

Review Article

Multi-Omics Insights into Systemic Molecular Alterations and Emerging Therapeutic Targets in Diabetic Retinopathy

Ruba A. Zenati  Reem A. Qannita, Ahmad Y. Abuhelwa  Mohammad A.Y. Alqudah  Shereen M. Aleidi, Waseem El-Huneidi  Eman Abu-Gharbieh, Zainab M. AlShareef, Karem H. Alzoubi  Adnane Guella, Yasser Bustanji, Mohammad H. Semreen 

Received (first version): 14-Apr-2026

Accepted: 03-Jun- 2026

Published online: 05-Aug-2026

Abstract

Diabetic retinopathy (DR) is a vision-threatening complication of diabetes mellitus and one of the leading causes of blindness among working-age adults globally. It is characterised by progressive microvascular damage, neurodegeneration, and inflammation within the retina. Current clinical management relies on anti-vascular endothelial growth factor (VEGF) therapy and laser photocoagulation, which target late-stage vascular changes but fail to address the underlying systemic and molecular drivers of early disease. Moreover, diagnostic strategies remain largely imaging-based, with limited integration of molecular biomarkers that could facilitate early detection or personalized intervention. In recent years, multi-omics technologies, including metabolomics and proteomics, have emerged as powerful tools to unravel the complex molecular landscape of DR. These approaches have identified key dysregulated pathways, like the polyol pathway, and chronic inflammatory signalling, alongside candidate biomarkers in plasma, and retinal tissue. Despite this progress, clinical translation remains limited due to heterogeneity in findings and gaps in longitudinal validation. This review integrates recent multi-omics discoveries relevant to DR pathogenesis, highlights emerging systemic and retinal biomarkers, and evaluates potential therapeutic targets beyond VEGF. By integrating evidence from human and animal studies, we outline how omics-informed insights can inform risk stratification, guide therapies, and support a shift toward personalized management of DR.

Keywords: Diabetic retinopathy; Diabetes mellitus; Multi-omics; Metabolomics; Proteomics; Biomarkers.

Ruba A. Zenati. Research Institute for Medical and Health Sciences, University of Sharjah, Sharjah 27272, United Arab Emirates; Department of Medicinal Chemistry, College of Pharmacy, University of Sharjah, 27272, United Arab Emirates. u23107609@sharjah.ac.ae

Reem A. Qannita. Research Institute for Medical and Health Sciences, University of Sharjah, Sharjah 27272, United Arab Emirates. U22103857@sharjah.ac.ae

Ahmad Y. Abuhelwa. Research Institute for Medical and Health Sciences, University of Sharjah, Sharjah 27272, United Arab Emirates; Department of Pharmacy Practice and Pharmacotherapeutics, College of Pharmacy, The University of Sharjah, Sharjah, 27272, United Arab Emirates. ahmad.abuhelwa@sharjah.ac.ae

Mohammad A. Y. Alqudah. Department of Pharmacy Practice and Pharmacotherapeutics, College of Pharmacy, The University of Sharjah, Sharjah, 27272, United Arab Emirates; Department of Clinical Pharmacy, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, 22110, Jordan. malqudah@sharjah.ac.ae

Shereen M. Aleidi. Research Institute for Medical and Health Sciences, University of Sharjah, Sharjah 27272, United Arab Emirates; Department of Medicinal Chemistry, College of Pharmacy, University of Sharjah, 27272, United Arab Emirates; Department of Biopharmaceutics and Clinical Pharmacy, School of Pharmacy, The University of Jordan, Amman 11942, Jordan. saleidi@sharjah.ac.ae

Waseem El-Huneidi. Research Institute for Medical and Health Sciences, University of Sharjah, Sharjah 27272, United Arab Emirates; Department of Basic Medical Sciences, College of Medicine, University of Sharjah,

Sharjah 27272, United Arab Emirates. welhuneidi@sharjah.ac.ae

Eman Abu-Gharbieh. Research Institute for Medical and Health Sciences, University of Sharjah, Sharjah 27272, United Arab Emirates; Department of Clinical Sciences, College of Medicine, University of Sharjah, Sharjah 27272, United Arab Emirates; Department of Biopharmaceutics and Clinical Pharmacy, School of Pharmacy, The University of Jordan, Amman 11942, Jordan. eabugharbieh@sharjah.ac.ae

Zainab M. AlShareef. Research Institute for Medical and Health Sciences, University of Sharjah, Sharjah 27272, United Arab Emirates; Department of Basic Medical Sciences, College of Medicine, University of Sharjah, Sharjah 27272, United Arab Emirates. zalshareef@sharjah.ac.ae

Karem H. Alzoubi. Department of Pharmaceutical Sciences, College of Pharmacy, QU Health, Qatar University, Doha 2173, Qatar. karemalzoubi@yahoo.com

Adnane Guella. Nephrology Department, University Hospital Sharjah, Sharjah 27272, United Arab Emirates. guella@gmail.com

Yasser Bustanji. Research Institute for Medical and Health Sciences, University of Sharjah, Sharjah 27272, United Arab Emirates; Department of Basic Medical Sciences, College of Medicine, University of Sharjah, Sharjah 27272, United Arab Emirates; Department of Biopharmaceutics and Clinical Pharmacy, School of Pharmacy, The University of Jordan, Amman 11942, Jordan. ybustanji@sharjah.ac.ae

Mohammad H. Semreen*. Department of Medicinal Chemistry, College of Pharmacy, University of Sharjah, Sharjah 27272, United Arab Emirates; Research Institute for Medical and Health Sciences, University of Sharjah, Sharjah 27272, United Arab Emirates. msemreen@sharjah.ac.ae



INTRODUCTION

Diabetic retinopathy (DR) is one of the most common microvascular complications of diabetes mellitus and remains a leading cause of visual impairment and blindness worldwide¹. With the global diabetes burden projected to reach 783 million cases by 2045², the incidence of DR is expected to reach 129.84 million by 2030 and 160.50 million by 2045, posing a significant socioeconomic and healthcare challenge³⁻⁵. Despite advances in diabetes management and ophthalmologic screening, many patients progress to advanced stages of DR, including proliferative DR (PDR) and diabetic macular edema, both of which can severely compromise vision and quality of life^{6,7}.

The pathogenesis of DR is complex and multifactorial, driven primarily by chronic hyperglycemia and characterized by a cascade of biochemical and molecular events affecting the retinal vasculature, neurons, and supporting glial cells⁸. These changes include increased oxidative stress⁹, chronic low-grade inflammation⁹, mitochondrial dysfunction¹⁰, activation of angiogenic factors such as vascular endothelial growth factor (VEGF) and disruption of the blood retinal barrier¹¹. Over the past decades, several metabolic pathways have been implicated in DR progression, including the polyol pathway¹², advanced glycation end-product (AGE) accumulation¹³, protein kinase C (PKC) activation¹⁴, and the hexosamine biosynthesis pathway¹⁵. Each of these pathways contributes to cellular damage through unique and overlapping mechanisms, complicating efforts to develop effective single-target therapies.

Currently, the management of DR relies heavily on anti-VEGF agents and laser photocoagulation. While these therapies have demonstrated clinical benefits, they are often limited by incomplete response, high treatment burden, and lack of efficacy in the early stages of disease¹⁶. Moreover, these approaches do not address the underlying systemic and molecular alterations that precede overt retinal damage. This has led to growing interest in identifying upstream molecular targets and reliable circulating biomarkers that could enable earlier diagnosis and more effective, personalized interventions.

Recent advances in omics technologies have revolutionized the ability to characterize systemic molecular alterations associated with complex diseases such as DR. These high-throughput platforms enable the discovery of novel metabolic and protein signatures in blood, vitreous fluid, or retinal tissue, offering insights into disease mechanisms and therapeutic response¹⁷. When integrated with systems biology approaches, multi-omics can provide a holistic view of disease progression and help prioritize therapeutic targets based on network connectivity and clinical relevance¹⁸.

In this review, we provide a distinct and updated perspective on diabetic retinopathy by emphasizing the integration

of multi-omics approaches, rather than isolated single-omics studies, to elucidate pathway crosstalk and system-level disease mechanisms. We focus specifically on therapeutic targets beyond VEGF, highlighting upstream metabolic, inflammatory, and neurodegenerative pathways that contribute to disease initiation and progression. Furthermore, we discuss the emerging role of omics-informed biomarkers for early diagnosis, patient stratification, and prediction of therapeutic response. Through this integrative framework, the review aims to support the development of pathway-driven, precision-based, and patient-centered strategies for the prevention and management of diabetic retinopathy.

PATHOPHYSIOLOGY OF DIABETIC RETINOPATHY

DR is initiated and sustained by chronic hyperglycemia, which triggers a complex interplay of biochemical, cellular, and vascular alterations within the retina¹⁹. The disease progresses through a spectrum from non-proliferative diabetic retinopathy (NPDR), marked by microaneurysms, hemorrhages, and vascular leakage, to proliferative diabetic retinopathy (PDR), characterized by neovascularization and fibrovascular proliferation²⁰. Understanding the pathophysiological mechanisms that drive this progression is critical for identifying therapeutic targets beyond current anti-angiogenic approaches.

Hyperglycemia and Metabolic Stress

Persistent hyperglycemia is the primary driver of DR and exerts its deleterious effects through several interconnected metabolic pathways²¹. These include increased flux through the polyol pathway, accumulation of Advanced Glycation End Products (AGEs) activation of Protein Kinase C (PKC), and enhancement of the hexosamine biosynthesis pathway (HBP)²². These pathways converge to promote oxidative stress, inflammation, and vascular dysfunction, which are central features of DR pathology.

Oxidative Stress and Mitochondrial Dysfunction

Chronic hyperglycemia triggers an excessive production of reactive oxygen species (ROS), which disrupts the redox balance essential for cellular stability. This oxidative stress exerts damaging effects on multiple retinal cell types, including endothelial cells, pericytes, neurons, and glial cells, all of which are critical for maintaining retinal structure and function²³. The overabundance of ROS leads to lipid peroxidation, DNA damage, and protein oxidation, ultimately compromising cellular integrity²⁴. At the core of this process lies mitochondrial dysfunction, driven by elevated glucose metabolism that overloads the electron transport chain and reduces mitochondrial efficiency²⁵. As mitochondria fail to produce sufficient adenosine triphosphate (ATP), energy deficits occur, further aggravating ROS generation. This establishes a vicious cycle of oxidative stress and mitochondrial impairment, perpetuating cellular injury. Over time, these molecular disturbances culminate in apoptotic cell death, microvascular degeneration, and neurodegeneration²⁶.



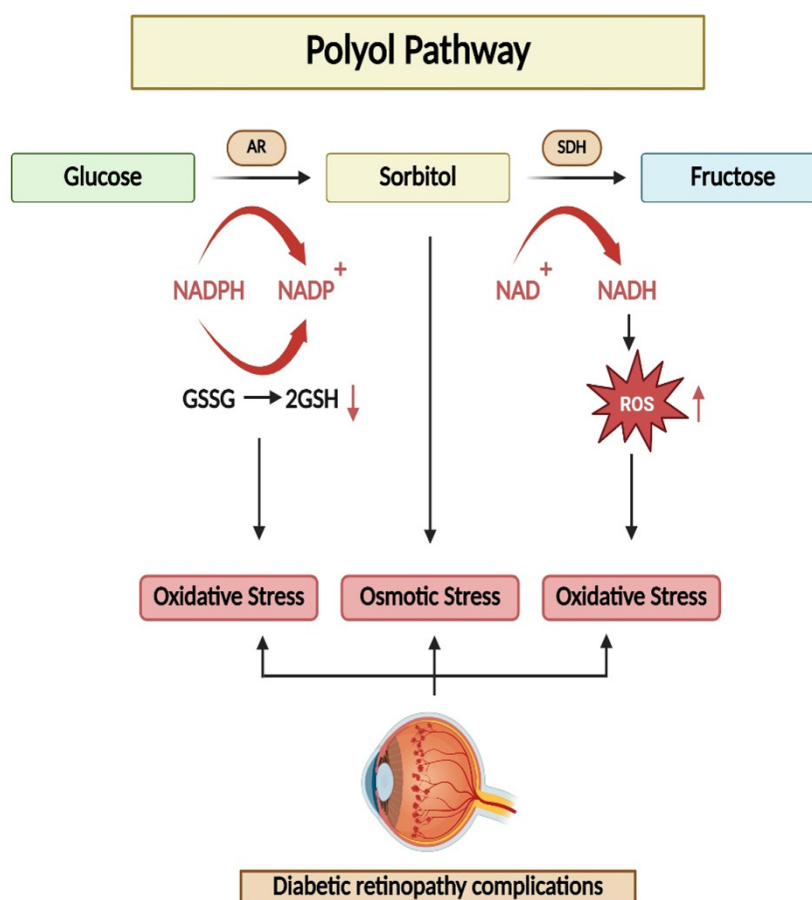


Figure 1. Polyol Pathway Mediated Oxidative and Osmotic Stress in Diabetic Retinopathy.

