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**Cellular targets in diabetic retinopathy therapy**

Rodríguez ML *et al*. Cellular targets in DR

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**Abstract**

Despite the existence of treatment for diabetes, inadequate metabolic control triggers the appearance of chronic complications such as diabetic retinopathy. Diabetic retinopathy is considered a multifactorial disease of complex etiology in which oxidative stress and low chronic inflammation play essential roles. Chronic exposure to hyperglycemia triggers a loss of redox balance that is critical for the appearance of neuronal and vascular damage during the development and progression of the disease. Current therapies for the treatment of diabetic retinopathy are used in advanced stages of the disease and are unable to reverse the retinal damage induced by hyperglycemia. The lack of effective therapies without side effects means there is an urgent need to identify an early action capable of preventing the development of the disease and its pathophysiological consequences in order to avoid loss of vision associated with diabetic retinopathy. Therefore, in this review we propose different therapeutic targets related to the modulation of the redox and inflammatory status that, potentially, can prevent the development and progression of the disease.

**Key Words:** Diabetic retinopathy; Oxidative stress; Inflammation; Cellular target; Diabetic macular edema

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**Core Tip:** The identification of potential therapeutic targets related to oxidative stress and low chronic inflammation induced in diabetic retinopathy (DR) may be crucial in developing therapeutic approaches for preventing the development of DR. Hence, we focus on the antioxidant role of nuclear factor erythroid 2-related factor 2, low and chronic inflammatory conditions developed in DR, modulation of lipid peroxidation, activation of glucagon-like peptide-1 receptor, the classical biochemical pathways altered under hyperglycemia, and epigenetic alterations.

**INTRODUCTION**

Diabetes mellitus is a metabolic disorder associated with hyperglycemia. The global prevalence of diabetes in adults 20-79 years of age, including both type 1 and type 2 diabetes, diagnosed, and undiagnosed, was estimated at 463 million in 2019. Based on the estimation, by 2045 a projected 700 million adults will have diabetes[1]. Although diabetes is a pathology with multiple systemic consequences, the loss of metabolic control in particular is not effectively controlled in many patients and that triggers the development of long-term damage of various organs, including the retina. In fact, diabetic retinopathy (DR) is the greatest cause of preventable blindness in the working age population and the most frequent ocular pathology caused by diabetes[2]. Its prevalence increases as the number of diabetic patients increases, depends on the duration of the disease, and on inadequate glycemic control. It has also been associated with the presence of hypertension, and was estimated to affect 2.6 million people in 2015 and projected to affect 3.2 million adults by 2020[2,3].

Cellular aerobic metabolism induces the physiological production of reactive oxygen species (ROS), which are molecular actors in the regulation of normal cell signaling. The production of ROS is countered by antioxidant enzymatic and nonenzymatic machinery enabling a homeostatic redox balance. However, the balance may be easily altered by a pathological condition. Glucose metabolism linked to reduction in antioxidant defenses triggers an oxidant environment in body tissues exposed to chronic hyperglycemia[4]. Although the blood-retinal barrier (BRB) makes the tissue a privileged place, as the retina is protected from the escape of circulating toxins, its cellular components are extremely sensitive to alterations in oxygen level[5]. In fact, the imbalance in redox homeostasis induced by diabetes triggers neuronal retinal cell death and pericyte cell death followed by an increase in the vascular permeability, and cumulative molecular damage leading to development and progression of DR to advanced stages[2,6-8]. Because of this, oxidative stress is considered a major cause of DR development.

The complex and extensive harmful effects of ROS contribute to the neurovascular complications observed in the retina. In this review, we focus on the main cellular targets affected by oxidative stress. The affects lead to cellular dysfunction and are potential therapeutic targets to avoid the development and progression of DR. Among hyperglycemia abnormalities closely associated with oxidative stress we highlight the key role of the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) and its importance in the modulation of oxidative stress, the increased accumulation of advanced glycation end products (AGEs), polyol and hexosamine pathways and protein kinase C activation, lipid peroxidation, activation of glucagon-like peptide-1 receptor (GLP1R), and alteration of the epigenetic status[2,9].

**THE IMPORTANCE OF LOOKING FOR NEW THERAPEUTIC TARGETS IN DIABETIC RETINOPATHY: ACTUAL THERAPIES**

As DR is most often asymptomatic, the pathology can be significantly advanced when the patients suffer a loss of vision. Therefore, an early diagnosis is necessary to detect the first signs before the disease progresses to more serious stages[10]. In the early stages of DR, with the objective being to prevent its development or stop its progression, the only therapeutic strategy is a strict control of risk factors, mainly blood glucose and blood pressure[11]. Overall, treatment is applicable in very advanced stages of the pathology and when DR affects the macula, triggering diabetic macular edema (DME), which is the most common cause of blindness induced by chronic hyperglycemia. The main interventions for DR and DME include ocular and systemic pharmacotherapy, with conventional laser therapy as the secondary treatment option, although it remains the first-line option when the cost and burden of drug treatment are considered, and vitreoretinal surgery[12,13]. The decision to use one or other of the treatments depends on the specific clinical situation of the patient.

***Pharmacotherapy***

The evidence that inflammation plays a critical role when DR affects the macula, triggering DME, has opened new avenues and targets for developing new treatments. There are many anti-inflammatory therapies, such as intravitreal glucocorticoids, topical nonsteroidal anti-inflammatory drugs (NSAIDs), inflammatory molecule inhibitors, renin-angiotensin system blockers, and natural anti-inflammatory therapies that can reduce the use of anti-neovascularizing agents in the treatment of DR, but more studies are needed[6]. Despite these therapies, the most important class of drugs are those that decrease the effects of vascular endothelial growth factor (VEGF), and corticosteroids[14].

***Anti-VEGF treatment***

Intravitreal injections of anti-VEGF drugs are the treatment par excellence for DR and its angiogenic complications. The monoclonal antibody ranibizumab (Lucentis®), the long-acting antibody bevacizumab (Avastin®), the aptamer pegaptanib (Macugen®), and the recombinant fusion protein aflibercept (Eylea®) are the anti-VEGF agents most frequently used to treat DME. The drugs, do not affect the pathogenesis of DR and must be administered for years as frequent intravitreal injections, estimated to be around 12-15 injections in the first 3 years of treatment[15-17]. They are also associated with adverse effects such as susceptibility to the development of endophthalmitis, vitreous floaters, and transient increase in intraocular pressure[18].

***Administration of corticosteroids***

Acknowledging the role of inflammatory processes in the pathogenesis of DR, anti-inflammatory drugs are an attractive option for the treatment of the disease[19]. Hence, the anti-inflammatory and anti-angiogenic effects associated with corticosteroids have led to their inclusion in the treatment of DR and DME. Several mediators of inflammation are upregulated in DR. The mediators, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and VEGF have a key role in pathogenesis and can be modulated by corticosteroids[20]. The effects of corticosteroids include the reduction of vascular permeability and the breakdown of the BRB, prevention of leukocyte adhesion to vascular walls, suppression of VEGF gene transcription and translation, and the rapid decrease of DME[21].

The main mode of administration is intravitreal injection, which avoids the limitations of BRB. However, treatment-associated adverse effects of steroids include cataracts, high intraocular pressure, and glaucoma. Less frequent side effects, such as vitreous hemorrhage, retinal detachment, and endophthalmitis are related to the injection[22,23]. Moreover, short-term effects and transient efficacy are limiting factors in the application of this treatment, and new injections it is often required at various time intervals based on the steroid half-life. Currently, DME is treated with several different steroids, including fluocinolone, triamcinolone, and dexamethasone[24]. Side effects associated with chronic use and the need for repeat injections have brought about the development of new methods of intraocular administration, such as sustained release from an intravitreal implant. Slow-release formulations are used to avoid reinjection, which allows the use small quantities of corticosteroids, which results in fewer side effects[25]. Both nonbiodegradable and biodegradable devices are available. In biodegradable devices, the polymers degrade slowly over time, thus avoiding the need for surgery to remove the implant, in contrast to the nonbiodegradable ones[26].

***Laser therapy***

Over the past 30 years, the most successful means of delaying the progression of DR has been focal, grid, or panretinal photocoagulation (PRP) laser treatment[27]. In the treatment of proliferative DR, the use of PRP reduces oxygen requirements and decreases retinal neovascularization. PRP eliminates the hypoxic retina and/or increases the diffusion of O2 found in the choroid to supplement the affected retinal circulation. Furthermore, laser therapy decreases the formation of vasoproliferative agents and inhibits neovascularization. The procedure uses scattered laser spots of 200-500 µm in the peripheral retina, avoiding the central macula. In the case of DME, the laser spots are applied in the regions of the macular area with microaneurysms in order to decrease exudation[28].

The use of laser therapy plays an important role in controlling diabetes mellitus-related retinal disease and is generally used in situations in which the use of pharmacotherapy is contraindicated, there is poor monitoring of patient visits, if the response to anti-VEGF treatment is ineffective, or if the patient is pregnant[13]. Although PRP treatment can effectively control neovascularization and prevent blindness, it is unable to restore vision and has its own damaging effects on vision[29]. The destructive capacity of laser therapy permanently damages the cells, thus producing side effects that affect the deterioration of vision, such as loss of contrast sensitivity, decreased night vision, color vision, visual field, and the appearance of DME[30]. In certain situations, the prior use of laser photocoagulation and intravitreal anti-VEGF agents induce fibrotic changes in preexisting retinal neovascularization, causing tractional retinal detachment with the need for early surgery to avoid permanent blindness[31].

***Surgical intervention***

Surgical intervention is used in cases that show no response to pharmacological treatment, laser, or combined therapy, as well as in the most severe cases of DME. Therefore, vitrectomy is indicated in situations such as vitreous hemorrhages that do not disappear, tractional detachment of the retina in proliferative DR, and anomalies in the vitreoretinal interface that prevent the resolution of DME[32]. To facilitate the intervention, an intravitreal injection of an anti-VEGF agent like bevacizumab, ranibizumab, or aflibercept, is included as a preoperative complement in patients with no contraindications, as they cause a rapid involution of active neovascularization[33].

Surgical vitrectomy entailing the removal of most of the vitreous body and hyaloid membrane has shown a series of benefits, such as decreased growth of fibrovascular membranes caused by the absence of proliferation in scaffolds, increased intraocular cytokine turnover, and removal of mechanical barriers that hinder the exit of metabolites and fluids and obstruct intravitreal drug delivery through intraretinal penetration[34]. However, because of individual variability in the surgical anatomy that each case presents, diabetic vitrectomy continues to be one of the most difficult conditions to treat. In addition, it has postoperative consequences such as rhegmatogenous retinal detachment, development of cataracts, proliferation of diabetic fibrovascular membranes, vitreous hemorrhage, appearance of epiretinal membranes, elevated intraocular pressure, and neurovascular glaucoma[35-37].

All these treatments are expensive, uncomfortable for the patient, have limited effectiveness because of the administration protocols, and are associated with a significant number of side effects[38]. Despite benefits in slowing the progression of DR and improving vision, damage to the retinal blood vessels the function of neuronal cells is irreversible[2]. Even after the advances made in the treatment of retinopathy, many patients still progress to advanced stages of disease. It is necessary, therefore, to investigate new therapeutic approaches capable of both delaying and preventing the appearance of the first stages of DR.

**RETINOCELLULAR ALTERATIONS IN DIABETIC RETINOPATHY DEVELOPMENT**

Oxidative stress has been defined as an imbalance between the production and the removal of free radicals, which leads to their accumulation. The most common free radicals are ROS, such as the superoxide anion (O2•−), hydrogen peroxide (H2O2), the peroxyl radical (ROO•), and the hydroxyl radical (•OH). These oxygen-derived molecules are very reactive and generally toxic to cells[39,40]. Under physiological conditions, free radicals are normally and continuously produced. Low to moderate levels of free radicals support normal cellular metabolism, proliferation, differentiation, immune system regulation, and vascular remodeling[2,41]. Intracellular ROS levels are controlled by enzymes including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) and nonenzymatic species like glutathione (GSH), thioredoxin, NADPH, α-tocopherol, ascorbic acid and β-carotene, which constitute an antioxidant defenses system. Oxidative stress leads to the accumulation of ROS because of excessive production or inefficient removal. ROS can modify the structure of proteins, lipids, carbohydrates, and nucleic acids, thus affecting their function[2].

Oxidative stress plays a critical role in the pathogenesis of DR. The retina has high metabolic activity, high oxygen partial pressure from the blood in the choroid, and it is highly exposed to bright light. All these factors, together with the oxidative environment induced by hyperglycemia in diabetes, cause an increased level of ROS in the retina[42-44]. ROS overproduction in the retina triggers cell death, retinal ischemia, retinal neovascularization, and DME[45]. Furthermore, various mutations of detoxifying enzymes that have a significant role in DR development, such as CAT or SOD, have been reported[46]. This suggest that hyperglycemia-induced oxidative stress is one of the main causes of DR[45,47,48]. Therefore, some treatments of DR are based in the inhibition of ROS generation, neutralization of free radicals, or the reinforcement of the antioxidant defense system[39].

