



Review

Glyoxalase System as a Therapeutic Target against Diabetic Retinopathy

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Abstract: Hyperglycemia, a defining characteristic of diabetes, combined with oxidative stress, results in the formation of advanced glycation end products (AGEs). AGEs are toxic compounds that have adverse effects on many tissues including the retina and lens. AGEs promote the formation of reactive oxygen species (ROS), which, in turn, boost the production of AGEs, resulting in positive feedback loops, a vicious cycle that compromises tissue fitness. Oxidative stress and the accumulation of AGEs are etiologically associated with the pathogenesis of multiple diseases including diabetic retinopathy (DR). DR is a devastating microvascular complication of diabetes mellitus and the leading cause of blindness in working-age adults. The onset and development of DR is multifactorial. Lowering AGEs accumulation may represent a potential therapeutic approach to slow this sight-threatening diabetic complication. To set DR in a physiological context, in this review we first describe relations between oxidative stress, formation of AGEs, and aging in several tissues of the eye, each of which is associated with a major age-related eye pathology. We summarize mechanisms of AGEs generation and anti-AGEs detoxifying systems. We specifically feature the potential of the glyoxalase system in the retina in the prevention of AGEs-associated damage linked to DR. We provide a comparative analysis of glyoxalase activity in different tissues from wild-type mice, supporting a major role for the glyoxalase system in the detoxification of AGEs in the retina, and present the manipulation of this system as a therapeutic strategy to prevent the onset of DR.

Keywords: diabetic retinopathy; oxidative stress; glycation; aging; glyoxalase

1. Oxidative Stress is Related to Many Age-Related Eye Diseases

Age-related eye diseases such as cataract, age-related macular degeneration (AMD), glaucoma, and diabetic retinopathy (DR) are the main causes of progressive and irreversible vision loss worldwide [1]. Loss of vision caused by these diseases diminishes quality of life [2–4]. The World Health Organization (WHO) reported that in 2010, there were 285 million people visually impaired, of which 39 million were blind, and that by 2050 it is estimated that the number will triple, exacerbating the enormous personal and public health burdens [5,6].

The pathogenesis of age-related eye diseases is complex and depends on many factors, some of which remain to be identified. However, it is clear that oxidative stress and the resultant dysfunctional cellular moieties are pathoetiologic for the development of age-related eye diseases [7–10].

Oxidative stress is defined as the generation of excess reactive oxygen species (ROS) beyond the capacity of the biological systems that detoxify these reactive free radicals [11]. Examples of ROS include hydrogen peroxide (H_2O_2), superoxide ($\text{O}_2^{\bullet-}$), and nitric oxide (NO). These imbalances lead to oxidative alterations of cellular macromolecular targets such as DNA, RNA, lipids, proteins, and carbohydrates, and eventually to dysfunction and degeneration of tissues [12–14]. In proteins, carbonyls are formed by the Fenton reaction of oxidants with lysine, arginine, proline, and threonine residues of the protein side chains [15]. Typical sequelae of oxidation are the formation of protein aggregates and impaired activity of many proteins, both structural and catalytic [16–19].

Although not the focus of this review, additional biological oxidation processes involve the formation of oxidized lipid metabolites such as hydroxynonenal, and the oxidation of lipids such as low-density lipoproteins in which both the protein and the lipids undergo oxidative changes that can cause cholesterol accumulation [20]. There is also oxidative damage to DNA resulting in several mutagenic lesions including 2-hydroxy adenine, 8-oxoadenine, 5-hydroxycytosine, cytosine glycol, thymine, and glycol [21].

A significant literature indicates that several eye tissues are particularly vulnerable to oxidative stress. Retinal photoreceptor cells and retinal ganglion cells have a large number of mitochondria, sustain high exposure to light and have a high rate of metabolism. As might be anticipated for a tissue in which light energy is transformed to chemical and then electrical impulses, which are chemically transmitted to the brain, retinal photoreceptor cells and retinal ganglion cells are susceptible to oxidative stress [8]. Outside the neurosensory retina, the retinal pigment epithelium (RPE) is also highly sensitive to photo-oxidative stress. The RPE is a single layer of cells located between photoreceptors and the choroid that plays key roles in the maintenance of photoreceptors. Oxidative stress is pathoetiologic in the RPE degeneration associated with the onset of AMD [9].

Oxidative stress is also pathoetiologic in other ocular tissues: (1) Damage to cell membrane fibers, lenticular proteins, photoreceptors, and DNA in most cells, (2) angiogenesis, endothelial dysfunction, and cell apoptosis, (3) loss of lens transparency by disrupting electrolyte balance homeostasis, and (4) increase of intraocular pressure and associated glaucoma [10]. Thus, it is not surprising that multiple ocular diseases have been linked to oxidative stress. These include retinitis pigmentosa, AMD, glaucoma, cataract, and others [12,22,23]. For example, intraocular pressure seems to increase with oxidative stress and accumulation of ROS in retinal ganglion cells [24]. In addition, glaucoma patients have diminished levels of antioxidant biomarkers such as vitamin E [23]. Crabb et al. using mass spectrometry, observed several oxidized proteins in drusen, extracellular deposits accumulated below the RPE on Bruch's membrane, from human AMD samples [25]. The accumulation of drusen is considered to be indicative of early AMD. Oxidative stress also plays a pathologic role in the onset and progression of cataracts. Crystallins, the major gene products in the eye lens, see sulfhydryls transformed to disulfides in cross-linked and aggregated cataractogenic moieties [26].

Based upon these associations between oxidative stress and the risk for cataract, AMD, glaucoma, and DR, many studies have tried to elucidate whether the dietary intake of nutrients with anti-oxidative properties could prevent different age-related eye diseases [27–30]. In the age-related eye diseases double-blinded placebo controlled study (AREDS), it was demonstrated that consumers of fruit and vegetable-rich diets, and people who consume supplements of vitamins C and E, as well as zinc and lutein (all involved in antioxidant function) are protected against AMD [27,31].

2. Advanced Glycation End Products: A Special Case of Oxidative Stress Found in Aged Eye Tissues and Throughout the Body

Advanced glycation end products (AGEs) are oxidation products of particular interest for this review because they are associated with multiple diseases of aging, including DR [32–37].

Dietary sugars or dicarbonyls generated from carbohydrate metabolism can be highly reactive and transform many biomolecules and structures through a process called glycation. This non-enzymatic process is initiated with the Maillard reaction, in which a reversible Schiff base is formed between the

carbonyl group of reducing sugars and free amino groups of proteins (Figure 1). These precursors undergo additional oxidations and rearrangements, resulting in the biogenesis of Amadori products. Depending on pH, Amadori products can rearrange to different types of dicarbonyls including 1,2-dicarbonyls such as methylglyoxal (MG) or 3-deoxyglucosone (3-DG), or 2,3-dicarbonyls such as 1-deoxyglucosone. The major glycating biologic reagent is MG, formed by the degradation of dihydroxyacetone phosphate and glyceraldehyde 3-phosphate, both glycolytic metabolites, as well as by the metabolism of threonine, the oxidation of ketone bodies, and upon degradation of glycated proteins [38]. Glyoxal and 3-DG are also highly reactive dicarbonyls formed during sugar metabolism [32,33]. These dicarbonyls are maintained at low levels under homeostatic conditions. Subsequent reactions result in methylglyoxal-derived hydroimidazolone 1 (MG-H1), N ϵ -carboxy-methyl-lysine (CML), N ϵ -carboxy-ethyl-lysine (CEL), pentosidine, glucosepane, and other types of AGEs [39] (Figure 1). As discussed later, AGE accumulation boosts the formation of ROS, resulting in increased production of AGEs. This vicious cycle of the oxidative formation of AGEs impacts cellular metabolism and contributes to hyperglycemia-induced tissue injury.