Inflammation and Leukostasis

Building upon the oxidative imbalance induced by chronic hyperglycemia, sustained activation of inflammatory signalling cascades further drives retinal injury in DR¹⁹. Hyperglycemia activates several pro-inflammatory signalling pathways, most notably nuclear factor-kappa B (NF-κB). Under hyperglycemic and oxidative stress conditions, NF-κB becomes aberrantly activated in retinal endothelial cells, pericytes, Müller glia, and microglia²⁷. This activation drives the transcription of numerous inflammatory mediators, including cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6); chemokines such as monocyte chemoattractant protein-1 (MCP-1/CCL2); and adhesion molecules including intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)²⁸. These inflammatory mediators act synergistically to promote leukostasis, a pathological hallmark of early DR characterized by the adhesion and entrapment of leukocytes within the retinal capillaries²⁹. In addition, pro-inflammatory cytokines disrupt tight junction proteins such as occludin and claudin-5, contributing to the

breakdown of the BRB and the development of vascular leakage and macular edema. TNF-α and IL-1β also stimulate the expression of matrix metalloproteinases (MMP-2 and MMP-9), which degrade extracellular matrix components, further weakening vascular integrity³⁰.

Neurodegeneration and Glial Activation

Retinal neurons exhibit early signs of degeneration, including dendritic pruning, apoptosis, and impaired synaptic function, often before overt vascular changes appear. Reactive gliosis, primarily involving Müller cells and microglia, amplifies inflammatory signalling within the neuroretina, contributing to excitotoxicity and neuronal death³¹. Progressive neuronal loss, particularly of ganglion and amacrine cells, contributes to deficits in contrast sensitivity and visual processing even at early disease stages. As inflammatory and oxidative processes compromise capillary integrity and perfusion, localized retinal hypoxia ensues. In response, retinal cells upregulate hypoxia-inducible factor-1 alpha (HIF-1α), leading to increased production of VEGF and angiopoietin-2 (Ang-2)³². These angiogenic factors drive neovascularization, but the resulting new vessels are fragile and prone to leakage or hemorrhage, worsening visual outcomes³³.

MOLECULAR AND METABOLIC PATHWAYS IMPLICATED IN DIABETIC RETINOPATHY

DR is driven by a network of complex molecular and biochemical mechanisms that underlie its pathogenesis. Several interconnected pathways have been identified as central contributors to disease initiation and progression. Among these, the polyol pathway, the hexosamine biosynthesis pathway, the accumulation of AGEs with subsequent receptor activation (RAGE), and PKC signaling represent the most prominent hyperglycemia-induced mechanisms³⁴.

Persistent hyperglycemia initiates a cascade of pathological events, including oxidative stress, chronic inflammation, neurodegenerative changes, and vascular dysfunction, all of which contribute to retinal injury³⁵. Insights from recent multi-omics studies have further expanded our understanding of these processes by uncovering transcriptional regulatory networks and potential therapeutic targets relevant to both DR and its advanced proliferative stage^{36,37}. Importantly, these molecular pathways do not act independently but are interconnected, amplifying one another to create a cycle of cellular stress and damage³⁸. The AGE-RAGE axis has emerged as a particularly critical mechanism, sustaining inflammation and tissue injury that extends beyond the retina to systemic diabetic complications³⁹. Emerging evidence also suggests that extrinsic factors, such as fluctuations in glucose levels, can modulate these pathways. Hypoglycemic episodes, for example, have been linked to the activation of hypoxia-inducible factors (HIFs), which further contribute to pathological angiogenesis in the retina⁴⁰. Collectively, these findings underscore the importance of understanding the molecular crosstalk that drives DR.

The Polyol Pathway

The polyol pathway is one of the earliest and most well-characterized metabolic routes triggered by hyperglycaemia, which has been repeatedly implicated in the development and progression of DR. Under physiological conditions, only small proportion of glucose is metabolised through this pathway because hexokinase, the primary enzyme of glycolysis, has a much higher affinity for glucose than aldose reductase (AR)⁴¹. However, when intracellular glucose rises during hyperglycemia, the flux through the polyol pathway increases dramatically, setting into motion a cascade of metabolic disturbances that compromise retinal homeostasis⁴².

As illustrated in Figure 1, the pathway proceeds in two enzymatic steps: AR reduces glucose to sorbitol using NADPH as a cofactor, and sorbitol dehydrogenase (SDH) subsequently oxidizes sorbitol to fructose using NAD⁺. While AR has low affinity but has high capacity for glucose, SDH

is characterized by high affinity but limited catalytic capacity. In hyperglycemic states, this kinetic imbalance results in the accumulation of sorbitol inside cells, particularly in insulin-independent tissues such as the retina, kidney, peripheral nerves, and vascular endothelium⁴³. Because sorbitol does not readily cross cell membranes, its accumulation increases intracellular osmotic pressure, disrupts ionic equilibrium, and compromises cell integrity. In parallel, the consumption of NADPH by AR depletes cellular antioxidant reserves, particularly the regeneration of reduced glutathione, thereby weakening the retina's capacity to counter oxidative stress. The subsequent SDH reaction further exacerbates the problem by altering the cytosolic NADH/NAD⁺ ratio, tipping the redox balance toward a pseudohypoxic state that favors ROS⁴⁴.

Under hyperglycaemic conditions, excess intracellular glucose is shunted into the polyol pathway, where AR converts glucose to sorbitol using NADPH as a cofactor. Sorbitol is subsequently oxidized to fructose by SDH, consuming NAD⁺. The accumulation of sorbitol and fructose leads to osmotic stress, oxidative imbalance due to NADPH depletion, and reduced glutathione regeneration. These changes contribute to increased ROS production, endothelial dysfunction, and neuronal damage, thereby promoting the progression of diabetic retinopathy.

These biochemical changes manifest as functional disturbances across multiple retinal cell types. In endothelial cells and pericytes, osmotic stress and oxidative imbalance impair mitochondrial function, suppress Na⁺/K⁺-ATPase activity, and initiate apoptotic signaling pathways, ultimately contributing to capillary degeneration and the formation of microaneurysms. In neurons and glial cells, increased oxidative stress and mitochondrial dysfunction disrupt neurotransmission, alter glial support, and promote cell death, contributing to the early neurodegenerative component of DR⁴⁵.

Evidence supporting the pathological significance of the polyol pathway in human DR is considerable. Immunohistochemical studies have demonstrated robust AR expression in human and rodent retinal endothelial cells, while organ-cultured human retinas exposed to high glucose accumulate sorbitol to levels comparable with diabetic rat retinas⁴⁴. More recently, metabolomic analyses of vitreous and retinal tissue from diabetic patients have confirmed elevated sorbitol and fructose levels, which correlate with microvascular alterations such as pericyte dropout, basement membrane thickening, and capillary non-perfusion⁴⁶. Moreover, genetic polymorphisms in the AR and SDH genes have been associated with increased risk and severity of DR, underscoring the clinical relevance of this pathway⁴⁷.

Importantly, the polyol pathway does not act in isolation but interacts with other hyperglycemia-induced injury cascades. NADPH depletion, for instance, favors activation of PKC isoforms and accelerates the formation of AGEs, both of which promote inflammation, vascular leakage, and neuronal stress. In this sense, the polyol pathway serves as a metabolic hub



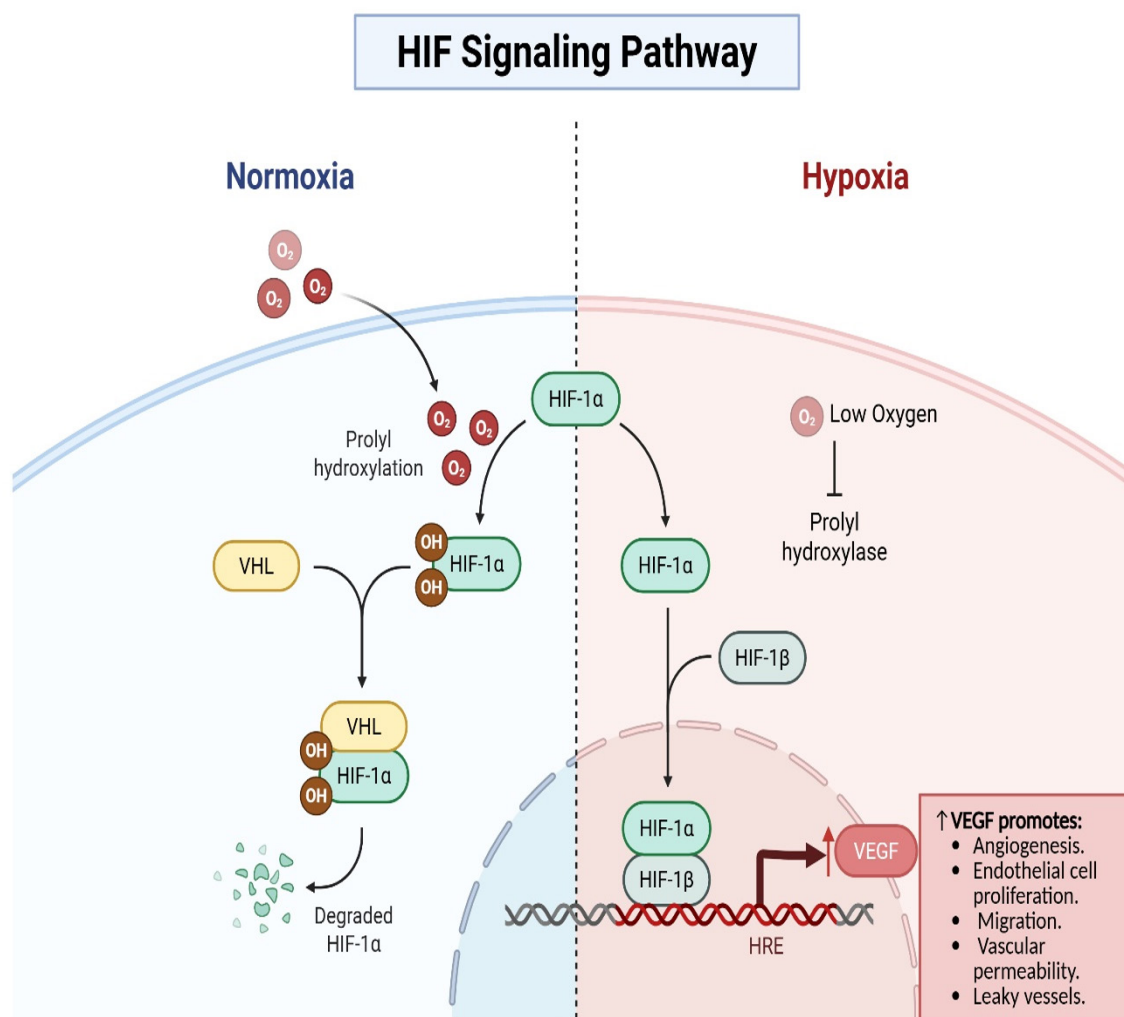


Figure 2. Hypoxia-Induced VEGF Upregulation in Diabetic Retinopathy.

that amplifies the downstream effects of hyperglycemia and integrates with parallel mechanisms of retinal injury⁴⁸.

Although significant insights have been gained into the polyol pathway's role in DR, some uncertainties remain. While osmotic stress has long been considered a primary driver of polyol-mediated injury, the modest increase in retinal sorbitol levels in diabetic models may not fully account for the extent of observed damage. This suggests that redox imbalance and oxidative stress are more decisive contributors than osmotic effects per se⁴⁹. Moreover, it is increasingly clear that the contribution of the polyol pathway varies between retinal cell types, highlighting the need for single-cell and multi-omics studies to dissect cell-specific vulnerabilities. Taken together, the polyol pathway remains a critical, though complex, mediator of hyperglycemia-induced retinal injury, serving as both an initiator of oxidative stress and a driver of metabolic crosstalk with other pathogenic cascades in DR.

Advanced Glycation End-products (AGEs) and the RAGE Axis

Chronic hyperglycemia creates a biochemical environment that favors the non-enzymatic glycation and oxidation of proteins, lipids, and nucleic acids, ultimately leading to the formation of AGEs⁵⁰. Early glycation events generate Schiff bases and Amadori products, which undergo further rearrangements to form irreversible AGEs such as carboxymethyllysine (CML), carboxyethyllysine (CEL), and pentosidine. These chemically stable compounds accumulate over time, particularly within long-lived proteins of the extracellular matrix and vasculature, including collagen and laminin, leading to stiffening and cross-linking of the basement membrane⁵¹. Such structural modifications compromise retinal vascular integrity and form the biochemical basis of microvascular dysfunction in DR.