***Oxidative stress and Nrf2***

Nrf2 is a transcription factor that activates the expression of various detoxifying and antioxidant defense genes in response to oxidative stress[49,50]. The functional activity of Nrf2 depends on whether it is located in the nucleus or in the cytoplasm. Under physiological conditions and in the absence of oxidative stress, Kelch-like enoyl-CoA hydratase-associated protein 1 (Keap1) sequesters Nrf2 in the cytoplasm and mediates its rapid ubiquitination and degradation, suppressing its transcriptional activity[51,52]. When there is an accumulation of ROS, Keap1 changes its conformational structure and releases Nrf2, which then translocates from the cytoplasm to the nucleus. Once there, Nrf2 binds to the antioxidant response element of a promoter region to initiate transcription of several genes encoding heme oxygenase 1 (HO-1), NAD(P)H dehydrogenase (quinone) 1, thioredoxin reductase, peroxiredoxins, SOD, CAT, GPx, GSH reductase (GR), GSH S-transferase (GST), and glutamate-cysteine ligase (GCL). These enzymes contribute to elimination of ROS and play a critical defensive role in cell homeostasis[2,50,53]. Nrf2 is an important cellular pathway that protects against oxidative stress in the retina[54,55]. In diabetes, Nrf2 increases in the retina but so does keap-1, which prevents Nrf2 from reaching the nucleus. Thus, Nrf2 nuclear level is decreased and the antioxidant defense system is compromised[55-57]. As a result, the activity of Nrf2-associated antioxidant enzymes like SOD, GR, GPx, and CAT in diabetes patients or glutamate-cysteine ligase in rat diabetes models[55,58,59]. Thus, the increased risk of developing DR in diabetes patients results from reduced antioxidant capability and the oxidative environment generated by hyperglycemia[2]. These studies also suggest that Keap1 knockdown would release Nrf2, which would move to the nucleus and activate the antioxidant defense system[54,55]. In addition to the regulation of the antioxidant response, Nrf2 regulates the inflammatory response in diabetes[60]. The response is mediated by nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and cyclooxygenase-2 (COX-2). When Nrf2 activity is reduced, there is an increase of proinflammatory cytokines because of the induction of NF-κB, which is associated with capillary cell apoptosis in diabetes *via* the overexpression of proapoptotic Bax or TNF-α[61-63]. In an experimental model of streptozotocin-induced diabetes, rutin, a flavonoid derivative of quercetin, protected against neuron damage in diabetes *via* the Nrf2/HO-1 and NF-κB signaling pathway, together with its anti-inflammatory action *via* COX-2 inhibition[2,64]. The data suggest that Nrf2 activation could be an important protective mechanism for diabetic complications, making it an especially attractive pharmacological target in the progression of DR[54]. Many studies suggest that natural compounds, including polyphenols, can reduce oxidative stress and inflammation through activating Nrf2 and the consequent antioxidant response[57].

Several publications have described the therapeutic potential of various polyphenols in diabetes, including those in green tea,, resveratrol, curcumin, quercetin, and tannins[65-73]. Pterostilbene (Pter), is a phenol that been shown to prevent early DR alterations *via* Nrf2 activation in an experimental rabbit model[47]. In addition to natural antioxidants, other molecules have been shown to activate Nrf2 in DR. One example is RS9, a derivative of the triterpenoid bardoxolone methyl, which was found to delay retinal degeneration by inhibiting inflammatory responses and increasing intrinsic antioxidant enzymes *via* activation of Nrf2[74]. Another triterpenoid derivative, dihydro-CDDO-trifluoroethyl amide (dh404) has been shown to protect the retina against diabetes-induced damage through the activation of Nrf2[75].

Another therapeutic approach in the treatment of DR is, as suggested above, is the inactivation of Keap1. Triterpenoids, salvianolic acids, and sulforaphane[76-79] have been shown to inactivate Keap1 by covalently modifying its reactive cysteine residues. As a consequence, Nrf2 is activated by its translocation into the nucleus and its downstream target genes are then activated, which prevents or reverts ROS-mediated toxicity[50].

***Inflammatory response***

Inflammation is a defensive process mediated by the host immune system in response to injury or stress. In DR, acute inflammation normally produces beneficial effects like tissue defense and repair. Chronic inflammation produces structural and molecular alterations in the retina that usually cause tissue damage and cell death[2]. The inflammatory response in the retina is caused by various factors like hyperglycemia, growth factors, AGEs, high levels of circulating or vitreous cytokines and chemokines, and ROS[80]. These factors induce intracellular signaling pathways, including the transcription factor NF-κB, which translocates into the nucleus to initiate the transcription of proinflammatory cytokines *i.e.* TNF-α, IL-1β, and IL-6; proinflammatory proteins such as COX-2 or the inducible isoform of nitric oxide synthetase (iNOS), and chemokines such as monocyte chemoattractant protein-1. The proinflammatory molecules play an important role in the recruitment and activation of monocytes and leukocytes[81,82]. Adhesion of leukocytes to the capillaries of the retina (leukostasis), together with the release of ROS and proinflammatory cytokines, leads to vascular permeability, BRB breakdown, and capillary pericyte loss. Thus, it is clear that chronic inflammation is critical for the development of DR, principally in the early stages[2,27,81,83].

Several studies have shown that there is an increase of proinflammatory molecules in the retina or vitreous humor of diabetic animals and patients. Those reported are VEGF, TNF-α, iNOS, COX-2, prostacyclin, insulin-like growth factor 1, NF-κB, placental growth factor, intercellular adhesion molecule-1, IL-1β, IL-2, IL-6 and IL-8[81,84-86]. The findings highlight the key role of inflammation in the development of DR. The detailed mechanisms involved in the inflammatory response in DR are not clear, but inhibition of some of the inflammatory mediators mentioned in the previous paragraphs has been shown to block DR development in animal models of diabetes[82,87-92]. NSAIDs, anti-VEGF, and anti-TNF-α agents diminish the progression of DR in humans because of their anti-inflammatory properties[93]. Systemic administration of specific COX-2 inhibitors could be a possible therapy, although COX-2 inhibitors increase the incidence of heart attack and stroke[94]. Nevertheless, in preclinical studies, topical administration was shown to reduce the signs of DR[95-97]. More studies on the beneficial effects of these molecules are needed.

Tetracyclines, such as minocycline and doxycycline, have immunomodulatory properties that include inhibiting the production of NO, COX, prostaglandins, IL-1β, TNF-α, and caspases[98-100]. In a single-center phase I/II clinical trial in five patients with DME, treatment with minocycline resulted in improved visual function, reduced central DME, and vascular leakage[101]. In another clinical trial, patients with severe nonproliferative or non-high-risk proliferative DR were treated with doxycycline, which resulted in an improvement of perimetric parameters compared with patients who received a placebo[102]. IL-6 is one of the most important proinflammatory cytokines present in the vitreous of DR patients. Various clinical studies have investigated the effect on DR of two IL-6 inhibitors, an antibody against IL-6 (EBI-031, clinicaltrials.gov ID: [NCT02842541](https://clinicaltrials.gov/ct2/show/NCT02842541)) and an antibody against the IL-6 receptor (tocilizumab, clinicaltrials.gov ID: [NCT02511067](https://clinicaltrials.gov/ct2/show/NCT02511067)) in patients with DME. Although they have not yet concluded, the studies have shown that IL-6 inhibitors can be effective in the management of non-infectious uveitis. Therefore, the roles of IL-6 inhibition could be more widely investigated in the management of retinal vascular diseases and non-uveitic DME[103]. The effect of anti-TNF-α therapy has also been studied in a few clinical cases but there are no conclusive data about the effects of these inhibitors in DR or DME[104]. The same is true of canakinumab, a selective IL-1β antibody[105].

***Alteration of biochemical pathways***

It has long been accepted that hyperglycemia induces the alteration of the biochemical pathways, such as an increased flux of advanced glycation end products/receptors (AGE/RAGE), the polyol pathway, protein kinase C (PKC) activation, the hexosamine pathway, and unbalancing redox status. The induction of ROS stimulates a low chronic inflammatory state that contributes to the development and progression of neurovascular dysfunction in DR[2]. The regulation of these molecular pathways therefore offers potential targets against DR.

Glucose and products generated by carbohydrate metabolism are able to transform proteins, lipids, or nucleic acids by glycation, triggering the formation of AGEs, a synthesis that is accelerated in the presence of ROS and redox-active transition metals[106,107]. In addition, the production of AGEs stimulates increased formation of oxidative species, resulting in positive feedback that contributes to the progression of the complications of diabetes[108]. AGEs have severe effects on retinal tissue, such as aberrant extracellular crosslinking of extracellular matrix proteins and increased vascular stiffness, which disturbs normal vascular function. AGEs also bind to various receptors in the plasma membrane (RAGE) and activate intracellular signaling cascades that trigger the release of proinflammatory cytokines and proangiogenic factors, with evident damage of neurovascular retinal structures[109]. As AGEs formation is closely related to oxidative stress, modulation of the antioxidant machinery is an attractive approach for preventing the development and progression of DR. The administration of curcumin to diabetic rats was shown to improve redox imbalance in the retina[110] and protect against effects of glycation[111]. Epigallocatechin 3-gallate, quercetin, kaempferol, and resveratrol are other examples of natural antioxidants able to diminish the production of AGEs[112-115]. In addition, drugs such as aminoguanidine have been shown to be effective inhibitors of AGE formation and to inhibit the development of DR[116,117]. However, adverse side- effects preclude their use in humans[108]. Aragonès *et al*[108] in their latest excellent paper, review the benefits of enhancing the detoxifying activity of the glyoxalase system, a main mechanism for detoxifying the intermediates and precursors of AGEs formation, to avoid glycation-derived damage in DR.

Under normoglycemic conditions, glucose is metabolized by the glycolytic pathway. However, in chronic hyperglycemia, excess glucose is reduced to sorbitol by the enzymatic action of aldose reductase. Sorbitol is then converted to fructose by sorbitol dehydrogenase. The two enzymes constitute an alternative route of glucose metabolism known as the polyol pathway, which is an important source of oxidative stress and AGE production[2]. In addition, sorbitol increases cellular osmolarity, triggering osmotic damage and cell death in retinal capillaries[118,119]. Although clinical trials have been inconclusive in the use of polyol pathway inhibitors to treat DR, its use as a potential therapeutic target in DR should not be ruled out[120,121]. In fact, the benefits of polyphenols for DR treatment is extended to inhibition of the polyol pathway. For example, Pter, a natural stilbene analog of resveratrol, in addition to promoting antioxidant defenses *via* Nrf2, inhibited aldose reductase and AGEs formation in a galactosemic rat model[47,122]. Another alternative route to glycolysis in hyperglycemia is the hexosamine pathway. Glutamine fructose-6-phosphate amidotransferase (GFAT) converts fructose-6-phosphate to N-acetylglucosamine-6-phosphate, which is a substrate of O-N-Acetyl-GluN transferase (OGT) and converted to uridine-5-diphosphate-N-acetylglucosamine (UDP-GlucNAc), a precursor of glycoproteins, glycolipids, proteoglycans, and glycosaminoglycans[123]. High levels of glucose and N-acetylglucosamine-6-phosphate activity inhibit glucose-6-phosphate dehydrogenase and low NADPH-dependent GSH production, triggering an increase in the level of H2O2[124]. Glucosamine administration or overexpression of GFAT also leads to H2O2 accumulation, highlighting the role of the hexosamine pathway in oxidative stress[125]. Moreover, OGT activity has been associated with altered *TGFβ* gene expression, which induces NADPH oxidase (NOX) activation, suppression of the antioxidant system, and mitochondrial ROS production[126-128]. In fact, antioxidant treatment has shown beneficial effects against some adverse consequences of the hexosamine pathway[125]. Various inhibitors of the hexosamine pathway, such as the antineoplastic azaserine, the anthraquinone rhein, and the lipid-soluble thiamine derivative benfotiamine, have been evaluated in experimental animal models. In addition to the hexosamine pathway, those agents inhibit AGE formation and the PKC pathway[129-131]. However, the effectiveness of this therapeutic approach in DR has not been shown in clinical trials.

Inhibition of the PKC pathway is of interest. PKCs comprise a family of cAMP-dependent protein kinases with multiple isoforms involved in the regulation of other proteins[2]. PKCs are activated when the second messenger is bound to its regulatory domain. Phosphatidylserine, calcium, and diacylglycerol (DAG) or phorbol esters are activators of PKC-α, β1, β2, and γ. Phosphatidylserine, DAG or phorbol 12-myristate 13-acetate (PMA) activate PKC-δ, ε, θ, and η, while PKC-ζ and -ι/λ are not activated by calcium, DAG or PMA[132]. Cysteine residues are abundant in the PKC structure which makes the regulatory domain susceptible to redox modulation[2]. In fact, hyperglycemia can activate some PKC isoforms directly through DAG, or indirectly by the oxidative stress generated through AGE production and the polyol pathway[133,134]. PKC contributes to redox injury of retinas exposed to chronic hyperglycemia at different levels, triggering the signs of DR. For example, PKC-β is an activator of NOX, and the overproduction of O2•− increases the formation of peroxynitrite to induce endothelial changes[135-137]. PKC-δ is involved in the death of capillary cells and perycites, with subsequent formation of microaneurysms[138,139]. PKC-β and PKC-ζ are involved in VEGF-dependent changes of the retinal barrier[140]. Moreover, PKC induces the overexpression of plasminogen activator-1 and the activation of NF- κB in vascular smooth muscle and endothelial cells, pericytes, and mesangial cells[134]. Inhibition of PKC has been considered as an effective approach to treat DR. The highly selective PKC-β inhibitor, ruboxistaurin mesylate, is one of the most studied. Initial clinical studies showed its potential in the prevention of vision loss induced by DR[141]. However, in 2007 the European Medicines Agency declared a minimum benefit in the treatment of moderately severe to severe non-proliferative DR[142]. In any case, knowledge of the role of the various isoforms of PKC is incomplete and offers another therapeutic target to be considered.

***Lipid alterations***

Lipids play a crucial role in the maintenance and development of retinal functions. The plasma membranes of the outer segments of retina photoreceptors contain high levels of polyunsaturated fatty acids (PUFAs). The most abundant PUFAs in the retina are ω3-docohexaenoic (DHA), ω3-eicosapentaenoic (EPA), and ω6-arachidonic (AA), with DHA being predominant[143-146]. The functions of PUFAs in the retina have been demonstrated in numerous studies. PUFA supplementation has protective and therapeutic effects against proliferative and degenerative retinal diseases, possibly resulting from their antioxidant and anti-inflammatory properties[147-150]. In addition, DHA deficiency has been associated with structural and functional abnormalities in the visual system[149]. ROS formed during oxidative stress can oxidize PUFAs because of the presence of susceptible carbon double bonds in the molecular structure[44,150]. The free radical chain reaction results in lipid peroxidation and acts to amplify the generation of lipid radical species, causing PUFA degradation into a variety of potentially harmful oxidation products[42,146]. The increase of ROS in DR, together with the high PUFA content in the membranes of the photoreceptors, triggers an increase of lipid peroxidation[42,44,151]. In fact, patients with DR have higher lipid peroxidation than those without retinal disease[151-153]. Moreover, a number of published papers indicate that lipid peroxidation has serious pathophysiological effects that contribute to the development of DR[149,154-158], and there is increasing evidence of the importance of products of lipid peroxidation as mediators in the development of neovascularization in DR[149,159,160].