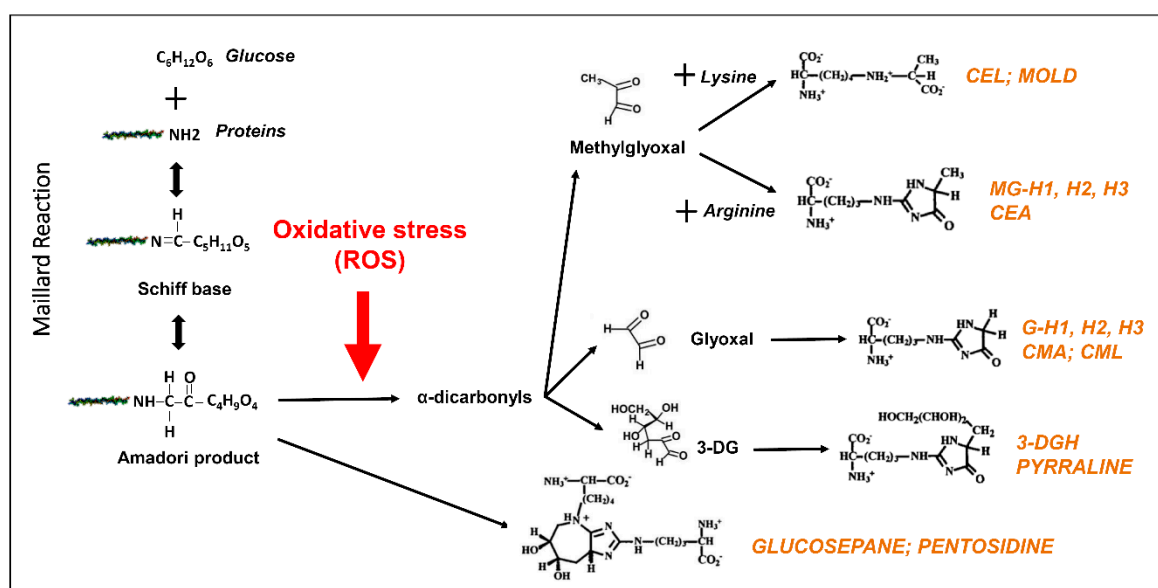


Figure 1. Production of advanced glycation end-products (AGEs) is accelerated under oxidative stress. AGEs are derived from sugars or dicarbonyls generated from carbohydrate metabolism through different chemical routes including the Maillard reaction. The excess of reactive oxidative species (ROS) promotes the production of different AGEs from dicarbonyls (methylglyoxal, glyoxal, or 3-deoxyglucosone (3-DG)). AGEs are highlighted in orange. CML: N ϵ -(carboxymethyl)-lysine; CMA: N ϵ -(carboxymethyl)-arginine; 3-DG: 3-deoxyglucosone; 3-DGH: GH-1,2,3: Glyoxal-derived hydroimidazolone; MGH-1,2,3: Methylglyoxal-derived hydroimidazolone; CEL: N ϵ -(carboxyethyl)-lysine; CEA: N ϵ -(carboxyethyl)-arginine; MOLD: Methylglyoxal-derived lysine dimer.

AGEs are accumulated throughout the body upon aging and particularly in diabetic patients. Three decades ago, different studies reported higher levels of different AGEs in diabetic tissues [40–43]. One of the best examples of glycation with aging is the extracellular matrix. Examination of interstitial collagen shows a gradual increase in AGEs upon aging [44] (Figure 2A). Another example of the relation between AGEs accumulation and aging occurs in eye tissues. Cataracts are perhaps the earliest example of pathobiology of AGEs in aged tissues [45]. Human lens crystallins become progressively yellow-brown pigmented with age as a result of the accumulation of Maillard products [46] (Figure 2B,C). The extensive modification of crystallins by glycation alters the dynamic state of crystallins, promotes their aggregation, and disrupts their chaperone function, contributing to cataractogenesis.

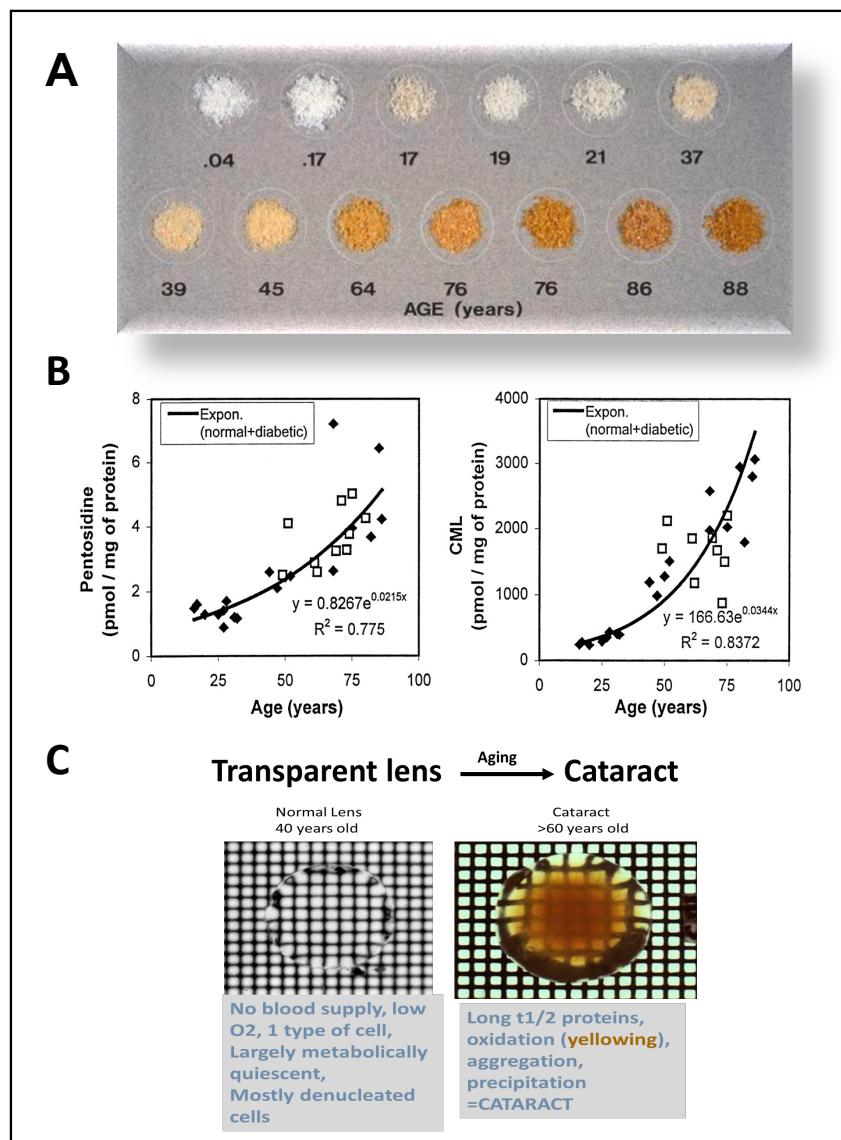


Figure 2. Aging promotes the accumulation of AGEs throughout the body. (A) Age-dependent changes in costal cartilage isolated at autopsy from donors of various ages (reprinted with permission of Dr. Baynes) [44]. (B) Level of pentosidine (left) and N ϵ -(carboxymethyl)-lysine (CML) (right) in lens crystallins from diabetic (■) and non-diabetic (♦) subjects as a function of age (reprinted with permission of Dr. Monnier) [46]. (C) Transillumination of isolated lenses: Normal lens from young donor (left) and cataractous lens from older donor (right). Lenses were placed in a culture dish that had a grid etched in its bottom surface. The dish was placed on the stage of a dissecting microscope and viewed with transmitted light. Note the degree of yellowing and opacity in the cataractous lens.