The pathological significance of AGEs is mainly mediated through interaction with the RAGE, which is expressed on retinal endothelial cells, pericytes, and microglia. Engagement of AGEs with RAGE initiates a complex cascade of intracellular signaling events, including activation of NF-

κB, MAPK, JAK–STAT, TGF-β, and PI3K–Akt pathways, as well as stimulation of NADPH oxidase, leading to increased oxidative stress⁵². These mechanisms regulate a wide range of cellular processes, including inflammation, apoptosis, autophagy, and angiogenesis, thereby integrating metabolic stress with vascular pathology⁵³.

Within the retina, AGE–RAGE engagement is particularly detrimental. Activation of NF-κB induces the expression of inflammatory cytokines and adhesion molecules, contributing to pericyte apoptosis and leukostasis, while simultaneously upregulating VEGF, which increases vascular permeability^{53,54}. Importantly, this interaction also enhances RAGE expression itself through positive feedback, amplifying the pathway's pathological effects.

A key mediator of AGE–RAGE signaling is oxidative stress. : The binding of AGEs to RAGE activates NADPH oxidase, leading to the overproduction of ROS. These ROS further stimulate NF-κB activity and proinflammatory cytokine release, which in turn upregulate NADPH oxidase expression, establishing a vicious cycle of inflammation and oxidative stress^{39,55}. In addition to NADPH oxidase, AGEs can also generate ROS via mitochondrial electron transport chain dysfunction, further fueling redox imbalance⁵⁶. The accumulation of ROS not only amplifies AGE-induced cellular injury but also promotes additional AGE formation and RAGE upregulation, creating a self-perpetuating loop that accelerates retinal degeneration⁵⁷.

Experimental evidence strongly supports the pathogenic significance of this axis. Periodic intravitreal injection of AGEs in animal models induces retinal hyperpermeability, leukostasis, and elevated ROS and RAGE expression⁵⁸. Interestingly, co-administration of pigment epithelium-derived factor (PEDF) has been shown to block these effects by suppressing AGE-mediated ROS generation, NF-κB activation, and VEGF overexpression, highlighting the therapeutic potential of targeting this pathway⁵⁹.

Importantly, proteomic and transcriptomic analyses of plasma, vitreous fluid, and retinal tissues from DR patients have revealed elevated AGE-modified proteins and upregulated RAGE expression, correlating strongly with disease severity and progression⁶⁰. Omics-based profiling has also highlighted AGE-derived metabolic intermediates as potential systemic biomarkers, reinforcing the clinical importance of the AGE–RAGE axis not only as a pathogenic driver but also as a measurable indicator of diabetic microvascular injury⁶¹.

Protein Kinase C (PKC) Pathway

Increased vascular permeability and pathological neovascularization are defined features of endothelial dysfunction in DR. Among the major molecular pathways implicated in these vascular complications, the PKC pathway

has emerged as a key mediator of hyperglycemia-induced cellular dysfunction. The PKC pathway has emerged as a pivotal mediator of hyperglycemia-induced vascular dysfunction in DR. PKC represents a family of multifunctional serine/threonine kinases that regulate numerous downstream signaling proteins and cellular processes. To date, at least twelve PKC isoforms have been identified and classified into three groups: classical (PKC-α, -β1/2, -γ), novel (PKC-δ, -ε, -η, -θ), and atypical (PKC-ζ, -ι/λ), based on their activation requirements⁶². Chronic hyperglycemia leads to increased intracellular diacylglycerol (DAG) levels, which serve as potent activators of PKC isoforms, particularly PKC-β and PKC-δ. Once activated, PKC exerts wide-ranging effects on retinal cells, influencing endothelial function, pericyte survival, extracellular matrix composition, and vascular tone⁶³.

Activation of PKC isoforms has profound vascular consequences. PKC alters endothelial permeability, promotes vasoconstriction, and amplifies angiogenic responses by upregulating VEGF and endothelin-1, while simultaneously reducing nitric oxide (NO) bioavailability^{63,64}. Additionally, PKC activation promotes leukocyte adhesion by increasing the expression of ICAM-1, thereby exacerbating retinal inflammation. Importantly, PKC augments the activity of NADPH oxidase, driving ROS generation and amplifying oxidative stress, a central pathogenic mechanism in DR⁶⁵. These vascular and oxidative effects contribute to microvascular constriction, blood flow dysregulation, and ultimately, breakdown of the blood–retinal barrier.

Beyond vascular tone and permeability, PKC activation influences several other processes central to DR progression. It stimulates mitogen-activated protein kinase (MAPK) pathways and the phosphorylation of transcription factors that regulate stress-responsive genes such as c-Jun kinases and heat shock proteins⁶⁶. Experimental studies further demonstrate that PKC-β acts as a signaling component for VEGF and a regulator of endothelial cell permeability, thereby promoting abnormal endothelial migration, proliferation, and tube formation which are hallmarks of retinal neovascularization^{63,67}. In contrast, in pericytes, PKC activation disrupts platelet-derived growth factor (PDGF) signaling, contributing to pericyte apoptosis and capillary destabilization⁶⁸.

Recent transcriptomic and phosphoproteomic profiling of diabetic retinas has mapped PKC-dependent signaling networks linking chronic PKC activation to extracellular matrix remodeling, basement membrane thickening, and increased expression of plasminogen activator inhibitor-1 (PAI-1)⁶⁹. These molecular alterations collectively contribute to microvascular constriction, blood flow dysregulation, and breakdown of the blood–retinal barrier, the pathophysiological foundation of DR.

Hexosamine Biosynthesis Pathway (HBP)

HBP represents a pivotal glucose-sensing metabolic route that links hyperglycemia to cellular dysfunction in DR. Although only a small fraction (2–5%) of intracellular glucose is diverted into this pathway, its biological impact is disproportionately large,



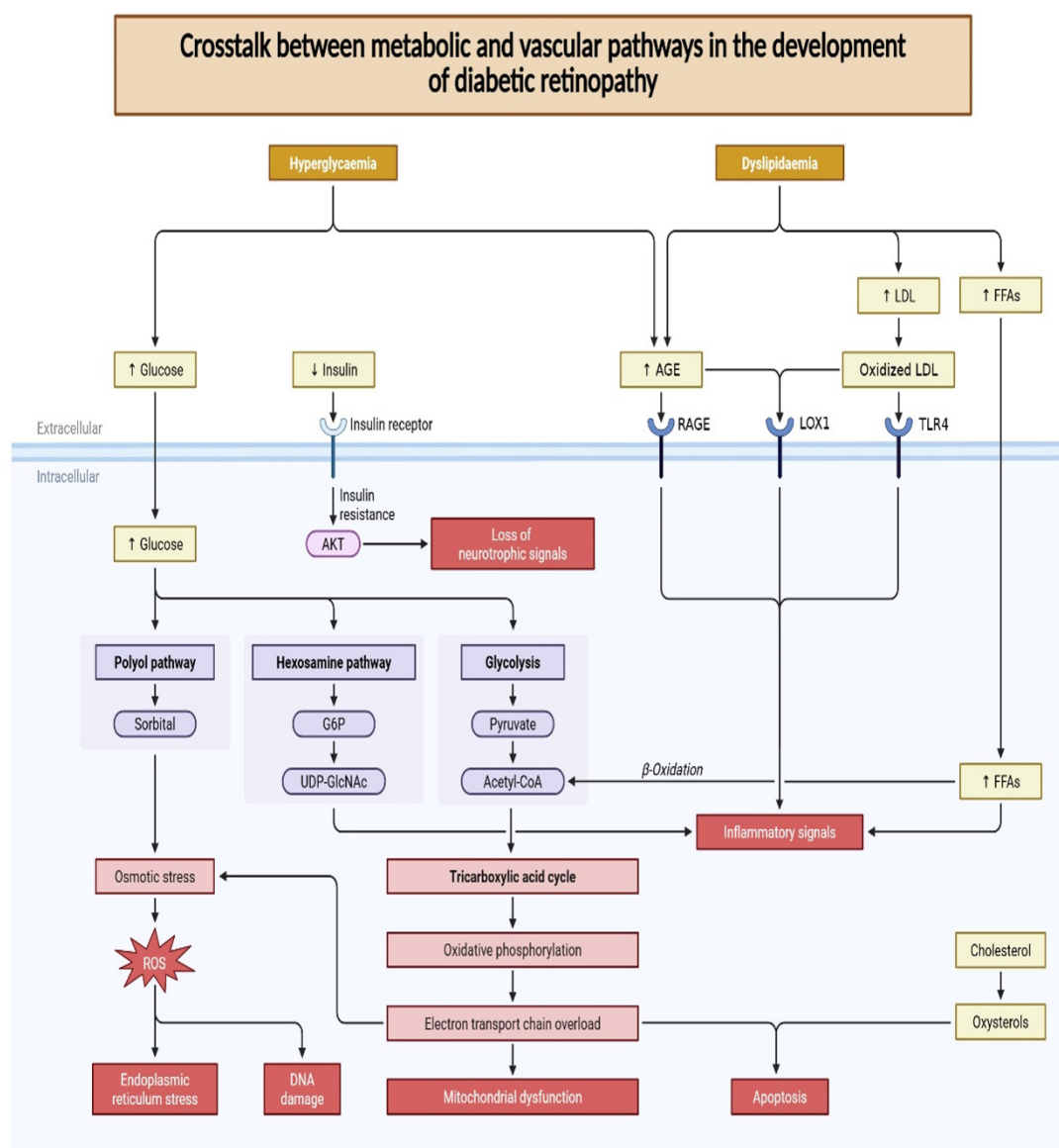


Figure 3. Schematic representation of the key mechanisms involved in diabetic retinopathy.

influencing a wide spectrum of gene and protein regulatory processes⁷⁰. Early studies demonstrated that hexosamine content is markedly increased in the retinal tissues of humans and experimental animals with diabetes, implicating this pathway in the pathogenesis of diabetic complications⁷¹.

Under hyperglycemic conditions, excess intracellular glucose is phosphorylated to glucose-6-phosphate, which can proceed through glycolysis, glycogen synthesis, or the HBP⁷². Within this pathway, fructose-6-phosphate is converted to glucosamine-6-phosphate by the rate-limiting enzyme glutamine:fructose-6-phosphate amidotransferase (GFAT), using glutamine as an amine donor⁷³. Through a series of enzymatic steps, this intermediate is ultimately converted into uridine diphosphate-N-acetylglucosamine (UDP-GlcNAc), the final product of the pathway. UDP-GlcNAc serves as the substrate for O-linked N-acetylglucosamine (O-GlcNAc) modification of nuclear

and cytoplasmic proteins, a reversible post-translational modification catalyzed by O-GlcNAc transferase (OGT)⁷⁴.

Activation of the HBP under chronic hyperglycemia alters the O-GlcNAcylation pattern of key transcription factors and signaling proteins, thereby reshaping gene expression and cellular signaling. Experimental inhibition of GFAT has been shown to block hyperglycemia-induced upregulation of transforming growth factor- β 1 (TGF- β 1) and plasminogen activator inhibitor-1 (PAI-1), two pivotal mediators of fibrosis and vascular dysfunction⁷¹. Mechanistically, these changes have been linked to the O-GlcNAc modification of the transcription factor Sp1, which enhances its ability to activate the PAI-1 promoter in vascular smooth muscle cells⁷¹. Thus, excessive flux through the HBP promotes transcriptional reprogramming that contributes to vascular remodeling, basement membrane thickening, and other microvascular

Table 1. Recent Metabolomics Studies in Diabetic Retinopathy.					
Study	Sample Type	Study Design	Platform Used	Biomarkers Identified	Key Findings
Nie et al., 2025 ¹⁰⁹	Aqueous	Cross-sectional	UPLC-MS	3-methylhistamine ↓, 5-HIAA ↑	Stage-specific markers distinguish NPDR from PDR
Hiller et al., 2025 ¹⁰⁸	Vitreous	Cross-sectional	HPLC-HRMS	Lysine ↑, carnitine ↑	Amino acids and energy metabolites increase with DR stage
Fang et al., 2025 ¹¹⁴	Serum	Cross-sectional	Targeted MS + Lipidomics	Sulfatide ST 24:1;O4 ↑, β-hydroxyisovalerate ↑	Metabolic-lipid profile stratifies DR stages
Zhang et al., 2025 ¹⁰²	Retina + serum	Animal model	LC-MS + RNA-seq	Sorbitol ↑, mannitol ↑	Polyol pathway metabolites linked to retinal injury
Zhou et al., 2024 ¹¹⁵	Serum	Cross-sectional	Untargeted LC-MS + MRM	L-tyrosine ↓, EPA ↓	Low L-tyrosine and EPA levels associated with DR
Ma et al., 2024 ¹¹¹	Plasma (genetic)	Mendelian randomization	GWAS data	Pyroglutamine ↓, stachydrine ↑, kynurenine ↑	Metabolites causally linked to DR risk
Yang et al., 2024 ¹¹⁰	Plasma	Prospective cohort	NMR	Lactate ↑, citrate ↓	Improved DR prediction with metabolite panel
Liu et al., 2024 ¹¹⁶	Vitreous	Case control	UHPLC-MS	Ferrous ascorbate ↑, arginine ↑	Altered amino acid and oxidative stress markers in DR
Yagin et al., 2024 ¹¹⁷	Serum	Cross-sectional	Targeted LC-MS + ML	Glycine ↑, creatinine ↑	ML model predicts DR stage from metabolite panel
Wang et al., 2024 ¹¹⁸	Blood (genetic)	Mendelian randomization	GWAS	HexCer ↑, acetylspermidine ↓	HexCer increase and acetylspermidine decrease DR risk
Fernandes Silva et al., 2023 ¹¹⁹	Serum	Longitudinal	Untargeted LC-MS	N-lactoyl amino acids ↑, citrulline ↓	Risk and protective metabolites for DR identified
Tomofuji et al., 2023 ¹²⁰	Serum	Case-control	Untargeted LC-MS	Cyclohexylamine ↑, stearoyl ethanolamide ↑	Fatty acid metabolites elevated in DR + DKD
Wang et al., 2023 ¹²¹	Plasma	Case control	HPLC-MS	Phenylacetylglycine ↑, arginine ↓	Early DR linked to gut-derived and nitric oxide metabolites
Ancel et al., 2023 ¹²²	Plasma	Cross-sectional	Untargeted LC-MS	Tryptophan ↓, ceramides ↑	Tryptophan deficiency and lipid remodeling in DR

abnormalities characteristic of DR⁷⁵.

