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Diabetic Retinopathy: Breaking the Barrier

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Abstract

Diabetic retinopathy (DR) remains a major complication of diabetes and a leading cause of blindness among adults worldwide. DR is a progressive disease affecting both type I and type II diabetic patients at any stage of the disease, and targets the retinal microvasculature. DR results from multiple biochemical, molecular and pathophysiological changes to the retinal vasculature, which affect both microcirculatory functions and ultimately photoreceptor function. Several neural, endothelial, and support cell (e.g., pericyte) mechanisms are altered in a pathological fashion in the hyperglycemic environment during diabetes that can disturb important cell surface components in the vasculature producing the features of progressive DR pathophysiology. These include loss of the glycocalyx, blood-retinal barrier dysfunction, increased expression of inflammatory cell markers and adhesion of blood leukocytes and platelets. Included in this review is a discussion of modifications that occur at or near the surface of the retinal vascular endothelial cells, and the consequences of these alterations on the integrity of the retina.

Keywords

Retina; Permeability; Oxidative stress; Diabetes; Blood-retinal barrier

1. INTRODUCTION

Diabetes is an epidemic health issue that currently afflicts millions of individuals worldwide. According to the Center for Disease Control and Prevention (CDC), 10% of US adults have diabetes, with a projected increase to one in three US adults having diabetes by 2050 [1]. This is an alarming trend considering the massive financial, physical, and emotional burden diabetes can have. Almost all individuals with insulin-dependent diabetes mellitus (IDDM) will develop some form of a macrovascular or microvascular complication within 15 years of their diagnosis, with a higher occurrence in type I than in type II diabetes [2]. Macrovascular complications of diabetes include coronary heart disease, peripheral artery disease, stroke, and many other disorders [3, 4]. Microvascular complications of diabetes

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can also lead to diabetic neuropathy, diabetic nephropathy, or diabetic retinopathy [3, 4]. Diabetic retinopathy is a serious complication of diabetes, and is the leading cause of new-onset blindness in working-age adults [5]. Although diabetes is characterized by hyperglycemia, DR will often continue to progress even after tight glycemic control is achieved [6, 7].

Typically, the time elapsed between an initial diagnosis of diabetes and the clinical detection of DR can be lengthy, the average being 10 years [8], during which major and frequently irreversible biochemical and physiological alterations take place within the diabetic retina. At some point, injury reaches a point of 'no return' where DR will become inevitable. Most of the current therapies are aimed at managing the symptoms of diabetes, or glucose levels. For type I diabetes, the standard treatment is insulin, while for type II diabetes, it can be one or a combination of sulfonylureas, thiazolidinediones, biguanides, alpha-glucosidase inhibitors, meglitinides, and insulin [9]. However, these drugs do not, for the most part, treat the underlying microvascular mechanisms contributing to DR. Studies have indicated that despite tight glycemic control, retinal microvascular complications will continue to develop and the factors leading to these complications will persist in DR [10–12]. This phenomenon has been termed 'metabolic memory' [13–15], where hyperglycemic vascular stresses continue even after the normalization of blood glucose levels. Many factors have been attributed to this phenomenon, such as advanced glycation end products [16], mitochondrial damage and oxidative stress [17], and epigenetic changes [7, 18, 19], all of which will be discussed in detail later in the review.

Diabetic retinopathy can be divided into two main stages, non-proliferative diabetic retinopathy (NPDR), and proliferative diabetic retinopathy (PDR), with NPDR further subdivided into three phases, mild NPDR, moderate NPDR, and severe NPDR (Figure 1). These stages are marked by progressive alterations to the retinal microvasculature that contribute to the pathology of DR. As shown in Figure 2, the blood vessels in the diabetic retina are more tortuous, and are prone to microaneurysms and microhemorrhages. The microcirculation suffers from deficient endothelial-dependent vasodilation [20–22], and the retina can have capillary dropout, leading to ischemic zones that are described as cotton-wool spots. Newly formed vessels in the diabetic retina are fragile and leaky to fluid and protein, which leads to macular edema (retinal thickening), blood pooling in the tissue, and substantial visual impairment [23].

Diabetic retinopathy can develop in either type 1 or type 2 diabetes, but is more common in type 1, because it begins earlier and consequently has a longer time course. Several factors are known to increase the risk of developing DR. These include the duration of diabetes, poor glycemic control, high blood pressure, high cholesterol, pregnancy, elevated glycated hemoglobin-A1c (HbA1c) and tobacco use [2]. In addition, there are many molecular players that contribute to the development of DR and can be considered as risk factors in its pathogenesis. These factors include elevated levels of Th1 cytokines and growth factors, which are elevated even prior to evidence of clinical manifestations of DR. Many of these factors (for example, vascular endothelial growth factor, VEGF) not only drive proliferation, but also lead to a loss of vascular integrity in proliferative DR. Moreover, connective tissue growth factor (CTGF, CCN2) plays a major role in the formation of fibrous tissue around the

microvessels, which leads to retinal detachment and tearing [24]. In addition, a recent study has shown a correlation between proliferative DR development and plasma erythropoietin (EPO), which was significantly increased [25, 26]. This result came to light after reports that DR deterioration accelerates after patients start hemodialysis, and the recent identification of EPO as an angiogenic factor independent of VEGF [26, 27].

2. DIABETIC RETINOPATHY: A HISTORICAL PERSPECTIVE

As early as 1552 BC, a 3rd Dynasty Egyptian papyrus written by the physician Hesy-Ra describes frequent urination as a symptom of what was later termed diabetes, and is the oldest record of it. Early Hindu writings ca. 1500 BC described that ants were attracted to the urine of people with a mysterious emaciating disease, most likely diabetes. Later, around 500 BC, the presence of sugar in the urine and its occurrence in obese individuals was linked. Aretaeus of Cappadocia, a renowned physician of the Pneumatic School who lived in Alexandria and Rome during the 2nd century AD [28], was the first to introduce the term diabetes. The term 'diabetes', which meant siphon, was started by Apollonius of Memphis, and is consistent with the polyuria associated with diabetes. Later, 1st century Greeks described diabetes as a melting disease in which the body was imagined to convert to urine.

However, not until the mid-19th century was the link between eye disease and diabetes firmly established. Apollinaire Bouchardat, a French ophthalmologist in Paris, reported the progress of visual loss in the absence of cataracts in diabetics in 1846 [29, 30]. This was reported as reversible, and most complications were eliminated with the control of diabetes through diet and exercise [28]. In 1855, the ophthalmologist Eduard Jäger in Austria built an ophthalmoscope that for the first time enabled him to observe diabetic macular changes that he recorded in fine detail in his now famous paintings [31]. An article by Henry Noyes (1869) supported the link between diabetes and maculopathy (the damage of the macula) [32], and was confirmed by another paper by Edward Nettleship (1872) in London. Nettleship's paper described details of diabetes-induced pathological retinal changes [28].

Proliferative DR was first described in 1876 by Wilhelm Manz, who described degeneration of the optic disc (the area where all the axons of the ganglion cells exit the retina to form the optic nerve[33]), and retinal adhesions [34]. By 1890, characteristics of DR had been nearly completely described, but not explained. Julius Hirschberg, a German ophthalmologist, first classified DR into its four stages with what he termed 1) 'retinitis centralis punctuate', 2) hemorrhagic form, 3) retinal infarction and 4) hemorrhagic glaucoma [35]. It was up to the English ophthalmologist Arthur Ballantyne to link DR to alterations and dysfunction of the retinal vasculature, and suggested the potential role of capillaries in propagating the disease [36]. All of these previous discoveries have led to our current understanding of the pathophysiology and natural progression of DR.

3. THE RETINA

The retina is considered part of the central nervous system, with the blood-retinal barrier sharing the characteristics of the blood-brain barrier. The support cells that contribute to the retinal barrier tightness are essentially similar to those of the main central nervous system

blood barrier [37]. The retina is a complex structure of neuronal circuitry and supporting cells, and is considered one of the most metabolically active tissues in the body with a high oxygen consumption [38]. The retina can be divided into different layers, starting with the inner limiting membrane that separates the vitreous humor from the retina, followed by the nerve fiber layer (NFL), ganglion cell layer (GCL), the inner plexiform layer (IPL), the inner nuclear layer (IPL), the outer plexiform layer (OPL), the outer nuclear layer (ONL), the photoreceptor layer (PR), and finally, the retinal pigmented epithelium (RPE) [39, 40].

During embryonic development, an outpocket of the diencephalon forms the optic vesicle, and later the optic cup [39], where the retina is evolved from the inner wall and the pigment epithelium is evolved from the outer wall of the optic cup [39]. All throughout the layers, an extensive vascular network supplies nutrients and oxygen to ensure proper functions, and can be divided into a superficial microvascular layer that has the main arterioles and venules, the middle capillary layer, and the deep capillary layer. In addition to delivering critical nutrients to the retinal tissue, the microvessels form a tight blood retinal barrier that limits the access of toxins and pathogens, and makes the retina an immune privileged tissue by restricting the migration of leukocytes to the surrounding tissue.

The blood-retinal barrier (BRB) consists of inner and outer components (iBRB and oBRB). The iBRB is formed by the tight junctions and adherens junctions between neighboring endothelial cells of the retinal microvasculature. The breakdown of the iBRB contributes to the development of DR due to the vulnerability of the neuronal retina, where leakage from the vasculature to the surrounding tissue can be detrimental to vision. The oBRB is established by the tight junctions between the retinal pigmented epithelial (RPE) cells, and separates the choroid from the neuronal retina. Tight junctions are composed of specialized proteins such as occludins, claudins, and zonula occludens (ZO-1, -2, and -3), which play an important role in maintaining the barrier function through the regulation of the transport of solutes and molecules through the endothelial and RPE cell layers [37, 41, 42]. Although both retinal endothelial cells and retinal epithelial cells have tight junctions, they differ in their organization and composition; epithelial tight junctions are more concentrated at the apical side of the cell, whereas endothelial tight junctions are more dispersed between adherens junctions and gap junctions. The adherens junction is comprised of vascular endothelial-cadherin (VE-cadherin) and its associated proteins, such as catenins and plakoglobin, both of which act as a linker protein anchoring the complex to the cytoskeleton [43]. Moreover, cell-cell junctions contain additional adhesion molecules, such as platelet endothelial cell adhesion molecule-1 (PECAM-1), that help in the maintenance the retinal endothelial barrier integrity [44]. Together, these adhesion molecules not only adhere cells together, but also can function as signaling molecules, scaffolds to other proteins, modulation of cell growth contact inhibition, and apoptosis [45–49].

4. MOLECULAR MECHANISMS OF DIABETIC RETINOPATHY

Hyperglycemia and genetic predisposition is associated with a variety of pathophysiological events identified in the progression of diabetic retinopathy [50]. To date, several major mechanisms are thought to induce retinal stress in DR, including 1) the polyol pathway, 2) non-enzymatic glycation, 3) activation of protein kinase C (PKC), 4) genetic factors, 5)

inflammation, and 3) oxidative stress: all of which have been implicated in the development of microvascular damage and retinopathy (Figure 3).

Because the retinal vasculature lacks autonomic innervation, and modulation of blood flow through the neuropil (an area that is comprised of unmyelinated axons, dendrites and glial cell processes and a minimum number of cell bodies [39]) is dependent on local signaling mechanisms, it is extremely susceptible to disturbances which interfere with normal exchange and blood flow distribution [51]. This section will cover the enzymatic and non-enzymatic changes that occur in the retina due to hyperglycemia, and the pathophysiological consequences of these alterations.