The role of lipid peroxidation in DR has been extensively studied, the determination of lipid peroxidation products, including aldehydes such as 4-hydroxynonenal (4-HNE) or malondialdehyde (MDA), and F2-isoprostanes (F2-IsoP) such as 8-iso-PGF2α in plasma, urine, or the retina[161]. 4-HNE, an end product of nonenzymatic lipid peroxidation of ω6 PUFAs like linoleic acid and amino acids, has been shown to be extremely reactive with DNA, RNA, and proteins in the retina[39,162-165]. Zhou *et al*[166] reported that 4-HNE activates the canonical WNT pathway through oxidative stress in a rat model, playing a pathogenic role in the development of DR. Previous studies by that group have shown that blockade of WNT signaling attenuated retinal inflammation and neovascularization in DR in humans and animal models[167]. In fact, inhibition of the WNT pathway by peroxisome proliferator-activated receptor alpha (PPARα) overexpression induced anti-inflammatory and antifibrosis effects[168]. The retinal protective role of PPARα has been demonstrated both *in vitro* and *in vivo*. Chronic hyperglycemia in experimental animal models of diabetes or treatment of retinal cell lines with high glucose concentrations reduces PPARα mRNA and protein expression levels. The use of PPARα agonists, such as fenofibrate, have been discussed as a treatment of DR by preventing microvascular damage[169,170]. Overexpression of PPARα was found to reduce ROS production, apoptosis induced by oxidative stress, and downregulation of NOX4 expression[171]. It also inhibited cell proliferation, migration, and had anti-angiogenic effects[172]. The data suggest that the WNT pathway and PPARα represent a new target for therapeutical intervention of DR[167].

Other studies suggest that 4-HNE retinal damage in DR could result from the induction of p53-mediated apoptosis in retinal pigment epithelial cells[173]. It has also been shown that 4-HNE attenuated β2-adrenoceptor-mediated vasodilation of rat retinal arterioles, which would contribute to the retinal vascular dysfunction observed in patients with diabetes mellitus[174].

Several studies of possible new treatments of DR have focusing on protecting effects damage associated with 4-HNE. Chiang *et al*[175] reported that fucoxanthin, a marine carotenoid extracted from seaweed, effectively protected against the effects of 4-HNE- and high glucose-induced DR in ARPE-19 human retinal epithelial cells through the antioxidant ability of this compound. Pter was also shown to reduce 4‑HNE levels in the retina of a rabbit model of type 1 diabetes mellitus, preventing early DR alterations[47]. MDA is a product of the peroxidative decomposition of PUFAs. It is a highly reactive molecule that forms covalent bonds with the amino acids of endogenous proteins[42,48]. MDA possesses cytotoxic, hepatotoxic, mutagenic, and genotoxic properties, and can alter proteins, DNA, RNA, and many other biomolecules[176,177]. MDA concentration as a final product of lipid oxidation is routinely determined by thiobarbituric acid assay or chromatography-mass spectrometry[176-178]. There are no studies of its mechanism of action in DR. It has only been used as a biomarker of lipid peroxidation in biological samples.

Since its discovery, F2-IsoP has become one of the most reliable biomarkers of lipid peroxidation and oxidative stress in in vitro studies and in animal models[179-181]. F2-IsoP comprises a family of prostaglandin-like compounds produced by nonenzymatic peroxidation of amino acids in membrane phospholipids[181]. One of the most studied F2-IsoP is 8-iso-PGF2α (also known as 8-epi-PGF2α or 15-F2t-isoprostane), which has been shown to be involved in inflammation and immunity in various diseases[48,181]. In DR, 8-iso-PGF2α is produced by COX activity and enzymatic oxidation of PGF2α[182]. It has been shown to be a potent vasoconstrictor in the retina by increasing thromboxane A2 formation through the activation of Ca2+ influx[182-184].

Further research is needed to clarify the pathophysiological activity of PUFA derivatives in DR. Nevertheless, it seems that inhibition of the formation of these highly cytotoxic molecules could be a possible therapeutic strategy for the management of DR. In fact, Pter has been recently reported to be able to restore the control levels of a large group of specific neuronal and retinal lipid peroxidation markers in diabetic rabbits[185]. This suggests that this polyphenol could protect the retina, preventing early lipid peroxidation damage in DR development.

***GLP1R***

In recent years, new pharmacological therapies have been developed as effective treatments for type 2 diabetes. Glucagon-like peptide 1 receptor agonists (GLP1RAs) have emerged as a safe treatment, and some agonists have been incorporated into the clinical guidelines of the American Diabetes Association and the European Association for the Study of Diabetes. Furthermore, preclinical studies have shown the benefits of GLP1R activation on diabetic vascular complications such as DR[186]. Actually, the benefits are broad. GLP1R activation, independent of homeostatic glycemic control, can reduce the harmful consequences of diabetes on the retina, such as oxidative stress, neurodegeneration, inflammation, BRB breakdown, or angiogenesis[187-190].

The AKT pathway is a target of GLP1R activation and is essential for retinal neuroprotection in early DR development[188]. AKT phosphorylates a number of heterogeneous substrates including E2 ubiquitin ligases, transcription factors, protein and lipid kinases, metabolic enzymes, *etc.*, showing that AKT not only regulates a physiological process, but also controls multiple cellular functions. The first AKT substrate reported was GSK3[191]. Inactivation of GSK3 by AKT-phosphorylation has been shown to regulate transcription factors such as Nrf2, which is needed for DR development[192]. Moreover, in vitro and in vivo studies have demonstrated the ability of GLP1 to protect neurons from aggregation by β-amyloid peptide and against AGEs, as well as being able to reduce hyperphosphorylation of the *tau* protein by regulating GSK3β. It is believed that the mechanism of action of GLP1 is the activation of the PI3K/AKT signaling pathway, which is capable of phosphorylating and inactivating GSK3β[193]. Although further studies are needed to understand the importance and possible modulation of PI3K/AKT/GSK3β/Nrf2 pathway by GLP1R, these observations allow us to develop hypotheses of the key effects that modulation of Nrf2 by GLP1R agonists have on DR development.

***Epigenetic modifications***

Although glycemic control may be achieved, chronic hyperglycemia during the first few months may be enough exposure to develop stable and heritable epigenetic modifications capable of altering gene expression and becoming a potential major factor of DR development[194]. The alterations occur on chromosomes without changes in the DNA sequence and are the basis of the known “metabolic memory”. The identified molecular mechanisms underlying these long-term effects act at different levels that include DNA methylation, post translational modifications of histones or regulation by noncoding (nc)RNAs[195]. For example, the low retinal histone acetylation of H3 induced by hyperglycemia for 6 mo did not recover after 6 mo of good glycemic control[196]. Likewise, euglycemia was unable to recover the DNA hypomethylation and unusual gene expression induced by hyperglycemia[197].

DNA methylation status is controlled by the activity of DNA methyltransferase (DNMT) enzymes that catalyze the transfer of a methyl group from S-adenosyl-L-methinione, and DNA demethylases. Imbalanced activity in diabetes, induces alterations in specific genes that triggers aberrant expression related to DR. For example, chronic hyperglycemia in the retina stimulates the binding of DNMT1 and the DNA demethylase ten-eleven-translocation (TET) 2 to the promoter of Ras-related C3 botulinum toxin substrate (Rac1)[198]. Methylation induced by DNMT1 is rapidly reversed by TET2, triggering hypomethylation of the promoter and allowing Rac1 transcription, which induces NOX, and relevant effectors in DR development[199]. In fact, the mitochondrial damage initiated by NOX-2 activation has been associated with early DR development while its inhibition protects endothelial retinal cells from diabetes-induced apoptosis[200].

Although diabetes induces a global state of DNA hypomethylation, different states of methylation for specific CpG islands are closely related to DR development. An increase in the expression and activity of DNMTs has been observed in DR[201-203]. Based on that, inhibition of DNMTs can be a possible protective therapy against the development of DR. For example, 5-aza-2'-deoxycytidine, a nonselective inhibitor of DNMTs, re-establishes the expression of genes hypermethylated by hyperglycemia and related to DR development, such as SOD2 and glutathione S-transferase theta 1 (GSTT1), which protects against oxidative stress[203].

Changes in the pattern of acetylation and methylation are the most studied post translational modifications of histones. Overall, the acetylation of histones H3 and H4 and di or tri-methylation of H3K4 are related to euchromatin status. Low acetylation and high methylation levels are associated with silent heterochromatin. Experimental models of DR have provided contradictory results for histone acetylation. For example, Zhong and Kowluru[196] revealed reduced global acetylation, but Kadiyala *et al*[204] observed augmented histone acetylation in diabetic retinas. So far, in vivo experimental results for histone acetylation in DR remain contradictory[194,205].

Histone methylation is associated with transcriptional activation or repression depending on the type of residue and the number of methyl groups. Hence, the methylation of H3K4, H3K48, and H3K79 have been considered activation marks, while that of H3K9 and H3K27 are associated with transcriptional repression[206]. For example, decreased levels of H3K4me1 and H3K4me3 at the GCL promoter in diabetic rats compromised Nrf2 binding, triggering low transcription of the enzyme and reduced levels of GSH in the retina[207]. Moreover, the overexpression of matrix metalloproteinase-9, a proapoptotic enzyme in the development of DR, is caused by a decrease in H3K9me2 and an increase in acetyl H3K9, which facilitates the binding of NF-κB p65[208].

Thus, hyperglycemia-induced differential histone methylation or acetylation appears to regulate expression of several genes in cellular pathways that contribute to the development of diabetic retinopathy. In fact, the polyisoprenylated benzophenone derivative garcinol, prevents histone acetylation involved in the metabolic memory in DR[209]. In that sense, histone deacetylase inhibitors like resveratrol, curcumin, and genistein are also being considered as targets for treatment of DR[210].

A low percentage of cellular transcribed RNA is ncRNA, RNA sequences with different but important cell functions. Long ncRNA and small ncRNA, such as circular RNA, or miRNA, are essential in the pathological processes of diabetic complications, including atherosclerosis, microvascular dysfunction, and DR[211]. The most well-studied are miRNAs[212], sequences of approximately 18-25 nucleotides partially complementary to mRNAs able to block their translation and activate their degradation in collaboration with the ribonucleoprotein complex RNA-induced silencing complex[213]. There are numerous examples of the importance of their role in DR. Experimental models of DR have shown that downregulation of miR126, miR-146a, and miR200b is associated with retinal neovascularization through increased VEGF production[214]. The expression of miR-20b-5p, a modulator of cell proliferation, apoptosis, differentiation, and angiogenesis, is upregulated in the retinal endothelial cells of diabetic rats and patients with DR, inducing a decrease in tight junction proteins that increases BRB permeability and the microvascular leakage observed in DR[215]. Although the expression and physiological function of circular RNA is not yet fully elucidated, the molecules serve as miRNA or RNA-binding protein sponges to modulate expression or translation of regulatory proteins[216]. Circular DNMT3B, a reducer of the expression of miR-20b-5p, is downregulated in diabetes and its overexpression improves the vascular dysfunction induced in diabetic retinas, an interesting potential strategy for treatment of DR[215]. The possibility of using siRNAs to target some miRNAs mentioned above has also been considered. However, no methods are currently available for in vivo treatments[209]. In addition, double-stranded miRNA mimics and anti-mRNA antisense oligodeoxyribonucleotide are being used to target specific miRNA in other diseases, and therefore can also be studied for the treatment of DR[210].

With the increase in evidence on the importance of epigenetic modifications in DR, a better understanding of their effects has great potential for establishing new targets against this pathology. Fortunately, advances are being made in the use of mimics and inhibitors in different chronic diseases and cancer that will undoubtedly contribute to a better understanding of the role of epigenetic changes in DR.

**CONCLUSION**

With the global increase in the prevalence of diabetes, an increase in associated complications such as DR is expected. Although in recent decades considerable advances have been made in the treatment of the disease, current therapeutic approaches focus on advanced stages in which the retina can present irreparable damage at the neuronal and vascular level. Furthermore, the recommended treatments for DR have serious limitations such us long-term side effects, the high cost involved, or patient discomfort. Hence the need for the development of new therapeutic approaches. Considering the current state of knowledge, treatments for diabetic retinopathy should go beyond acting on a single etiological cause such as neovascularization. New treatments should present a set of advantages that facilitate their administration without the need for special facilities. Ideal treatments would be noninvasive, effective, affordable, and accessible to the global population. Recognizing the importance of redox imbalance in the development and progression of DR offers a new direction for tackling the condition. One such option that should be explored is action directed at cellular targets that participate in modulating or altering the pathology, so that the progression of the disease can be delayed or even prevented.