Beyond its vascular effects, the HBP also contributes to retinal neurodegeneration. Increased glucose flux through this pathway has been reported to induce neuronal apoptosis via two converging mechanisms: through altered glycosylation of neuroprotective proteins and through disruption of insulin-Akt survival signaling⁷⁶. This dual insult highlights the broader impact of the HBP on the neurovascular unit, a key structural and functional component compromised in DR⁷⁶.

Clinically, increased GFAT activity has been positively correlated with HbA1c levels, linking HBP activation to systemic glycemic burden⁷¹. Elevated GFAT expression has also been observed in other diabetic complications such as nephropathy, though data regarding its expression in ocular tissues remain limited⁷⁴. Recent multi-omics studies have revealed aberrantly O-GlcNAcylated proteins in diabetic retinas, associated with endothelial dysfunction, pericyte apoptosis, leukostasis, and extracellular matrix accumulation⁷⁷.

The hyperactivation of the HBP fosters a pro-inflammatory and pro-fibrotic cellular environment by enhancing O-GlcNAcylation of proteins involved in oxidative stress responses, mitochondrial regulation, and VEGF signaling⁷². Through these interactions, the HBP amplifies the activity of other major pathogenic pathways in DR, including PKC and advanced glycation end-product-RAGE signaling, ultimately promoting inflammation, angiogenesis, and oxidative injury⁷⁷.

Inflammation and the NLRP3 Inflammasome

Chronic low-grade inflammation is a defining feature of DR and a central mechanism linking metabolic stress to retinal damage. Under hyperglycemic conditions, the retina is exposed to a hostile microenvironment characterized by excessive production of ROS, accumulation of AGEs, and persistent oxidative stress. These pathological cues converge to activate inflammatory signaling cascades that disrupt tissue homeostasis and accelerate both vascular and neuronal injury⁷⁸.

Among these inflammatory mediators, the NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome has emerged as a pivotal molecular complex integrating metabolic dysfunction with innate immune activation⁷⁹. The NLRP3 inflammasome consists of the sensor protein NLRP3, the adaptor ASC (apoptosis-associated speck-like protein containing a CARD), and the effector enzyme procaspase-1. Upon activation, NLRP3 recruits ASC and procaspase-1 into a supramolecular complex that triggers the autocatalytic activation of caspase-1⁸⁰. Active caspase-1 then cleaves the precursors of interleukin (IL)-1 β and IL-18 into their mature forms, which are secreted into the extracellular space, perpetuating inflammation and promoting endothelial dysfunction, leukocyte adhesion, and microvascular damage, which are hallmarks of DR⁸¹.

The activation of NLRP3 in DR is multifactorial and involves complex cellular and metabolic perturbations⁸². Persistent hyperglycemia induces mitochondrial overproduction of ROS, leading to oxidative damage and the release of mitochondrial DNA (mtDNA) into the cytosol, which directly binds and activates NLRP3⁸³. Concurrently, high extracellular glucose enhances ATP release from stressed or dying cells, which binds to purinergic P2X7 receptors on retinal endothelial cells and microglia, causing potassium efflux and calcium influx that serve as potent activation signals for NLRP3 assembly⁸⁴. In addition, lysosomal destabilization induced by crystalline deposits such as monosodium urate (formed when uric acid exceeds its solubility threshold under hyperglycemic conditions) results in the release of cathepsin B into the cytoplasm, further stimulating NLRP3 activation⁸⁵.

Once assembled, the NLRP3 inflammasome initiates caspase-1-mediated cleavage of gasdermin D (GSDMD), whose N-terminal fragment forms pores in the plasma membrane⁸⁶. This event facilitates the release of IL-1 β and IL-18, leading to a specific form of inflammatory cell death known as pyroptosis⁸⁷. The resulting amplification of inflammatory signaling disrupts the blood-retinal barrier (BRB), enhances vascular permeability, and accelerates pericyte loss and neuronal degeneration⁸².

Omics-based evidence supports the critical involvement of NLRP3 in DR pathogenesis. Transcriptomic and proteomic profiling of diabetic retinal tissues reveal significant upregulation of NLRP3, ASC, caspase-1, IL-1 β , and IL-18⁸⁸. In vitro studies demonstrate that high-glucose exposure suppresses pericyte proliferation and promotes apoptosis through the NLRP3/caspase-1/GSDMD axis⁸². Furthermore, in diabetic animal models, pharmacological inhibition of NLRP3 has been shown to reduce retinal inflammation and vascular leakage, while interventions targeting uric acid or monosodium urate accumulation can attenuate inflammasome-mediated retinal injury, highlighting the contribution of the NLRP3 pathway to DR pathogenesis^{82,89}.

Hypoxia and Angiogenesis Pathways

Hypoxia plays a pivotal role in the progression of DR, especially during its proliferative stage. Chronic hyperglycemia and microvascular damage contribute to capillary occlusion, reduced retinal perfusion, and localized ischemia, which are key factors in the pathogenesis of DR⁹⁰. The ensuing oxygen deprivation activates HIF-1 α , a master transcriptional regulator that orchestrates the cellular response to low oxygen tension. Under normoxic conditions, HIF-1 α is hydroxylated by prolyl hydroxylase enzymes and targeted for proteasomal degradation via the von Hippel-Lindau (VHL) complex⁹¹. However, during hypoxia, this degradation pathway is inhibited, allowing HIF-1 α to accumulate, translocate to the nucleus, and dimerize with HIF-1 β . The active HIF-1 complex binds to hypoxia-response elements (HREs) in the promoters of target genes, inducing the expression of multiple pro-angiogenic and pro-survival molecules (Figure 2)^{91,92}.

Table 2. Recent Proteomics Studies in Diabetic Retinopathy.

Study	Samples / Proteomic Approach	Key Findings
Liu et al., 2025 ¹³²	Vitreous from PDR patients with traction retinal detachment (TRD) vs. vitreous hemorrhage vs. macular hole (controls); label-free MS.	87 proteins significantly changed. Complement and coagulation cascades and actin cytoskeleton regulation were highly enriched in PDR. APOA4, CHI3L1 (YKL-40) and LTBP2 were significantly elevated in PDR-TRD vitreous (↑).
Zhang et al., 2025 ¹³³	Vitreous from PDR vs. idiopathic macular hole; Olink 92-cytokine panel profiling.	22 inflammatory proteins differentially expressed in PDR, with 21 upregulated (↑) and 1 down. Fibroblast growth factor 23 (FGF23) was highly elevated in PDR vitreous (↑). FGF23 was also upregulated in diabetic mice retinas and high glucose treated glial cells, and blocking FGF23 reduced angiogenesis in vitro, suggesting FGF23 as a new pro-angiogenic factor in DR.
Ma et al., 2025 ¹³⁴	Tear fluid from diabetic macular edema (DME) patients undergoing anti-VEGF therapy: comparing responders vs. non-responders; TMT proteomics with ELISA validation.	Found 3,121 tear proteins; DME responders (improved after anti-VEGF) had proteomic enrichment in extracellular matrix remodeling and resolution of inflammation, whereas non-responders showed persistent inflammatory and NOTCH/Wnt signaling activation. Key tear proteins correlated with treatment response: a balanced interplay of TIMP1 (tissue inhibitor of metalloproteinase, ↑ increased in responders) and MMP9 (↑ increased in non-responders) alongside IGFBP-3 (insulin-like growth factor binding protein, higher in responders) was observed. These findings suggest that stronger ECM stabilization and anti-inflammatory profiles in tears are associated with better therapeutic outcomes.
Jin et al., 2025 ¹³⁵	Aqueous humor from type 2 diabetics across DR stages (no-DR, NPDR, PDR) vs. non-diabetic cataract controls; DIA mass-spec with PRM validation.	Quantified 739 AH proteins, showing clear proteomic shifts with advancing DR. Complement and coagulation cascades were upregulated even in early DR (NPDR), along with changes in folate and selenium-binding proteins. Network analysis and PRM confirmed 83 proteins with stage-dependent expression; e.g. fibrinogen fragments (FGB/FGG), vitronectin, and retinol-binding protein 4 were higher in PDR (↑) than NPDR.
Oh et al., 2025 ¹³⁶	Aqueous humor (AH) from patients with NPDR, PDR, and neovascular glaucoma (NVG); TMT-based quantitative proteomics with targeted validation.	Identified 2,255 AH proteins, with functional enrichment in lens development, immune response, and lipid metabolism pathways. Validated 20 proteins with significant concentration changes across NPDR→PDR progression. For example, γ-crystallin S (CRYGS) was increased in PDR/NVG (↑) and correlated with cataract severity.
Brighenti et al., 2024 ¹³⁷	Vitreous from DR vs. macular pucker (control); LC-MS/MS profiling.	167 differentially expressed proteins with enrichment of glucose metabolism pathways. Aldo-keto reductase 1B1 (↑) in DR emerged as a potential therapeutic target.
Fan et al., 2024 ¹³⁸	Tear fluid from DR patients (n=46) vs. diabetic no-DR (n=28) vs. healthy controls (n=30); DIA proteomics with machine-learning biomarker selection.	Quantified 3,364 tear proteins; 235 were altered in DR vs healthy and 88 vs diabetics without DR. These dysregulated proteins are associated with retinal homeostasis, inflammation/immunity, oxidative stress, angiogenesis, and coagulation. A 3-protein panel – SIRT2, AOFB (amine oxidase B), and NUDT16 – was identified with high diagnostic power for DR (AUC ≈0.9). All three were elevated in tears of DR patients (↑).
Alanazi et al., 2024 ¹³⁹	Post-mortem vitreous from type 2 diabetics vs. non-diabetic donors; LC-MS/MS profiling.	22 proteins were significantly dysregulated in diabetic vitreous. Glycerolipid and histidine metabolism and Wnt signalling were altered. Proteins involved in calcium signalling and inflammation were markedly upregulated in diabetic vitreous (↑), suggesting aberrant calcium homeostasis and immune activation in DR.
Santos et al., 2023 ¹²⁹	Vitreous from PDR, dry AMD, and epiretinal membrane (control); label-free discovery + MRM validation.	118 proteins differed in PDR vitreous vs controls. PDR showed enrichment of complement and coagulation components (acute-phase proteins) and a relative deficit in certain structural/neuronal proteins. A panel of 15 discriminatory biomarkers was defined via multivariate analysis. Notable examples: complement C2 and prothrombin were elevated in PDR vitreous (↑), while an ECM factor opticin and neuroprotective amyloid-β were reduced (↓) in PDR. This highlights a shift toward inflammation/coagulation and loss of neuroprotective factors in DR.

Under normoxic conditions, prolyl hydroxylase enzymes promote HIF-1 α degradation through VHL-mediated ubiquitination, maintaining low VEGF expression. In diabetic retinopathy, retinal hypoxia inhibits the degradation of HIF-1 α , leading to its nuclear accumulation and the transcriptional activation of VEGF. The resulting VEGF overproduction drives angiogenesis, vascular leakage, and inflammation, contributing to disease progression.

Among these, VEGF is the most extensively studied mediator. VEGF promotes endothelial cell proliferation, migration, and vascular permeability, driving the formation of new but fragile and leaky vessels. This neovascularization is a hallmark of PDR and a significant cause of vision loss due to vitreous hemorrhage and tractional retinal detachment⁹³. In parallel, Ang-2 acts synergistically with VEGF by destabilizing existing vessels and sensitizing them to angiogenic stimuli, thereby facilitating pathological remodeling of the retinal vasculature. Elevated levels of both VEGF and Ang-2 have been detected in the vitreous humor of patients with advanced DR, correlating with disease severity⁹⁴.