The deleterious effect of glycative damage is cell-type dependent and the molecular consequences of AGEs accumulation occur at different levels in eye tissues [47]. Regarding DR, the involvement of AGEs in the pathogenesis of the disease is complex. Glycation of the extracellular matrix results in decreased elasticity and increased vascular stiffness, leading to abnormal vascular function due to rigidity of the vessel wall. AGEs can also exert an indirect effect by binding to different cellular receptors in the plasma membrane, triggering intracellular pathways such as NF- κ B activation via Ras-MAPK or RhoA/ROCK pathways [48]. As a result, changes in intracellular signaling cascades and cytopathological responses are triggered that include releases of pro-inflammatory cytokines and pro-angiogenic factors. This also leads to ROS generation, pericyte apoptosis, vascular inflammation, angiogenesis, changes in vasopermeability, and compromised blood–retinal barrier [47] (Figure 3).

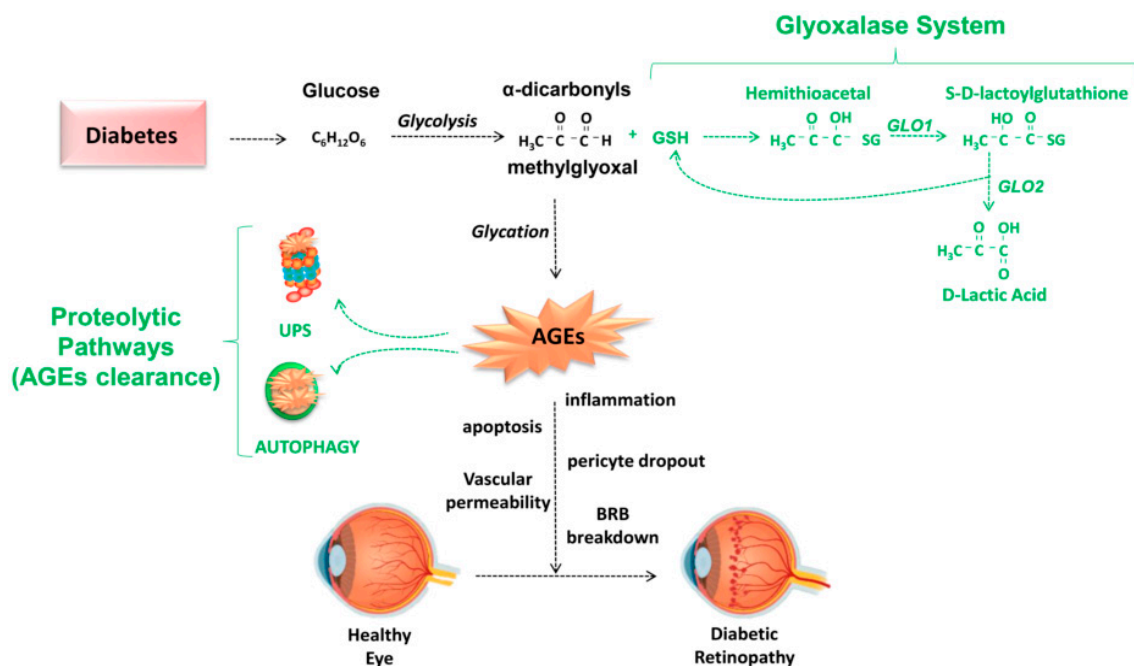


Figure 3. Schematic overview of detoxifying pathways against AGEs-derived damage in diabetic retinopathy (DR). Hyperglycemia-associated diabetes involves the abnormal formation of highly reactive α -dicarbonyls such as methylglyoxal which leads to accelerated AGEs formation. The glyoxalase system is a protective mechanism that slows down the synthesis of AGEs by limiting formation of dicarbonyls. Once formed, AGEs can be cleared by two proteolytic pathways: The ubiquitin-proteasome (UPS) system and autophagy. These protective mechanisms (highlighted in green) decline under diabetic conditions and with age. AGEs are pathologic features in the early stages of DR, impacting the function of neuroglial and vascular cells. AGEs-derived damage results in cellular and tissue dysfunction contributing to the onset of DR. BRB: Blood–retina barrier; GLO1: Glyoxalase 1; GLO2: Glyoxalase 2; GSH: Glutathione.

3. Role of Advanced Glycation End Products in the Pathogenesis of DR

Among the best studied pathologies related to oxidative stress are diabetes and DR, which occur in about 15% of those with long-lasting diabetes mellitus. DR is characterized by high levels of circulating sugars, high levels of oxidative stress, accumulation of AGEs, and microvascular damage [49]. DR is a leading cause of blindness in adults. During the early stage of DR, there is a loss of pericytes from capillaries, leading to the formation of acellular capillaries and retinal microaneurysms, along with thickening of the capillary basement membrane. Oxidative stress enhances damage to tight-junction complexes, causing vascular permeability, and blood–retinal barrier damage [50]. Together, these pathological changes result in irreversible damage to the blood–retinal barrier. Once the disease progresses to a late stage, neovascularization and bleeding can occur along with retinal detachment and macular edema, ultimately resulting in vision loss [51].

Several lines of evidence point to a relationship between oxidative stress and glycation-derived damage. Urinary 8-hydroxy-2'-deoxyguanosine, a marker for oxidative stress, was positively associated with glycated albumin levels in patients with type 2 diabetes, whereas improved glycemic control was associated with decreased levels of oxidative stress [52]. *In vitro* experiments showed that glycated human serum albumin protein promoted sustained ROS production in human endothelial cells. These findings support the hypotheses that there is a vicious cycle of oxidative stress, formation of AGEs, and production of more ROS. Furthermore, long-term oxidative stress induced by AGEs results in endothelial dysfunction which is associated with DR [53]. Indeed, oral administration of AGEs through a diet highly enriched in AGEs promoted oxidative stress, increasing inflammation and insulin resistance [54]. In addition, it has been shown that levels of H_2O_2 and $O_2^{\bullet-}$ are increased in the retinas

of diabetic rats, consistent with oxidative stress as a component of the pathobiology of DR [55,56]. Together, this experimental evidence indicates a relationship between elevated levels of ROS, AGEs, and DR.

Glycation is a critical biological process in the retina given that this ocular tissue has high metabolic activity whose activity depends on glucose demand. Such is the case in other neural tissues with the same embryological origin such as the cerebral cortex. The retina and cerebral cortex are exposed to comparable levels of blood glucose; however, the retina is more vulnerable to microvascular lesions derived from hyperglycemia. For example, in diabetic rats, intracellular concentrations of glucose increased significantly in the retina but not in the cerebral cortex, suggesting that a differential response of glucose uptake might contribute to the higher susceptibility of the retina to diabetes-induced microvascular complications [57].