**REFERENCES**

1 **Saeedi P**, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, Colagiuri S, Guariguata L, Motala AA, Ogurtsova K, Shaw JE, Bright D, Williams R; IDF Diabetes Atlas Committee. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract* 2019; **157**: 107843 [PMID: 31518657 DOI: 10.1016/j.diabres.2019.107843]

2 **Rodríguez ML**, Pérez S, Mena-Mollá S, Desco MC, Ortega ÁL. Oxidative Stress and Microvascular Alterations in Diabetic Retinopathy: Future Therapies. *Oxid Med Cell Longev* 2019; **2019**: 4940825 [PMID: 31814880 DOI: 10.1155/2019/4940825]

3 **Flaxman SR**, Bourne RRA, Resnikoff S, Ackland P, Braithwaite T, Cicinelli MV, Das A, Jonas JB, Keeffe J, Kempen JH, Leasher J, Limburg H, Naidoo K, Pesudovs K, Silvester A, Stevens GA, Tahhan N, Wong TY, Taylor HR; Vision Loss Expert Group of the Global Burden of Disease Study. Global causes of blindness and distance vision impairment 1990-2020: a systematic review and meta-analysis. *Lancet Glob Health* 2017; **5**: e1221-e1234 [PMID: 29032195 DOI: 10.1016/S2214-109X(17)30393-5]

4 **Asmat U**, Abad K, Ismail K. Diabetes mellitus and oxidative stress-A concise review. *Saudi Pharm J* 2016; **24**: 547-553 [PMID: 27752226 DOI: 10.1016/j.jsps.2015.03.013]

5 **Wangsa-Wirawan ND**, Linsenmeier RA. Retinal oxygen: fundamental and clinical aspects. *Arch Ophthalmol* 2003; **121**: 547-557 [PMID: 12695252 DOI: 10.1001/archopht.121.4.547]

6 **Semeraro F**, Morescalchi F, Cancarini A, Russo A, Rezzola S, Costagliola C. Diabetic retinopathy, a vascular and inflammatory disease: Therapeutic implications. *Diabetes Metab* 2019; **45**: 517-527 [PMID: 31005756 DOI: 10.1016/j.diabet.2019.04.002]

7 **Rangasamy S**, McGuire PG, Das A. Diabetic retinopathy and inflammation: novel therapeutic targets. *Middle East Afr J Ophthalmol* 2012; **19**: 52-59 [PMID: 22346115 DOI: 10.4103/0974-9233.92116]

8 **Ishida S**, Usui T, Yamashiro K, Kaji Y, Ahmed E, Carrasquillo KG, Amano S, Hida T, Oguchi Y, Adamis AP. VEGF164 is proinflammatory in the diabetic retina. *Invest Ophthalmol Vis Sci* 2003; **44**: 2155-2162 [PMID: 12714656 DOI: 10.1167/iovs.02-0807]

9 **Sinclair SH**, Schwartz SS. Diabetic Retinopathy-An Underdiagnosed and Undertreated Inflammatory, Neuro-Vascular Complication of Diabetes. *Front Endocrinol (Lausanne)* 2019; **10**: 843 [PMID: 31920963 DOI: 10.3389/fendo.2019.00843]

10 **Stitt AW**, Lois N, Medina RJ, Adamson P, Curtis TM. Advances in our understanding of diabetic retinopathy. *Clin Sci (Lond)* 2013; **125**: 1-17 [PMID: 23485060 DOI: 10.1042/CS20120588]

11 **Hernández C**, Simó-Servat A, Bogdanov P, Simó R. Diabetic retinopathy: new therapeutic perspectives based on pathogenic mechanisms. *J Endocrinol Invest* 2017; **40**: 925-935 [PMID: 28357783 DOI: 10.1007/s40618-017-0648-4]

12 **Calderon GD**, Juarez OH, Hernandez GE, Punzo SM, De la Cruz ZD. Oxidative stress and diabetic retinopathy: development and treatment. *Eye (Lond)* 2017; **31**: 1122-1130 [PMID: 28452994 DOI: 10.1038/eye.2017.64]

13 **Mansour SE**, Browning DJ, Wong K, Flynn HW Jr, Bhavsar AR. The Evolving Treatment of Diabetic Retinopathy. *Clin Ophthalmol* 2020; **14**: 653-678 [PMID: 32184554 DOI: 10.2147/OPTH.S236637]

14 **Bandello F**, Toni D, Porta M, Varano M. Diabetic retinopathy, diabetic macular edema, and cardiovascular risk: the importance of a long-term perspective and a multidisciplinary approach to optimal intravitreal therapy. *Acta Diabetol* 2020; **57**: 513-526 [PMID: 31749046 DOI: 10.1007/s00592-019-01453-z]

15 **Fogli S**, Del Re M, Rofi E, Posarelli C, Figus M, Danesi R. Clinical pharmacology of intravitreal anti-VEGF drugs. *Eye (Lond)* 2018; **32**: 1010-1020 [PMID: 29398697 DOI: 10.1038/s41433-018-0021-7]

16 **Sultan MB**, Zhou D, Loftus J, Dombi T, Ice KS; Macugen 1013 Study Group. A phase 2/3, multicenter, randomized, double-masked, 2-year trial of pegaptanib sodium for the treatment of diabetic macular edema. *Ophthalmology* 2011; **118**: 1107-1118 [PMID: 21529957 DOI: 10.1016/j.ophtha.2011.02.045]

17 **Diabetic Retinopathy Clinical Research Network**, Elman MJ, Qin H, Aiello LP, Beck RW, Bressler NM, Ferris FL 3rd, Glassman AR, Maturi RK, Melia M. Intravitreal ranibizumab for diabetic macular edema with prompt versus deferred laser treatment: three-year randomized trial results. *Ophthalmology* 2012; **119**: 2312-2318 [PMID: 22999634 DOI: 10.1016/j.ophtha.2012.08.022]

18 **Fallico M**, Maugeri A, Lotery A, Longo A, Bonfiglio V, Russo A, Avitabile T, Pulvirenti A, Furino C, Cennamo G, Barchitta M, Agodi A, Reibaldi M. Intravitreal anti-vascular endothelial growth factors, panretinal photocoagulation and combined treatment for proliferative diabetic retinopathy: a systematic review and network meta-analysis. *Acta Ophthalmol* 2020 [PMID: 33326183 DOI: 10.1111/aos.14681]

19 **Gologorsky D**, Thanos A, Vavvas D. Therapeutic interventions against inflammatory and angiogenic mediators in proliferative diabetic retinopathy. *Mediators Inflamm* 2012; **2012**: 629452 [PMID: 23028203 DOI: 10.1155/2012/629452]

20 **Kumar B**, Gupta SK, Saxena R, Srivastava S. Current trends in the pharmacotherapy of diabetic retinopathy. *J Postgrad Med* 2012; **58**: 132-139 [PMID: 22718058 DOI: 10.4103/0022-3859.97176]

21 **Kaštelan S**, Tomić M, Gverović Antunica A, Salopek Rabatić J, Ljubić S. Inflammation and pharmacological treatment in diabetic retinopathy. *Mediators Inflamm* 2013; **2013**: 213130 [PMID: 24288441 DOI: 10.1155/2013/213130]

22 **Quiram PA**, Gonzales CR, Schwartz SD. Severe steroid-induced glaucoma following intravitreal injection of triamcinolone acetonide. *Am J Ophthalmol* 2006; **141**: 580-582 [PMID: 16490518 DOI: 10.1016/j.ajo.2005.10.004]

23 **Pai A**, El Shafei MM, Mohammed OA, Al Hashimi M. Current concepts in intravitreal drug therapy for diabetic retinopathy. *Saudi J Ophthalmol* 2010; **24**: 143-149 [PMID: 23960892 DOI: 10.1016/j.sjopt.2010.06.003]

24 **Stewart MW**. Corticosteroid use for diabetic macular edema: old fad or new trend? *Curr Diab Rep* 2012; **12**: 364-375 [PMID: 22581206 DOI: 10.1007/s11892-012-0281-8]

25 **Kompella UB**, Kadam RS, Lee VH. Recent advances in ophthalmic drug delivery. *Ther Deliv* 2010; **1**: 435-456 [PMID: 21399724 DOI: 10.4155/TDE.10.40]

26 **Kuno N**, Fujii S. Biodegradable intraocular therapies for retinal disorders: progress to date. *Drugs Aging* 2010; **27**: 117-134 [PMID: 20104938 DOI: 10.2165/11530970-000000000-00000]

27 **Wang W**, Lo ACY. Diabetic Retinopathy: Pathophysiology and Treatments. *Int J Mol Sci* 2018; **19** [PMID: 29925789 DOI: 10.3390/ijms19061816]

28 **Ellis MP**, Lent-Schochet D, Lo T, Yiu G. Emerging Concepts in the Treatment of Diabetic Retinopathy. *Curr Diab Rep* 2019; **19**: 137 [PMID: 31748965 DOI: 10.1007/s11892-019-1276-5]

29 **Lee R**, Wong TY, Sabanayagam C. Epidemiology of diabetic retinopathy, diabetic macular edema and related vision loss. *Eye Vis (Lond)* 2015; **2**: 17 [PMID: 26605370 DOI: 10.1186/s40662-015-0026-2]

30 **Fong DS**, Girach A, Boney A. Visual side effects of successful scatter laser photocoagulation surgery for proliferative diabetic retinopathy: a literature review. *Retina* 2007; **27**: 816-824 [PMID: 17891003 DOI: 10.1097/IAE.0b013e318042d32c]

31 **Helbig H**. Surgery for diabetic retinopathy. *Ophthalmologica* 2007; **221**: 103-111 [PMID: 17380064 DOI: 10.1159/000098255]

32 **Sharma T**, Fong A, Lai TY, Lee V, Das S, Lam D. Surgical treatment for diabetic vitreoretinal diseases: a review. *Clin Exp Ophthalmol* 2016; **44**: 340-354 [PMID: 27027299 DOI: 10.1111/ceo.12752]

33 **Zhao LQ**, Zhu H, Zhao PQ, Hu YQ. A systematic review and meta-analysis of clinical outcomes of vitrectomy with or without intravitreal bevacizumab pretreatment for severe diabetic retinopathy. *Br J Ophthalmol* 2011; **95**: 1216-1222 [PMID: 21278146 DOI: 10.1136/bjo.2010.189514]

34 **Nakajima T**, Roggia MF, Noda Y, Ueta T. EFFECT OF INTERNAL LIMITING MEMBRANE PEELING DURING VITRECTOMY FOR DIABETIC MACULAR EDEMA: Systematic Review and Meta-analysis. *Retina* 2015; **35**: 1719-1725 [PMID: 26079478 DOI: 10.1097/IAE.0000000000000622]

35 **Belin PJ**, Parke DW 3rd. Complications of vitreoretinal surgery. *Curr Opin Ophthalmol* 2020; **31**: 167-173 [PMID: 32175941 DOI: 10.1097/ICU.0000000000000652]

36 **Lahey JM**, Francis RR, Kearney JJ. Combining phacoemulsification with pars plana vitrectomy in patients with proliferative diabetic retinopathy: a series of 223 cases. *Ophthalmology* 2003; **110**: 1335-1339 [PMID: 12867387 DOI: 10.1016/S0161-6420(03)00454-8]

37 **Khuthaila MK**, Hsu J, Chiang A, DeCroos FC, Milder EA, Setlur V, Garg SJ, Spirn MJ. Postoperative vitreous hemorrhage after diabetic 23-gauge pars plana vitrectomy. *Am J Ophthalmol* 2013; **155**: 757-763, 763.e1-763.e2 [PMID: 23317651 DOI: 10.1016/j.ajo.2012.11.004]

38 **Stitt AW**, Curtis TM, Chen M, Medina RJ, McKay GJ, Jenkins A, Gardiner TA, Lyons TJ, Hammes HP, Simó R, Lois N. The progress in understanding and treatment of diabetic retinopathy. *Prog Retin Eye Res* 2016; **51**: 156-186 [PMID: 26297071 DOI: 10.1016/j.preteyeres.2015.08.001]

39 **Kang Q**, Yang C. Oxidative stress and diabetic retinopathy: Molecular mechanisms, pathogenetic role and therapeutic implications. *Redox Biol* 2020; **37**: 101799 [PMID: 33248932 DOI: 10.1016/j.redox.2020.101799]

40 **Slater TF**. Free-radical mechanisms in tissue injury. *Biochem J* 1984; **222**: 1-15 [PMID: 6383353 DOI: 10.1042/bj2220001]

41 **Sies H**, Berndt C, Jones DP. Oxidative Stress. *Annu Rev Biochem* 2017; **86**: 715-748 [PMID: 28441057 DOI: 10.1146/annurev-biochem-061516-045037]

42 **Kersten E**, Paun CC, Schellevis RL, Hoyng CB, Delcourt C, Lengyel I, Peto T, Ueffing M, Klaver CCW, Dammeier S, den Hollander AI, de Jong EK. Systemic and ocular fluid compounds as potential biomarkers in age-related macular degeneration. *Surv Ophthalmol* 2018; **63**: 9-39 [PMID: 28522341 DOI: 10.1016/j.survophthal.2017.05.003]

43 **Tokarz P**, Kaarniranta K, Blasiak J. Role of antioxidant enzymes and small molecular weight antioxidants in the pathogenesis of age-related macular degeneration (AMD). *Biogerontology* 2013; **14**: 461-482 [PMID: 24057278 DOI: 10.1007/s10522-013-9463-2]

44 **Cai J**, Nelson KC, Wu M, Sternberg P Jr, Jones DP. Oxidative damage and protection of the RPE. *Prog Retin Eye Res* 2000; **19**: 205-221 [PMID: 10674708 DOI: 10.1016/s1350-9462(99)00009-9]

45 **Wu Y**, Tang L, Chen B. Oxidative stress: implications for the development of diabetic retinopathy and antioxidant therapeutic perspectives. *Oxid Med Cell Longev* 2014; **2014**: 752387 [PMID: 25180070 DOI: 10.1155/2014/752387]

46 **Priščáková P**, Minárik G, Repiská V. Candidate gene studies of diabetic retinopathy in human. *Mol Biol Rep* 2016; **43**: 1327-1345 [PMID: 27730450 DOI: 10.1007/s11033-016-4075-y]

47 **Millán I**, Desco MDC, Torres-Cuevas I, Pérez S, Pulido I, Mena-Mollá S, Mataix J, Asensi M, Ortega ÁL. Pterostilbene Prevents Early Diabetic Retinopathy Alterations in a Rabbit Experimental Model. *Nutrients* 2019; **12** [PMID: 31892189 DOI: 10.3390/nu12010082]

48 **Al-Shabrawey M**, Smith S. Prediction of diabetic retinopathy: role of oxidative stress and relevance of apoptotic biomarkers. *EPMA J* 2010; **1**: 56-72 [PMID: 23199041 DOI: 10.1007/s13167-010-0002-9]

49 **Kensler TW**, Wakabayashi N, Biswal S. Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. *Annu Rev Pharmacol Toxicol* 2007; **47**: 89-116 [PMID: 16968214 DOI: 10.1146/annurev.pharmtox.46.120604.141046]

50 **Batliwala S**, Xavier C, Liu Y, Wu H, Pang IH. Involvement of Nrf2 in Ocular Diseases. *Oxid Med Cell Longev* 2017; **2017**: 1703810 [PMID: 28473877 DOI: 10.1155/2017/1703810]

51 **Canning P**, Sorrell FJ, Bullock AN. Structural basis of Keap1 interactions with Nrf2. *Free Radic Biol Med* 2015; **88**: 101-107 [PMID: 26057936 DOI: 10.1016/j.freeradbiomed.2015.05.034]

52 **McMahon M**, Itoh K, Yamamoto M, Hayes JD. Keap1-dependent proteasomal degradation of transcription factor Nrf2 contributes to the negative regulation of antioxidant response element-driven gene expression. *J Biol Chem* 2003; **278**: 21592-21600 [PMID: 12682069 DOI: 10.1074/jbc.M300931200]

53 **Itoh K**, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, Oyake T, Hayashi N, Satoh K, Hatayama I, Yamamoto M, Nabeshima Y. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochem Biophys Res Commun* 1997; **236**: 313-322 [PMID: 9240432 DOI: 10.1006/bbrc.1997.6943]

54 **Xu Z**, Wei Y, Gong J, Cho H, Park JK, Sung ER, Huang H, Wu L, Eberhart C, Handa JT, Du Y, Kern TS, Thimmulappa R, Barber AJ, Biswal S, Duh EJ. NRF2 plays a protective role in diabetic retinopathy in mice. *Diabetologia* 2014; **57**: 204-213 [PMID: 24186494 DOI: 10.1007/s00125-013-3093-8]

55 **Zhong Q**, Mishra M, Kowluru RA. Transcription factor Nrf2-mediated antioxidant defense system in the development of diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2013; **54**: 3941-3948 [PMID: 23633659 DOI: 10.1167/iovs.13-11598]

56 **Mishra M**, Zhong Q, Kowluru RA. Epigenetic modifications of Keap1 regulate its interaction with the protective factor Nrf2 in the development of diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2014; **55**: 7256-7265 [PMID: 25301875 DOI: 10.1167/iovs.14-15193]

57 **Nabavi SF**, Barber AJ, Spagnuolo C, Russo GL, Daglia M, Nabavi SM, Sobarzo-Sánchez E. Nrf2 as molecular target for polyphenols: A novel therapeutic strategy in diabetic retinopathy. *Crit Rev Clin Lab Sci* 2016; **53**: 293-312 [PMID: 26926494 DOI: 10.3109/10408363.2015.1129530]

58 **Kowluru RA**, Kennedy A. Therapeutic potential of anti-oxidants and diabetic retinopathy. *Expert Opin Investig Drugs* 2001; **10**: 1665-1676 [PMID: 11772276 DOI: 10.1517/13543784.10.9.1665]

59 **Haskins K**, Bradley B, Powers K, Fadok V, Flores S, Ling X, Pugazhenthi S, Reusch J, Kench J. Oxidative stress in type 1 diabetes. *Ann N Y Acad Sci* 2003; **1005**: 43-54 [PMID: 14679039 DOI: 10.1196/annals.1288.006]

60 **Negi G**, Kumar A, Joshi RP, Sharma SS. Oxidative stress and Nrf2 in the pathophysiology of diabetic neuropathy: old perspective with a new angle. *Biochem Biophys Res Commun* 2011; **408**: 1-5 [PMID: 21439933 DOI: 10.1016/j.bbrc.2011.03.087]

61 **Li W**, Khor TO, Xu C, Shen G, Jeong WS, Yu S, Kong AN. Activation of Nrf2-antioxidant signaling attenuates NFkappaB-inflammatory response and elicits apoptosis. *Biochem Pharmacol* 2008; **76**: 1485-1489 [PMID: 18694732 DOI: 10.1016/j.bcp.2008.07.017]

62 **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; **37**: 1169-1180 [PMID: 14703729 DOI: 10.1080/10715760310001604189]

63 **Romeo G**, Liu WH, Asnaghi V, Kern TS, Lorenzi M. Activation of nuclear factor-kappaB induced by diabetes and high glucose regulates a proapoptotic program in retinal pericytes. *Diabetes* 2002; **51**: 2241-2248 [PMID: 12086956 DOI: 10.2337/diabetes.51.7.2241]

64 **Mittal R**, Kumar A, Singh DP, Bishnoi M, Nag TC. Ameliorative potential of rutin in combination with nimesulide in STZ model of diabetic neuropathy: targeting Nrf2/HO-1/NF-kB and COX signalling pathway. *Inflammopharmacology* 2018; **26**: 755-768 [PMID: 29094308 DOI: 10.1007/s10787-017-0413-5]

65 **Sun W**, Liu X, Zhang H, Song Y, Li T, Liu X, Liu Y, Guo L, Wang F, Yang T, Guo W, Wu J, Jin H, Wu H. Epigallocatechin gallate upregulates NRF2 to prevent diabetic nephropathy via disabling KEAP1. *Free Radic Biol Med* 2017; **108**: 840-857 [PMID: 28457936 DOI: 10.1016/j.freeradbiomed.2017.04.365]

66 **Cheng AS**, Cheng YH, Chiou CH, Chang TL. Resveratrol upregulates Nrf2 expression to attenuate methylglyoxal-induced insulin resistance in Hep G2 cells. *J Agric Food Chem* 2012; **60**: 9180-9187 [PMID: 22917016 DOI: 10.1021/jf302831d]

67 **Palsamy P**, Subramanian S. Resveratrol protects diabetic kidney by attenuating hyperglycemia-mediated oxidative stress and renal inflammatory cytokines via Nrf2-Keap1 signaling. *Biochim Biophys Acta* 2011; **1812**: 719-731 [PMID: 21439372 DOI: 10.1016/j.bbadis.2011.03.008]

68 **Bucolo C**, Drago F, Maisto R, Romano GL, D'Agata V, Maugeri G, Giunta S. Curcumin prevents high glucose damage in retinal pigment epithelial cells through ERK1/2-mediated activation of the Nrf2/HO-1 pathway. *J Cell Physiol* 2019; **234**: 17295-17304 [PMID: 30770549 DOI: 10.1002/jcp.28347]

69 **Pugazhenthi S**, Akhov L, Selvaraj G, Wang M, Alam J. Regulation of heme oxygenase-1 expression by demethoxy curcuminoids through Nrf2 by a PI3-kinase/Akt-mediated pathway in mouse beta-cells. *Am J Physiol Endocrinol Metab* 2007; **293**: E645-E655 [PMID: 17535857 DOI: 10.1152/ajpendo.00111.2007]

70 **Zhang X**, Liang D, Guo L, Liang W, Jiang Y, Li H, Zhao Y, Lu S, Chi ZH. Curcumin protects renal tubular epithelial cells from high glucose-induced epithelial-to-mesenchymal transition through Nrf2-mediated upregulation of heme oxygenase-1. *Mol Med Rep* 2015; **12**: 1347-1355 [PMID: 25823828 DOI: 10.3892/mmr.2015.3556]

71 **Park JY**, Han X, Piao MJ, Oh MC, Fernando PM, Kang KA, Ryu YS, Jung U, Kim IG, Hyun JW. Hyperoside Induces Endogenous Antioxidant System to Alleviate Oxidative Stress. *J Cancer Prev* 2016; **21**: 41-47 [PMID: 27051648 DOI: 10.15430/JCP.2016.21.1.41]

72 **Liu XF**, Hao JL, Xie T, Malik TH, Lu CB, Liu C, Shu C, Lu CW, Zhou DD. Nrf2 as a target for prevention of age-related and diabetic cataracts by against oxidative stress. *Aging Cell* 2017; **16**: 934-942 [PMID: 28722304 DOI: 10.1111/acel.12645]

73 **Laddha AP**, Kulkarni YA. Tannins and vascular complications of Diabetes: An update. *Phytomedicine* 2019; **56**: 229-245 [PMID: 30668344 DOI: 10.1016/j.phymed.2018.10.026]

74 **Nakagami Y**, Hatano E, Inoue T, Yoshida K, Kondo M, Terasaki H. Cytoprotective Effects of a Novel Nrf2 Activator, RS9, in Rhodopsin Pro347Leu Rabbits. *Curr Eye Res* 2016; **41**: 1123-1126 [PMID: 26430824 DOI: 10.3109/02713683.2015.1078362]

75 **Deliyanti D**, Alrashdi SF, Tan SM, Meyer C, Ward KW, de Haan JB, Wilkinson-Berka JL. Nrf2 Activation Is a Potential Therapeutic Approach to Attenuate Diabetic Retinopathy. *Invest Ophthalmol Vis Sci* 2018; **59**: 815-825 [PMID: 29411009 DOI: 10.1167/iovs.17-22920]

76 **Liu X**, Ward K, Xavier C, Jann J, Clark AF, Pang IH, Wu H. The novel triterpenoid RTA 408 protects human retinal pigment epithelial cells against H2O2-induced cell injury via NF-E2-related factor 2 (Nrf2) activation. *Redox Biol* 2016; **8**: 98-109 [PMID: 26773873 DOI: 10.1016/j.redox.2015.12.005]

77 **Zhang H**, Liu YY, Jiang Q, Li KR, Zhao YX, Cao C, Yao J. Salvianolic acid A protects RPE cells against oxidative stress through activation of Nrf2/HO-1 signaling. *Free Radic Biol Med* 2014; **69**: 219-228 [PMID: 24486344 DOI: 10.1016/j.freeradbiomed.2014.01.025]

78 **Guerrero-Beltrán CE**, Calderón-Oliver M, Pedraza-Chaverri J, Chirino YI. Protective effect of sulforaphane against oxidative stress: recent advances. *Exp Toxicol Pathol* 2012; **64**: 503-508 [PMID: 21129940 DOI: 10.1016/j.etp.2010.11.005]

79 **Li S**, Yang H, Chen X. Protective effects of sulforaphane on diabetic retinopathy: activation of the Nrf2 pathway and inhibition of NLRP3 inflammasome formation. *Exp Anim* 2019; **68**: 221-231 [PMID: 30606939 DOI: 10.1538/expanim.18-0146]

80 **Al-Kharashi AS**. Role of oxidative stress, inflammation, hypoxia and angiogenesis in the development of diabetic retinopathy. *Saudi J Ophthalmol* 2018; **32**: 318-323 [PMID: 30581303 DOI: 10.1016/j.sjopt.2018.05.002]

81 **Rübsam A**, Parikh S, Fort PE. Role of Inflammation in Diabetic Retinopathy. *Int J Mol Sci* 2018; **19** [PMID: 29565290 DOI: 10.3390/ijms19040942]

82 **Tang J**, Kern TS. Inflammation in diabetic retinopathy. *Prog Retin Eye Res* 2011; **30**: 343-358 [PMID: 21635964 DOI: 10.1016/j.preteyeres.2011.05.002]

83 **Kern TS**. Contributions of inflammatory processes to the development of the early stages of diabetic retinopathy. *Exp Diabetes Res* 2007; **2007**: 95103 [PMID: 18274606 DOI: 10.1155/2007/95103]

84 **ValdezGuerrero AS**, Quintana-Pérez JC, Arellano-Mendoza MG, Castañeda-Ibarra FJ, Tamay-Cach F, Alemán-González-Duhart D. Diabetic Retinopathy: Important Biochemical Alterations and the Main Treatment Strategies. *Can J Diabetes* 2020 [PMID: 33341391 DOI: 10.1016/j.jcjd.2020.10.009]

85 **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**: e108508 [PMID: 25329075 DOI: 10.1371/journal.pone.0108508]

86 **Simó-Servat O**, Hernández C, Simó R. Usefulness of the vitreous fluid analysis in the translational research of diabetic retinopathy. *Mediators Inflamm* 2012; **2012**: 872978 [PMID: 23028204 DOI: 10.1155/2012/872978]

87 **Li Q**, Verma A, Han PY, Nakagawa T, Johnson RJ, Grant MB, Campbell-Thompson M, Jarajapu YP, Lei B, Hauswirth WW. Diabetic eNOS-knockout mice develop accelerated retinopathy. *Invest Ophthalmol Vis Sci* 2010; **51**: 5240-5246 [PMID: 20435587 DOI: 10.1167/iovs.09-5147]

88 **Vallejo S**, Palacios E, Romacho T, Villalobos L, Peiró C, Sánchez-Ferrer CF. The interleukin-1 receptor antagonist anakinra improves endothelial dysfunction in streptozotocin-induced diabetic rats. *Cardiovasc Diabetol* 2014; **13**: 158 [PMID: 25518980 DOI: 10.1186/s12933-014-0158-z]

89 **Zheng L**, Du Y, Miller C, Gubitosi-Klug RA, Kern TS, Ball S, Berkowitz BA. Critical role of inducible nitric oxide synthase in degeneration of retinal capillaries in mice with streptozotocin-induced diabetes. *Diabetologia* 2007; **50**: 1987-1996 [PMID: 17583794 DOI: 10.1007/s00125-007-0734-9]

90 **Joussen AM**, Poulaki V, Mitsiades N, Kirchhof B, Koizumi K, Döhmen S, Adamis AP. Nonsteroidal anti-inflammatory drugs prevent early diabetic retinopathy via TNF-alpha suppression. *FASEB J* 2002; **16**: 438-440 [PMID: 11821258 DOI: 10.1096/fj.01-0707fje]

91 **Zheng L**, Howell SJ, Hatala DA, Huang K, Kern TS. Salicylate-based anti-inflammatory drugs inhibit the early lesion of diabetic retinopathy. *Diabetes* 2007; **56**: 337-345 [PMID: 17259377 DOI: 10.2337/db06-0789]

92 **Vincent JA**, Mohr S. Inhibition of caspase-1/interleukin-1beta signaling prevents degeneration of retinal capillaries in diabetes and galactosemia. *Diabetes* 2007; **56**: 224-230 [PMID: 17192486 DOI: 10.2337/db06-0427]

93 **Rosberger DF**. Diabetic retinopathy: current concepts and emerging therapy. *Endocrinol Metab Clin North Am* 2013; **42**: 721-745 [PMID: 24286948 DOI: 10.1016/j.ecl.2013.08.001]

94 **Kim SJ**, Flach AJ, Jampol LM. Nonsteroidal anti-inflammatory drugs in ophthalmology. *Surv Ophthalmol* 2010; **55**: 108-133 [PMID: 20159228 DOI: 10.1016/j.survophthal.2009.07.005]

95 **Kern TS**, Miller CM, Du Y, Zheng L, Mohr S, Ball SL, Kim M, Jamison JA, Bingaman DP. Topical administration of nepafenac inhibits diabetes-induced retinal microvascular disease and underlying abnormalities of retinal metabolism and physiology. *Diabetes* 2007; **56**: 373-379 [PMID: 17259381 DOI: 10.2337/db05-1621]

96 **Amrite AC**, Ayalasomayajula SP, Cheruvu NP, Kompella UB. Single periocular injection of celecoxib-PLGA microparticles inhibits diabetes-induced elevations in retinal PGE2, VEGF, and vascular leakage. *Invest Ophthalmol Vis Sci* 2006; **47**: 1149-1160 [PMID: 16505053 DOI: 10.1167/iovs.05-0531]

97 **Yüksel B**, Karti Ö, Kusbeci T. Topical nepafenac for prevention of post-cataract surgery macular edema in diabetic patients: patient selection and perspectives. *Clin Ophthalmol* 2017; **11**: 2183-2190 [PMID: 29269999 DOI: 10.2147/OPTH.S132810]

98 **Garrido-Mesa N**, Zarzuelo A, Gálvez J. Minocycline: far beyond an antibiotic. *Br J Pharmacol* 2013; **169**: 337-352 [PMID: 23441623 DOI: 10.1111/bph.12139]

99 **Krady JK**, Basu A, Allen CM, Xu Y, LaNoue KF, Gardner TW, Levison SW. Minocycline reduces proinflammatory cytokine expression, microglial activation, and caspase-3 activation in a rodent model of diabetic retinopathy. *Diabetes* 2005; **54**: 1559-1565 [PMID: 15855346 DOI: 10.2337/diabetes.54.5.1559]

100 **Bernardino AL**, Kaushal D, Philipp MT. The antibiotics doxycycline and minocycline inhibit the inflammatory responses to the Lyme disease spirochete Borrelia burgdorferi. *J Infect Dis* 2009; **199**: 1379-1388 [PMID: 19301981 DOI: 10.1086/597807]

101 **Cukras CA**, Petrou P, Chew EY, Meyerle CB, Wong WT. Oral minocycline for the treatment of diabetic macular edema (DME): results of a phase I/II clinical study. *Invest Ophthalmol Vis Sci* 2012; **53**: 3865-3874 [PMID: 22589436 DOI: 10.1167/iovs.11-9413]

102 **Scott IU**, Jackson GR, Quillen DA, Larsen M, Klein R, Liao J, Holfort S, Munch IC, Gardner TW. Effect of doxycycline vs placebo on retinal function and diabetic retinopathy progression in patients with severe nonproliferative or non-high-risk proliferative diabetic retinopathy: a randomized clinical trial. *JAMA Ophthalmol* 2014; **132**: 535-543 [PMID: 24604308 DOI: 10.1001/jamaophthalmol.2014.93]

103 **Karkhur S**, Hasanreisoglu M, Vigil E, Halim MS, Hassan M, Plaza C, Nguyen NV, Afridi R, Tran AT, Do DV, Sepah YJ, Nguyen QD. Interleukin-6 inhibition in the management of non-infectious uveitis and beyond. *J Ophthalmic Inflamm Infect* 2019; **9**: 17 [PMID: 31523783 DOI: 10.1186/s12348-019-0182-y]

104 **Tsilimbaris MK**, Panagiotoglou TD, Charisis SK, Anastasakis A, Krikonis TS, Christodoulakis E. The use of intravitreal etanercept in diabetic macular oedema. *Semin Ophthalmol* 2007; **22**: 75-79 [PMID: 17564925 DOI: 10.1080/08820530701418243]

105 **Stahel M**, Becker M, Graf N, Michels S. SYSTEMIC INTERLEUKIN 1β INHIBITION IN PROLIFERATIVE DIABETIC RETINOPATHY: A Prospective Open-Label Study Using Canakinumab. *Retina* 2016; **36**: 385-391 [PMID: 26218500 DOI: 10.1097/IAE.0000000000000701]

106 **Ahmed N**. Advanced glycation endproducts--role in pathology of diabetic complications. *Diabetes Res Clin Pract* 2005; **67**: 3-21 [PMID: 15620429 DOI: 10.1016/j.diabres.2004.09.004]

107 **Gkogkolou P**, Böhm M. Advanced glycation end products: Key players in skin aging? *Dermatoendocrinol* 2012; **4**: 259-270 [PMID: 23467327 DOI: 10.4161/derm.22028]

108 **Aragonès G**, Rowan S, G Francisco S, Yang W, Weinberg J, Taylor A, Bejarano E. Glyoxalase System as a Therapeutic Target against Diabetic Retinopathy. *Antioxidants (Basel)* 2020; **9** [PMID: 33143048 DOI: 10.3390/antiox9111062]

109 **Xu J**, Chen LJ, Yu J, Wang HJ, Zhang F, Liu Q, Wu J. Involvement of Advanced Glycation End Products in the Pathogenesis of Diabetic Retinopathy. *Cell Physiol Biochem* 2018; **48**: 705-717 [PMID: 30025404 DOI: 10.1159/000491897]

110 **Kowluru RA**, Kanwar M. Effects of curcumin on retinal oxidative stress and inflammation in diabetes. *Nutr Metab (Lond)* 2007; **4**: 8 [PMID: 17437639 DOI: 10.1186/1743-7075-4-8]

111 **Sajithlal GB**, Chithra P, Chandrakasan G. Effect of curcumin on the advanced glycation and cross-linking of collagen in diabetic rats. *Biochem Pharmacol* 1998; **56**: 1607-1614 [PMID: 9973181 DOI: 10.1016/s0006-2952(98)00237-8]

112 **Sampath C**, Rashid MR, Sang S, Ahmedna M. Green tea epigallocatechin 3-gallate alleviates hyperglycemia and reduces advanced glycation end products via nrf2 pathway in mice with high fat diet-induced obesity. *Biomed Pharmacother* 2017; **87**: 73-81 [PMID: 28040599 DOI: 10.1016/j.biopha.2016.12.082]

113 **Bhuiyan MN**, Mitsuhashi S, Sigetomi K, Ubukata M. Quercetin inhibits advanced glycation end product formation via chelating metal ions, trapping methylglyoxal, and trapping reactive oxygen species. *Biosci Biotechnol Biochem* 2017; **81**: 882-890 [PMID: 28388357 DOI: 10.1080/09168451.2017.1282805]

114 **Kishore L**, Kaur N, Singh R. Effect of Kaempferol isolated from seeds of Eruca sativa on changes of pain sensitivity in Streptozotocin-induced diabetic neuropathy. *Inflammopharmacology* 2018; **26**: 993-1003 [PMID: 29159712 DOI: 10.1007/s10787-017-0416-2]

115 **Hajizadeh-Sharafabad F**, Sahebkar A, Zabetian-Targhi F, Maleki V. The impact of resveratrol on toxicity and related complications of advanced glycation end products: A systematic review. *Biofactors* 2019; **45**: 651-665 [PMID: 31185146 DOI: 10.1002/biof.1531]

116 **Bolton WK**, Cattran DC, Williams ME, Adler SG, Appel GB, Cartwright K, Foiles PG, Freedman BI, Raskin P, Ratner RE, Spinowitz BS, Whittier FC, Wuerth JP; ACTION I Investigator Group. Randomized trial of an inhibitor of formation of advanced glycation end products in diabetic nephropathy. *Am J Nephrol* 2004; **24**: 32-40 [PMID: 14685005 DOI: 10.1159/000075627]

117 **Hammes HP**, Brownlee M, Edelstein D, Saleck M, Martin S, Federlin K. Aminoguanidine inhibits the development of accelerated diabetic retinopathy in the spontaneous hypertensive rat. *Diabetologia* 1994; **37**: 32-35 [PMID: 8150227 DOI: 10.1007/BF00428774]

118 **Gabbay KH**. Hyperglycemia, polyol metabolism, and complications of diabetes mellitus. *Annu Rev Med* 1975; **26**: 521-536 [PMID: 238458 DOI: 10.1146/annurev.me.26.020175.002513]

119 **Gabbay KH**. The sorbitol pathway and the complications of diabetes. *N Engl J Med* 1973; **288**: 831-836 [PMID: 4266466 DOI: 10.1056/NEJM197304192881609]

120 **Hotta N**, Kawamori R, Fukuda M, Shigeta Y; Aldose Reductase Inhibitor-Diabetes Complications Trial Study Group. Long-term clinical effects of epalrestat, an aldose reductase inhibitor, on progression of diabetic neuropathy and other microvascular complications: multivariate epidemiological analysis based on patient background factors and severity of diabetic neuropathy. *Diabet Med* 2012; **29**: 1529-1533 [PMID: 22507139 DOI: 10.1111/j.1464-5491.2012.03684.x]

121. A randomized trial of sorbinil, an aldose reductase inhibitor, in diabetic retinopathy. Sorbinil Retinopathy Trial Research Group. *Arch Ophthalmol* 1990; **108**: 1234-1244 [PMID: 2119168 DOI: 10.1001/archopht.1990.01070110050024]

122 **Dodda D**, Rama Rao A, Veeresham C. In vitro and in vivo evaluation of pterostilbene for the management of diabetic complications. *J Ayurveda Integr Med* 2020; **11**: 369-375 [PMID: 30459079 DOI: 10.1016/j.jaim.2018.01.003]

123 **Yang Y**, Hayden MR, Sowers S, Bagree SV, Sowers JR. Retinal redox stress and remodeling in cardiometabolic syndrome and diabetes. *Oxid Med Cell Longev* 2010; **3**: 392-403 [PMID: 21307645 DOI: 10.4161/oxim.3.6.14786]

124 **Horal M**, Zhang Z, Stanton R, Virkamäki A, Loeken MR. Activation of the hexosamine pathway causes oxidative stress and abnormal embryo gene expression: involvement in diabetic teratogenesis. *Birth Defects Res A Clin Mol Teratol* 2004; **70**: 519-527 [PMID: 15329829 DOI: 10.1002/bdra.20056]

125 **Kaneto H**, Xu G, Song KH, Suzuma K, Bonner-Weir S, Sharma A, Weir GC. Activation of the hexosamine pathway leads to deterioration of pancreatic beta-cell function through the induction of oxidative stress. *J Biol Chem* 2001; **276**: 31099-31104 [PMID: 11390407 DOI: 10.1074/jbc.M104115200]

126 **Goldberg H**, Whiteside C, Fantus IG. O-linked β-N-acetylglucosamine supports p38 MAPK activation by high glucose in glomerular mesangial cells. *Am J Physiol Endocrinol Metab* 2011; **301**: E713-E726 [PMID: 21712532 DOI: 10.1152/ajpendo.00108.2011]

127 **Ighodaro OM**. Molecular pathways associated with oxidative stress in diabetes mellitus. *Biomed Pharmacother* 2018; **108**: 656-662 [PMID: 30245465 DOI: 10.1016/j.biopha.2018.09.058]

128 **Liu RM**, Desai LP. Reciprocal regulation of TGF-β and reactive oxygen species: A perverse cycle for fibrosis. *Redox Biol* 2015; **6**: 565-577 [PMID: 26496488 DOI: 10.1016/j.redox.2015.09.009]

129 **Rajapakse AG**, Ming XF, Carvas JM, Yang Z. The hexosamine biosynthesis inhibitor azaserine prevents endothelial inflammation and dysfunction under hyperglycemic condition through antioxidant effects. *Am J Physiol Heart Circ Physiol* 2009; **296**: H815-H822 [PMID: 19136606 DOI: 10.1152/ajpheart.00756.2008]

130 **Zheng JM**, Zhu JM, Li LS, Liu ZH. Rhein reverses the diabetic phenotype of mesangial cells over-expressing the glucose transporter (GLUT1) by inhibiting the hexosamine pathway. *Br J Pharmacol* 2008; **153**: 1456-1464 [PMID: 18264122 DOI: 10.1038/bjp.2008.26]

131 **Hammes HP**, Du X, Edelstein D, Taguchi T, Matsumura T, Ju Q, Lin J, Bierhaus A, Nawroth P, Hannak D, Neumaier M, Bergfeld R, Giardino I, Brownlee M. Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. *Nat Med* 2003; **9**: 294-299 [PMID: 12592403 DOI: 10.1038/nm834]

132 **Geraldes P**, King GL. Activation of protein kinase C isoforms and its impact on diabetic complications. *Circ Res* 2010; **106**: 1319-1331 [PMID: 20431074 DOI: 10.1161/CIRCRESAHA.110.217117]

133 **Zong H**, Ward M, Stitt AW. AGEs, RAGE, and diabetic retinopathy. *Curr Diab Rep* 2011; **11**: 244-252 [PMID: 21590515 DOI: 10.1007/s11892-011-0198-7]

134 **Koya D**, King GL. Protein kinase C activation and the development of diabetic complications. *Diabetes* 1998; **47**: 859-866 [PMID: 9604860 DOI: 10.2337/diabetes.47.6.859]

135 **Lei S**, Su W, Liu H, Xu J, Xia ZY, Yang QJ, Qiao X, Du Y, Zhang L, Xia Z. Nitroglycerine-induced nitrate tolerance compromises propofol protection of the endothelial cells against TNF-α: the role of PKC-β2 and NADPH oxidase. *Oxid Med Cell Longev* 2013; **2013**: 678484 [PMID: 24396568 DOI: 10.1155/2013/678484]

136 **Chalupsky K**, Cai H. Endothelial dihydrofolate reductase: critical for nitric oxide bioavailability and role in angiotensin II uncoupling of endothelial nitric oxide synthase. *Proc Natl Acad Sci USA* 2005; **102**: 9056-9061 [PMID: 15941833 DOI: 10.1073/pnas.0409594102]

137 **Kowluru RA**, Abbas SN. Diabetes-induced mitochondrial dysfunction in the retina. *Invest Ophthalmol Vis Sci* 2003; **44**: 5327-5334 [PMID: 14638734 DOI: 10.1167/iovs.03-0353]

138 **Geraldes P**, Hiraoka-Yamamoto J, Matsumoto M, Clermont A, Leitges M, Marette A, Aiello LP, Kern TS, King GL. Activation of PKC-delta and SHP-1 by hyperglycemia causes vascular cell apoptosis and diabetic retinopathy. *Nat Med* 2009; **15**: 1298-1306 [PMID: 19881493 DOI: 10.1038/nm.2052]

139 **Enge M**, Bjarnegård M, Gerhardt H, Gustafsson E, Kalén M, Asker N, Hammes HP, Shani M, Fässler R, Betsholtz C. Endothelium-specific platelet-derived growth factor-B ablation mimics diabetic retinopathy. *EMBO J* 2002; **21**: 4307-4316 [PMID: 12169633 DOI: 10.1093/emboj/cdf418]

140 **Jiang Y**, Zhang Q, Steinle JJ. Beta-adrenergic receptor agonist decreases VEGF levels through altered eNOS and PKC signaling in diabetic retina. *Growth Factors* 2015; **33**: 192-199 [PMID: 26115368 DOI: 10.3109/08977194.2015.1054990]

141 **Gálvez MI**. Rubosixtaurin and other PKC inhibitors in diabetic retinopathy and macular edema. Review. *Curr Diabetes Rev* 2009; **5**: 14-17 [PMID: 19199893 DOI: 10.2174/157339909787314167]

142 **Deissler HL**, Lang GE. The Protein Kinase C Inhibitor: Ruboxistaurin. *Dev Ophthalmol* 2016; **55**: 295-301 [PMID: 26501476 DOI: 10.1159/000431204]

143 **Eynard AR**, Repossi G. Role of ω3 polyunsaturated fatty acids in diabetic retinopathy: a morphological and metabolically cross talk among blood retina barriers damage, autoimmunity and chronic inflammation. *Lipids Health Dis* 2019; **18**: 114 [PMID: 31092270 DOI: 10.1186/s12944-019-1049-9]

144 **Liu A**, Chang J, Lin Y, Shen Z, Bernstein PS. Long-chain and very long-chain polyunsaturated fatty acids in ocular aging and age-related macular degeneration. *J Lipid Res* 2010; **51**: 3217-3229 [PMID: 20688753 DOI: 10.1194/jlr.M007518]

145 **Skowronska-Krawczyk D**, Chao DL. Long-Chain Polyunsaturated Fatty Acids and Age-Related Macular Degeneration. *Adv Exp Med Biol* 2019; **1185**: 39-43 [PMID: 31884586 DOI: 10.1007/978-3-030-27378-1\_7]

146 **Tanito M,** Anderson RE. Dual roles of polyunsaturated fatty acids in retinal physiology and pathophysiology associated with retinal degeneration. *Clin Lipidol* 2009; **4**: 821-827 [DOI: 10.2217/clp.09.65]

147 **Suzumura A**, Terao R, Kaneko H. Protective Effects and Molecular Signaling of n-3 Fatty Acids on Oxidative Stress and Inflammation in Retinal Diseases. *Antioxidants* (Basel) 2020; **9**: 920 [PMID: 32993153 DOI: 10.3390/antiox9100920]

148 **Behl T**, Kotwani A. Omega-3 fatty acids in prevention of diabetic retinopathy. *J Pharm Pharmacol* 2017; **69**: 946-954 [PMID: 28481011 DOI: 10.1111/jphp.12744]

149 **SanGiovanni JP**, Chew EY. The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina. *Prog Retin Eye Res* 2005; **24**: 87-138 [PMID: 15555528 DOI: 10.1016/j.preteyeres.2004.06.002]

150 **Nowak JZ**. Oxidative stress, polyunsaturated fatty acids-derived oxidation products and bisretinoids as potential inducers of CNS diseases: focus on age-related macular degeneration. *Pharmacol Rep* 2013; **65**: 288-304 [PMID: 23744414 DOI: 10.1016/s1734-1140(13)71005-3]

151 **Polak M**, Zagórski Z. Lipid peroxidation in diabetic retinopathy. *Ann Univ Mariae Curie Sklodowska Med* 2004; **59**: 434-437 [PMID: 16146026]

152 **Mondal LK**, Bhaduri G, Bhattacharya B. Biochemical scenario behind initiation of diabetic retinopathy in type 2 diabetes mellitus. *Indian J Ophthalmol* 2018; **66**: 535-540 [PMID: 29582815 DOI: 10.4103/ijo.IJO\_1121\_17]

153 **Pan HZ**, Zhang H, Chang D, Li H, Sui H. The change of oxidative stress products in diabetes mellitus and diabetic retinopathy. *Br J Ophthalmol* 2008; **92**: 548-551 [PMID: 18369071 DOI: 10.1136/bjo.2007.130542]

154 **Al-Shabrawey M**, Bartoli M, El-Remessy AB, Ma G, Matragoon S, Lemtalsi T, Caldwell RW, Caldwell RB. Role of NADPH oxidase and Stat3 in statin-mediated protection against diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2008; **49**: 3231-3238 [PMID: 18378570 DOI: 10.1167/iovs.08-1754]

155 **Al-Shabrawey M**, Bartoli M, El-Remessy AB, Platt DH, Matragoon S, Behzadian MA, Caldwell RW, Caldwell RB. Inhibition of NAD(P)H oxidase activity blocks vascular endothelial growth factor overexpression and neovascularization during ischemic retinopathy. *Am J Pathol* 2005; **167**: 599-607 [PMID: 16049343 DOI: 10.1016/S0002-9440(10)63001-5]

156 **Armstrong D**, al-Awadi F. Lipid peroxidation and retinopathy in streptozotocin-induced diabetes. *Free Radic Biol Med* 1991; **11**: 433-436 [PMID: 1797628 DOI: 10.1016/0891-5849(91)90161-u]

157 **Davì G**, Ciabattoni G, Consoli A, Mezzetti A, Falco A, Santarone S, Pennese E, Vitacolonna E, Bucciarelli T, Costantini F, Capani F, Patrono C. In vivo formation of 8-iso-prostaglandin f2alpha and platelet activation in diabetes mellitus: effects of improved metabolic control and vitamin E supplementation. *Circulation* 1999; **99**: 224-229 [PMID: 9892587 DOI: 10.1161/01.cir.99.2.224]

158 **Augustin AJ**, Spitznas M, Koch F, Grus F, Böker T. Indicators of oxidative tissue damage and inflammatory activity in epiretinal membranes of proliferative diabetic retinopathy, proliferative vitreoretinopathy and macular pucker. *Ger J Ophthalmol* 1995; **4**: 47-51 [PMID: 7728110]

159 **Ayalasomayajula SP**, Kompella UB. Celecoxib, a selective cyclooxygenase-2 inhibitor, inhibits retinal vascular endothelial growth factor expression and vascular leakage in a streptozotocin-induced diabetic rat model. *Eur J Pharmacol* 2003; **458**: 283-289 [PMID: 12504784 DOI: 10.1016/s0014-2999(02)02793-0]

160 **Li T**, Hu J, Du S, Chen Y, Wang S, Wu Q. ERK1/2/COX-2/PGE2 signaling pathway mediates GPR91-dependent VEGF release in streptozotocin-induced diabetes. *Mol Vis* 2014; **20**: 1109-1121 [PMID: 25324681]

161 **Njie-Mbye YF**, Kulkarni-Chitnis M, Opere CA, Barrett A, Ohia SE. Lipid peroxidation: pathophysiological and pharmacological implications in the eye. *Front Physiol* 2013; **4**: 366 [PMID: 24379787 DOI: 10.3389/fphys.2013.00366]

162 **Esterbauer H**, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic Biol Med* 1991; **11**: 81-128 [PMID: 1937131 DOI: 10.1016/0891-5849(91)90192-6]

163 **Van Kuijk FJ**, Holte LL, Dratz EA. 4-Hydroxyhexenal: a lipid peroxidation product derived from oxidized docosahexaenoic acid. *Biochim Biophys Acta* 1990; **1043**: 116-118 [PMID: 2138035 DOI: 10.1016/0005-2760(90)90118-h]

164 **Esterbauer H**. Cytotoxicity and genotoxicity of lipid-oxidation products. *Am J Clin Nutr* 1993; **57**: 779S-785S; discussion 785S-786S [PMID: 8475896 DOI: 10.1093/ajcn/57.5.779S]

165 **Csala M**, Kardon T, Legeza B, Lizák B, Mandl J, Margittai É, Puskás F, Száraz P, Szelényi P, Bánhegyi G. On the role of 4-hydroxynonenal in health and disease. *Biochim Biophys Acta* 2015; **1852**: 826-838 [PMID: 25643868 DOI: 10.1016/j.bbadis.2015.01.015]

166 **Zhou T**, Zhou KK, Lee K, Gao G, Lyons TJ, Kowluru R, Ma JX. The role of lipid peroxidation products and oxidative stress in activation of the canonical wingless-type MMTV integration site (WNT) pathway in a rat model of diabetic retinopathy. *Diabetologia* 2011; **54**: 459-468 [PMID: 20978740 DOI: 10.1007/s00125-010-1943-1]

167 **Chen Y**, Hu Y, Zhou T, Zhou KK, Mott R, Wu M, Boulton M, Lyons TJ, Gao G, Ma JX. Activation of the Wnt pathway plays a pathogenic role in diabetic retinopathy in humans and animal models. *Am J Pathol* 2009; **175**: 2676-2685 [PMID: 19893025 DOI: 10.2353/ajpath.2009.080945]

168 **Cheng R**, Ding L, He X, Takahashi Y, Ma JX. Interaction of PPARα With the Canonic Wnt Pathway in the Regulation of Renal Fibrosis. *Diabetes* 2016; **65**: 3730-3743 [PMID: 27543085 DOI: 10.2337/db16-0426]

169 **Yao PL**, Peavey J, Malek G. Leveraging Nuclear Receptors as Targets for Pathological Ocular Vascular Diseases. *Int J Mol Sci* 2020; **21** [PMID: 32326149 DOI: 10.3390/ijms21082889]

170 **ACCORD Study Group.**; ACCORD Eye Study Group, Chew EY, Ambrosius WT, Davis MD, Danis RP, Gangaputra S, Greven CM, Hubbard L, Esser BA, Lovato JF, Perdue LH, Goff DC Jr, Cushman WC, Ginsberg HN, Elam MB, Genuth S, Gerstein HC, Schubart U, Fine LJ. Effects of medical therapies on retinopathy progression in type 2 diabetes. *N Engl J Med* 2010; **363**: 233-244 [PMID: 20587587 DOI: 10.1056/NEJMoa1001288]

171 **Ding L**, Cheng R, Hu Y, Takahashi Y, Jenkins AJ, Keech AC, Humphries KM, Gu X, Elliott MH, Xia X, Ma JX. Peroxisome proliferator-activated receptor α protects capillary pericytes in the retina. *Am J Pathol* 2014; **184**: 2709-2720 [PMID: 25108226 DOI: 10.1016/j.ajpath.2014.06.021]

172 **Hu Y**, Chen Y, Ding L, He X, Takahashi Y, Gao Y, Shen W, Cheng R, Chen Q, Qi X, Boulton ME, Ma JX. Pathogenic role of diabetes-induced PPAR-α down-regulation in microvascular dysfunction. *Proc Natl Acad Sci USA* 2013; **110**: 15401-15406 [PMID: 24003152 DOI: 10.1073/pnas.1307211110]

173 **Sharma A**, Sharma R, Chaudhary P, Vatsyayan R, Pearce V, Jeyabal PV, Zimniak P, Awasthi S, Awasthi YC. 4-Hydroxynonenal induces p53-mediated apoptosis in retinal pigment epithelial cells. *Arch Biochem Biophys* 2008; **480**: 85-94 [PMID: 18930016 DOI: 10.1016/j.abb.2008.09.016]

174 **Mori A**, Takei T, Sakamoto K, Nakahara T, Ishii K. 4-Hydroxy-2-nonenal attenuates β2-adrenoceptor-mediated vasodilation of rat retinal arterioles. *Naunyn Schmiedebergs Arch Pharmacol* 2015; **388**: 575-582 [PMID: 25693977 DOI: 10.1007/s00210-015-1099-0]

175 **Chiang YF**, Chen HY, Chang YJ, Shih YH, Shieh TM, Wang KL, Hsia SM. Protective Effects of Fucoxanthin on High Glucose- and 4-Hydroxynonenal (4-HNE)-Induced Injury in Human Retinal Pigment Epithelial Cells. *Antioxidants (Basel)* 2020; **9** [PMID: 33255669 DOI: 10.3390/antiox9121176]

176 **Esterbauer H**, Cheeseman KH. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. *Methods Enzymol* 1990; **186**: 407-421 [PMID: 2233308 DOI: 10.1016/0076-6879(90)86134-h]

177 **Ayala A**, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid Med Cell Longev* 2014; **2014**: 360438 [PMID: 24999379 DOI: 10.1155/2014/360438]

178 **Cheeseman KH**, Beavis A, Esterbauer H. Hydroxyl-radical-induced iron-catalysed degradation of 2-deoxyribose. Quantitative determination of malondialdehyde. *Biochem J* 1988; **252**: 649-653 [PMID: 3421915 DOI: 10.1042/bj2520649]

179 **Morrow JD**, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts LJ 2nd. A series of prostaglandin F2-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. *Proc Natl Acad Sci USA* 1990; **87**: 9383-9387 [PMID: 2123555 DOI: 10.1073/pnas.87.23.9383]

180 **Gopaul NK**, Anggård EE, Mallet AI, Betteridge DJ, Wolff SP, Nourooz-Zadeh J. Plasma 8-epi-PGF2 alpha levels are elevated in individuals with non-insulin dependent diabetes mellitus. *FEBS Lett* 1995; **368**: 225-229 [PMID: 7628610 DOI: 10.1016/0014-5793(95)00649-t]

181 **Galano JM**, Lee YY, Oger C, Vigor C, Vercauteren J, Durand T, Giera M, Lee JC. Isoprostanes, neuroprostanes and phytoprostanes: An overview of 25years of research in chemistry and biology. *Prog Lipid Res* 2017; **68**: 83-108 [PMID: 28923590 DOI: 10.1016/j.plipres.2017.09.004]

182 **Van't Erve TJ**, Lih FB, Jelsema C, Deterding LJ, Eling TE, Mason RP, Kadiiska MB. Reinterpreting the best biomarker of oxidative stress: The 8-iso-prostaglandin F2α/prostaglandin F2α ratio shows complex origins of lipid peroxidation biomarkers in animal models. *Free Radic Biol Med* 2016; **95**: 65-73 [PMID: 26964509 DOI: 10.1016/j.freeradbiomed.2016.03.001]

183 **Lahaie I**, Hardy P, Hou X, Hasséssian H, Asselin P, Lachapelle P, Almazan G, Varma DR, Morrow JD, Roberts LJ 2nd, Chemtob S. A novel mechanism for vasoconstrictor action of 8-isoprostaglandin F2 alpha on retinal vessels. *Am J Physiol* 1998; **274**: R1406-R1416 [PMID: 9612409 DOI: 10.1152/ajpregu.1998.274.5.R1406]

184 **Hou X**, Roberts LJ 2nd, Gobeil F Jr, Taber D, Kanai K, Abran D, Brault S, Checchin D, Sennlaub F, Lachapelle P, Varma D, Chemtob S. Isomer-specific contractile effects of a series of synthetic f2-isoprostanes on retinal and cerebral microvasculature. *Free Radic Biol Med* 2004; **36**: 163-172 [PMID: 14744628 DOI: 10.1016/j.freeradbiomed.2003.10.024]

185 **Torres-Cuevas I**, Millán I, Asensi M, Vento M, Oger C, Galano JM, Durand T, Ortega ÁL. Analysis of Lipid Peroxidation by UPLC-MS/MS and Retinoprotective Effects of the Natural Polyphenol Pterostilbene. *Antioxidants (Basel)* 2021; **10** [PMID: 33498744 DOI: 10.3390/antiox10020168]

186 **Simó R**, Hernández C. GLP-1R as a Target for the Treatment of Diabetic Retinopathy: Friend or Foe? *Diabetes* 2017; **66**: 1453-1460 [PMID: 28533296 DOI: 10.2337/db16-1364]

187 **Hölscher C**. Potential role of glucagon-like peptide-1 (GLP-1) in neuroprotection. *CNS Drugs* 2012; **26**: 871-882 [PMID: 22938097 DOI: 10.2165/11635890-000000000-00000]

188 **Hernández C**, Bogdanov P, Corraliza L, García-Ramírez M, Solà-Adell C, Arranz JA, Arroba AI, Valverde AM, Simó R. Topical Administration of GLP-1 Receptor Agonists Prevents Retinal Neurodegeneration in Experimental Diabetes. *Diabetes* 2016; **65**: 172-187 [PMID: 26384381 DOI: 10.2337/db15-0443]

189 **Zeng Y**, Yang K, Wang F, Zhou L, Hu Y, Tang M, Zhang S, Jin S, Zhang J, Wang J, Li W, Lu L, Xu GT. The glucagon like peptide 1 analogue, exendin-4, attenuates oxidative stress-induced retinal cell death in early diabetic rats through promoting Sirt1 and Sirt3 expression. *Exp Eye Res* 2016; **151**: 203-211 [PMID: 27212443 DOI: 10.1016/j.exer.2016.05.002]

190 **Fan Y**, Liu K, Wang Q, Ruan Y, Ye W, Zhang Y. Exendin-4 alleviates retinal vascular leakage by protecting the blood-retinal barrier and reducing retinal vascular permeability in diabetic Goto-Kakizaki rats. *Exp Eye Res* 2014; **127**: 104-116 [PMID: 24910901 DOI: 10.1016/j.exer.2014.05.004]

191 **Cross DA**, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* 1995; **378**: 785-789 [PMID: 8524413 DOI: 10.1038/378785a0]

192 **Manning BD**, Toker A. AKT/PKB Signaling: Navigating the Network. *Cell* 2017; **169**: 381-405 [PMID: 28431241 DOI: 10.1016/j.cell.2017.04.001]

193 **Rowlands J**, Heng J, Newsholme P, Carlessi R. Pleiotropic Effects of GLP-1 and Analogs on Cell Signaling, Metabolism, and Function. *Front Endocrinol (Lausanne)* 2018; **9**: 672 [PMID: 30532733 DOI: 10.3389/fendo.2018.00672]

194 **Kowluru RA**. Diabetic retinopathy, metabolic memory and epigenetic modifications. *Vision Res* 2017; **139**: 30-38 [PMID: 28700951 DOI: 10.1016/j.visres.2017.02.011]

195 **García-Giménez JL**, Mena-Mollá S, Beltrán-García J, Sanchis-Gomar F. Challenges in the analysis of epigenetic biomarkers in clinical samples. *Clin Chem Lab Med* 2017; **55**: 1474-1477 [PMID: 28301317 DOI: 10.1515/cclm-2016-1162]

196 **Zhong Q**, Kowluru RA. Role of histone acetylation in the development of diabetic retinopathy and the metabolic memory phenomenon. *J Cell Biochem* 2010; **110**: 1306-1313 [PMID: 20564224 DOI: 10.1002/jcb.22644]

197 **Olsen AS**, Sarras MP Jr, Leontovich A, Intine RV. Heritable transmission of diabetic metabolic memory in zebrafish correlates with DNA hypomethylation and aberrant gene expression. *Diabetes* 2012; **61**: 485-491 [PMID: 22228713 DOI: 10.2337/db11-0588]

198 **Duraisamy AJ**, Mishra M, Kowluru A, Kowluru RA. Epigenetics and Regulation of Oxidative Stress in Diabetic Retinopathy. *Invest Ophthalmol Vis Sci* 2018; **59**: 4831-4840 [PMID: 30347077 DOI: 10.1167/iovs.18-24548]

199 **Sahajpal N**, Kowluru A, Kowluru RA. The Regulatory Role of Rac1, a Small Molecular Weight GTPase, in the Development of Diabetic Retinopathy. *J Clin Med* 2019; **8** [PMID: 31277234 DOI: 10.3390/jcm8070965]

200 **Kowluru RA**, Kowluru A, Veluthakal R, Mohammad G, Syed I, Santos JM, Mishra M. TIAM1-RAC1 signalling axis-mediated activation of NADPH oxidase-2 initiates mitochondrial damage in the development of diabetic retinopathy. *Diabetologia* 2014; **57**: 1047-1056 [PMID: 24554007 DOI: 10.1007/s00125-014-3194-z]

201 **Kreuz S**, Fischle W. Oxidative stress signaling to chromatin in health and disease. *Epigenomics* 2016; **8**: 843-862 [PMID: 27319358 DOI: 10.2217/epi-2016-0002]

202 **Mishra M**, Kowluru RA. The Role of DNA Methylation in the Metabolic Memory Phenomenon Associated With the Continued Progression of Diabetic Retinopathy. *Invest Ophthalmol Vis Sci* 2016; **57**: 5748-5757 [PMID: 27787562 DOI: 10.1167/iovs.16-19759]

203 **Xie MY**, Yang Y, Liu P, Luo Y, Tang SB. 5-aza-2'-deoxycytidine in the regulation of antioxidant enzymes in retinal endothelial cells and rat diabetic retina. *Int J Ophthalmol* 2019; **12**: 1-7 [PMID: 30662833 DOI: 10.18240/ijo.2019.01.01]

204 **Kadiyala CS**, Zheng L, Du Y, Yohannes E, Kao HY, Miyagi M, Kern TS. Acetylation of retinal histones in diabetes increases inflammatory proteins: effects of minocycline and manipulation of histone acetyltransferase (HAT) and histone deacetylase (HDAC). *J Biol Chem* 2012; **287**: 25869-25880 [PMID: 22648458 DOI: 10.1074/jbc.M112.375204]

205 **Corso-Díaz X**, Jaeger C, Chaitankar V, Swaroop A. Epigenetic control of gene regulation during development and disease: A view from the retina. *Prog Retin Eye Res* 2018; **65**: 1-27 [PMID: 29544768 DOI: 10.1016/j.preteyeres.2018.03.002]

206 **Gensous N**, Franceschi C, Santoro A, Milazzo M, Garagnani P, Bacalini MG. The Impact of Caloric Restriction on the Epigenetic Signatures of Aging. *Int J Mol Sci* 2019; **20** [PMID: 31022953 DOI: 10.3390/ijms20082022]

207 **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; **75**: 129-139 [PMID: 25016074 DOI: 10.1016/j.freeradbiomed.2014.07.001]

208 **Kowluru RA**, Mishra M. Contribution of epigenetics in diabetic retinopathy. *Sci China Life Sci* 2015; **58**: 556-563 [PMID: 26025281 DOI: 10.1007/s11427-015-4853-0]

209 **Zhang X**, Zhao L, Hambly B, Bao S, Wang K. Diabetic retinopathy: reversibility of epigenetic modifications and new therapeutic targets. *Cell Biosci* 2017; **7**: 42 [PMID: 28815013 DOI: 10.1186/s13578-017-0167-1]

210 **Kowluru RA**, Kowluru A, Mishra M, Kumar B. Oxidative stress and epigenetic modifications in the pathogenesis of diabetic retinopathy. *Prog Retin Eye Res* 2015; **48**: 40-61 [PMID: 25975734 DOI: 10.1016/j.preteyeres.2015.05.001]

211 **Jin J**, Wang X, Zhi X, Meng D. Epigenetic regulation in diabetic vascular complications. *J Mol Endocrinol* 2019; **63**: R103-R115 [PMID: 31600719 DOI: 10.1530/JME-19-0170]

212 **Lambert M**, Benmoussa A, Provost P. Small Noncoding RNAs Derived From Eukaryotic Ribosomal RNA. *Noncoding RNA* 2019; **5** [PMID: 30720712 DOI: 10.3390/ncrna5010016]

213 **Creugny A**, Fender A, Pfeffer S. Regulation of primary microRNA processing. *FEBS Lett* 2018; **592**: 1980-1996 [PMID: 29683487 DOI: 10.1002/1873-3468.13067]

214 **McArthur K**, Feng B, Wu Y, Chen S, Chakrabarti S. MicroRNA-200b regulates vascular endothelial growth factor-mediated alterations in diabetic retinopathy. *Diabetes* 2011; **60**: 1314-1323 [PMID: 21357793 DOI: 10.2337/db10-1557]

215 **Zhu K**, Hu X, Chen H, Li F, Yin N, Liu AL, Shan K, Qin YW, Huang X, Chang Q, Xu GZ, Wang Z. Downregulation of circRNA DMNT3B contributes to diabetic retinal vascular dysfunction through targeting miR-20b-5p and BAMBI. *EBioMedicine* 2019; **49**: 341-353 [PMID: 31636010 DOI: 10.1016/j.ebiom.2019.10.004]

216 **Yu CY**, Kuo HC. The emerging roles and functions of circular RNAs and their generation. *J Biomed Sci* 2019; **26**: 29 [PMID: 31027496 DOI: 10.1186/s12929-019-0523-z]

**Footnotes**

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**Table 1 Summary of alterations, targets, and novel therapies**

|  |  |  |  |
| --- | --- | --- | --- |
| **Contributors in DR development** | **Retinal alterations** | **Targets** | **Possible novel therapies** |
| ROS accumulation | Low nuclear levels of Nrf2, antioxidant enzymes activities, and GLP1R expression. Retinal cell death, retinal ischemia, retinal neovascularization, DME | Nrf2 activation, Keap1 knockdown, inhibition and/or neutralization of ROS generation, GLP1R activation, reinforcement of the antioxidant defense system | Green tea polyphenols, resveratrol, curcumin, quercetin, tannins, pterostilbene, GLP1R agonist, RS9, dh404, triterpenoids, salvianolic acids, sulforaphane |
| Synthesis of proinflammatory molecules | Vascular permeability, BRB breakdown, capillary pericyte loss, neovascularization | Inhibition of inflammatory pathways | COX-2 inhibitors, tetracyclines (minocycline and doxycycline), IL-6 inhibitors (EBI-031 and tocilizumab), anti-TNF-α therapy, canakinumab (selective IL-1β antibody), fenofibrate (PPARα agonist) |
| Increased production of AGE/RAGE | Aberrant extracellular crosslinking of extracellular matrix proteins, increased vascular stiffness, release of proinflammatory cytokines and proangiogenic factors | Low the production of AGEs | Curcumin, epigallocatechin 3-gallate, quercetin, kaempferol and resveratrol |
| Activation of the polyol pathway | Retinal capillary osmotic damage and cell death | Inhibition of the polyol pathway | Pterostilbene |
| Increased flux through the hexosamine pathway | Neuro-vascular dysfunctions | Inhibition of the hexosamine pathway | Azaserine (antineoplastic), rhein (anthraquinone), benfotiamine (lipid-soluble thiamine derivative) |
| Activation of the PKC pathway | Endothelial alterations, cell demise of capillary cells and pericytes, formation of microaneurysms, VEGF-dependent retinal barrier alterations | Inhibition of PKC pathway | Ruboxistaurin mesylate (PKC-β inhibitor) |
| Lipid peroxidation | Generation of lipid radical species, apoptosis in retinal pigment epithelial cells, retinal vascular dysfunction, development of neovascularization | Inhibition of the formation of lipid peroxides in the retina | Fucoxanthin, pterostilbene |
| DNA methylation | Increased expression and activity of DNMTs | Inhibition of DNMTs | 5-aza-2'-deoxycytidine |
| Histone methylation and acetylation | Decreased levels of H3K4me1 and H3K4me3 at glutamate-cysteine ligase promoter or decreased levels of H3K9me2 and increased levels in acetyl H3K9 | Regulation of histone methylation/acetylation | Garcinol, resveratrol, curcumin, genistein |
| Regulation by ncRNA (miRNA and circular RNA) | Downregulation of miR126, miR-146a, and miR200b; retinal upregulation of miR-20b-5p, neovascularization and microvascular leakage | Modulation of miRNAs expression, overexpression of circular DNMT3B | siRNAs,  double-stranded miRNA mimics and anti-mRNA antisense oligodeoxyribonucleotide |

AGE/RAGE: Advanced glycation end products/receptors; BRB: Blood-retinal barrier; COX-2: Cyclooxygenase-2; dh404: Dihydro-CDDO-trifluoroethyl amide; DME: Diabetic macular edema; DNMT: DNA methyltransferases; GLP1R: Glucagon-like peptide-1 receptor; IL: Interleukin; Keap1: Kelch-like enoyl-CoA hydratase associated protein 1; miRNA: microRNA; ncRNA: noncoding RNAs; Nrf2: Nuclear factor erythroid 2-related factor 2; PKC: Protein kinase C; PPARα: Peroxisome proliferator-activated receptor α; ROS: Reactive oxygen species; TNF-α: Tumor necrosis factor α; VEGF: Vascular endothelial growth factor.