4.1 The polyol pathway

In the face of persistently elevated extracellular glucose levels, intracellular glucose levels also will rise, and the polyol pathway, a secondary pathway for glucose metabolism, becomes activated. This pathway is a two-step metabolic pathway where glucose is first converted to sorbitol, and then to fructose [52, 53]. In the first step, aldose reductase (AR) reduces glucose to sorbitol, an alcohol, in an NADPH-dependent fashion. Sorbitol eventually is metabolized to fructose by sorbitol dehydrogenase, which uses NAD^+ as a cofactor. This enzymatic pathway can have serious detrimental effects on metabolism and can induce cell injury. Sorbitol results in an osmotic damage to the retinal vascular endothelium, loss of pericytes, and thickening of the basement membrane [8, 54]. As a polyhydroxylated, and a strongly hydrophilic alcohol, sorbitol will accumulate in the cell where it is trapped within the cytoplasm and initiates an osmotic shock [52, 55, 56] leading to a hyperosmolar stress within the retina. Osmotic shock also affects the retinal pigmented epithelium (RPE). Cell shrinkage is a consequence of cellular hyperosmolar stress, and is recovered by cell swelling, which utilizes the regulatory volume increase (RVI) mechanism [57]. Moreover, osmoprotective genes such as the taurine transporter, aldose reductase, the betaine/GABA transporter, the *myo*inositol transporter, and glycerophosphocholine (GPC), are transactivated by the transcription factor TonEBP/NFAT5 as a response to hyperosmolar stress, and leads to late phases of RVI. In addition to osmoadaptation, cellular responses to hyperosmolar stress include apoptosis, DNA damage, oxidative stress, cell cycle arrest, mitochondrial adaptation, and inhibition of transcription and translation [58]. Moreover, altered expression of stress protein, and cytoskeleton rearrangements occur as a result of hyperosmolar stress [58]. Aquaporin 4 (AQP4) expression is reduced due to hyperosmotic stress, probably due to the ubiquitination-independent proteasome pathway in RPEs [59]. Hyperosmolar stress also leads to the reorganization of RPE cell cytoskeleton [60].

In addition, increased sorbitol pathway activity has been known to generate oxidative stress that contributes to diabetic complications [61, 62], due to the depletion of important antioxidants, in particular, glutathione (GSH), ascorbate, and taurine [61, 63]. Fructose generated by the sorbitol pathway is a more potent glycation agent than glucose [64]. In a postmortem electron microscopic study of diabetic eyes, BRB disruption and increased aldose reductase expression in the vascular cells retinal vascular endothelial cells and Müller cells were found, which suggests that aldose reductase-induced intracellular accumulation of sorbitol in vascular cells might contribute to the BRB breakdown in diabetes [65].

Moreover, since AR uses NADPH as a cofactor, it means there will be less NADPH available to be used by glutathione reductase to reduce and regenerate glutathione (GSH), the most important reductant and antioxidant in this environment. Consequently, both neural and endothelial cells will become more susceptible to oxidative damage because of a reduced ability to adapt to oxidative stress [66]. In addition, the ratio of NADH/NAD⁺ will increase due to the consumption of NAD⁺ by sorbitol dehydrogenase. This reduction in NAD⁺ as a result of hyperglycemia is referred to as pseudohypoxia, where in spite of a normal oxygen partial pressure, the tissue mimics a hypoxic condition, such as the activation of HIF-1 α , leading to many disturbances in cell signaling and function [67, 68]. Furthermore, the phosphorylation of fructose to fructose-3-phosphate, and its subsequent metabolism to 3-deoxyglucose, both of which are strong glycating agents, leads to the formation of advanced glycation end products (AGEs) that can further alter and damage cells and components of the cellular environment [69, 70]. DR therapy studies are currently aimed at identifying effective inhibitors for AR without promoting adverse side effects. However, several AR inhibitors were withdrawn from clinical trials due to either their non-specificity, where they react with other analogous enzymes, or their adverse side effects by impairing AR detoxification functions [71]. This suggests the need for the development of more specific AR inhibitors by identifying specific binding sites. In a study where the investigators were looking at the molecular structure of AR2 [72], which is considered an isoenzyme of AR1 [73, 74], they identified a site where the inhibitors can be targeted, leaving the catalytic site free for its detoxification functions [72].

4.2 Advanced glycation end products

Advanced glycation end products (AGEs) are post-translationally modified proteins and lipids that are non-enzymatically glycosylated and oxidized after exposure to aldose sugars [75]. AGEs have been implicated in vision loss, especially in the development of cataracts, macular degeneration, and DR [76]. Modification of hormones, cytokines, and extracellular matrix by AGEs disrupts their functions and leads to vascular damage [77]. AGEs affect cellular functions by cross-linking key molecules in the basement membrane, or initiating signaling cascades that alter cell functions (upregulation of NF- κ B, NADPH oxidase, mitogen-activated protein kinases (MAPK), intercellular adhesion molecule-1 (ICAM-1), VEGF, cytokines, and a decrease in nitric oxide (NO)) [69].

The formation of AGEs depends on multiple factors, such as hyperglycemia, turnover of proteins, and oxidative stress [77, 78]. In the Maillard reaction, Schiff bases and Amadori products (1-amino-1-deoxyketos) are the precursors to AGEs. Schiff bases form by the reversible chemical reaction between glucose and the amino groups of proteins. In the event of continuous glucose exposure, Schiff bases undergo rearrangement to the more stable Amadori product [79]. Highly reactive carbonyl group intermediates resulting from Amadori reorganization accumulate causing carbonyl stress [80, 81]. The reaction between these intermediates and the amino, sulfhydryl, and guanidine functional groups of proteins leads to its denaturation and cross-linking, while the interaction of the carbonyl intermediates with the lysine and arginine functional groups of proteins leads to the formation of stable AGE compounds [82–84].

AGEs have multiple receptors such as RAGEs, which interact with AGEs initiating cellular signaling cascades that disrupt cellular functions. RAGEs belong to the immunoglobulin superfamily of receptors [85], consisting of a 332 amino acid extracellular domain, a single transmembrane domain, and a highly charged 43 amino acid cytosolic tail [86]. In addition, the increase in AGEs will promote the expression of RAGEs in a positive feedback loop manner [75]. AGEs also play a role in the inhibition of DNA synthesis, increases in VEGF mRNA, increases in NF- κ B expression in the vascular endothelium, and promotion of apoptosis in retinal pericytes [87]. In addition, there is strong evidence linking N-epsilon-carboxy methyl-lysine, which is a product of AGEs, in the development of non-proliferative retinopathy in type II diabetes, where its increased levels were observed [88]. Currently, new and improved technologies are being developed to detect tissue damage caused by AGEs, such as pulse wave analysis that is used as a vascular dysfunction marker, and skin autofluorescence that detects long-term accumulation of AGEs [89]. In addition, pigment epithelium derived factor (PEDF) has been shown to inhibit AGE-induced ROS and oxidative stress in pericytes, which may suggest the use of PEDF as an effective therapeutic agent in the prevention of pericyte dropout that contributes to the disruption of BRB [90, 91].

4.3 Protein Kinase C (PKC) Pathway Activation

PKC, which belongs to the AGC kinase family (cAMP-dependent protein kinase A (PKA), cGMP-dependent protein kinase G (PKG), lipid-activated protein kinase C (PKC)), are a group of at least 12 isoforms that are widely distributed in mammalian tissues [92, 93]. PKC-induced phosphorylation of serine and threonine residue of various target proteins, such as transcription factors, receptors, and enzymes, triggers a pathophysiological response [94]. In DR, PKC activation can result in an increase in permeability, pericyte dropout, changes in retinal blood flow, and abnormal angiogenesis [95–97]. Moreover, PKC activation can result in an augmented response to other pathophysiological conditions, such as shear stress [98] and hypoxia [99]. The endogenous activator of PKC is diacylglycerol (DAG), which has elevated levels in vascular tissues, such as the retina, under hyperglycemic conditions [100]. During hyperglycemia, glucose enters the retinal endothelial cells via the GLUT 1 transporter, where it is metabolized by glycolysis, leading to the accumulation of the metabolic intermediate glyceraldehyde-3-phosphate (G-3-P), and the de novo synthesis of DAG [101]. Persistent and excessive activation of several PKC isoforms by DAG operates as a third common pathway mediating tissue injury resulting from diabetes-induced ROS. Evidence suggests that the enhanced activity of PKC isoforms also could result from the interaction between AGEs and their cell-surface receptors [102, 103]. Overactivity of PKC has been implicated in the decreased NO production in smooth muscle cells [104]. Moreover, the activation of PKC by high glucose induces the expression of the permeability-enhancing factor VEGF in vascular endothelial cells and smooth muscle cells [97, 105]. In addition, activation of PKC may contribute to the accumulation of microvascular matrix proteins by inducing the expression of TGF- β_1 [106, 107]. Tight junctions form an essential structural component of the blood–brain barrier (BBB) and the blood–retinal barrier (BRB)[108]. Studies suggest that increased vascular permeability in diabetic retinopathy is due to altered regulation of one or more tight junction proteins, which can be regulated directly through modification of these proteins or indirectly through effects

on the cytoskeleton. Increased vascular permeability associated with the induction of diabetes may be mediated by chronic activation of PKC [109].

PKC has multiple isoforms with a wide variety of functions in multiple biological systems. These isoforms have been derived from multiple genes and alternative splicing of mRNA transcripts [110], and have been characterized according to their cofactors and structure. Classic or conventional PKC (cPKC: cPKC- α , - β_I , - β_{II} , and - γ) are activated by DAG, calcium, and phosphatidylserine (PS) or phorbol esters such as phorbol 12-myristate 13-acetate (PMA). Novel PKC (nPKC: nPKC- δ , - ϵ , - θ and - η) are activated by DAG, PS, and PMA, however, they are calcium independent. Atypical PKC (aPKC: aPKC- ζ , - λ , and - ξ) do not require DAG, calcium, nor PMA for activation [93, 111–113]. The retina expresses various isoforms of PKC. Among these different isoforms, studies have shown an increase in the activity of PKC- β_I and β_{II} relative to other isoforms [100, 114], which implicates their role in the early diabetes-induced complications in the retina. In addition, PKC- α , - γ , and - δ had an increased activity under hyperglycemic conditions, albeit to a lesser degree than PKC- β_I and β_{II} . Moreover, the inhibition of PKC- ξ decreased retinal vascular leakage by attenuating VEGF and AGE-induced decrease of tight junction proteins [115]. When clinical doses of VEGF were intravitreally injected, it resulted in rapid activation of PKC, and the membrane translocation of PKC- α , PKC- β , and PKC- δ leading to an increase in retinal vascular permeability [116]. These findings indicate a substantial role of diabetes-induced PKC activation in DR pathogenesis.

4.4 Inflammation

Molecular and physiological changes consistent with inflammation occur during DR. Hyperglycemia is considered a proinflammatory environment, where inducible nitric oxide synthase (iNOS), leukotrienes, and cyclooxygenase -2 (COX-2) are upregulated [117]. Using iNOS knockout mice, investigators were able to demonstrate a direct role between iNOS and the development of DR in its early stages [118]. Moreover, it has been reported that patients treated for rheumatoid arthritis with salicylate-based drugs had lower incidences of DR [119, 120], indicating a possible role for anti-inflammatory drugs in modulating DR progression. In addition to hyperglycemic conditions, the alteration in retinal fatty acid metabolism, possibly via AGE/RAGE interactions and the activation of the MAPK pathway, can lead to chronic inflammation due to a decrease in retinal n-3 polyunsaturated fatty acids (PUFAs), such as docosahexanoic acid (DHA) [121]. Moreover, the increase in n6 essential fatty acid (EFA) levels, such as with linoleic acid in diabetes, may stimulate inflammation in the diabetic retina [122, 123]. In addition to its possible role in dyslipidemia, AGE/RAGE interactions may lead to vascular endothelial cell activation, upregulation of ICAM-1 and E-selectin expression, and increased leukostasis [124]. Moreover, AGEs upregulate monocyte chemoattractant protein-1 (MCP-1) levels leading to increased T-cell adhesion to endothelial cells [125]. Thus, AGEs/RAGEs are important players in promoting inflammation in DR. Hypoxia in the diabetic retina, which may develop due to capillary blockage or dropout, is an important and causative factor in developing retinopathy. Ischemia in the diabetic retina leads to the upregulation of chemokines such as MCP-1 that attracts macrophages into the hypoxic tissue. Moreover, the upregulation of TNF- α by hypoxia-activated macrophages and microglia induces vascular

endothelial cells to release MCP-1, VEGF, and IL-8, which exacerbates the inflammatory response in the diabetic retina [126].