The reasons for the high vulnerability of the retina to glycative stress remain unclear but some evidence indicates that the regulation of glucose uptake could be key. In the retina, the glucose delivery from systemic circulation occurs across retinal capillaries and the RPE. Glucose transport is mainly mediated by the sodium-independent glucose transporters (GLUTs). There are fourteen GLUTs, of which five are well characterized [58]. GLUT1 and GLUT3 are expressed in all cell types (including retina, lens, brain, vascular endothelium). GLUT2 is mainly expressed in the liver, kidney, intestine, and in beta cells of the pancreas. GLUT4 is found in the heart, adipose, and skeletal muscle. Transport of glucose via GLUT1 is insulin independent, thus GLUT1 is always “open”, allowing unimpeded transport of glucose. GLUT1 is expressed in retina and retinal capillaries, although the highest levels are found in the RPE. Thus, the RPE appears to be the major route for glucose delivery from the choriocapillaris to the neural retina [59]. Changes in the levels of this glucose transporter have been associated with the development of DR [60]. Levels of GLUT1 decreased in the retina of diabetic-induced rats; however, the expression of GLUT1 in the RPE was not affected by diabetes, suggesting that the trans-epithelial transport of glucose was not compromised [61–63]. The limited capacity of retinal endothelial cells to modulate glucose uptake makes them highly sensitive to the detrimental consequences of hyperglycemia in diabetes.

There are several plasma membrane proteins with the capacity to bind AGEs. The best studied receptor for AGEs in the context of DR is the receptor for advanced glycation end products (RAGE), also called AGER [64]. The binding of AGEs to RAGE is engaged in vital cellular processes such as inflammation, apoptosis, or proliferation and associated with the development of different human diseases. It is reported that RAGE-dependent signaling plays a major role in microvascular diabetic complications. In the eye, RAGE is expressed in multiple cells including pericytes, endothelial cells, microglia, Müller glia, and retinal pigmented epithelium cells, and expression of RAGE is increased under diabetic conditions [65,66]. This would appear to exacerbate the influx of glucose. Consistent with RAGE function, upon diabetes induction, RAGE knockout mice had reduced acellular capillary formation and showed less retinal vasopermeability, microglial activation, and Müller cell gliosis [67].

A vast amount of literature associates glycation and AGEs with the progression of DR. AGEs found in skin were shown to predict the risk of DR progression [68,69]. Intravenous administration of AGEs increased retinal vascular leakage *in vivo* by stimulating the expression of vascular endothelial growth factor (VEGF) and decreasing levels of the antiangiogenic pigment epithelium-derived factor (PEDF) [70].

Compounds that inhibit the formation of AGEs are reportedly effective against DR in preclinical settings [71]. An inhibitor of AGEs formation, aminoguanidine, significantly reduced serum AGEs and prevented the development of diabetes-induced basement membrane expansion in retinal capillaries of diabetic rats [72]. Aminoguanidine also reportedly reduced the number of acellular capillaries and abnormal microthrombus formations [73–75]. In addition, two different randomized, double-blind placebo-controlled trials reported that two different AGEs inhibitors, aminoguanidine and pimagedine, slow the progression of diabetic complications including DR [76,77]. Unfortunately, adverse side-effects of these AGEs inhibitors preclude their use in humans, and clinical trials for most of AGEs inhibitors have been discontinued. There is, therefore, a need to identify other means of lowering toxic AGEs.

4. Protective Mechanisms against Glycation-Derived Damage

4.1. Antioxidant Enzymes, Antioxidants, and Signaling

Since the biogenesis of AGEs involves oxidative stress, it might be anticipated that an ability to scavenge ROS would provide a first line of defense. Such enzymatic capacities are provided by antioxidant enzymes such as catalases, H₂O₂-detoxifying enzymes, and superoxide dismutases, as well as glutathione recycling enzymes, glutathione reductases, and glutathione peroxidases [78]. There are also non-enzymatic antioxidants, the most prevalent and most potent of which are ascorbate and glutathione (GSH). In the eye, these are present at mM concentrations, many times the levels in the blood [79–82]. GSH provides a reducing cellular environment. However, it is also a critical component of the glyoxalase system. It also regulates the ubiquitin proteolytic pathway (discussed later in the review). The GSH/GSSG ratio establishes cellular redox status, with consequences for many metabolic processes. Thus, scavenging ROS might be a useful strategy to diminish the production of AGEs in our tissues.

In addition to ascorbate, other non-enzymatic ROS-scavengers, albeit less effective on a per molecule basis, include vitamin E, carotenoids, and flavonoids. Several natural compounds with antioxidant properties have attracted attention with regard to DR, including multiple polyphenols, some of which have anti-glycating potential. For example, administration of curcumin to streptozotocin-induced diabetic rats enhanced the antioxidant capacity in the retina [55] and there was a protective effect on the glycation and crosslinking of collagen [83]. In addition, flavonols such as quercetin, catechin, or kaempferol were shown to diminish AGEs formation [84–86]. Treatment of hepatic cells with a hydroxytyrosol-enriched extract from olive leaves reduced protein carbonylation and the formation of AGEs [87]. Several studies also showed attenuating effects for resveratrol on the production of AGEs or the RAGE receptor in cell culture, animal models, and human studies (reviewed in [88]).

Among the pathobiological mechanisms by which AGEs enhance ROS production are perturbations in cellular signaling associated with the interaction between AGEs and RAGE. The production of free radicals by NADPH-oxidase and the mitochondrial electron transport system was shown to involve the AGEs–RAGE axis. The stimulation of the AGEs–RAGE signaling generates ROS by activating NADPH oxidases, augmenting the intracellular levels of H₂O₂, O₂^{•−}, and NO [89–91]. Interestingly, AGEs-induced upregulation of H₂O₂ production, along with mitochondrial dysfunction, resulted in apoptosis in ARPE-19, an RPE-derived cell line from normal eyes [92].

Several studies found that the upregulation of AGE-Receptor 1 (AGER1) inhibited the activity of NADPH oxidase, thereby weakening ROS production [93,94]. AGER1 is linked to sirtuin1 (SIRT1) [54,95]. In this context, deacetylation of NFκB by SIRT1 reduced the NFκB-mediated proinflammatory response [54]. However, long-term exposure to AGEs reduced AGER1 and SIRT1 expression, causing oxidative stress, inflammation, and insulin resistance in several tissues [54]. Another study showed that the dietary limitation of AGEs in type 2 diabetes mellitus patients diminished insulin resistance and increased expression of SIRT1 and AGER1 [94].

In sum, glycative stress contributes to the pathogenesis of different human diseases, including DR, through the interaction of oxidative stress and AGE accumulation.

4.2. Detoxifying Mechanisms against AGEs: Glyoxalase System, Aldehyde Dehydrogenases (ALDH), Aldoketoreductases (AKR), DJ-1/Park7, and Aldol Condensations

There are also specific protective pathways with the capacity to detoxify reactive dicarbonyls formed during sugar metabolism [96–98]. Once AGEs are formed, most are irreversible, so these protective mechanisms diminish the accumulation of AGEs in our tissues through the clearance of AGEs intermediates.