Recent multi-omics studies have expanded the understanding of angiogenic regulation beyond VEGF alone. These analyses reveal upregulation of alternative angiogenic mediators such as placental growth factor (PlGF), galectin-1, and secretogranin III, all of which contribute to endothelial activation, extracellular matrix remodeling, and leukocyte recruitment⁹⁵. PlGF, a member of the VEGF family, acts through VEGFR-1 to potentiate VEGF signaling, while galectin-1 promotes angiogenesis and vascular permeability via H-Ras and ERK1/2 pathway activation⁹⁵. These findings suggest that DR-associated neovascularization results from a coordinated network of angiogenic signals rather than VEGF alone, which may explain the limited efficacy of monotherapy in some patients⁹⁶.

Crosstalk Between Pathways and Systemic Effects

The pathogenesis of DR is not driven by a single molecular event but rather by a complex network of interrelated biochemical and signaling pathways. Although individual mechanisms such as activation of the polyol pathway, accumulation of AGEs, PKC activation, hexosamine pathway flux, inflammation, and hypoxia-induced angiogenesis have been studied extensively, it is now evident that these pathways are tightly interconnected⁹⁷. Crosstalk among them creates a self-perpetuating cycle of oxidative stress, inflammation, and vascular dysfunction that underlies the progressive nature of DR (Figure 3).

Under hyperglycaemic conditions, excessive ROS generation through the polyol, AGE, and PKC pathways triggers local inflammation and cellular damage. This feedback loop exacerbates vascular permeability, neural impairment, retinal hypoxia, and neovascularization. The interconnected processes of neurodegeneration, inflammation, and vascular dysfunction progress simultaneously, culminating in the onset and progression of diabetic retinopathy.

Oxidative stress serves as a central hub linking these pathways. Excessive production of ROS, generated primarily through the polyol pathway and mitochondrial dysfunction, can activate downstream signaling cascades including PKC and the AGE–RAGE axis. The activation of PKC enhances NADPH oxidase activity, which further amplifies ROS generation, while AGEs interacting with RAGE stimulate NF- κ B signaling, promoting inflammatory cytokine release and endothelial injury⁹⁸. These feedback loops not only exacerbate vascular permeability and pericyte loss but also potentiate hypoxia, which in turn stabilizes HIF-1 α and upregulates VEGF expression, promoting pathological angiogenesis. Thus, oxidative stress acts both as a trigger and an effector, bridging metabolic and inflammatory responses in the diabetic retina⁹⁸.

Inflammation also plays an integrative role in this pathological network. Pro-inflammatory cytokines such as IL-1 β , TNF- α , and IL-6, released via NLRP3 inflammasome activation or AGE–RAGE signaling, can modulate HIF-1 α stability and VEGF transcription, further fueling angiogenesis and vascular leakage⁷⁸. Simultaneously, inflammatory signaling influences glucose metabolism and mitochondrial function, thereby feeding back into the regulation of oxidative and metabolic stress. These interdependent processes highlight how inflammatory and metabolic pathways converge to drive the neurovascular dysfunction characteristic of DR. This system-level understanding underscores the importance of combination therapeutic strategies that simultaneously target multiple pathogenic pathways, rather than focusing on a single molecular axis. Agents that modulate oxidative stress, inflammation, and angiogenesis in parallel may offer greater efficacy and durability in halting DR progression. Additionally, the integration of circulating biomarkers derived from proteomic or metabolomic profiling into clinical practice holds promise for non-invasive monitoring of disease progression and assessment of personalized therapy response.

MULTI-OMICS APPROACHES IN DIABETIC RETINOPATHY

The complexity of DR pathogenesis necessitates a system-level approach for a comprehensive understanding of its pathogenesis. Traditional single-marker studies are often insufficient to capture the heterogeneity and multifactorial nature of DR. In this context, multi-omics technologies, which include metabolomics and proteomics, provide a powerful toolkit to dissect disease mechanisms, discover novel biomarkers, and identify potential therapeutic targets⁹⁹⁻¹⁰¹.

Metabolomics in Diabetic Retinopathy

Metabolomics, the high-throughput profiling of small-molecule metabolites, has provided new insights into the pathophysiology of DR and potential biomarkers (Table 1). Untargeted metabolomics studies across various biofluids (plasma, serum, vitreous, aqueous humor, tears) consistently report disturbances in energy metabolism, polyol pathway intermediates, amino acids, and lipids in DR. For example,



Table 3. Therapeutic targets and intervention strategies in diabetic retinopathy.				
Target/Pathway	Mechanism	Example therapeutic agent(s)/approach	Development stage	References
VEGF signaling	Reduces vascular permeability and neovascularization	Ranibizumab, aflibercept, bevacizumab	Clinical	151
Corticosteroid-responsive inflammation	Suppresses cytokines, leukostasis; stabilizes BRB	Dexamethasone implant; fluocinolone acetonide implant	Clinical (DME)	152
Polyol pathway (aldose reductase)	Sorbitol accumulation; NADPH depletion → oxidative stress	Aldose reductase inhibitors (e.g., epalrestat)	Clinical (limited/region-specific) / preclinical in DR context	153
AGE–RAGE axis	NF-κB activation, ROS amplification, vascular inflammation	AGE formation inhibitors; RAGE decoys (sRAGE); downstream ROS/NF-κB modulation	Preclinical	154
Gene therapy (anti-angiogenic expression)	Long-term intraocular expression to reduce injection burden	AAV-mediated anti-VEGF/anti-angiogenic constructs	Clinical trials (investigational)	155

diabetic retinas show accumulation of glycolytic and polyol metabolites (glucose, sorbitol, fructose) linked to oxidative stress¹⁰². Altered tricarboxylic acid (TCA) cycle intermediates, such as succinate and fumarate, which reflect hypoxic stress, have also been observed in the plasma of DR patients^{103,104}. Several studies report reduced levels of branched-chain amino acids (BCAAs) and specific polyunsaturated fatty acids correlating with DR progression^{105,106}. Dysregulation of arginine metabolism is a recurring finding; DR patients have elevated plasma arginine and citrulline, implicating the nitric oxide pathway and endothelial dysfunction¹⁰⁷. Consistently, polyol pathway metabolites (sorbitol, fructose) and downstream oxidative products are higher in DR, linking hyperglycemia to retinal microvascular damage¹⁰². Metabolomic changes in ocular fluids mirror systemic alterations: in the vitreous humor of DR eyes, antioxidant metabolites, such as ascorbate and taurine, are markedly depleted (108), whereas amino acids (e.g., lysine, arginine) and lipid derivatives accumulate with disease severity¹⁰⁸. Similarly, aqueous humor studies find perturbations in amino acid and nucleotide metabolites (e.g. 5-hydroxytryptophan, hypoxanthine) between NPDR and PDR¹⁰⁹. These metabolic derangements highlight oxidative stress, inflammation, and mitochondrial dysfunction as key features of DR. Importantly, metabolomics holds promise for non-invasive biomarkers. Blood-based metabolite “fingerprints” can predict the onset and progression of DR beyond traditional risk factors¹¹⁰. For instance, a recent plasma NMR study identified a panel of 118 metabolites (including glucose, lactate, citrate) that significantly improved the prediction of future DR in diabetic cohorts. Some metabolites (like lactate and citrate) even correlated with retinal microvascular damage, underscoring their pathogenic relevance¹¹⁰. Other studies have pinpointed

metabolites with protective vs. deleterious associations: e.g. higher circulating levels of specific phospholipids (like 1-oleoylglycerophosphoethanolamine) and antioxidants (e.g. erythritol) appear protective, whereas elevated levels of molecules like kynurenine, butyrylcarnitine, and stachydrine are linked to greater DR risk¹¹¹. Metabolomic signatures have also been a link to specific DR lesions. For example, serum lipidomic profiles in DR patients with extensive hard exudates exhibit lower levels of very-long-chain ceramides and shifts in sphingomyelins^{112,113}. Taken together, these findings suggest that metabolomic alterations both reflect and contribute to DR’s pathogenesis, providing insight into disease mechanisms and potential therapeutic targets.

Proteomics in Diabetic Retinopathy

Proteomics enables the global profiling of proteins in tissues or biofluids, measuring their abundance, modifications, and interactions on a large scale¹²³. In DR, untargeted mass-spectrometry proteomics can identify thousands of proteins in retinal samples or ocular fluids without prior hypotheses¹²⁴. Using such approaches, studies have found dysregulation of multiple protein pathways in DR (Table 2). For example, inflammatory proteins are altered, including the damage-associated S100A8/A9 (calgranulin) complex, C-reactive protein (CRP), and various complement components¹²⁵. These relate to the chronic inflammation in the DR’s microenvironment. Similarly, angiogenic factors are highly elevated: notably, VEGF and angiopoietin-2 (ANG2) rise in the diabetic vitreous and drive pathological neovascularization¹²⁶. Another pro-angiogenic mediator, galectin-1, is markedly upregulated in vitreous and retinal tissue of proliferative DR, in correlation with VEGF levels¹²⁷. Proteomic analyses also highlight changes in oxidative

stress enzymes, including superoxide dismutase, peroxiredoxins and glutathione-related detoxifying enzymes in diabetic retinal tissue, reflecting an imbalance in redox homeostasis under hyperglycemia¹²⁸. Recent proteomic studies of vitreous fluid and retinal biopsies from DR patients have uncovered protein networks involved in extracellular matrix (ECM) remodelling, leukocyte migration/inflammation, and even mitochondrial dysfunction¹²⁹. For instance, DR vitreous exhibits an enrichment of complement and coagulation proteins in the acute inflammatory and ECM-modifying pathways, alongside deficits in certain structural and neuroprotective proteins¹²⁹. These findings suggest that retinal microvasculature damage in DR is accompanied by remodeling of the ECM and inflammatory cell infiltration, as well as metabolic stress on retinal cells. Notably, plasma proteomics is gaining traction as a minimally invasive method for detecting DR-related protein changes. Circulating levels of certain retinal injury markers, such as S100A8/A9, CRP, and ICAM-1, correlate with DR severity¹³⁰. Additionally, large-scale plasma proteome screens have identified risk biomarkers for DR years before the onset of retinal symptoms¹³¹.

THERAPEUTIC TARGETS AND INTERVENTIONS IN DIABETIC RETINOPATHY

Current therapeutic approaches primarily target the VEGF axis. Anti-VEGF agents, including ranibizumab, aflibercept, and bevacizumab, have revolutionized DR management by reducing vascular leakage, resolving macular edema, and stabilizing vision¹⁴⁰. However, these standard therapies have significant limitations, including suboptimal responses in some patients, the need for frequent invasive treatments, and a focus on late-stage vascular changes rather than early molecular drivers of the disease¹⁴¹. Moreover, prolonged VEGF inhibition may impair physiological vascular maintenance and neuronal survival in the retina, emphasizing the need for more selective or combination therapies¹⁴².

In this context, combination therapies are increasingly recognized as a rational approach, aiming to simultaneously suppress pathological angiogenesis while modulating upstream metabolic and inflammatory mechanisms. For example, in clinical practice, intravitreal anti-VEGF agents are already combined with corticosteroids, such as dexamethasone implant, in selected patients with diabetic macular edema to enhance anti-inflammatory effects and improve anatomical outcomes¹⁴³. Emerging evidence further supports the potential of combining anti-VEGF therapy with agents targeting oxidative stress, inflammation, or metabolic dysregulation, particularly in patients who exhibit incomplete responses to monotherapy¹⁴⁴.

Consequently, emerging treatments are targeting the upstream pathogenic mechanisms of DR chronic inflammation, oxidative stress, retinal neurodegeneration, and the metabolic imbalances induced by hyperglycemia⁹⁸. Novel approaches aim to modulate these pathways: for example, inhibiting the polyol pathway with aldose reductase inhibitors such as epalrestat, which reduces intracellular sorbitol accumulation and

mitigates oxidative injury¹⁴⁵. PKC pathway, particularly PKC- β , has also emerged as a critical mediator of vascular dysfunction. ruboxistaurin, a selective PKC- β inhibitor, demonstrated efficacy in reducing macular edema and delaying disease progression in clinical trials. However, despite its mechanistic appeal, it did not achieve regulatory approval due to modest overall benefit¹⁴⁶. Another pivotal axis in DR pathogenesis is the AGEs-RAGE. Inhibitors of AGE formation, such as aminoguanidine, have shown protective effects in preclinical retinal models, while sRAGE decoy receptors attenuate inflammation and vascular permeability^{147,148}. However, despite encouraging preclinical data, clinical translation has been limited by issues of efficacy and safety. More recently, attention has shifted toward targeting downstream pathways activated by AGE-RAGE signaling, including oxidative stress modulators and anti-inflammatory agents, as well as exploiting Age-related metabolites as biomarkers for precision medicine approaches¹⁴⁷.