4.5 Genetic Modifications

Many factors contribute to the pathogenesis of DR, such as the duration of diabetes, poor blood glucose control, and high blood pressure. In addition to metabolic and physiologic factors, genetic factors can influence the susceptibility and severity of the disease. Even with optimal glucose and blood pressure control, DR can still develop in some patients, while it does not manifest at all in others even with uncontrolled glucose levels. In proliferative DR, it has been shown that heritability estimates range from 25–50% [127]. Moreover, the increased prevalence of DR among certain ethnic groups indicates a genetic susceptibility. Thus, identifying the genetic factors that can affect the prevalence of DR among at risk groups will lead to a better screening and early intervention in DR. In the past two decades, many studies have identified genetic factors that can affect the prevalence of DR. These studies employed three main research strategies: Linkage studies, which is based on the principle of genetic recombination and the analysis of shared alleles between family members, Genome-wide Association Studies (GWAS), and candidate gene studies, which looks at genes involved in DR pathogenesis. In a study using genome-wide linkage analysis in the Pima Indian population [128] and in Mexican Americans [129], investigators found evidence suggesting a possible linkage to chromosome 1p36. However, none of the genes located on this chromosome, such as peptidyl arginine deiminases (PADI) 1, 2, 3, 4 and 6, CASP-9, CLCN-Ka and CLCN-Kb, were associated with DR. Additionally, investigators found an association with the proximal end of chromosome 12 that contains multiple genes involved in DR such as WNT5B (adipogenesis), and chromosome 3 that contains genes related to retinal disease such as ROBO2 (retinal development) and PROS1 (retinopathy of prematurity). However, none of these associations were statistically significant. Candidate gene studies were focused on genetic variants related to diabetes development, specifically, metabolic pathways such as the polyol pathway, AGE formation, and VEGF. Meta-analysis studies have revealed a significant variation in the *AKR1B1* gene. This gene encodes aldo-keto reductase family 1 member B1, which is the rate-limiting enzyme of the polyol pathway [130, 131]. A Finnish group found an association between the polymorphism in *SUV39H2* and diabetic microvascular complications, including retinopathy [132].

In addition to heritable factors, DNA modification due to lifestyle or disease can affect the outcome of DR. In diabetes, epigenetic modifications impair Nrf2-mediated retinal GSH biosynthesis, where H3K4me2 at *Gclc-ARE4* is increased and H3K4me3 and H3K4me1 are decreased. Altered H3K4 methylation at *Gclc-ARE4* continues after the reversal of hyperglycemia. The regulation of H3K4 methylation maintains GSH levels in DR [133]. Hyperglycemia reduces histone H3 dimethyl lysine 9 (H3K9me2) and increases acetyl H3K9 (Ac-H3K9) levels, which facilitates the recruitment of p65 at the retinal MMP-9 promoter, leading to MMP-9 activation and mitochondrial damage [136]. Moreover, recent studies have shown that retinal Sod2 histone methylation has a significant role in DR development, more importantly, in the phenomenon known as metabolic memory that is associated with its sustained progression [134]. These findings have suggested a role of histone modification in the development of DR.

In addition to DNA methylation and histone modification, small non-coding RNA that can regulate the expression of genes post-transcriptionally by binding to their target gene have been shown to either increase or decrease in their expression during DR, indicating a possible role for DR progression. miR-126 and miR-200b are downregulated during DR, which is associated with the upregulation of VEGF and fibronectin [135]. Moreover, miR146a, miR146b, miR-155, and miR-132, which are NF- κ B-responsive, were upregulated under hyperglycemic conditions in retinal endothelial cells, indicating a possible role in modulating inflammation in DR [136].

4.6 Oxidative Stress

Many of the factors that can contribute to DR pathogenesis may stem from hyperglycemia-induced oxidative stress, which is the imbalance between reactive oxygen species (ROS) formation and elimination [137]. Under normal physiological conditions, ROS helps the body to destroy foreign microorganisms that can damage cells. However, if ROS levels increase, it can lead to cellular damage via lipid peroxidation, DNA modification, protein misfolding and destruction, and mitochondrial damage [138–142]. Hyperglycemia promotes the formation of ROS by disturbing glycolysis and the citric acid cycle pathways. Increased glucose concentration leads to increased NADH and FADH₂ production. NADH and FADH₂ act as electron donors in the mitochondrial transport chain, resulting in adenosine triphosphate (ATP) production, and the reduction of oxygen to superoxide radicals. ROS can activate the other pathways resulting in DR pathogenesis via the activation of the poly-ADP-ribose polymerase (PARP) pathway and the downregulation of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) activity [143, 144].

Many reports have indicated increased superoxide in DR. Retinal superoxide production was increased in a streptozotocin (STZ) rat model of type I diabetes following two months of hyperglycemia, as well as in retinal endothelial cells exposed to high glucose concentrations [145]. Moreover, superoxide levels were increased in the photoreceptors of STZ mice in both light- and dark-adapted eyes [146]. The activity of the enzyme responsible for converting superoxide into oxygen and hydrogen peroxide (superoxide dismutase (SOD)) has been reported to decrease in STZ-induced diabetic rats [147]. Diabetic retinopathy involves alterations in oxygen delivery, oxygen consumption, and superoxide production, with the interdependence of these mechanisms still yet to be determined. Pro-oxidant and pro-apoptotic thioredoxin-interacting protein (TXNIP) was shown to be highly upregulated in DR and by high glucose (HG) in retinal cells in culture. TXNIP binds to thioredoxin (Trx) inhibiting its oxidant scavenging and thiol reducing capacity [148]. Furthermore, oxidative stress has been shown to have a neurodegenerative effect in the diabetic retinas of STZ mice [149, 150].

5. Microvascular damage in DR

Many factors contribute to the pathogenesis of DR, where endothelial cell surface modifications, increased plasma leakage due to the breakdown of the BRB (Figure 5), and abnormal neovascularization may increase the severity of DR leading to eventual vision loss.

The following section will look at the factors leading to microvascular damage in DR, and the therapies and drugs being studied and developed to reverse or limit its progression.

5.1 The molecular mechanisms leading to BRB breakdown

The retina is an important structure that facilitates visual function, thus, any damage to its microvasculature that can lead to BRB breakdown can be detrimental. Plasma components leaking into the surrounding tissue results in edema, and damages the neuronal elements of the retina. Therefore, an understanding of the molecular mechanisms leading to BRB breakdown, as summarized in Figure 4, will help in developing better therapies to limit DR progression.

5.1.1 The kallikrein-kinin system—During the more severe and progressive stages of DR, retinal function declines in part due to an increase in vascular injury. One of the key contributors is the activation of the plasma kallikrein-kinin system (KKS). The KKS consists of two distinct proteolytic pathways, plasma kallikrein (PK) and tissue kallikrein (TK). PK originates in the liver, and is released into the plasma where it forms a complex with high molecular weight kininogen (HMWK), and circulates throughout the body. This complex has high binding affinity to endothelial cells, leading to PK activation and bradykinin production [151]. On the other hand, TK is expressed in a wide range of tissues, including the eye [152]. However, it is expressed at a low to non-detectable concentration in the vitreous fluid of patients with proliferative DR, unlike PK, which has a high activity in those patients [153]. PK and TK share a similarity in that upon activation, they both promote the production of highly potent kinin peptides; however, they differ in many aspects such as substrate specificity, tissue distribution, and regulatory mechanisms [154].

The KKS modulates important functions that participate in the development of DR such as inflammation, innate immunity, thrombosis, and angiogenesis [154–158]. The peptide hormone bradykinin (1–9) is the primary mediator of the actions of the KKS, and is a member of a family of kinins that includes kallidin, T-kinin, and des-Arg-kinins [159]. Kinins exert their biological functions through the activation of two cell surface G-protein receptors, the B1 and B2 receptors. The B2 receptor is constitutively expressed in the retina, while B1 receptors are usually not expressed under physiological conditions, but become upregulated following inflammation, diabetes, and tissue injury in the retina through a mechanism that involves oxidative stress [159, 160].

The kinin receptors B1 and B2 also are implicated in retinal blood barrier breakdown and the increase of plasma extravasation in DR [159]. The activation of the KKS of intraocular plasma promotes increased retinal vascular permeability, vasodilation, and edema [157]. The intravitreal administration of recombinant plasma kallikrein induces retinal vascular leakage and hemorrhage, while the administration of B1 and B2 receptor agonists induces retinal edema [161, 162]. Moreover, it was found that hemorrhage can enhance retinal vascular permeability, and leukocyte adhesion and migration, through the activation of PK, where these responses were attenuated by PK inhibition.

The previous findings indicate that the KKS can be a promising therapeutic target for reducing the development of DR. The B1 receptor non-peptide antagonist FOV-2304 has

been shown to reduce retinal vascular permeability, leukocyte adhesion and migration, and upregulation of proinflammatory components [161, 162]. In addition, a recent study has indicated that the B1 receptor antagonist R-954 significantly reduces vascular permeability, kallikrein activity, and NO [163].

On the other hand, TK has been shown to normalize retinal vascular permeability by decreasing NO levels, normalizing nitrite and nitrate levels, and increasing VEGF in the diabetic rat retina [164]. In addition, TK inhibits neovascularization by inhibiting VEGF165-induced tube formation, proliferation, and phosphorylation of VEGF receptor-2 [165].

One of the mechanisms that may activate the kallikrein-kinin system is carbonic anhydrase (CA). CA is a ubiquitous enzyme that catalyzes the interconversion of carbon dioxide and bicarbonate to regulate pH of tissue and to help the transport of carbon dioxide. In the human eye, four isoforms of CA have been found (CA I, II, IV and XIV). CA I is localized in retinal endothelial cells, and has been implicated in increasing retinal permeability [166]. The comparative proteomic analysis of the vitreous in non-diabetic, diabetic without retinopathy, and proliferative diabetic retinopathy (PDR) subjects revealed that the vitreous concentration of CA-I in the PDR group was 15.3 and 8.2 times higher than that of the non-diabetic and the diabetic without retinopathy groups, respectively [166]. The authors postulated that in diabetic retinopathy, increased CA elevates intraocular pH, which in turn activates the kallikrein-kinin system, and the subsequent bradykinin receptor activation leading to BRB breakdown.

5.1.2 Vascular Endothelial Growth Factor (VEGF)—VEGF has long been known as a potent angiogenic factor, and in recent studies, it has been shown to be a strong vascular permeability factor [167]. Among the different isoforms, VEGF165 is the predominant isoform with the most functional bioavailability [168]. Under hypoxic conditions, hypoxia inducible factor-1 α (HIF-1 α) is stabilized and binds with HIF-1 β leading to the formation of the HIF complex and its translocation to the nucleus. HIF, in turn, will bind to the hypoxia response element (HRE) of the VEGF gene leading to an increase in its expression [169–172]. The upregulation of VEGF during DR due to chronic hypoxia leads to increased vascular permeability through both transcellular and paracellular pathways [173, 174]. Both tight and adherens junctions are affected by VEGF in the paracellular pathway, where the expression of the tight junction proteins ZO-1 and occludin are reduced [175, 176]. Phosphorylation of tight junction proteins mediated by VEGF is also responsible for the increase in vascular permeability [177]. VEGF-dependent activation of PKC- ζ leads to the phosphorylation and subsequent ubiquitination of occludin, and the reorganization of tight junctions, leading to increased vascular permeability [178]. In addition, VEGF induces the phosphorylation of VE-cadherin, a major component of adherens junctions between endothelial cells, leading to their disorganization, disruption of BRB, and increased vascular permeability [177].

Due to its role in vascular permeability and neovascularization, VEGF is a therapeutic target for the treatment and management of proliferative DR. Sac-0601, a pseudo-sugar derivative of cholesterol, blocked VEGF-induced actin stress fiber formation, stabilized the cortical actin ring in retinal endothelial cells, inhibited occludin degradation, and reduced retinal

vascular permeability and leakage in diabetic mice [179]. In addition, ranibizumab, bevacizumab, and aflibercept are anti-VEGF treatments that are currently in use to treat diabetic macular edema and proliferative DR.