The primary mechanism for detoxifying these reactive dicarbonyls is the glyoxalase system. This converts highly reactive MG into far less reactive D-lactate [99] (Figure 3). This process involves the sequential activity of two enzymes, glyoxalase 1 (GLO1) and glyoxalase 2 (GLO2), and a catalytic

amount of GSH. First, GSH reacts spontaneously with the aldehyde of the dicarbonyl to form a hemithioacetal adduct. Then, GLO1 catalyzes the formation of *S*-D-lactoylglutathione. In the second enzymatic step, GLO2 catalyzes the reaction of *S*-D-lactoylglutathione into *D*-lactate, regenerating GSH. It is important to note that GLO1 activity is proportional to GSH levels, and that its activity decreases if cellular cytosolic GSH is diminished, as upon oxidative stress [100]. Unfortunately, such diminution of GSH also compromises the function of the ubiquitin proteasome system, diminishing the cellular capacity to degrade AGEs and cope with glycation-derived damage [19]. There are additional substrates metabolized via this pathway, including glyoxal, phenylglyoxal, and hydroxypyruvaldehyde [101]. Of note, GLO1 is the rate-limiting enzyme, catalyzing the first detoxification step in the glyoxalase system, and its activity is required to prevent the accumulation of these reactive α -oxoaldehydes [102]. Thus, GLO1 has a key protective role against glycative stress-induced AGEs formation.

The structure of glyoxalase is informative. Briefly, the human GLO1 translation product contains 184 amino acids. It is a dimeric protein of molecular mass 42 kDa and contains one zinc ion per subunit. Its structure has two domains per subunit and there are two active sites per protein formed by amino acid residues from the apposed subunit such that the monomer is inactive. The human *GLO1* gene is diallelic (on 6p21.2) and encodes two similar subunits in heterozygotes that result in the dimeric holoenzyme. The two alleles, *GLO1* and *GLO2*, give two similar subunits and three dimers: allozymes GLO 1-1, GLO 1-2, and GLO 2-2. The only amino acid difference between the expression products of the two *GLO1* alleles is at position 111 (Ala¹¹¹ or Glu¹¹¹). Some studies showed association of this polymorphism with the variations of diabetes and its vascular complications [103]. In addition, in stage 5 renal failure patients on hemodialysis, the Glu111Glu homozygote was associated with increased prevalence of cardiovascular disease and peripheral vascular disease [104].

Transcriptional regulation of *GLO1* is only partially understood, but it is known that the *GLO1* sequence contains multiple regulatory elements. These include a metal-response element (MRE), insulin-response element (IRE), and early gene 2 factor isoform (E2F4) and activating enhancer-binding protein 2 α (AP-2 α) binding sites [98,105]. IRE and MRE functionalities were validated in reporter assays where insulin and zinc chloride exposure produced a 2-fold increased transcriptional response [105]. Similar functional activities were shown for E2F and AP-2 α [106,107]. As discussed later, an antioxidant-response element (ARE) in exon 1 of *Glo1* enhances its transcription through the nuclear factor erythroid 2-related factor 2 (NRF2) oxidative stress-responsive transcriptional system [108].

Several systems appear to operate in the absence of glyoxalase activity, albeit their biological importance is largely unexplored. Other alternative routes for detoxification of dicarbonyls are aldehyde dehydrogenases (ALDHs), aldo-keto reductases (AKRs), the Parkinson-associated protein DJ-1, and scavenging by acetoacetate to form 3-hydroxyhexane-2,5-dione (3-HHD) [97,109].

AKRs are a large protein superfamily that is responsible for the reduction of aldehydes and ketones into primary and secondary alcohols. AKRs metabolize MG to hydroxyacetone or lactaldehyde. Transgenic expression of both human (*AKR7A2*) and mouse (*Akr7a5*) AKR in hamster fibroblasts cells protected against MG-induced cytotoxicity, suggesting that AKRs are able to detoxify MG and decrease AGE levels [110–112]. The role of AKR1B3 has also been studied in the hearts of induced diabetic *Akr1b3*-null mice, and it was observed that these mice had increased levels of MG and AGEs [112]. Human studies showed that aldose reductase activity (AKR1B1) contributed to the detoxification of MG in tissues where the protein was overexpressed and the levels of GSH were low [113]. An increase of AKR1B3 activity was observed in Schwann cells lacking glyoxalase activity, suggesting a compensatory relationship between these systems [114]. The theme of compensatory relationships between GLO1 activity and levels of various glycolytic metabolites will be noted in other systems, below.

ALDHs also metabolize MG, by oxidation to pyruvate. MG treatment induced ALDH expression in wild-type mouse Schwann cells [114]. Additionally, in both zebrafish and mouse models lacking glyoxalase activity, ALDH activity was induced, apparently as a compensatory mechanism [115,116].

3-deoxyglucosone (3-DG) is another highly reactive dicarbonyl formed during sugar metabolism. Although the physiological relevance of 3-deoxyglucosone (3-DG) in DR remains to be established,

the 3-DG metabolite formed by ALDH1A1, 2-keto-3-deoxygluconic acid, was elevated in plasma and erythrocytes of diabetic patients [117]. High ALDH1A1 activity was also found in lung, testis and liver but is negligible in other tissues [118]. Interestingly, aldehyde detoxifying capacity is found in retina, however ALDHs were found to be downregulated in diabetic conditions [119].

Another protein with anti-glycation activity is DJ-1, also known as Parkinson's disease protein 7 (PARK7) [120,121]. DJ-1 was shown to have glyoxalase activity *in vitro*, converting MG into lactic acid, in the absence of GSH, and preventing MG-induced tissue damage in *C. elegans* [122]. In addition, DJ-1 was shown to repair methylglyoxal- and glyoxal-glycated proteins *in vitro*. This deglycase activity removes early-stage MG adducts from protein side chains and prevents the formation of irreversible AGEs [123]. However, no contribution of DJ-1 to MG accumulation was observed in DJ-1 knockdown *Drosophila* cells and DJ-1 knockout flies [124]. A recent finding shows that DJ-1 may function as a relevant DNA deglycase [125] and recent studies observed a similar deglycase function for DJ-1 on DNA-wrapped histone proteins [126,127].

It was also reported that the ketone body acetoacetate decreased MG via a non-enzymatic conversion during diabetic and dietary ketosis [128]. Indeed, acetoacetate was able to scavenge endogenous MG in a non-enzymatic aldol reaction [129]. This has great importance since physiological ketosis produces high levels of acetoacetate and this condition may prevent diabetes progression [130].

4.3. Proteolytic Pathways: The Last Line of Defense against Glycation-Derived Damage

Which other mechanisms limit the accumulation of AGEs-modified proteins? Although AGEs are irreversible adducts and cross-links in our tissues, these can be removed through different proteolytic capacities. AGEs are substrates of intracellular protein degradation pathways and two major proteolytic capacities are suggested to contribute to the clearance of AGEs: the ubiquitin proteasome system (UPS) and autophagy [96,131–135] (Figure 3).

As for other cargos, AGEs-modified proteins were shown to be ubiquitinated [132]. Ubiquitin is a 76 amino acid protein that when conjugated to a protein substrate can facilitate degradation of that substrate by the proteasome. Obsolete or damaged proteins are tagged with ubiquitin and these ubiquitinated substrates are degraded by the proteasome. The ubiquitin proteolytic system operates mainly on soluble substrates. Several lines of evidence point to a relevant role for the UPS in the clearance of AGEs. Pharmacological proteasomal inhibition boosts the accumulation of AGEs *in vitro* in RPE-derived cells [132]. However, excessive glycativ stress decreases proteasomal capacity via the formation of intermolecular crosslinks that, consequently, lead to accelerated accumulation of AGEs [136–138].