Beyond conventional pharmacological strategies, emerging therapeutic modalities are gaining increasing attention. Gene therapy approaches, most commonly based on adeno-associated viral (AAV) vectors, are being explored to enable sustained intraocular expression of anti-angiogenic or neuroprotective factors, with the potential to reduce treatment burden¹⁴⁹. In parallel, RNA-based therapies, including small interfering RNA (siRNA) and antisense oligonucleotides, offer the possibility of selectively silencing disease-relevant transcripts involved in angiogenic and inflammatory signalling¹⁵⁰. In addition, accumulating mechanistic evidence implicating innate immune activation in DR has prompted interest in inflammasome-targeted interventions. Pharmacological inhibition of the NLRP3 inflammasome or modulation of downstream IL-1 β /IL-18 signalling has demonstrated protective effects in experimental models, reducing retinal inflammation, vascular leakage, and neurovascular injury⁸². Although these advanced approaches remain largely investigational in the context of DR, they align closely with current molecular insights and represent promising components of future, multi-targeted therapeutic strategies. The major therapeutic targets, representative interventions, and their translational status are summarized in Table 3.

CHALLENGES AND FUTURE PERSPECTIVES

Despite considerable progress in delineating the molecular mechanisms of DR, translating these findings into effective clinical applications remains a substantial challenge. A major challenge is the dependence on retinal imaging modalities such as fundus photography, optical coherence tomography (OCT), and fluorescein angiography for diagnosis and disease staging¹⁵⁶. These tools are effective for detecting structural and vascular changes but are typically limited to identifying DR after clinically visible damage has occurred. As early DR is often asymptomatic and subclinical, many patients remain undiagnosed until moderate or advanced stages, limiting the potential for early intervention. Although several omics-based studies have identified promising molecular biomarkers, none



have yet been integrated into routine clinical practice due to a lack of validation across large, diverse cohorts. This unmet need underscores the importance of identifying reliable, non-invasive biomarkers that can serve as predictive tools to detect disease onset and progression before irreversible vision loss ensues¹⁵⁷.

Another major obstacle lies in the current treatments of DR. Anti-VEGF agents, while effective in treating diabetic macular edema and proliferative DR, address only the angiogenic component of the disease¹⁵⁸. They have little impact on early-stage or non-angiogenic DR, which involves neurodegeneration, inflammation, and metabolic dysfunction. Notably, prophylactic anti-VEGF therapy in eyes with non-proliferative DR can slow progression to proliferative disease, but it does not improve visual acuity in early stages. This highlights the need to address early pathology, thereby preventing microvascular and neural damage before vision is compromised. Moreover, anti-VEGF therapy by itself has a high burden: patients require frequent intravitreal injections due to the drugs' short duration, with diminishing benefits if adherence lapses. Anti-VEGF monotherapy thus neither fully halts DR progression nor addresses other pathways like chronic inflammation or oxidative stress. There is a recognized need for multi-targeted strategies that go beyond VEGF¹⁵⁹.

Despite these challenges, multiple promising avenues are emerging. Personalized and predictive medicine is a key area of growth, with omics-derived biomarkers offering the potential to stratify patients by risk, disease subtype, and therapeutic response. Additionally, longitudinal cohort studies are crucial for capturing the temporal dynamics of disease progression and validating biomarker panels across diverse populations. These data, when integrated through systems biology approaches, it can provide a network-level understanding of DR pathogenesis and therapeutic response.

CONCLUSION

DR is a complex and progressive complication of diabetes, involving a wide range of biological disturbances, including vascular dysfunction, inflammation, oxidative stress, and neurodegeneration. Although current treatments such as anti-VEGF therapies have improved visual outcomes in advanced disease, they do not effectively prevent early retinal damage or address the full spectrum of underlying molecular abnormalities. As a result, there is a pressing need for earlier, more targeted interventions. Recent advances in multi-omics technologies have deepened our understanding of the systemic and retinal changes that drive DR. These approaches have revealed critical pathways and identified circulating and tissue-specific biomarkers with potential for early diagnosis, risk stratification, and treatment monitoring. Moreover, emerging therapeutic strategies targeting upstream mechanisms such as the polyol pathway, PKC activation, AGE–RAGE signalling, and chronic inflammation offer new opportunities for more comprehensive disease management.

AUTHOR CONTRIBUTIONS

Conceptualization M.H.S. and R.A.Z.; methodology, M.H.S., R.A.Z., and R.A.Q.; writing original draft, R.A.Z., and R.A.Q.; writing, review and editing, R.A.Z., R.A.Q., A.Y.A., M.A.Y.A., S.M.A., W.E.H., E.A.G., Z.M.A., K.H.A., A.G., Y.B., and M.H.S. All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Kropp M, Golubnitschaja O, Mazurakova A, Koklesova L, Sargheini N, Vo T-TKS, et al. Diabetic retinopathy as the leading cause of blindness and early predictor of cascading complications—risks and mitigation. 2023;14(1):21-42.
2. Hossain MJ, Al-Mamun M, Islam MRJHSR. Diabetes mellitus, the fastest growing global public health concern: Early detection should be focused. 2024;7(3):e2004.
3. Yuan D, Xu Y, Xue L, Zhang W, Gu L, Liu QJH. Resveratrol protects against diabetic retinal ganglion cell damage by activating the Nrf2 signaling pathway. 2024;10(9).
4. Yang C, Ma Y, Yao M, Jiang Q, Xue JFIE. Causal relationships between blood metabolites and diabetic retinopathy: a two-sample Mendelian randomization study. 2024;15:1383035.
5. Ashraf M, Sampani K, Rageh A, Silva PS, Aiello LP, Sun JKJJo. Interaction between the distribution of diabetic retinopathy lesions and the association of optical coherence tomography angiography scans with diabetic retinopathy severity. 2020;138(12):1291-7.
6. Chaudhary S, Zaveri J, Becker NJD-a-M. Proliferative diabetic retinopathy (PDR). 2021;67(5):101140.
7. Zhang J, Zhang J, Zhang C, Zhang J, Gu L, Luo D, et al. Diabetic macular edema: current understanding, molecular mechanisms and therapeutic implications. 2022;11(21):3362.
8. Ciorba AL, Saber S, Abdelhamid AM, Keshk N, Elnaghy F, Elmorsy EA, et al. Diabetic Retinopathy in Focus: Update on Treatment Advances, Pharmaceutical Approaches, and New Technologies. 2025:107307.
9. Cecilia O-M, José Alberto C-G, José N-P, Ernesto Germán C-M, Ana Karen L-C, Luis Miguel R-P, et al. Oxidative stress as the main target in diabetic retinopathy pathophysiology. 2019;2019(1):8562408.
10. Xu X, Pang Y, Fan XJST, Therapy T. Mitochondria in oxidative stress, inflammation and aging: from mechanisms to therapeutic

- advances. 2025;10(1):190.
11. Abcouwer SFJoc, immunology c. Angiogenic factors and cytokines in diabetic retinopathy. 2013(11):1.
12. Lorenzi MJJoDR. The polyol pathway as a mechanism for diabetic retinopathy: attractive, elusive, and resilient. 2007;2007(1):061038.
13. Chen Y, Meng Z, Li Y, Liu S, Hu P, Luo EJMM. Advanced glycation end products and reactive oxygen species: uncovering the potential role of ferroptosis in diabetic complications. 2024;30(1):141.
14. Xia L, Wang H, Munk S, Frecker H, Goldberg HJ, Fantus IG, et al. Reactive oxygen species, PKC- β 1, and PKC- ζ mediate high-glucose-induced vascular endothelial growth factor expression in mesangial cells. 2007;293(5):E1280-E8.
15. Kang Q, Dai H, Jiang S, Yu LJFiN. Advanced glycation end products in diabetic retinopathy and phytochemical therapy. 2022;9:1037186.
16. Bahr TA, Bakri SJL. Update on the management of diabetic retinopathy: anti-VEGF agents for the prevention of complications and progression of nonproliferative and proliferative retinopathy. 2023;13(5):1098.
17. Lei Y, Guo J, He S, Yan HJC. Essential role of multi-omics approaches in the study of retinal vascular diseases. 2022;12(1):103.
18. Ivanisevic T, Sewduth RNJP. Multi-omics integration for the design of novel therapies and the identification of novel biomarkers. 2023;11(4):34.
19. Wei L, Sun X, Fan C, Li R, Zhou S, Yu HJFiC, et al. The pathophysiological mechanisms underlying diabetic retinopathy. 2022;10:963615.
20. Poshtchaman F, Dehnabi A, Poshtchaman Z, Birjandi BJMCP. HbA1C, proliferative and non-proliferative retinopathy in diabetic patients. 2023;6(3):100371.
21. Giri B, Dey S, Das T, Sarkar M, Banerjee J, Dash SKJB, et al. Chronic hyperglycemia mediated physiological alteration and metabolic distortion leads to organ dysfunction, infection, cancer progression and other pathophysiological consequences: An update on glucose toxicity. 2018;107:306-28.
22. Dai X, Hui X, Xi MJB, Pharmacotherapy. Critical factors driving diabetic retinopathy pathogenesis and a promising interventional strategy. 2025;189:118106.
23. Bhatti JS, Sehrawat A, Mishra J, Sidhu IS, Navik U, Khullar N, et al. Oxidative stress in the pathophysiology of type 2 diabetes and related complications: Current therapeutics strategies and future perspectives. 2022;184:114-34.
24. Juan CA, Pérez de la Lastra JM, Plou FJ, Pérez-Lebeña EIJoms. The chemistry of reactive oxygen species (ROS) revisited: outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. 2021;22(9):4642.
25. Kim J-a, Wei Y, Sowers JRJCr. Role of mitochondrial dysfunction in insulin resistance. 2008;102(4):401-14.
26. Pessoa J, Duarte AIJNRR. Overcoming mitochondrial dysfunction in neurodegenerative diseases. 2023;18(7):1486-8.
27. Ding X, Sun Z, Guo Y, Tang W, Shu Q, Xu GJAoTM. Inhibition of NF- κ B ameliorates aberrant retinal glia activation and inflammatory responses in streptozotocin-induced diabetic rats. 2023;11(5):197.
28. Ramos H, Hernandez C, Simo R, Simo-Servat OJIJoMS. Inflammation: the link between neural and vascular impairment in the diabetic retina and therapeutic implications. 2023;24(10):8796.
29. Zhang W, Liu H, Rojas M, Caldwell RW, Caldwell RBJI. Anti-inflammatory therapy for diabetic retinopathy. 2011;3(5):609-28.
30. Klok JA, Friedrichson B, Wülfroth P, Henning R, Zacharowski KJC. Microvascular leakage as therapeutic target for ischemia and reperfusion injury. 2023;12(10):1345.
31. Callan A, Jha S, Valdez L, Tsin AJCVP. Cellular and molecular mechanisms of neuronal degeneration in early-stage diabetic retinopathy. 2024;22(5):301-15.
32. Willard AL, Herman IMJJo. Vascular complications and diabetes: current therapies and future challenges. 2012;2012(1):209538.
33. Stitt AW, Curtis TM, Chen M, Medina RJ, McKay GJ, Jenkins A, et al. The progress in understanding and treatment of diabetic retinopathy. 2016;51:156-86.
34. Kang Q, Yang CJRb. Oxidative stress and diabetic retinopathy: Molecular mechanisms, pathogenetic role and therapeutic implications. 2020;37:101799.
35. Zhu H, Li B, Huang T, Wang B, Li S, Yu K, et al. Update in the molecular mechanism and biomarkers of diabetic retinopathy. 2025;1871(5):167758.
36. Cai F-h, Qian F-c, Li B-l, Li L-d, Liao B-h, Yu Z-m, et al. DiabetesOmic: A comprehensive multi-omics diabetes database. 2025.
37. Yi G, Li Z, Sun Y, Ma X, Wang Z, Chen J, et al. Integration of multi-omics transcriptome-wide analysis for the identification of novel therapeutic drug targets in diabetic retinopathy. 2024;22(1):1146.
38. Błaszczewicz M, Walulik A, Florek K, Górecki I, Sławatyniec O, Gomułka KJB. Advances and perspectives in relation to the molecular basis of diabetic retinopathy—a review. 2023;11(11):2951.
39. Zhou M, Zhang Y, Shi L, Li L, Zhang D, Gong Z, et al. Activation and modulation of the AGEs-RAGE axis: implications for inflammatory pathologies and therapeutic interventions—a review. 2024;206:107282.
40. Guo C, Deshpande M, Niu Y, Kachwala I, Flores-Bellver M, Megarity H, et al. HIF-1 α accumulation in response to transient hypoglycemia may worsen diabetic eye disease. 2023;42(1).
41. Thakur S, Gupta SK, Ali V, Singh P, Verma MJAPR. Aldose Reductase: a cause and a potential target for the treatment of diabetic complications. 2021;44(7):655-67.