5.1.3 Inflammation—Inflammation plays a major role in the pathogenesis and progression of DR where several inflammatory cytokines and chemokines participate in the breakdown of BRB in the diabetic retina. Hyperglycemia leads to the formation of AGEs, ROS, as well as NOS dysregulation, which activates NF- κ B. NF- κ B will, in turn, promote the expression of inflammatory cytokines (example, TNF- α , IL-1 β , IL-6) and chemokines (example, CCL2, CCL5, CCL12). These cytokines and chemokines induce the disorganization and redistribution of junctional proteins in the diabetic retinal microvasculature, leading to increased permeability and extravasation of plasma. TNF- α promotes the down-regulation of tight junction proteins such as claudin-5 and ZO-1 in a PKC- ζ dependent manner [180], which was prevented by the administration of the TNF- α blocker Etanercept, or PKC- ζ inhibitors [180]. In addition, in TNF- α knockout rats, leukocyte adhesion and migration, apoptosis, and BRB breakdown was significantly reduced [181]. Moreover, IL-1 β , a multifunctional proinflammatory cytokine, induces barrier dysfunction through leukocyte recruitment. IL-1 β levels are elevated in the diabetic retina of animal models [182]. High glucose upregulates the expression of IL-1 β , and through a positive feedback mechanism, IL-1 β induces its own expression in the retinal endothelium, Müller cells, and astrocytes. IL-1 β in turn will upregulate the expression of cytokines such as TNF- α , and promote leukocyte recruitment and the release of histamine, leading to BRB breakdown [183]. A study has shown that delivering a viral anti-inflammatory M013 protein (TatM013) significantly reduced the level of IL-1 β and infiltrating monocytes in an endotoxin-induced uveitis mouse model, which suggests that this therapy can be generalized for various inflammatory diseases such as DR [184].

Chemokines also play a key role in propagating the inflammatory effects on the diabetic retina. They act as chemoattractants for chemokines whereby their influx is stimulated by specific chemokines and their receptors [185]. CCL2 is a chemokine that plays a role in mediating diabetic retinal vascular permeability [185] and has been shown to be elevated in the vitreous of patients with DR [186, 187]. CCL2 affects the BRB indirectly by recruiting monocytes, causing its breakdown. Once activated, monocytes localized at the extravascular space differentiate to macrophages, releasing cytokines and growth factors including VEGF, Ang-II, and TNF- α that play a role in the breakdown of BRB as discussed earlier. Diabetic CCL2 knock-out mice show significantly reduced retinal vascular leakage and monocyte trafficking [185], thus, therapies targeting CCL2 can be promising in managing and treating DR.

5.1.4 Pericyte dropout in DR—Pericytes are specialized contractile mesenchymal cells of mesodermal origin that play a role similar to smooth muscle cells in larger blood vessels [188]. They exist in close proximity to the endothelial cells where they cover retinal capillaries and share a basement membrane. Endothelial cells and pericytes are present in the retina at an almost 1:1 ratio. Their high number in the retina, which is higher than in the brain, contributes to the tightness of the blood-retinal barrier [189]. Communication between

pericytes and endothelial cells is mediated by diverse molecules such as angiopoietin, transforming growth factor- β (TGF- β_1), platelet derived growth factor- β (PDGF- β) and sphingosine-1-phosphate (S1P) [189–193]. One of the earliest pathological changes in DR is pericyte loss in the retina [194]. Hyperglycemia, AGEs, basement membrane thickening, and hypertension trigger pericyte apoptosis and dropout [188, 189, 195]. In addition to the breakdown of the BRB, pericyte loss will promote pathologic angiogenesis due to the role of pericytes in regulating endothelial cell proliferation.

5.2 Endothelial cell surface modifications in diabetic retinopathy

Hyperglycemia is associated with multiple changes that occur to the endothelial cells lining the retinal microvasculature in DR. These changes contribute to the progression and advancement of proliferative DR that may lead to vision loss. Endothelial cell surface modifications mediate inflammation, vascular blockage, angiogenesis, and lesion formation. This section will discuss the major modifications leading to endothelial cell dysfunction and how they contribute to DR.

The glycocalyx, a layer of proteoglycans, glycoproteins, and soluble proteins covering the endothelium (Figure 4), is involved in the protective capacity of the vessel wall. It acts as an interface between the endothelial cells and the blood, and has multiple functions including its role as a mechanotransducer (e.g., shear-induced production of NO), a barrier, and an inhibitor of platelet and leukocyte adhesion [196, 197]. Hyperglycemia induces endothelial dysfunction leading to glycocalyx shedding. In addition, the glycocalyx can be cleaved by matrix metalloproteinases (MMPs), particularly MMP-2 and MMP-9, which have been implicated in the pathology of diabetic retinopathy [196, 198]. The thickness of the retinal glycocalyx has been found to be significantly decreased by diabetes in a human study [199] and in a rat study, and in the latter, an increase in leukocyte adhesion was observed in the areas of markedly degraded glycocalyx [200]. In diabetes, the volume of the glycocalyx is decreased by 50% systemically, with increasing glycocalyx components in plasma [201]. In both types of diabetes, shedding of heparan sulfate (HS) chains of endothelial cells occurs due to low insulin and high glucose levels [202]. Additionally, under high glucose conditions, endothelial cells increase the rate of heparanase secretion, which leads to more HS degradation [201]. High glucose also affects the synthesis and/or metabolism of HS. Moreover, it has been shown that cultured endothelial cells in high glucose media were unable to align under shear stress [201]. The lack of the endothelial hydraulic conductivity (Lp) response in shear stress, and the lack of alignment is due to the decreased phosphorylation of endothelial-derived nitric oxide synthase (eNOS), which leads to decreased NO production [201, 203]. In addition to its role in endothelial alignment, NO plays a role in inhibiting MMP-2 and MMP-9; therefore, the decreased NO levels lead to an increase in these MMPs [204]. These findings indicate that the loss of the glycocalyx due to hyperglycemia in the diabetic retina can mediate increased leukocyte adhesion, decreased NO availability, and increased ROS production and oxidative stress.

Moreover, the upregulation of leukocyte adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) on the diabetic retinal endothelium makes it more adhesive to leukocytes, leading to increased inflammatory responses, capillary plugging, and the

development of acellular vessels. In addition, one major player of leukocyte migration to the tissue is platelet endothelial cell adhesion molecule-1 (PECAM-1). PECAM-1 is a 130 kDa transmembrane glycoprotein that belongs to the IgG superfamily of receptors with an immunoreceptor tyrosine-like inhibitory motif (ITIM) domain [205]. It is highly expressed at the intercellular junction of endothelial cells, and lumen-facing areas of the blood vessels, and has an integral role in cellular adhesion and transendothelial migration of leukocytes. PECAM-1 levels were reported to be decreased in both in vivo and in-vitro models of DR [206, 207]. Leukocytes that adhere to capillaries may have more difficulty migrating into the tissue without PECAM-1, and therefore, PECAM-1 loss may promote capillary plugging and eventual capillary loss.

6. CONCLUSION

We are in the midst of a serious worldwide increase in diabetes that calls for immediate action to prevent retinopathy before the advanced stages of the disease. Diabetic retinopathy is the leading cause of new-onset blindness in working-age adults of industrialized countries, where it causes major financial, physical, and emotional burdens. DR is a multifactorial disease, where many factors come into play such as hyperglycemia, inflammation, oxidative stress, and endothelial dysfunction. Moreover, the vascular pathology of DR arises because of the complex interaction between cytokines, chemokines, growth factors, ROS, and AGEs that are upregulated due to hyperglycemia. A better understanding of the mechanisms of these abnormalities will lead to the development of novel therapies for the treatment of DR. Retinal vascular leakage due to the breakdown of the blood-retinal barrier, and the endothelial cell surface changes that lead to increased leukocyte adhesion, can be important therapeutic targets for the treatment of DR. There are multiple promising drugs and therapies that are currently being investigated, which provides hope for finding an effective, long-term medical intervention to preserve sight and improve the quality of life for patients with DR.

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References

1. Retinal Conditions - Common Eye Disorders|Vision Health Initiative (VHI). 2014. cited; Available from: http://www.cdc.gov/visionhealth/basic_information/eye_disorders_retinal.htm
2. Yau JW, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, et al. Global prevalence and major risk factors of diabetic retinopathy. *Diabetes Care*. 2012 Mar; 35(3):556–64. [PubMed: 22301125]
3. Fowler MJ. Microvascular and Macrovascular Complications of Diabetes. 2008 Apr 01.2008
4. Morello CM. Etiology and natural history of diabetic retinopathy: an overview. *Am J Health Syst Pharm*. 2007 Sep 1; 64(17 Suppl 12):S3–7.
5. Klein BE. Overview of epidemiologic studies of diabetic retinopathy. *Ophthalmic Epidemiol*. 2007 Jul-Aug;14(4):179–83. [PubMed: 17896294]
6. Madsen-Bouterse SA, Mohammad G, Kanwar M, Kowluru RA. Role of Mitochondrial DNA Damage in the Development of Diabetic Retinopathy, and the Metabolic Memory Phenomenon Associated with Its Progression. 2010 Jul 20.2010 <http://dxdoiorg/101089/ars20092932>.
7. Villeneuve LM, Natarajan R. The role of epigenetics in the pathology of diabetic complications. 2010 Jul 01.2010

8. Espaillat, A. Diabetic Eye Disease: A Comprehensive Review. Thorofare, NJ: Slack incorporated; 2012.
9. Jonas D, Van Scoyoc E, Gerrald K, Wines R, Amick H, Triplette M, et al. Drug Class Review: Newer Diabetes Medications, TZDs, and Combinations: Final Original Report. 2011 Feb.2011
10. Group TDCaCTeODIaCR. Retinopathy and Nephropathy in Patients with Type 1 Diabetes Four Years after a Trial of Intensive Therapy — NEJM. N Engl J Med 2000. 2000; 342:381–9.
11. Nathan DM. Group ftDER. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study at 30 Years: Overview. 2014 Jan 01.2014
12. White NH, Sun W, Cleary PA, Danis RP, Davis MD, Hainsworth DP, et al. Prolonged effect of intensive therapy on the risk of retinopathy complications in patients with type 1 diabetes mellitus: 10 years after the Diabetes Control and Complications Trial. Arch Ophthalmol. 2008 Dec; 126(12):1707–15. [PubMed: 19064853]
13. Drzewoski J, Kasznicki J, Trojanowski Z. The role of “metabolic memory” in the natural history of diabetes mellitus. Pol Arch Med Wewn. 2009 Jul-Aug;119(7–8):493–500. [PubMed: 19776690]
14. Ceriello A. Hypothesis: the “metabolic memory”, the new challenge of diabetes. Diabetes Res Clin Pract. 2009 Dec; 86(Suppl 1):S2–6. [PubMed: 20115927]
15. Ceriello A, Ihnat MA, Thorpe JE. Clinical review 2: The “metabolic memory”: is more than just tight glucose control necessary to prevent diabetic complications? J Clin Endocrinol Metab. 2009 Feb; 94(2):410–5. [PubMed: 19066300]
16. Chilelli NC, Burlina S, Lapolla A. AGEs, rather than hyperglycemia, are responsible for microvascular complications in diabetes: a “glycoxidation-centric” point of view. Nutr Metab Cardiovasc Dis. 2013 Oct; 23(10):913–9. [PubMed: 23786818]
17. Kowluru RA. Mitochondria damage in the pathogenesis of diabetic retinopathy and in the metabolic memory associated with its continued progression. Curr Med Chem. 2013; 20(26): 3226–33. [PubMed: 23745551]
18. Kowluru RA, Santos JM, Mishra M. Epigenetic modifications and diabetic retinopathy. Biomed Res Int. 2013; 2013:635284. [PubMed: 24286082]
19. Perrone L, Matrone C, Singh LP. Epigenetic modifications and potential new treatment targets in diabetic retinopathy. J Ophthalmol. 2014; 2014:789120. [PubMed: 25165577]
20. Hein TW, Xu W, Xu X, Kuo L. Acute and Chronic Hyperglycemia Elicit JIP1/JNK-Mediated Endothelial Vasodilator Dysfunction of Retinal Arterioles. Invest Ophthalmol Vis Sci. 2016 Aug; 57(10):4333–40. [PubMed: 27556216]
21. Hein TW, Potts LB, Xu W, Yuen JZ, Kuo L. Temporal development of retinal arteriolar endothelial dysfunction in porcine type 1 diabetes. Invest Ophthalmol Vis Sci. 2012 Dec; 53(13):7943–9. [PubMed: 23139282]
22. Nguyen TT, Kawasaki R, Wang JJ, Kreis AJ, Shaw J, Vilser W, et al. Flicker light-induced retinal vasodilation in diabetes and diabetic retinopathy. Diabetes Care. 2009 Nov; 32(11):2075–80. [PubMed: 19641162]
23. Fong DS, Aiello L, Gardner TW, King GL, Blankenship G, Cavallerano JD, et al. Diabetic Retinopathy. 2003 Jan 01.2003
24. Van Geest RJ, Lesnik-Oberstein SY, Tan HS, Mura M, Goldschmeding R, Van Noorden CJ, et al. A shift in the balance of vascular endothelial growth factor and connective tissue growth factor by bevacizumab causes the angiofibrotic switch in proliferative diabetic retinopathy. Br J Ophthalmol. 2012 Apr; 96(4):587–90. [PubMed: 22289291]
25. Gholamhossein Y, Behrouz H, Asghar Z. Diabetic retinopathy risk factors: plasma erythropoietin as a risk factor for proliferative diabetic retinopathy. Korean J Ophthalmol. 2014 Oct; 28(5):373–8. [PubMed: 25276078]
26. Takagi H, Watanabe D, Suzuma K, Kurimoto M, Suzuma I, Ohashi H, et al. Novel role of erythropoietin in proliferative diabetic retinopathy. Diabetes Res Clin Pract. 2007 Sep; 77(Suppl 1):S62–4. [PubMed: 17481772]
27. Wen CT, He T, Xing YQ. Erythropoietin promotes retinal angiogenesis in a mouse model. Mol Med Rep. 2014 Dec; 10(6):2979–84. [PubMed: 25269825]
28. Kalantzis G, Angelou M, Poulakou-Rebelakou E. Diabetic retinopathy: an historical assessment. Hormones (Athens). 2006 Jan-Mar;5(1):72–5. [PubMed: 16728388]