Autophagy targets cargos for degradation and can operate on insoluble substrates, including organelles such as mitochondria. Autophagy requires macromolecular assemblies and organelles to identify, sequester, and eventually degrade substrates via the lysosome. Both proteolytic routes, autophagy and UPS, are functionally cooperative and a deficiency of one of these pathways triggers the upregulation of the other [139,140]. Several reports support a vital role for autophagy in the removal of AGEs. Pharmacological blockade of autophagy *in vitro* induced higher accumulation of AGEs in RPE cells [132]. Accumulation of AGEs was observed in kidney tubules of diabetic autophagy-deficient mice [131]. Importantly, mice lacking autophagy in the RPE showed increased levels of oxidized and glycated proteins and were predisposed to develop AMD-phenotypes and retinal degeneration [133].

In sum, a significant literature supports a critical role for the UPS and autophagy in maintaining non-toxic, homeostatic levels of AGEs. Unfortunately, the function of both proteolytic pathways declines with extensive glycativ stress and upon aging in many tissues [141], resulting in intracellular accumulation of protein aggregates (also glycated conjugates) and dysfunctional organelles in aged tissues [132,142,143]. We propose that deficits of these pathways in diabetic conditions could contribute to the accumulation and deposition of AGE-modified proteins in the retina, thereby contributing to DR. To date, there is scarce information about how these proteolytic pathways remove AGEs. This thwarts development of strategies to lower AGEs accumulation by boosting proteolytic capacities (Figure 4).

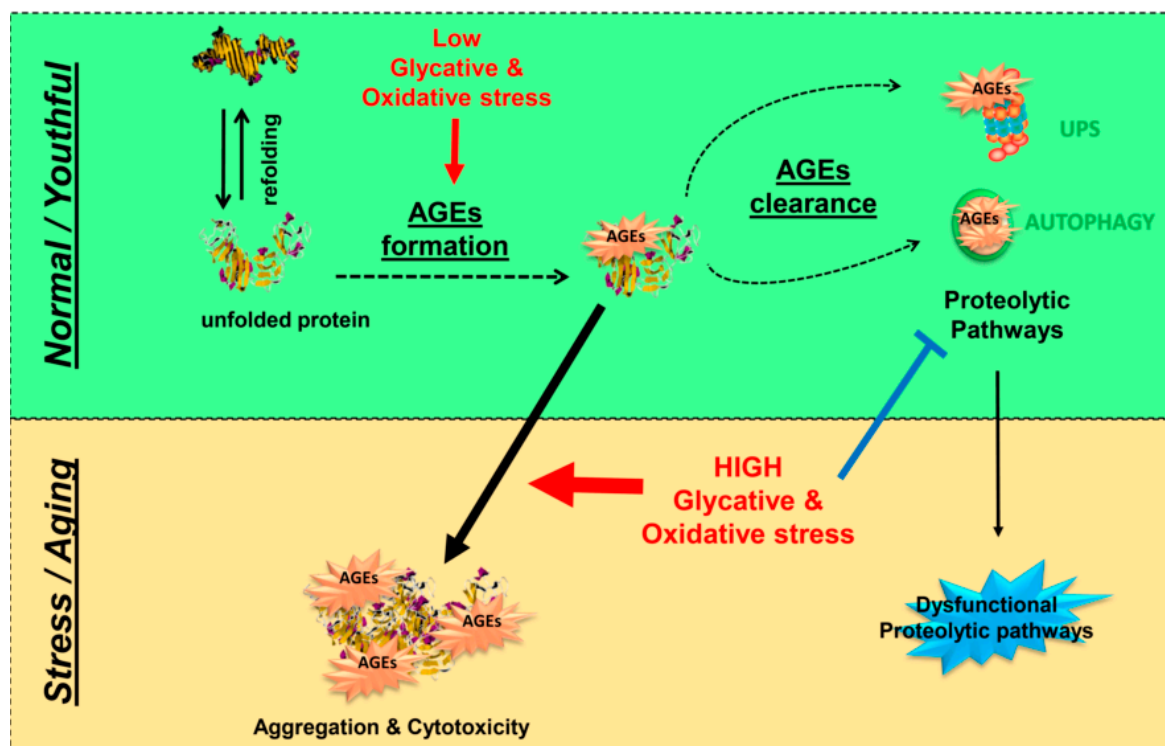


Figure 4. Proteolytic pathways are the last line of defense against AGE-derived proteotoxicity. Under homeostatic conditions (green box), different proteolytic pathways (UPS and autophagy) act to avoid the accumulation of toxic AGEs. Under high levels of glycative and oxidative stress and/or aging (orange box), the production of AGEs is boosted and tissue fitness is compromised as result of glycation-derived protein aggregation and cytotoxicity. Proteolytic capacities are insufficient to lower AGEs levels because of age-related changes in UPS and autophagy along with the inhibitory effect of glycative stress on proteolytic function.

4.4. Protective Role of NRF2 against Glycation-Derived Damage and Modulation of GLO1

NRF2 is an essential transcription factor for genes encoding a number of detoxification enzymes that contain one or more antioxidant response elements (AREs) in their regulatory regions. Examples of genes with AREs include glutathione S-transferases, UDP-glucuronosyltransferases, aldo-keto reductases, and NAD(P)H:quinone oxidoreductase 1. NRF2 acts indirectly by upregulating genes that metabolize and excrete the causative agents and byproducts of oxidative stress [144].

NRF2 is highly conserved from mammalian species to chicken and zebrafish, particularly within six regions designated the Neh1–6 domains [145]. The Neh1 domain contains the cnc-bZIP region, which dictates dimerization partners and confers DNA binding specificity. The Neh4 and Neh5 domains act cooperatively to bind the coactivator CREB-binding protein, thereby activating transcription [146].

Under physiological conditions, NRF2 is in the cytoplasm in a complex with KEAP1, a substrate adaptor protein for the cullin-3-dependent E3 ubiquitin ligase complex. This directs NRF2 for degradation by the 26S proteasome [147,148]. Under oxidative stress, the complex dissociates and NRF2 translocates into the nucleus and upregulates several antioxidant genes, including those related to MG metabolism, as well as genes required for glutathione synthesis [149–151].

Several studies investigated the effect of NRF2 activators on MG and AGEs formation and deposition. Hepatic, brain, heart, kidney, and lung *Glo1* mRNA and protein were decreased in *Nrf2*-knockout mice [108]. Compounds that increase *GLO1* expression and activity, such as sulforaphane or trans-resveratrol, decreased cellular and extracellular concentrations of MG and MG-derived protein adducts [152–156]. In addition, the binding of NRF2 to the *Glo1*-ARE increases expression of *Glo1*; however, the inflammatory activation of NF- κ B (nuclear factor κ B) by NRF2 could diminish *Glo1*

expression [157]. Of note, NF- κ B directly can also downregulate GLO1 activity, and the inhibition thus takes place at both functional and transcriptional levels [158].

Other transcription factors can modulate the expression of *Glo1*. Under hypoxia conditions, *Glo1* expression is inversely regulated by HIF1 α (hypoxia-inducible factor 1 α), an important physiological driver of dicarbonyl stress [159]. In addition, *Glo1* is acetylated and probably deacetylated by cytosolic sirtuin-2 [160,161], and its expression may be decreased by activation of the RAGE [127,162].

Several additional observations regarding the above control pathways and GLO1 are noteworthy. GLO1 downregulation is linked to activation of the RAGE receptor that is involved in pro-inflammatory signaling and the development of vascular complications of diabetes [127,162]. Upon glycative stress, there is increased GLO1 ubiquitination and degradation in high glucose concentration media *in vitro* [163]. Downregulation of NRF2 signaling is linked to decreased expression of *Glo1* and reduction of NRF2-antioxidant pathway signaling is associated with inflammation [108,157]. Nevertheless, even in Nrf2-knockout mice, GLO1 protein is still present in the retina and can even be moderately increased through dietary treatment, suggesting that NRF2 is just one of many different regulatory nodes for *Glo1*, at least in this specific ocular tissue [164]. The molecular mechanisms behind the increased retinal GLO1 stability in mice lacking NRF2 remain unknown.