42. Mathebula SDJAV, Health E. Biochemical changes in diabetic retinopathy triggered by hyperglycaemia: A review. 2018;77(1):1-7.
43. Safi SZ, Qvist R, Kumar S, Batumalaie K, Ismail ISJBri. Molecular mechanisms of diabetic retinopathy, general preventive strategies, and novel therapeutic targets. 2014;2014(1):801269.
44. Yan LJAm, medicine e. Redox imbalance stress in diabetes mellitus: Role of the polyol pathway. 2018;1(1):7-13.
45. Gómez-Jiménez V, Burggraaf-Sánchez de las Matas R, Ortega ÁLJA. Modulation of oxidative stress in diabetic retinopathy: Therapeutic role of natural polyphenols. 2025;14(7):875.
46. Wang R, Jian Q, Hu G, Du R, Xu X, Zhang FJM. Integrated metabolomics and transcriptomics reveal metabolic patterns in retina of STZ-induced diabetic retinopathy mouse model. 2022;12(12):1245.
47. Sienkiewicz-Szłapka E, Fiedorowicz E, Król-Grzymała A, Kordulewska N, Rozmus D, Cieślińska A, et al. The role of genetic polymorphisms in diabetic retinopathy: Narrative review. 2023;24(21):15865.
48. Chalke SD, Kale PPJCDT. Combinational approaches targeting neurodegeneration, oxidative stress, and inflammation in the treatment of diabetic retinopathy. 2021;22(16):1810-24.
49. Jian Q, Wu Y, Zhang FJC. Metabolomics in diabetic retinopathy: from potential biomarkers to molecular basis of oxidative stress. 2022;11(19):3005.
50. Zgutka K, Tkacz M, Tomasiak P, Tarnowski MJJoms. A role for advanced glycation end products in molecular ageing. 2023;24(12):9881.
51. Khan MN. Advanced Glycation End Products: Origins and Role in Aging. 2020.
52. Bhattacharya R, Alam MR, Kamal MA, Seo KJ, Singh LRJFimn. AGE-RAGE axis culminates into multiple pathogenic processes: a central road to neurodegeneration. 2023;16:1155175.
53. Xu J, Chen L-J, Yu J, Wang H-J, Zhang F, Liu Q, et al. Involvement of advanced glycation end products in the pathogenesis of diabetic retinopathy. 2018;48(2):705-17.
54. Lu Z, Fan B, Li Y, Zhang YBEO. RAGE plays key role in diabetic retinopathy: a review. 2023;22(1):128.
55. Abedini A, Cao P, Plesner A, Zhang J, He M, Derk J, et al. RAGE binds preamyloid IAPP intermediates and mediates pancreatic β cell proteotoxicity. 2018;128(2):682-98.
56. Mathieu C, Gillard P, Benhalima KJNRE. Insulin analogues in type 1 diabetes mellitus: getting better all the time. 2017;13(7):385-99.
57. He W, Tang P, Lv HJFil. Targeting oxidative stress in diabetic retinopathy: mechanisms, pathology, and novel treatment approaches. 2025;16:1571576.
58. Zhu X-f, Zou H-dJBRI. PEDF in diabetic retinopathy: a protective effect of oxidative stress. 2012;2012(1):580687.
59. Hammes H-PJD. Diabetic retinopathy: hyperglycaemia, oxidative stress and beyond. 2018;61(1):29-38.
60. Schori C, Trachsel C, Grossmann J, Zygoula I, Barthelmes D, Grimm CJlo, et al. The proteomic landscape in the vitreous of patients with age-related and diabetic retinal disease. 2018;59(4):AMD31-AMD40.
61. Han X, Zhang L, Kong L, Tong M, Shi Z, Li XM, et al. Comprehensive metabolic profiling of diabetic retinopathy. 2023;233:109538.
62. Tan J, Xiao A, Yang L, Tao Y-L, Shao Y, Zhou QJWJoD. Diabetes and high-glucose could upregulate the expression of receptor for activated C kinase 1 in retina. 2024;15(3):519.
63. Pan D, Xu L, Guo MJFIE. The role of protein kinase C in diabetic microvascular complications. 2022;13:973058.
64. Geraldine P, King GLJCr. Activation of protein kinase C isoforms and its impact on diabetic complications. 2010;106(8):1319-31.
65. Lenin R, Thomas SM, Gangaraju RJCPd. Endothelial activation and oxidative stress in neurovascular defects of the retina. 2018;24(40):4742-54.
66. Salvatore T, Pafundi PC, Galiero R, Rinaldi L, Caturano A, Vetrano E, et al. Can metformin exert as an active drug on endothelial dysfunction in diabetic subjects? 2020;9(1):3.
67. Mima A, Kitada M, Geraldine P, Li Q, Matsumoto M, Mizutani K, et al. Glomerular VEGF resistance induced by PKC δ /SHP-1 activation and contribution to diabetic nephropathy. 2012;26(7):2963.
68. Hou Z, Wang X, Cai J, Zhang J, Hassan A, Auer M, et al. Platelet-derived growth factor subunit B signaling promotes pericyte migration in response to loud sound in the cochlear stria vascularis. 2018;19(4):363-79.
69. Azad N, Agrawal L, Emanuele NV, Klein R, Bahn GD, McCarren M, et al. Association of PAI-1 and fibrinogen with diabetic retinopathy in the Veterans Affairs Diabetes Trial (VADT). 2014;37(2):501-6.
70. Roy BJMS. Pathophysiological mechanisms of diabetes-induced macrovascular and microvascular complications: The role of oxidative stress. 2025;13(3):87.
71. Wang Y, Eshwaran R, Beck SC, Hammes H-P, Wieland T, Feng YJMM. Contribution of the hexosamine biosynthetic pathway in the hyperglycemia-dependent and-independent breakdown of the retinal neurovascular unit. 2023;73:101736.
72. Li Y, Liu Y, Liu S, Gao M, Wang W, Chen K, et al. Diabetic vascular diseases: molecular mechanisms and therapeutic strategies. 2023;8(1):152.
73. Liu X, Cai YD, Chiu JCJJoBC. Regulation of protein O-GlcNAcylation by circadian, metabolic, and cellular signals. 2024;300(2):105616.
74. Qi B, Chen Y, Chai S, Lu X, Kang LJDM. O-linked β -N-acetylglucosamine (O-GlcNAc) modification: Emerging pathogenesis and a

- therapeutic target of diabetic nephropathy. 2025;42(2):e15436.
75. Rohilla M, Bansal S, Garg A, Dhiman S, Dhankhar S, Saini M, et al. Discussing pathologic mechanisms of Diabetic retinopathy & therapeutic potentials of curcumin and β -glucogallin in the management of Diabetic retinopathy. 2023;169:115881.
 76. Aldosari DI, Malik A, Alhomida AS, Ola MSJFin. Implications of diabetes-induced altered metabolites on retinal neurodegeneration. 2022;16:938029.
 77. Dong W, Imdad L, Xu S, Wang Y, Liu C, Song S, et al. O-GlcNAc Modification Is a Promising Therapeutic Target for Diabetic Retinopathy. 2024;25(11):6286.
 78. Tang L, Xu G-T, Zhang J-FJNrr. Inflammation in diabetic retinopathy: possible roles in pathogenesis and potential implications for therapy. 2023;18(5):976-82.
 79. Yang M, Wang X, Han Y, Li C, Wei L, Yang J, et al. Targeting the NLRP3 inflammasome in diabetic nephropathy. 2021;28(42):8810-24.
 80. Plowman TJ, Shah MH, Fernandez E, Christensen H, Aiges M, Ramana KVJCM. Role of innate immune and inflammatory responses in the development of secondary diabetic complications. 2023;23(9):901-20.
 81. Paik S, Kim JK, Silwal P, Sasakawa C, Jo E-KJC, immunology m. An update on the regulatory mechanisms of NLRP3 inflammasome activation. 2021;18(5):1141-60.
 82. Zheng X, Wan J, Tan GJFii. The mechanisms of NLRP3 inflammasome/pyroptosis activation and their role in diabetic retinopathy. 2023;14:1151185.
 83. Pereira CA, Carlos D, Ferreira NS, Silva JF, Zannotto CZ, Zamboni DS, et al. Mitochondrial DNA promotes NLRP3 inflammasome activation and contributes to endothelial dysfunction and inflammation in type 1 diabetes. 2020;10:1557.
 84. Kong H, Zhao H, Chen T, Song Y, Cui YJCD, Disease. Targeted P2X7/NLRP3 signaling pathway against inflammation, apoptosis, and pyroptosis of retinal endothelial cells in diabetic retinopathy. 2022;13(4):336.
 85. Karasawa T, Takahashi MJl, regeneration. The crystal-induced activation of NLRP3 inflammasomes in atherosclerosis. 2017;37(1):18.
 86. Zhao H, Dai Y, Li Y, Li J, Li HJCD, Disease. TNFSF15 inhibits progression of diabetic retinopathy by blocking pyroptosis via interacting with GSDME. 2024;15(2):118.
 87. Zhao M, Li S, Matsubara JAJFim. Targeting pyroptotic cell death pathways in retinal disease. 2022;8:802063.
 88. Chen H, Zhang X, Liao N, Mi L, Peng Y, Liu B, et al. Enhanced expression of NLRP3 inflammasome-related inflammation in diabetic retinopathy. 2018;59(2):978-85.
 89. Ge K, Wang Y, Li P, Li M, Zhang W, Dan H, et al. Down-expression of the NLRP3 inflammasome delays the progression of diabetic retinopathy. 2022;139:104265.
 90. Bi J, Zhou W, Tang ZJB, Pharmacotherapy. Pathogenesis of diabetic complications: Exploring hypoxic niche formation and HIF-1 α activation. 2024;172:116202.
 91. Qannita RA, Alalami AI, Harb AA, Aleidi SM, Taneera J, Abu-Gharbieh E, et al. Targeting hypoxia-inducible factor-1 (HIF-1) in cancer: emerging therapeutic strategies and pathway regulation. 2024;17(2):195.
 92. Zhang Q, Yan Q, Yang H, Wei WJG, diseases. Oxygen sensing and adaptability won the 2019 Nobel Prize in Physiology or medicine. 2019;6(4):328-32.
 93. Vaz-Pereira S, Morais-Sarmiento T, Esteves Marques RJlJor, vitreous. Optical coherence tomography features of neovascularization in proliferative diabetic retinopathy: a systematic review. 2020;6(1):26.
 94. Tsai T, Alwees M, Asaad MA, Theile J, Kakkassery V, Dick HB, et al. Increased Angiopoietin-1 and-2 levels in human vitreous are associated with proliferative diabetic retinopathy. 2023;18(1):e0280488.
 95. Nguyen QD, De Falco S, Behar-Cohen F, Lam WC, Li X, Reichhart N, et al. Placental growth factor and its potential role in diabetic retinopathy and other ocular neovascular diseases. 2018;96(1):e1-e9.
 96. Ji L, Waduge P, Wu Y, Huang C, Kaur A, Oliveira P, et al. Secretogranin III selectively promotes vascular leakage in the deep vascular plexus of diabetic retinopathy. 2023;24(13):10531.
 97. Feldman EL, Callaghan BC, Pop-Busui R, Zochodne DW, Wright DE, Bennett DL, et al. Diabetic neuropathy. 2019;5(1):41.
 98. Seo H, Park S-J, Song MJC. Diabetic retinopathy (DR): mechanisms, current therapies, and emerging strategies. 2025;14(5):376.
 99. Hassanein MM, Hagyousif YA, Zenati RA, Al-Hroub HM, Khan FM, Abuhelwa AY, et al. Metabolomics insights into doxorubicin and 5-fluorouracil combination therapy in triple-negative breast cancer: a xenograft mouse model study. 2025;11:1517289.
 100. Abufares HI, Zenati RA, Soares NC, El-Huneidi W, Dahabiyeh LA, Al-Hroub HM, et al. A non-targeted metabolomics comparative study on plasma of pfizer and sinopharm COVID-19 vaccinated individuals, assessed by (TIMS-QTOF) mass spectrometry. 2024;10(15)
 101. Alqudah MA, Yaseen MM, Alzoubi KH, Al-Husein BA, Bardaweel SK, Abuhelwa AY, et al. Metabolomic Analysis, Antiproliferative, Anti-Migratory, and Anti-Invasive Potential of Amlodipine in Lung Cancer Cells. 2025:1215-29.
 102. Zhang X, Liu Y, Xia M, Yang M, Wu Y, Zhang FJB, et al. Multi-omics analysis of potential metabolic networks linking peripheral metabolic changes to inflammatory retinal conditions in STZ-induced early diabetic retinopathy. 2025;43:102182.
 103. Wang Z-y, Yang F-y, Cai S-W, Tian W, Xie R-r, Sun R, et al. Plasma metabolomic profiling of diabetic macular edema. 2025;15(1):10012.