29. Joslin EP. Apollinaire Bouchardat 1806–1886. Diabetes Its Medical and Cultural History. 1989:359–62.
30. Chast F, Slama G. Apollinaire Bouchardat and diabetes. Hist Sci Med. 2007 Jul-Sep;41(3):287–301. [PubMed: 18348493]
31. Fischer F. The First Case of Diabetic Retinopathy. Diabetes Its Medical and Cultural History. 1989:363–9.
32. Noyes HD. Retinitis in Glycosuria. Trans Am Ophthalmol Soc. 1868; 1(4–5):71–5.
33. Digre, KB. Practical Viewing of the Optic Disc. Burlington, MA: Elsvire Science; 2003.
34. Fleming NBB. Case of Retinitis Proliferans without History. Proc R Soc Med. 1928 Sep; 21(11): 1805–6. [PubMed: 19986647]
35. Tattersall. Diabetes: The Biography. Oxford University Press; 2009.
36. Ballantyne AJ. THE OCULAR MANIFESTATIONS OF SPONTANEOUS SUBARACHNOID HAEMORRHAGE. Br J Ophthalmol. 1943 Sep; 27(9):383–414. [PubMed: 18169953]
37. Minagar A, Alexander JS. Blood-brain barrier disruption in multiple sclerosis. Mult Scler. 2003 Dec; 9(6):540–9. [PubMed: 14664465]
38. Trick GL, Berkowitz BA. Retinal oxygenation response and retinopathy. Progress in retinal and eye research. 2005 Mar; 24(2):259–74. [PubMed: 15610976]
39. Purves D, Augustine GJ, Fitzpatrick D, Katz LC, LaMantia A-S, McNamara JO, et al. The Retina. 2001; 2001
40. Saidha S, Syc SB, Ibrahim MA, Eckstein C, Warner CV, Farrell SK, et al. Primary retinal pathology in multiple sclerosis as detected by optical coherence tomography. Brain. 2011 Feb; 134(Pt 2):518–33. [PubMed: 21252110]
41. Kevil CG, Oshima T, Alexander B, Coe LL, Alexander JS. H(2)O(2)-mediated permeability: role of MAPK and occludin. Am J Physiol Cell Physiol. 2000 Jul; 279(1):C21–30. [PubMed: 10898713]
42. Blaschuk OW, Oshima T, Gour BJ, Symonds JM, Park JH, Kevil CG, et al. Identification of an occludin cell adhesion recognition sequence. Inflammation. 2002 Aug; 26(4):193–8. [PubMed: 12184633]
43. Dejana E, Orsenigo F, Lampugnani MG. The role of adherens junctions and VE-cadherin in the control of vascular permeability. J Cell Sci. 2008 Jul; 121(Pt 13):2115–22. [PubMed: 18565824]
44. Dejana E. Endothelial cell-cell junctions: happy together. Nat Rev Mol Cell Biol. 2004 Apr; 5(4): 261–70. [PubMed: 15071551]
45. Tzima E, Irani-Tehrani M, Kiosses WB, Dejana E, Schultz DA, Engelhardt B, et al. A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. Nature. 2005; 437(7057):426–31. [PubMed: 16163360]
46. Dejana E, Bazzoni G, Lampugnani MG. Vascular endothelial (VE)-cadherin: only an intercellular glue? Exp Cell Res. 1999 Oct 10; 252(1):13–9. [PubMed: 10502395]
47. Ilan N, Mahooti S, Rimm DL, Madri JA. PECAM-1 (CD31) functions as a reservoir for and a modulator of tyrosine-phosphorylated beta-catenin. J Cell Sci. 1999 Sep; 112(Pt 18):3005–14. [PubMed: 10462517]
48. Ilan N, Mohsenin A, Cheung L, Madri JA. PECAM-1 shedding during apoptosis generates a membrane-anchored truncated molecule with unique signaling characteristics. Faseb j. 2001 Feb; 15(2):362–72. [PubMed: 11156952]
49. Newman PJ, Newman DK. Signal transduction pathways mediated by PECAM-1: new roles for an old molecule in platelet and vascular cell biology. Arterioscler Thromb Vasc Biol United States. 2003:953–64.
50. Cai J, Boulton M. The pathogenesis of diabetic retinopathy: old concepts and new questions. Eye (Lond). 2002 May; 16(3):242–60. [PubMed: 12032713]
51. Bergua A, Kapsreiter M, Neuhuber WL, Reitsamer HA, Schrod F. Innervation pattern of the preocular human central retinal artery. Exp Eye Res. 2013 May; 110:142–7. [PubMed: 23220730]
52. Lorenzi M. The polyol pathway as a mechanism for diabetic retinopathy: attractive, elusive, and resilient. Exp Diabetes Res. 2007; 2007:61038. [PubMed: 18224243]

53. Safi SZ, Qvist R, Kumar S, Batumalaie K, Ismail IS. Molecular Mechanisms of Diabetic Retinopathy, General Preventive Strategies, and Novel Therapeutic Targets. *Biomed Res Int.* 2014; 2014:801269. [PubMed: 25105142]
54. Gabbay KH. The sorbitol pathway and the complications of diabetes. *N Engl J Med.* 1973 Apr 19; 288(16):831–6. [PubMed: 4266466]
55. Kitada M, Zhang Z, Mima A, King GL. Molecular mechanisms of diabetic vascular complications. *J Diabetes Investig.* 2010 Jun 1; 1(3):77–89.
56. Tarr JM, Kaul K, Chopra M, Kohner EM, Chibber R. Pathophysiology of diabetic retinopathy. *ISRN Ophthalmol.* 2013; 2013:343560. [PubMed: 24563789]
57. Hoffmann EK, Lambert IH, Pedersen SF. Physiology of Cell Volume Regulation in Vertebrates. 2009 Jan 01.2009
58. Burg MB, Ferraris JD, Dmitrieva NI. Cellular response to hyperosmotic stresses. *Physiol Rev.* 2007 Oct; 87(4):1441–74. [PubMed: 17928589]
59. Willermain F, Janssens S, Arsenijevic T, et al. Department of Ophthalmology CS-PaB, Brussels, Belgium, Laboratory of Pathophysiological and Nutritional Biochemistry ULdB, Brussels, Belgium. Osmotic stress decreases aquaporin-4 expression in the human retinal pigment epithelial cell line, ARPE-19. *International Journal of Molecular Medicine.* 2014; 34(2):533–8. [PubMed: 24888368]
60. Omori K, Fujiseki Y, Suzukawa J, Inagaki C. Regulation of the expression of lysyl oxidase mRNA in cultured rabbit retinal pigment epithelium cells. *Matrix Biol.* 2002 Jun; 21(4):337–48. [PubMed: 12128071]
61. Obrosova IG. Increased sorbitol pathway activity generates oxidative stress in tissue sites for diabetic complications. *Antioxid Redox Signal.* 2005 Nov-Dec;7(11–12):1543–52. [PubMed: 16356118]
62. Obrosova IG, Kador PF. Aldose reductase/polyol inhibitors for diabetic retinopathy. *Curr Pharm Biotechnol.* 2011 Mar 1; 12(3):373–85. [PubMed: 20939801]
63. Cheng HM, González RG. The effect of high glucose and oxidative stress on lens metabolism, aldose reductase, and senile cataractogenesis. *Metabolism.* 1986 Apr; 35(4 Suppl 1):10–4. [PubMed: 3083198]
64. Suarez G, Rajaram R, Oronsky AL, Gawinowicz MA. Nonenzymatic glycation of bovine serum albumin by fructose (fructation). Comparison with the Maillard reaction initiated by glucose. *J Biol Chem.* 1989 Mar 5; 264(7):3674–9. [PubMed: 2537288]
65. Viores SA, Van Niel E, Swerdloff JL, Campochiaro PA. Electron microscopic immunocytochemical demonstration of blood-retinal barrier breakdown in human diabetics and its association with aldose reductase in retinal vascular endothelium and retinal pigment epithelium. *Histochem J.* 1993 Sep; 25(9):648–63. [PubMed: 8226103]
66. Barnett PA, Gonzalez RG, Chylack LT Jr, Cheng HM. The effect of oxidation on sorbitol pathway kinetics. *Diabetes.* 1986 Apr; 35(4):426–32. [PubMed: 3956880]
67. Williamson JR, Chang K, Frangos M, Hasan KS, Ido Y, Kawamura T, et al. Hyperglycemic pseudohypoxia and diabetic complications. *Diabetes.* 1993 Jun; 42(6):801–13. [PubMed: 8495803]
68. Gomes AP, Price NL, Ling AJ, Moslehi JJ, Montgomery MK, Rajman L, et al. Declining NAD(+) induces a pseudohypoxic state disrupting nuclear-mitochondrial communication during aging. *Cell.* 2013 Dec 19; 155(7):1624–38. [PubMed: 24360282]
69. Singh VP, Bali A, Singh N, Jaggi AS. Advanced glycation end products and diabetic complications. *Korean J Physiol Pharmacol.* 2014 Feb; 18(1):1–14. [PubMed: 24634591]
70. Kandarakis SA, Piperi C, Topouzis F, Papavassiliou AG. Emerging role of advanced glycation-end products (AGEs) in the pathobiology of eye diseases. *Prog Retin Eye Res.* 2014 Sep;42:85–102. [PubMed: 24905859]
71. Alexiou P, Pegklidou K, Chatzopoulou M, Nicolaou I, Demopoulos VJ. Aldose reductase enzyme and its implication to major health problems of the 21(st) century. *Curr Med Chem.* 2009; 16(6): 734–52. [PubMed: 19199934]

72. Kumar H, Shah A, Sobhia ME. Novel insights into the structural requirements for the design of selective and specific aldose reductase inhibitors. *J Mol Model*. 2012 May; 18(5):1791–9. [PubMed: 21833829]
73. Wermuth B, Burgisser H, Bohren K, von Wartburg JP. Purification and characterization of human-brain aldose reductase. *Eur J Biochem*. 1982 Oct; 127(2):279–84. [PubMed: 6814912]
74. Ris MM, von Wartburg JP. Heterogeneity of NADPH-dependent aldehyde reductase from human and rat brain. *Eur J Biochem*. 1973 Aug 1; 37(1):69–77. [PubMed: 4147090]
75. Schmidt AM, Hori O, Brett J, Yan SD, Wautier JL, Stern D. Cellular receptors for advanced glycation end products. Implications for induction of oxidant stress and cellular dysfunction in the pathogenesis of vascular lesions. *Arterioscler Thromb*. 1994 Oct; 14(10):1521–8. [PubMed: 7918300]
76. Kandarakis SA, Piperi C, Topouzis F, Papavassiliou AG. Emerging role of advanced glycation-end products (AGEs) in the pathobiology of eye diseases. *Prog Retin Eye Res*. 2014 Sep; 42c:85–102.
77. Brownlee M. Advanced protein glycosylation in diabetes and aging. *Annu Rev Med*. 1995; 46:223–34. [PubMed: 7598459]
78. Schmidt AM, Hori O, Brett J, Yan SD, Wautier JL, Stern D. Cellular receptors for advanced glycation end products. Implications for induction of oxidant stress and cellular dysfunction in the pathogenesis of vascular lesions. 1994 Oct 01. 1994
79. Sharma SD, Pandey BN, Mishra KP, Sivakami S. Amadori product and age formation during nonenzymatic glycosylation of bovine serum albumin in vitro. *J Biochem Mol Biol Biophys*. 2002 Aug; 6(4):233–42. [PubMed: 12186738]
80. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes*. 1999 Jan; 48(1):1–9. [PubMed: 9892215]
81. Suzuki D, Miyata T, Saotome N, Horie K, Inagi R, Yasuda Y, et al. Immunohistochemical evidence for an increased oxidative stress and carbonyl modification of proteins in diabetic glomerular lesions. *J Am Soc Nephrol*. 1999 Apr; 10(4):822–32. [PubMed: 10203367]
82. Lo TW, Westwood ME, McLellan AC, Selwood T, Thornalley PJ. Binding and modification of proteins by methylglyoxal under physiological conditions. A kinetic and mechanistic study with N alpha-acetylarginine, N alpha-acetylcysteine, and N alpha-acetyllysine, and bovine serum albumin. *J Biol Chem*. 1994 Dec 23; 269(51):32299–305. [PubMed: 7798230]
83. Frye EB, Degenhardt TP, Thorpe SR, Baynes JW. Role of the Maillard reaction in aging of tissue proteins. Advanced glycation end product-dependent increase in imidazolium cross-links in human lens proteins. *J Biol Chem*. 1998 Jul 24; 273(30):18714–9. [PubMed: 9668043]
84. Ahmed MU, Thorpe SR, Baynes JW. Identification of N epsilon-carboxymethyllysine as a degradation product of fructoselysine in glycated protein. *J Biol Chem*. 1986 Apr 15; 261(11):4889–94. [PubMed: 3082871]
85. Schmidt AM, Vianna M, Gerlach M, Brett J, Ryan J, Kao J, et al. Isolation and characterization of two binding proteins for advanced glycosylation end products from bovine lung which are present on the endothelial cell surface. *J Biol Chem*. 1992 Jul 25; 267(21):14987–97. [PubMed: 1321822]
86. Schmidt AM, Yan SD, Yan SF, Stern DM. The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. *J Clin Invest*. 2001:949–55.
87. Maeda S, Matsui T, Ojima A, Takeuchi M, Yamagishi S. Sulforaphane inhibits advanced glycation end product-induced pericyte damage by reducing expression of receptor for advanced glycation end products. *Nutr Res*. 2014 Sep; 34(9):807–13. [PubMed: 25241332]
88. Choudhuri S, Dutta D, Sen A, Chowdhury IH, Mitra B, Mondal LK, et al. Role of N-epsilon-carboxy methyl lysine, advanced glycation end products and reactive oxygen species for the development of nonproliferative and proliferative retinopathy in type 2 diabetes mellitus. *Mol Vis*. 2013; 19:100–13. [PubMed: 23378723]
89. Lyons TJ, Basu A. Biomarkers in diabetes: hemoglobin A1c, vascular and tissue markers. *Transl Res*. 2012 Apr; 159(4):303–12. [PubMed: 22424433]
90. Sheikpranbabu S, Haribalaganesh R, Gurunathan S. Pigment epithelium-derived factor inhibits advanced glycation end-products-induced cytotoxicity in retinal pericytes. *Diabetes Metab*. 2011 Dec; 37(6):505–11. [PubMed: 21636306]

91. Genuth S, Sun W, Cleary P, Sell DR, Dahms W, Malone J, et al. Glycation and carboxymethyllysine levels in skin collagen predict the risk of future 10-year progression of diabetic retinopathy and nephropathy in the diabetes control and complications trial and epidemiology of diabetes interventions and complications participants with type 1 diabetes. *Diabetes*. 2005 Nov; 54(11):3103–11. [PubMed: 16249432]
92. Koya D, King GL. Protein kinase C activation and the development of diabetic complications. *Diabetes*. 1998 Jun; 47(6):859–66. [PubMed: 9604860]
93. Newton AC. Regulation of the ABC kinases by phosphorylation: protein kinase C as a paradigm. *Biochem J*. 2003 Mar; 370(Pt 2):361–71. [PubMed: 12495431]
94. Nishizuka Y. The molecular heterogeneity of protein kinase C and its implications for cellular regulation. *Nature*. 1988 Aug; 334(6184):661–5. [PubMed: 3045562]
95. Huang Q, Yuan Y. Interaction of PKC and NOS in signal transduction of microvascular hyperpermeability. *Am J Physiol*. 1997 Nov; 273(5 Pt 2):H2442–51. [PubMed: 9374783]
96. Park JY, Takahara N, Gabriele A, Chou E, Naruse K, Suzuma K, et al. Induction of endothelin-1 expression by glucose: an effect of protein kinase C activation. *Diabetes*. 2000 Jul; 49(7):1239–48. [PubMed: 10909984]
97. Williams B, Gallacher B, Patel H, Orme C. Glucose-induced protein kinase C activation regulates vascular permeability factor mRNA expression and peptide production by human vascular smooth muscle cells in vitro. *Diabetes*. 1997 Sep; 46(9):1497–503. [PubMed: 9287052]
98. Morita T, Kurihara H, Maemura K, Yoshizumi M, Nagai R, Yazaki Y. Role of Ca²⁺ and protein kinase C in shear stress-induced actin depolymerization and endothelin 1 gene expression. *Circ Res*. 1994 Oct; 75(4):630–6. [PubMed: 7923609]
99. Aiello LP, Northrup JM, Keyt BA, Takagi H, Iwamoto MA. Hypoxic regulation of vascular endothelial growth factor in retinal cells. *Arch Ophthalmol*. 1995 Dec; 113(12):1538–44. [PubMed: 7487623]
100. Shiba T, Inoguchi T, Sportsman JR, Heath WF, Bursell S, King GL. Correlation of diacylglycerol level and protein kinase C activity in rat retina to retinal circulation. *Am J Physiol*. 1993 Nov; 265(5 Pt 1):E783–93. [PubMed: 8238505]
101. Xia P, Inoguchi T, Kern TS, Engerman RL, Oates PJ, King GL. Characterization of the mechanism for the chronic activation of diacylglycerol-protein kinase C pathway in diabetes and hypergalactosemia. *Diabetes*. 1994 Sep; 43(9):1122–9. [PubMed: 8070612]
102. Sakaguchi M, Murata H, Yamamoto K, Ono T, Sakaguchi Y, Motoyama A, et al. TIRAP, an adaptor protein for TLR2/4, transduces a signal from RAGE phosphorylated upon ligand binding. *PLoS One*. 2011; 6(8):e23132. [PubMed: 21829704]
103. Gerald P, King GL. Activation of protein kinase C isoforms and its impact on diabetic complications. *Circ Res*. 2010 Apr; 106(8):1319–31. [PubMed: 20431074]
104. Kizub IV, Klymenko KI, Soloviev AI. Protein kinase C in enhanced vascular tone in diabetes mellitus. *Int J Cardiol*. 2014 Jun; 174(2):230–42. [PubMed: 24794552]
105. Rask-Madsen C, King GL. Differential regulation of VEGF signaling by PKC- α and PKC- ϵ in endothelial cells. *Arterioscler Thromb Vasc Biol*. 2008 May; 28(5):919–24. [PubMed: 18323518]
106. Ha H, Yu MR, Lee HB. High glucose-induced PKC activation mediates TGF- β 1 and fibronectin synthesis by peritoneal mesothelial cells. *Kidney Int*. 2001 Feb; 59(2):463–70. [PubMed: 11168928]
107. Wu D, Peng F, Zhang B, Ingram AJ, Kelly DJ, Gilbert RE, et al. PKC- β 1 mediates glucose-induced Akt activation and TGF- β 1 upregulation in mesangial cells. *J Am Soc Nephrol*. 2009 Mar; 20(3):554–66. [PubMed: 19211711]
108. Klaassen I, Van Noorden CJ, Schlingemann RO. Molecular basis of the inner blood-retinal barrier and its breakdown in diabetic macular edema and other pathological conditions. *Prog Retin Eye Res*. 2013 May; 34:19–48. [PubMed: 23416119]
109. Titchenell PM, Lin CM, Keil JM, Sundstrom JM, Smith CD, Antonetti DA. Novel atypical PKC inhibitors prevent vascular endothelial growth factor-induced blood-retinal barrier dysfunction. *Biochem J*. 2012 Sep 15; 446(3):455–67. [PubMed: 22721706]

110. Housey GM, O'Brian CA, Johnson MD, Kirschmeier P, Weinstein IB. Isolation of cDNA clones encoding protein kinase C: evidence for a protein kinase C-related gene family. *Proc Natl Acad Sci U S A*. 1987 Feb; 84(4):1065–9. [PubMed: 3469647]
111. Steinberg SF. Structural basis of protein kinase C isoform function. *Physiol Rev*. 2008 Oct; 88(4): 1341–78. [PubMed: 18923184]
112. Corbalán-García S, Gómez-Fernández JC. Protein kinase C regulatory domains: the art of decoding many different signals in membranes. *Biochim Biophys Acta*. 2006 Jul; 1761(7):633–54. [PubMed: 16809062]
113. Mukherjee A, Roy S, Saha B, Mukherjee D. Spatio-Temporal Regulation of PKC Isoforms Imparts Signaling Specificity. *Front Immunol*. 2016; 7:45. [PubMed: 26925059]
114. Inoguchi T, Battan R, Handler E, Sportsman JR, Heath W, King GL. Preferential elevation of protein kinase C isoform beta II and diacylglycerol levels in the aorta and heart of diabetic rats: differential reversibility to glycemic control by islet cell transplantation. *Proc Natl Acad Sci U S A*. 1992 Nov; 89(22):11059–63. [PubMed: 1438315]
115. Song HB, Jun HO, Kim JH, Yu YS, Kim KW. Suppression of protein kinase C- ζ attenuates vascular leakage via prevention of tight junction protein decrease in diabetic retinopathy. *Biochem Biophys Res Commun*. 2014 Jan; 444(1):63–8. [PubMed: 24434146]
116. Aiello LP, Bursell SE, Clermont A, Duh E, Ishii H, Takagi C, et al. Vascular endothelial growth factor-induced retinal permeability is mediated by protein kinase C in vivo and suppressed by an orally effective beta-isoform-selective inhibitor. *Diabetes*. 1997 Sep; 46(9):1473–80. [PubMed: 9287049]
117. Du Y, Sarthy VP, Kern TS. Interaction between NO and COX pathways in retinal cells exposed to elevated glucose and retina of diabetic rats. *Am J Physiol Regul Integr Comp Physiol*. 2004 Oct; 287(4):R735–41. [PubMed: 15371279]
118. Leal EC, Manivannan A, Hosoya K, Terasaki T, Cunha-Vaz J, Ambrósio AF, et al. Inducible nitric oxide synthase isoform is a key mediator of leukostasis and blood-retinal barrier breakdown in diabetic retinopathy. *Invest Ophthalmol Vis Sci*. 2007 Nov; 48(11):5257–65. [PubMed: 17962481]
119. POWELL ED, FIELD RA. DIABETIC RETINOPATHY AND RHEUMATOID ARTHRITIS. *Lancet*. 1964 Jul; 2(7349):17–8. [PubMed: 14149196]
120. Bartels CM, Wong JC, Johnson SL, Thorpe CT, Barney NP, Sheibani N, et al. Rheumatoid arthritis and the prevalence of diabetic retinopathy. *Rheumatology (Oxford)*. 2015 Aug; 54(8): 1415–9. [PubMed: 25731768]
121. Tikhonenko M, Lydic TA, Wang Y, Chen W, Opreanu M, Sochacki A, et al. Remodeling of retinal Fatty acids in an animal model of diabetes: a decrease in long-chain polyunsaturated fatty acids is associated with a decrease in fatty acid elongases Elovl2 and Elovl4. *Diabetes*. 2010 Jan; 59(1): 219–27. [PubMed: 19875612]
122. Decsi T, Szabó E, Burus I, Marosvölgyi T, Kozári A, Erhardt E, et al. Low contribution of n-3 polyunsaturated fatty acids to plasma and erythrocyte membrane lipids in diabetic young adults. *Prostaglandins Leukot Essent Fatty Acids*. 2007 Mar; 76(3):159–64. [PubMed: 17321122]
123. Capozzi ME, McCollum GW, Cousins DB, Penn JS. Linoleic Acid is a Diabetes-relevant Stimulator of Retinal Inflammation in Human Retinal Muller Cells and Microvascular Endothelial Cells. *J Diabetes Metab*. 2016 Dec; 7(12)
124. Moore TC, Moore JE, Kaji Y, Frizzell N, Usui T, Poulaki V, et al. The role of advanced glycation end products in retinal microvascular leukostasis. *Invest Ophthalmol Vis Sci*. 2003 Oct; 44(10): 4457–64. [PubMed: 14507893]
125. Inagaki Y, Yamagishi S, Okamoto T, Takeuchi M, Amano S. Pigment epithelium-derived factor prevents advanced glycation end products-induced monocyte chemoattractant protein-1 production in microvascular endothelial cells by suppressing intracellular reactive oxygen species generation. *Diabetologia*. 2003 Feb; 46(2):284–7. [PubMed: 12627328]
126. Yoshida S, Yoshida A, Ishibashi T. Induction of IL-8, MCP-1, and bFGF by TNF-alpha in retinal glial cells: implications for retinal neovascularization during post-ischemic inflammation. *Graefes Arch Clin Exp Ophthalmol*. 2004 May; 42(5):409–13. [PubMed: 15029502]