5. The Use of Glyoxalase 1 in Animal Models

As previously explained, AGEs precursors are mainly detoxified by the glyoxalase system and, given the impact of AGEs on age-related pathologies, there is a need to develop strategies to counteract the accumulation of these toxic compounds. In the last few years, the manipulation of GLO1 activity in both animals and cell lines has demonstrated the causal involvement of MG and AGEs in several diseases.

In *Glo1* knockdown cell culture and animal models, an increase in free MG and toxic AGEs was described, but compensatory mechanisms were also observed [165,166]. Giacco et al. showed that in non-diabetic mice, knockdown of *Glo1* increased MG modification of glomerular proteins and oxidative stress to diabetic levels, causing alterations in kidney morphology indistinguishable from those caused by diabetes [167]. Surprisingly, in cell culture and animal models where *Glo1* was knocked out using CRISPR-Cas9 technology, there was no observed increase in MG accumulation [114,116,126,168]. In addition, these healthy *Glo1* knockout animal models did not present defects during development, while genetic deletion of *Glo1* was shown to be embryonically lethal in mice [169]. MGO treatment of cells lacking *Glo1* also revealed a decreased median lethal concentration for exogenous MG [114,126], indicating compensatory mechanisms for the loss of *Glo1*. Indeed, the authors demonstrated in this study that the deglycase protein DJ-1 may play a role in limiting the accumulation of MG-H1 on chromatin in cells lacking *Glo1*. Conversely, *Drosophila melanogaster* and *Danio rerio* *Glo1* knockout models showed an increase in tissue MG [115,170]. Moraru et al. observed increased MG levels, lipid accumulation in tissues, increased blood glucose, and decreased insulin sensitivity in *Drosophila melanogaster* *Glo1* knockout [170]. Consistent with these observations, Lodd et al. found under high nutrient intake increased MG levels driving insulin resistance and hyperglycemia in *Danio rerio* *Glo1* knockout [115].

Other studies have used the gene overexpression of *Glo1* in order to evaluate the biological impact of the glyoxalase system. The overexpression of *Glo1* reduced basal MG concentration, prevented mitochondrial protein modification, and enhanced lifespan in worms [171]. Similarly, *Glo1* overexpression reduced baseline MG concentration in the brain of mice [172]. In diabetic mice, *Glo1* overexpression also prevented diabetes-induced increases in MG modification of glomerular proteins, reduced oxidative stress, and prevented the development of diabetic kidney pathology, despite unchanged levels of hyperglycemia [165]. In agreement with mouse studies, rat models overexpressing human GLO1 led decreased MG levels, less AGEs formation, and reduced renal and endothelial dysfunction in response to induced diabetes compared with wild-type littermates [173–175]. Recently, GLO1 and aldose reductase were found to be upregulated in patients protected against

diabetic nephropathy [176], suggesting that the manipulation of the glyoxalase system could be a potential therapeutic strategy to prevent the onset of AGEs-related diseases.

In addition, diabetes and its microvascular complications (nephropathy, retinopathy, and neuropathy), are associated with elevated levels of MG and reduced levels of GLO1 expression and activity. Some studies showed that overexpression of *Glo1* in transgenic rats and mice could prevent the development of nephropathy, retinopathy, and neuropathy [167,174,175]. In vitro studies showed increased levels of MG and decreased GLO1 activity in endothelial cells when cultured in high glucose concentration media [102,177,178]. In contrast, overexpression of *Glo1* in endothelial cells under the same conditions prevented increased formation of AGEs [102]. Additionally, microvascular complications of diabetes linked to high glucose concentration in retinal pericytes were prevented by overexpression of *Glo1* [179]. In animal models, *Glo1* expression was decreased in the kidney of obese (db/db) diabetic mice, in the kidney and the sciatic nerve of streptozotocin-induced diabetic mice, in the kidney and liver of streptozotocin-induced diabetic Sprague–Dawley rats, in streptozotocin-induced diabetic Wistar rats, and in streptozotocin-induced rats overexpressing the renin-angiotensin system in extra-renal tissues [180–184]. By contrast, GLO1 activity was increased in red blood cells of streptozotocin-induced diabetic C57BL/6 mice, compared to non-diabetic controls [185] and GLO1 activity was increased in the red blood cells of patients with type 1 diabetes and type 2 diabetes, compared to healthy control subjects [186]. Patients with diabetes and microvascular complications had significantly higher GLO1 activity in red blood cells compared to patients without complications. These findings suggest a compensatory increase in GLO1 activity in response to elevated MG concentration [186] and that the response of GLO1 under diabetic conditions may be cell- and tissue-dependent.

5.1. The Decline of Glyoxalase 1 Activity with Age

Aging is characterized by a reduction in the functional properties of cells, tissues, and whole organs, starting with the impairment of major cellular homeostatic processes, including mitochondrial function, proteostasis, and stress-scavenging systems [166,187,188].

GLO1 expression and activity are modified upon aging and in age-related diseases including diabetes and its microvascular complications such as DR. Dicarbonyl stress contributes to aging through the age-related decline in GLO1 activity [171,186,189–196]. The first study that showed a causal link between aging and GLO1 was presented by Morcos et al. [171]. They found a marked decline of GLO1 expression and activity in *C. elegans* with age. Moreover, overexpression of *Glo1* was associated with prolonged lifespan, whereas *Glo1* silencing decreased lifespan, demonstrating that a decrease in GLO1 activity increases mitochondrial ROS production, thereby limiting lifespan [171]. In mice, Sharma–Luthra et al. found tissue specific differences. They showed that GLO1 activity diminished in liver and spleen with age, but increased in kidneys to maximum levels at 24 months [191]. GLO1 activity in rat tissues was decreased with age, as well as by hypoxia in young rats [192]. In humans, several studies have been conducted to investigate the impact of aging on GLO1. Most of them reported a decline of GLO1 activity in multiple tissues, such as arterial tissues, lens, brain, and red blood cells, with age [186,193–196].

5.2. Glyoxalase 1 Activity in Ocular Tissues

In spite of the vast literature on non-ocular tissues, there is scarce information about the role of the glyoxalase system in ocular tissues. However, increasing evidence points to a link between alteration of the glyoxalase system and the development of DR. A polymorphism that alters *GLO1* promoter activity has been linked to retinopathy in type 2 diabetic patients [197]. Expression of *GLO1* and *GLO2* are downregulated in patients with DR, indicating that a failure of this detoxifying system in humans may be involved in retinopathy [198,199]. Glyoxalase activity is reported *in vitro* to promote pericyte survival under hyperglycemic conditions [179] and retinal extracts from a mouse model protected from hyperglycemia-evoked vasoregression showed higher GLO1 activity [200]. Interestingly, a transgenic rat model overexpressing *Glo1* inhibits retinal AGE formation and prevents

DR lesions [174]. In sum, upregulation of GLO1 appears to reduce retinal AGEs in diabetic rats and ameliorate AGEs-related pathologies.