104. Wang X, Liu W, Zheng X, Yang MMJFiE. New insights of potential biomarkers in diabetic retinopathy: integrated multi-omic analyses. 2025;16:1595207.
105. Hiller JK, Sand ES, Rootwelt H, Vassli AØ, Lumi X, Moe MC, et al. Metabolic deficiencies in the vitreous of diabetic retinopathy: exploring the potential of dietary supplementation to address key metabolite imbalances. 2025:2997-3007.
106. Diniz TG, de Assis CS, de Sousa BRV, Batista KS, Silva AS, de Queiroga Evangelista IW, et al. Metabolomic analysis of retinopathy stages and amputation in type 2 diabetes. 2024;61:158-67.
107. Peters KS, Rivera E, Warden C, Harlow PA, Mitchell SL, Calcutt MW, et al. Plasma arginine and citrulline are elevated in diabetic retinopathy. 2022;235:154-62.
108. Hiller JK, Sandås EM, Rootwelt H, Vassli AØ, Lumi X, Moe MC, et al. Metabolomic biomarkers in vitreous humor: unveiling the molecular landscape of diabetic retinopathy progression. 2025;11(1):58.
109. Nie Z, Pang S, Wang Z, Yang M, Zhang X, Guo H, et al. Aqueous Humor Metabolomics in Different Stages of Diabetic Retinopathy Based on Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry. 2025:3941-53.
110. Yang S, Liu R, Xin Z, Zhu Z, Chu J, Zhong P, et al. Plasma Metabolomics identifies key metabolites and improves prediction of diabetic retinopathy: development and validation across multinational cohorts. 2024;131(12):1436-46.
111. Ma L, Dong Y, Li Z, Meng J, Zhao B, Wang QJSR. Relationship between circulating metabolites and diabetic retinopathy: a two-sample Mendelian randomization analysis. 2024;14(1):4964.
112. Shen Y, Wang H, Fang J, Liu K, Xu XJH. Novel insights into the mechanisms of hard exudate in diabetic retinopathy: Findings of serum lipidomic and metabolomics profiling. 2023;9(4).
113. He M, Hou G, Liu M, Peng Z, Guo H, Wang Y, et al. Lipidomic studies revealing serological markers associated with the occurrence of retinopathy in type 2 diabetes. 2024;22(1):448.
114. Fang J, Wang H, Niu T, Xing X, Shi X, Jiang Y, et al. Mass spectrometry-based metabolomic and lipidomic profiling stratifies stages of diabetic retinopathy. 2025:112423.
115. Zhou X, Hou G, Wang X, Peng Z, Yin X, Yang J, et al. Metabolomic studies reveal and validate potential biomarkers of diabetic retinopathy in two Chinese datasets with type 2 diabetes: a cross-sectional study. 2024;23(1):439.
116. Liu J, Liu S, Hui P, Teng S, Xie J, Sun YJBo. Ferrous ascorbate as a potential biomarker for diabetic retinopathy: a vitreous humour metabolomics study. 2024;24(1):270.
117. Yagin FH, Colak C, Algarni A, Gormez Y, Guldogan E, Ardigò LPJD. Hybrid explainable artificial intelligence models for targeted metabolomics analysis of diabetic retinopathy. 2024;14(13):1364.
118. Wang Z, Lu B, Zhang L, Xia Y, Shao X, Zhong SJJDR. Causality of Blood Metabolites on Proliferative Diabetic Retinopathy: Insights From a Genetic Perspective. 2024;2024(1):6828908.
119. Fernandes Silva L, Hokkanen J, Vangipurapu J, Oravilahti A, Laakso MJTJoCE, Metabolism. Metabolites as risk factors for diabetic retinopathy in patients with type 2 diabetes: a 12-year follow-up study. 2024;109(1):100-6.
120. Tomofuji Y, Suzuki K, Kishikawa T, Shojima N, Hosoe J, Inagaki K, et al. Identification of serum metabolome signatures associated with retinal and renal complications of type 2 diabetes. 2023;3(1):5.
121. Wang X, Yang S, Yang G, Lin J, Zhao P, Ding J, et al. Novel risk score model for non-proliferative diabetic retinopathy based on untargeted metabolomics of venous blood. 2023;14:1180415.
122. Ancel P, Martin JC, Doukbi E, Houssays M, Gascon P, Righini M, et al. Untargeted multiomics approach coupling lipidomics and metabolomics profiling reveals new insights in diabetic retinopathy. 2023;24(15):12053.
123. Zenati RA, Soares NC, Alniss HY, Al-Hroub HM, El-Awady R, Abuhelwa AY, et al. Untargeted Multiomics of LNCaP Cell Line Treated with a Novel DNA Minor Groove Binder and/or Doxorubicin Using Mass Spectrometry. 2025.
124. Kustatscher G, Collins T, Gingras A-C, Guo T, Hermjakob H, Ideker T, et al. Understudied proteins: opportunities and challenges for functional proteomics. 2022;19(7):774-9.
125. Youngblood H, Robinson R, Sharma A, Sharma SJJoms. Proteomic biomarkers of retinal inflammation in diabetic retinopathy. 2019;20(19):4755.
126. Klaassen I, Avery P, Schlingemann RO, Steel DHJSr. Vitreous protein networks around ANG2 and VEGF in proliferative diabetic retinopathy and the differential effects of aflibercept versus bevacizumab pre-treatment. 2022;12(1):21062.
127. Abu El-Asrar AM, Ahmad A, Allegaert E, Siddiquei MM, Alam K, Gikandi PW, et al. Galectin-1 studies in proliferative diabetic retinopathy. 2020;98(1):e1-e12.
128. Haydinger CD, Oliver GF, Ashander LM, Smith JRJA. Oxidative stress and its regulation in diabetic retinopathy. 2023;12(8):1649.
129. Santos FM, Ciordia S, Mesquita J, Cruz C, Sousa JPCe, Passarinha LA, et al. Proteomics profiling of vitreous humor reveals complement and coagulation components, adhesion factors, and neurodegeneration markers as discriminatory biomarkers of vitreoretinal eye diseases. 2023;14:1107295.
130. Lim RR, Vaidya T, Gadde SG, Yadav NK, Sethu S, Hainsworth DP, et al. Correlation between systemic S100A8 and S100A9 levels and severity of diabetic retinopathy in patients with type 2 diabetes mellitus. 2019;13(2):1581-9.
131. Yang S, Xin Z, Xiong R, Zhu Z, Li H, Chen Y, et al. Proteome atlas for mechanistic discovery and risk prediction of diabetic retinopathy. 2025;16(1):9636.
132. Liu Y, Wei R, Tang Y, Sun S, Zhang W, Zhou Y, et al. Identification of unique biomarkers for proliferative diabetic retinopathy with

- tractional retinal detachment by proteomics profiling of vitreous humor. 2025;15(1):13456.
133. Zhang J, Wang Y, Wu X, Zhang S, Cai D, Feng Y, et al. Vitreous Olink Proteomics Uncovers Novel Inflammatory Proteins in Patients with Proliferative Diabetic Retinopathy. 2025;24(9):4653-64.
 134. Ma I-H, Hsu Y-L, Wu H-Y, Hsieh Y-T, Pan S-HJOPR. Comparative Tear Fluid Proteomics to Explore Treatment Responsiveness in Diabetic Macular Edema: A Pilot Study. 2025;24(10):4925-34.
 135. Jin Y, Liu J, Zhang X, Zhang L, Cui Y, Luo X, et al. Stage-dependent proteomic alterations in aqueous humor of diabetic retinopathy patients based on data-independent acquisition and parallel reaction monitoring. 2025;23(1):476.
 136. Oh JW, Ahn SJ, Jung JH, Kim TW, Kim KPJM, Proteomics C. Proteomic Analysis of Aqueous Humor Identified Clinically Relevant Molecular Targets for Neovascular Complications in Diabetic Retinopathy. 2025;24(4).
 137. Brighenti T, Neri G, Mazzola M, Tomé G, Scalfati M, Peroni D, et al. Comparative proteomic analysis of human vitreous in rhegmatogenous retinal detachment and diabetic retinopathy reveals a common pathway and potential therapeutic target. 2024;21(1):63.
 138. Fan Z, Hu Y, Chen L, Lu X, Zheng L, Ma D, et al. Multiplatform tear proteomic profiling reveals novel non-invasive biomarkers for diabetic retinopathy. 2024;38(8):1509-17.
 139. Alanazi AH, Shan S, Narayanan SP, Somanath PRJL. Comparative Proteomic Analysis of Type 2 Diabetic versus Non-Diabetic Vitreous Fluids. 2024;14(7):883.
 140. Cheema AA, Cheema HR, Cheema RJC. Diabetic macular edema management: a review of anti-vascular endothelial growth factor (VEGF) therapies. 2024;16(1).
 141. Ross EL, Hutton DW, Stein JD, Bressler NM, Jampol LM, Glassman AR, et al. Cost-effectiveness of aflibercept, bevacizumab, and ranibizumab for diabetic macular edema treatment: analysis from the diabetic retinopathy clinical research network comparative effectiveness trial. 2016;134(8):888-96.
 142. Cao Y, Langer R, Ferrara NJNRDD. Targeting angiogenesis in oncology, ophthalmology and beyond. 2023;22(6):476-95.
 143. Hatamnejad A, Orr S, Dadak R, Khanani A, Singh R, Choudhry NJAO. Anti-VEGF and steroid combination therapy relative to anti-VEGF mono therapy for the treatment of refractory DME: A systematic review of efficacy and meta-analysis of safety. 2024;102(3):e204-e14.
 144. Chen F, Wang X, Chen M, Xin M, Xian YJFIE. Efficacy of dexamethasone intravitreal implant combined with anti-VEGF drugs in the treatment of diabetic macular edema: a systematic review and meta-analysis. 2025;16:1673371.
 145. Steele JW, Faulds D, Goa KLJD, aging. Epalrestat: a review of its pharmacology, and therapeutic potential in late-onset complications of diabetes mellitus. 1993;3(6):532-55.
 146. Kawano T, Inokuchi J, Eto M, Murata M, Kang J-HJP. Activators and inhibitors of protein kinase C (PKC): their applications in clinical trials. 2021;13(11):1748.
 147. Wang Z, Zhang N, Lin P, Xing Y, Yang NJFiE. Recent advances in the treatment and delivery system of diabetic retinopathy. 2024;15:1347864.
 148. Chen Q, Liu L, Ke W, Li X, Xiao H, Li YJBED. Association between the soluble receptor for advanced glycation end products and diabetes mellitus: systematic review and meta-analysis. 2024;24(1):232.
 149. Xia X, Guo XJFiM. Adeno-associated virus vectors for retinal gene therapy in basic research and clinical studies. 2023;10:1310050.
 150. Dui W, Xiaobin Z, Haifeng Z, Lijuan D, Wenhui H, Zhengfeng Z, et al. Harnessing RNA therapeutics: novel approaches and emerging strategies for cardiovascular disease management. 2025;12:1546515.
 151. Lombardo M, Maccauro C, D'Ambrosio M, Grossi M, Missiroli F, Cesareo M, et al. Aflibercept, ranibizumab, and bevacizumab for macular neovascularization secondary to age-related macular degeneration: a retrospective OCT-angiography study. 2025;11(1):111.
 152. Urbančič M, Gardašević Topčić IJCO. Dexamethasone implant in the management of diabetic macular edema from clinician's perspective. 2019:829-40.
 153. Dănilă A-I, Ghenciu LA, Stoicescu ER, Bolintineanu SL, Iacob R, Săndesc M-A, et al. Aldose reductase as a key target in the prevention and treatment of diabetic retinopathy: a comprehensive review. 2024;12(4):747.
 154. Lin H, Yang Y, Wang X, Chung M, Zhang L, Cai S, et al. Targeting the AGEs-RAGE axis: pathogenic mechanisms and therapeutic interventions in diabetic wound healing. 2025;12:1667620.
 155. Han N, Xu X, Liu Y, Luo GJMTM, Development C. AAV2-antiVEGFscFv gene therapy for retinal neovascularization. 2023;31.
 156. Chen Q, Chen Y, Mou K, Zhang MJB. Liquid Biopsy Combined with Multi-Omics Approaches in Diagnosis, Management, and Progression of Diabetic Retinopathy. 2025;13(6):1306.
 157. Frudd K, Sivaprasad S, Raman R, Krishnakumar S, Revathy YR, Turowski PJAO. Diagnostic circulating biomarkers to detect vision-threatening diabetic retinopathy: Potential screening tool of the future? 2022;100(3):e648-e68.
 158. Sivaprasad S, Cheung CMG, Gliem M, Wykoff CC, Zippel N, Ishida S, et al. New targets in diabetic retinopathy: addressing limitations of current treatments through the Sema3A/Nrp1 pathway. 2025:1-9.
 159. Zhu Q, Pan X, Du Z, Ying J, Hu Y, Yi Q, et al. From Monotherapy to Combination Strategies: Redefining Treatment Approaches for Multiple-Cause Macular Edema. 2025:887-97.