127. Arar NH, Freedman BI, Adler SG, Iyengar SK, Chew EY, Davis MD, et al. Heritability of the severity of diabetic retinopathy: the FIND-Eye study. *Invest Ophthalmol Vis Sci*. 2008 Sep; 49(9):3839–45. [PubMed: 18765632]
128. Looker HC, Nelson RG, Chew E, Klein R, Klein BE, Knowler WC, et al. Genome-wide linkage analyses to identify Loci for diabetic retinopathy. *Diabetes*. 2007 Apr; 56(4):1160–6. [PubMed: 17395753]
129. Hallman DM, Boerwinkle E, Gonzalez VH, Klein BE, Klein R, Hanis CL. A genome-wide linkage scan for diabetic retinopathy susceptibility genes in Mexican Americans with type 2 diabetes from Starr County, Texas. *Diabetes*. 2007 Apr; 56(4):1167–73. [PubMed: 17251272]
130. Donaghue KC, Margan SH, Chan AK, Holloway B, Silink M, Rangel T, et al. The association of aldose reductase gene (AKR1B1) polymorphisms with diabetic neuropathy in adolescents. *Diabet Med*. 2005 Oct; 22(10):1315–20. [PubMed: 16176189]
131. Abhary S, Burdon KP, Laurie KJ, Thorpe S, Landers J, Goold L, et al. Aldose reductase gene polymorphisms and diabetic retinopathy susceptibility. *Diabetes Care*. 2010 Aug; 33(8):1834–6. [PubMed: 20424224]
132. Syreeni A, El-Osta A, Forsblom C, Sandholm N, Parkkonen M, Tarnow L, et al. Genetic examination of SETD7 and SUV39H1/H2 methyltransferases and the risk of diabetes complications in patients with type 1 diabetes. *Diabetes*. 2011 Nov; 60(11):3073–80. [PubMed: 21896933]
133. Mishra M, Zhong Q, Kowluru RA. Epigenetic modifications of Nrf2-mediated glutamate-cysteine ligase: implications for the development of diabetic retinopathy and the metabolic memory phenomenon associated with its continued progression. *Free Radic Biol Med*. 2014 Oct; 75:129–39. [PubMed: 25016074]
134. Zhong Q, Kowluru RA. Epigenetic modification of Sod2 in the development of diabetic retinopathy and in the metabolic memory: role of histone methylation. *Invest Ophthalmol Vis Sci*. 2013 Jan; 54(1):244–50. [PubMed: 23221071]
135. Silva VA, Polesskaya A, Sousa TA, Corrêa VM, André ND, Reis RI, et al. Expression and cellular localization of microRNA-29b and RAX, an activator of the RNA-dependent protein kinase (PKR), in the retina of streptozotocin-induced diabetic rats. *Mol Vis*. 2011; 17:2228–40. [PubMed: 21897745]
136. Kowluru RA, Koppolu P, Chakrabarti S, Chen S. Diabetes-induced activation of nuclear transcriptional factor in the retina, and its inhibition by antioxidants. *Free Radic Res*. 2003 Nov; 37(11):1169–80. [PubMed: 14703729]
137. Betteridge DJ. What is oxidative stress? *Metabolism*. 2000 Feb; 49(2 Suppl 1):3–8.
138. Mugge A. The role of reactive oxygen species in atherosclerosis. *Z Kardiol*. 1998 Nov; 87(11):851–64. [PubMed: 9885178]
139. Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circ Res*. 2010 Oct 29; 107(9):1058–70. [PubMed: 21030723]
140. Hulsmans M, Van Dooren E, Holvoet P. Mitochondrial reactive oxygen species and risk of atherosclerosis. *Curr Atheroscler Rep*. 2012 Jun; 14(3):264–76. [PubMed: 22350585]
141. Dizdaroglu M, Jaruga P, Birincioglu M, Rodriguez H. Free radical-induced damage to DNA: mechanisms and measurement. *Free Radic Biol Med*. 2002 Jun; 32(11):1102–15. [PubMed: 12031895]
142. Salvi A, Carrupt P, Tillement J, Testa B. Structural damage to proteins caused by free radicals: assessment, protection by antioxidants, and influence of protein binding. *Biochem Pharmacol*. 2001 May; 61(10):1237–42. [PubMed: 11322927]
143. Du X, Matsumura T, Edelstein D, Rossetti L, Zsengellér Z, Szabó C, et al. Inhibition of GAPDH activity by poly(ADP-ribose) polymerase activates three major pathways of hyperglycemic damage in endothelial cells. *J Clin Invest*. 2003 Oct; 112(7):1049–57. [PubMed: 14523042]
144. Zheng L, Szabó C, Kern TS. Poly(ADP-ribose) polymerase is involved in the development of diabetic retinopathy via regulation of nuclear factor-kappaB. *Diabetes*. 2004 Nov; 53(11):2960–7. [PubMed: 15504977]
145. Du Y, Miller CM, Kern TS. Hyperglycemia increases mitochondrial superoxide in retina and retinal cells. *Free Radic Biol Med*. 2003 Dec; 35(11):1491–9. [PubMed: 14642397]

146. Du Y, Veenstra A, Palczewski K, Kern TS. Photoreceptor cells are major contributors to diabetes-induced oxidative stress and local inflammation in the retina. *Proc Natl Acad Sci U S A*. 2013 Oct; 110(41):16586–91. [PubMed: 24067647]
147. Eshaq RS, Wright WS, Harris NR. Oxygen delivery, consumption, and conversion to reactive oxygen species in experimental models of diabetic retinopathy. *Redox Biol*. 2014:661–6. [PubMed: 24936440]
148. Singh LP. Thioredoxin Interacting Protein (TXNIP) and Pathogenesis of Diabetic Retinopathy. *J Clin Exp Ophthalmol*. 2013 Aug;5:4.
149. Sasaki M, Ozawa Y, Kurihara T, Kubota S, Yuki K, Noda K, et al. Neurodegenerative influence of oxidative stress in the retina of a murine model of diabetes. *Diabetologia*. 2010 May; 53(5):971–9. [PubMed: 20162412]
150. Ozawa Y, Kurihara T, Sasaki M, Ban N, Yuki K, Kubota S, et al. Neural degeneration in the retina of the streptozotocin-induced type 1 diabetes model. *Exp Diabetes Res*. 2011; 2011:108328. [PubMed: 22144984]
151. Motta G, Rojkaer R, Hasan AA, Cines DB, Schmaier AH. High molecular weight kininogen regulates prekallikrein assembly and activation on endothelial cells: a novel mechanism for contact activation. *Blood*. 1998 Jan 15; 91(2):516–28. [PubMed: 9427705]
152. Ma JX, Song Q, Hatcher HC, Crouch RK, Chao L, Chao J. Expression and cellular localization of the kallikrein-kinin system in human ocular tissues. *Exp Eye Res*. 1996 Jul; 63(1):19–26. [PubMed: 8983960]
153. Pinna A, Emanuelli C, Dore S, Salvo M, Madeddu P, Carta F. Levels of human tissue kallikrein in the vitreous fluid of patients with severe proliferative diabetic retinopathy. *Ophthalmologica*. 2004 Jul-Aug;218(4):260–3. [PubMed: 15258415]
154. Webb JG. The kallikrein/kinin system in ocular function. *J Ocul Pharmacol Ther*. 2011 Dec; 27(6):539–43. [PubMed: 22136090]
155. Phipps JA, Feener EP. The kallikrein-kinin system in diabetic retinopathy: lessons for the kidney. *Kidney Int*. 2008 May; 73(10):1114–9. [PubMed: 18272958]
156. Phipps JA, Jobling AI, Greferath U, Fletcher EL, Vessey KA. Alternative pathways in the development of diabetic retinopathy: the renin-angiotensin and kallikrein-kinin systems. *Clin Exp Optom*. 2012 May; 95(3):282–9. [PubMed: 22594546]
157. Liu J, Feener EP. Plasma kallikrein-kinin system and diabetic retinopathy. *Biol Chem*. 2013 Mar; 394(3):319–28. [PubMed: 23362193]
158. Bhat M, Pouliot M, Couture R, Vaucher E. The kallikrein-kinin system in diabetic retinopathy. *Prog Drug Res*. 2014; 69:111–43. [PubMed: 25130041]
159. Abdouh M, Talbot S, Couture R, Hassessian HM. Retinal plasma extravasation in streptozotocin-diabetic rats mediated by kinin B(1) and B(2) receptors. *Br J Pharmacol*. 2008 May; 154(1):136–43. [PubMed: 18311190]
160. Abdouh M, Khanjari A, et al. Guy-Bernier Research Centre MRH, Montréal, PQ Canada H1T 2M4, Ophthalmology Do, Guy-Bernier Research Centre MRH, Montréal, PQ Canada H1T 2M4, Sciences B. Early upregulation of kinin B1 receptors in retinal microvessels of the streptozotocin-diabetic rat. *British Journal of Pharmacology*. 2003; 140(1):33–40. [PubMed: 12967932]
161. Pruneau D, Belichard P, Sahel JA, Combal JP. Targeting the kallikrein-kinin system as a new therapeutic approach to diabetic retinopathy. *Curr Opin Investig Drugs*. 2010 May; 11(5):507–14.
162. Hookes L. Association for Research in Vision and Ophthalmology (ARVO)--2010 Annual Meeting. For Sight: The Future of Eye and Vision Research--part 1. *IDrugs*. 2010 Jul; 13(7):427–9. [PubMed: 20582862]
163. Catanzaro O, Labal E, Andornino A, Capponi JA, Di Martino I, Sirois P. Blockade of early and late retinal biochemical alterations associated with diabetes development by the selective bradykinin B1 receptor antagonist R-954. *Peptides*. 2012 Apr; 34(2):349–52. [PubMed: 22369720]
164. Kato N, Hou Y, Lu Z, Lu C, Nagano H, Suzuma K, et al. Kallidinogenase normalizes retinal vasopermeability in streptozotocin-induced diabetic rats: potential roles of vascular endothelial