In order to explore the role of the glyoxalase system in the eye, we dissected retina, RPE/choroid, and lens, along with other non-ocular tissues from 2-month-old wild-type C57BL/6J mice. We quantified the GLO1 activity in cytosolic extracts of tissues, as previously reported [201]. As expected, we found activity in all tissues analyzed and the relative order of GLO1 activity was retina > liver > kidney > brain > heart > RPE/choroid > lens (Figure 5A). These results are consistent with previous publications in non-ocular tissues [116,184]. Regarding ocular tissues, we observed the highest activity of GLO1 in the retina of mice compared to the lens or RPE/choroid (Figure 5B). Glyoxalase activity is clearly tissue-dependent and retinal activity was about 9-fold and 13-fold greater than in the RPE/choroid and lens, respectively (Figure 5C,D). Clearly, there is even region-specific localization within tissues. This finding is highly relevant because avoiding the formation and accumulation of AGEs is especially important in highly differentiated tissues such as the retina or lens where the glycation damage cannot be diluted by cellular division [96,132]. Of note, when compared to non-ocular tissues, the retinal rate of detoxification was about 2-fold, 8.5-fold, 3.5-fold, and 4.5-fold greater than liver, heart, kidney, and brain, respectively (Figure 5E–H). From a teleological perspective, the high level of retinal GLO1 activity suggests an important protective role against AGE-derived damage in retina.

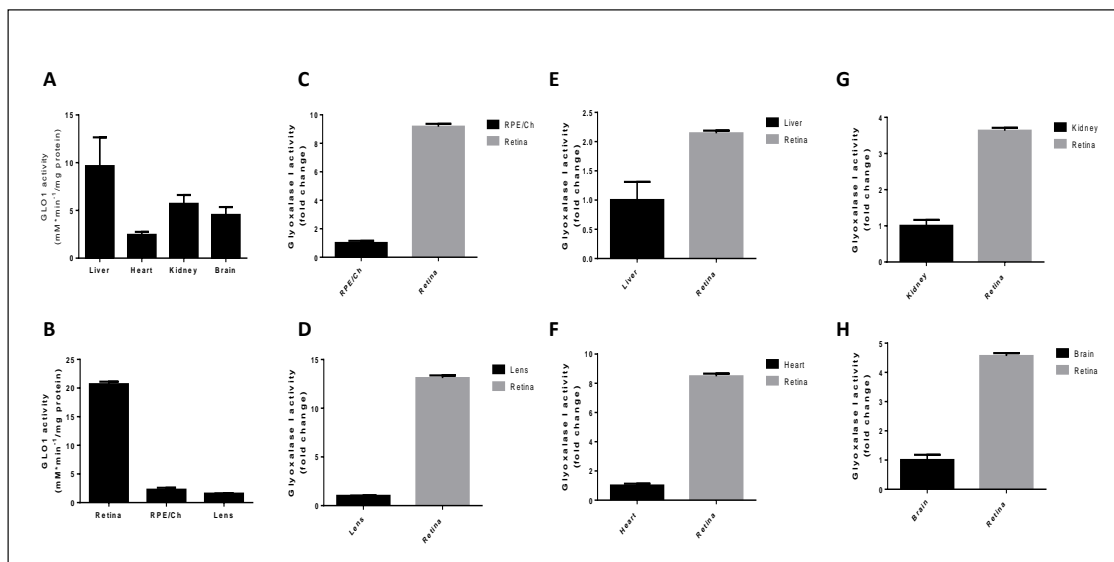


Figure 5. Evaluation of glyoxalase system activity in ocular and non-ocular tissues. Glyoxalase I activity was determined spectrophotometrically using 1 mL quartz cuvettes by following the initial rate of formation of S-D-lactoylglycylglutathione. The assay mixture containing the glycating reagent MG and reduced GSH was equilibrated at room temperature for 10 min, to ensure hemithioacetal formation. The reaction was initiated by the addition of 20 μ g of cytosolic extract and the A240 was monitored immediately and over the course of 5 min. The reaction rate was determined by following the increase in absorbance at 240 nm for which $\Delta\epsilon_{240} = 2.86 \text{ mM}^{-1} \text{ cm}^{-1}$. (A,B) Glyoxalase I activity was assayed in (A) non-ocular tissues and (B) ocular tissues and activity was expressed as milliunits per milligram of protein where one unit of GLO1 activity was the amount of enzyme which catalyzes the formation of 1 μ mol S-D-lactoylglycylglutathione per min under assay conditions. (C–H) Retinal glyoxalase I activity was compared to (C) RPE/choroid, (D) lens, (E) liver, (F) heart, (G) kidney, and (H) brain. Fold change was calculated relative to each tissue and values represent the mean \pm standard error of the mean of 4 independent experiments from the GLO1 activity assay.

Several cautions are appropriate with regard to expectations of GLO1 overexpression, particularly in the lens. GLO1 may be found and function primarily in the epithelial layers where glucose is received from the aqueous or vitreous humors that nurture the lens. The epithelial layers comprise a minority

of lens tissue; thus, the concentration of GLO1 might be significantly higher in the lens epithelium. This may explain glycation-related browning of lenses, particularly in the lens core, upon aging (Figure 2C). Given that the glyoxalase system declines in efficacy with age [189], enhancement of GLO1 activity might represent a therapeutic strategy to counteract the accumulation of these toxic compounds in the lens and retina.

The use of transcriptional modulators of GLO1 has been proposed as a potential intervention to support healthy aging and fight AGEs-related diseases. Metformin, an oral glucose-lowering agent for type 2 diabetes, increases GLO1 activity and was shown to lower circulating MG levels in patients with type 2 diabetes [202]. Candesartan, a synthetic drug that stimulates glyoxalase activity, reduced retinal acellular capillaries and attenuated inflammation and diabetic retinal vascular pathology [182]. In addition, dietary compounds including trans-resveratrol, fisetin, mangiferin, cyanidin, hesperetin, or sulforaphane possess stimulating GLO1-properties and lower MG and MG-derived adducts [152–156,203–208]. Proteomic analysis identified GLO1 as a protein differentially expressed in cells treated with sulforaphane [209]. Sulforaphane inhibited AGEs-derived pericyte damage [210] and delayed diabetes-induced retinal photoreceptor cell degeneration in streptozotocin-injected mice [211]. In addition, pyridoxamine, an MG scavenger that inhibits AGEs formation but also increases GLO1 activity [212], prevented the development of retinopathy in streptozotocin-induced diabetic rats [213]. There is a growing interest in drug discovery and high-throughput screening systems will identify novel regulators and small molecules with GLO1-stimulating properties [214], opening a possibility to treat DR. Clearly, further research is required to define nutritional and pharmacological approaches to overcome the progression of DR associated with glycative stress.

6. Conclusions

Currently there is no cure for DR. Modern therapies include anti-angiogenic strategies or surgery for retinal detachment, which cannot restore vision but only ameliorate further deterioration of the retina. A vast amount of literature stresses the pathogenic role of glycation and AGEs in the molecular basis of this eye-related disease. Based on this literature, lowering AGEs might be a potential therapeutic strategy against DR. Despite enormous effort to date, no AGEs inhibitors have reached clinical use. Exploiting the glyoxalase system and the discovery of compounds that enhance this detoxifying activity represent a therapeutic alternative to fight glycation-derived damage, under diabetic and non-diabetic conditions. As documented here, glyoxalase activity is highest in the retina, suggesting a significant role in eye physiology. The capacity of this detoxifying route is thought to decline with age. We propose that targeting the glyoxalase system through nutritional or pharmacological enhancers might be an alternative to fight this sight-threatening diabetic complication, aiming to reduce the economic burden caused by DR.

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References

1. Stevens, G.A.; White, R.A.; