisher.com
- Copyright related enquiries: service@antpublisher.com