- growth factor and nitric oxide. *Eur J Pharmacol.* 2009 Mar 15; 606(1–3):187–90. [PubMed: 19374851]
165. Nakamura S, Morimoto N, Tsuruma K, Izuta H, Yasuda Y, Kato N, et al. Tissue kallikrein inhibits retinal neovascularization via the cleavage of vascular endothelial growth factor-165. *Arterioscler Thromb Vasc Biol.* 2011 May; 31(5):1041–8. [PubMed: 21293011]
 166. Gao B-B, Clermont A, Rook S, Fonda SJ, Srinivasan VJ, Wojtkowski M, et al. Extracellular carbonic anhydrase mediates hemorrhagic retinal and cerebral vascular permeability through prekallikrein activation. *Nature Medicine* 2007. 2007 Jan 28; 13(2):181–8.
 167. Marti HH. Vascular Endothelial Growth Factor. 2000; 2000
 168. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med.* 2003 Jun; 9(6):669–76. [PubMed: 12778165]
 169. Dehne N, Kerkweg U, Flohe SB, Brune B, Fandrey J. Activation of hypoxia-inducible factor 1 in skeletal muscle cells after exposure to damaged muscle cell debris. *Shock.* 2011 Jun; 35(6):632–8. [PubMed: 21283061]
 170. Dery MA, Michaud MD, Richard DE. Hypoxia-inducible factor 1: regulation by hypoxic and non-hypoxic activators. *Int J Biochem Cell Biol.* 2005 Mar; 37(3):535–40. [PubMed: 15618010]
 171. Rudnicki M, Faine LA, Dehne N, Namgaladze D, Ferderbar S, Weinlich R, et al. Hypoxia inducible factor-dependent regulation of angiogenesis by nitro-fatty acids. *Arterioscler Thromb Vasc Biol.* 2011 Jun; 31(6):1360–7. [PubMed: 21454809]
 172. Semenza GL. HIF-1: mediator of physiological and pathophysiological responses to hypoxia. *J Appl Physiol* (1985). 2000 Apr; 88(4):1474–80. [PubMed: 10749844]
 173. Lee SW, Kim WJ, Choi YK, Song HS, Son MJ, Gelman IH, et al. SSeCKS regulates angiogenesis and tight junction formation in blood-brain barrier. *Nat Med.* 2003 Jul; 9(7):900–6. [PubMed: 12808449]
 174. Weidemann A, Krohne TU, Aguilar E, Kurihara T, Takeda N, Dorrell MI, et al. Astrocyte hypoxic response is essential for pathological but not developmental angiogenesis of the retina. *Glia.* 2010 Aug; 58(10):1177–85. [PubMed: 20544853]
 175. Wang C, Poon S, Murali S, Koo CY, Bell TJ, Hinkley SF, et al. Engineering a vascular endothelial growth factor 165-binding heparan sulfate for vascular therapy. *Biomaterials.* 2014 Aug; 35(25):6776–86. [PubMed: 24854095]
 176. Frey T, Antonetti DA. Alterations to the blood-retinal barrier in diabetes: cytokines and reactive oxygen species. *Antioxid Redox Signal.* 2011 Sep 1; 15(5):1271–84. [PubMed: 21294655]
 177. Esser S, Lampugnani MG, Corada M, Dejana E, Risau W. Vascular endothelial growth factor induces VE-cadherin tyrosine phosphorylation in endothelial cells. *J Cell Sci.* 1998 Jul; 111(Pt 13):1853–65. [PubMed: 9625748]
 178. Murakami T, Frey T, Lin C, Antonetti DA. Protein kinase c β phosphorylates occludin regulating tight junction trafficking in vascular endothelial growth factor-induced permeability in vivo. *Diabetes.* 2012 Jun; 61(6):1573–83. [PubMed: 22438576]
 179. Maharjan S, Lee S, Agrawal V, Choi HJ, Maeng YS, Kim K, et al. Sac-0601 prevents retinal vascular leakage in a mouse model of diabetic retinopathy. *Eur J Pharmacol.* 2011 Apr; 657(1–3):35–40. [PubMed: 21296064]
 180. Aveleira CA, Lin CM, Abcouwer SF, Ambrosio AF, Antonetti DA. TNF- α signals through PKC ζ /NF- κ B to alter the tight junction complex and increase retinal endothelial cell permeability. *Diabetes.* 2010 Nov; 59(11):2872–82. [PubMed: 20693346]
 181. Huang H, Gandhi JK, Zhong X, Wei Y, Gong J, Duh EJ, et al. TNF α is required for late BRB breakdown in diabetic retinopathy, and its inhibition prevents leukostasis and protects vessels and neurons from apoptosis. *Invest Ophthalmol Vis Sci.* 2011 Mar; 52(3):1336–44. [PubMed: 21212173]
 182. Krady JK, Basu A, Allen CM, Xu Y, LaNoue KF, Gardner TW, et al. Minocycline reduces proinflammatory cytokine expression, microglial activation, and caspase-3 activation in a rodent model of diabetic retinopathy. *Diabetes.* 2005 May; 54(5):1559–65. [PubMed: 15855346]
 183. Bamforth SD, Lightman SL, Greenwood J. Interleukin-1 β -induced disruption of the retinal vascular barrier of the central nervous system is mediated through leukocyte recruitment and histamine. *Am J Pathol.* 1997 Jan; 150(1):329–40. [PubMed: 9006348]

184. Ildefonso CJ, Jaime H, Rahman MM, Li Q, Boye SE, Hauswirth WW, et al. GENE DELIVERY OF A VIRAL ANTI-INFLAMMATORY PROTEIN TO COMBAT OCULAR INFLAMMATION. *Hum Gene Ther*. 2014 Nov 24.
185. Rangasamy S, McGuire PG, Franco Nitta C, Monickaraj F, Oruganti SR, Das A. Chemokine mediated monocyte trafficking into the retina: role of inflammation in alteration of the blood-retinal barrier in diabetic retinopathy. *PLoS One*. 2014; 9(10):e108508. [PubMed: 25329075]
186. Capeans C, De Rojas MV, Lojo S, Salorio MS. C-C chemokines in the vitreous of patients with proliferative vitreoretinopathy and proliferative diabetic retinopathy. *Retina*. 1998; 18(6):546–50. [PubMed: 9869464]
187. Banerjee S, Savant V, Scott RA, Curnow SJ, Wallace GR, Murray PI. Multiplex bead analysis of vitreous humor of patients with vitreoretinal disorders. *Invest Ophthalmol Vis Sci*. 2007 May; 48(5):2203–7. [PubMed: 17460280]
188. Beltramo E, Porta M. Pericyte loss in diabetic retinopathy: mechanisms and consequences. *Curr Med Chem*. 2013; 20(26):3218–25. [PubMed: 23745544]
189. Hammes HP. Pericytes and the pathogenesis of diabetic retinopathy. *Horm Metab Res*. 2005 Apr; 37(Suppl 1):39–43. [PubMed: 15918109]
190. Kolar P. Pathophysiology of diabetic retinopathy. *Vnitr Lek*. 2013 Mar; 59(3):173–6. [PubMed: 23713183]
191. Hu YB, Zhang JK, Sun ZY, Yuan ZG, Liu YM, Mao CJ, et al. Retinal cell apoptosis, microvascular changes and expression of connective tissue growth factor in experimental diabetic rats. *Zhonghua Yan Ke Za Zhi*. 2011 Jun; 47(6):521–6. [PubMed: 21914267]
192. Feenstra DJ, Yego EC, Mohr S. Modes of Retinal Cell Death in Diabetic Retinopathy. *J Clin Exp Ophthalmol*. 2013 Oct 1; 4(5):298. [PubMed: 24672740]
193. Hammes HP, Lin J, Wagner P, Feng Y, Vom Hagen F, Krzizok T, et al. Angiopoietin-2 causes pericyte dropout in the normal retina: evidence for involvement in diabetic retinopathy. *Diabetes*. 2004 Apr; 53(4):1104–10. [PubMed: 15047628]
194. Cogan DG, Toussaint D, Kuwabara T. Retinal vascular patterns. IV. Diabetic retinopathy. *Arch Ophthalmol*. 1961 Sep; 66:366–78. [PubMed: 13694291]
195. Van Geest RJ, Leeuwis JW, Dendooven A, Pfister F, Bosch K, Hoebe KA, et al. Connective tissue growth factor is involved in structural retinal vascular changes in long-term experimental diabetes. *J Histochem Cytochem*. 2014 Feb; 62(2):109–18. [PubMed: 24217924]
196. Nieuwdorp M, Haeften TWv, Gouverneur MCLG, Mooij HL, Lieshout MHPv, Levi M, et al. Loss of Endothelial Glycocalyx During Acute Hyperglycemia Coincides With Endothelial Dysfunction and Coagulation Activation In Vivo. 2006 Feb 01. 2006
197. Lipowsky HH, Gao L, Lescanic A. Shedding of the endothelial glycocalyx in arterioles, capillaries, and venules and its effect on capillary hemodynamics during inflammation. *Am J Physiol Heart Circ Physiol*. 2011 Dec; 301(6):H2235–45. [PubMed: 21926341]
198. Wisniewska-Kruk J, Klaassen I, Vogels IM, Magno AL, Lai CM, Van Noorden CJ, et al. Molecular analysis of blood-retinal barrier loss in the Akimba mouse, a model of advanced diabetic retinopathy. *Exp Eye Res*. 2014 May; 122:123–31. [PubMed: 24703908]
199. Broekhuizen LN, Lemkes BA, Mooij HL, Meuwese MC, Verberne H, Holleman F, et al. Effect of sulodexide on endothelial glycocalyx and vascular permeability in patients with type 2 diabetes mellitus. *Diabetologia*. 2010 Dec; 53(12):2646–55. [PubMed: 20865240]
200. Kumase F, Morizane Y, Mohri S, Takasu I, Ohtsuka A, Ohtsuki H. Glycocalyx degradation in retinal and choroidal capillary endothelium in rats with diabetes and hypertension. *Acta Med Okayama*. 2010 Oct; 64(5):277–83. [PubMed: 20975760]
201. Lopez-Quintero SV, Cancel LM, Pierides A, Antonetti D, Spray DC, Tarbell JM. High glucose attenuates shear-induced changes in endothelial hydraulic conductivity by degrading the glycocalyx. *PLoS One*. 2013; 8(11):e78954. [PubMed: 24260138]
202. Bhattacharjee PS, Huq TS, Potter V, Young A, Davenport IR, Graves R, et al. High-glucose-induced endothelial cell injury is inhibited by a Peptide derived from human apolipoprotein E. *PLoS One*. 2012; 7(12):e52152. [PubMed: 23284911]
203. Tarbell JM. Shear stress and the endothelial transport barrier. *Cardiovasc Res*. 2010 Jul 15; 87(2):320–30. [PubMed: 20543206]

204. Liu CP, Yeh JL, Wu BN, Chai CY, Chen IJ, Lai WT. KMUP-3 attenuates ventricular remodelling after myocardial infarction through eNOS enhancement and restoration of MMP-9/TIMP-1 balance. *Br J Pharmacol*. 2011 Jan; 162(1):126–35. [PubMed: 20840538]
205. Privratsky JR, Newman DK, Newman PJ. PECAM-1: conflicts of interest in inflammation. *Life Sci*. 2010 Jul 17; 87(3–4):69–82. [PubMed: 20541560]
206. Cheung AKH, Fung MKL, Lo ACY, Lam TTL, So KF, Chung SSM, et al. Aldose Reductase Deficiency Prevents Diabetes-Induced Blood-Retinal Barrier Breakdown, Apoptosis, and Glial Reactivation in the Retina of db/db Mice. 2005 Nov 01.2005
207. Cao Y, Feng B, Chen S, Chu Y, Chakrabarti S. Mechanisms of endothelial to mesenchymal transition in the retina in diabetes. *Invest Ophthalmol Vis Sci*. 2014; 55(11):7321–31. [PubMed: 25335984]

Figure 1.

Diabetic retinopathy (DR) can be divided into two main stages: non-proliferative DR (NPDR), and proliferative DR (PDR), with NPDR further subdivided into mild NPDR, moderate NPDR, and severe NPDR.

Figure 2.

Diabetic retinopathy is marked by microvascular dysfunction, including abnormal neovascularization, hemorrhages, microaneurysms, and cotton wool spots, that will eventually, if not treated, lead to vision loss.

Figure 3.

A summary of the factors, not necessarily independent of each other, leading to the development of diabetic retinopathy.

Figure 4. Diabetes-induced retinal microvascular damage, including blood retinal barrier breakdown, can be induced by the factors summarized in this figure.

Figure 5. Normally, as shown in panel A, the retinal endothelium has an intact glycocalyx and tight junctions. However, in diabetic retinopathy, the endothelium has increased permeability and possibly loses a portion of the glycocalyx (Panel B).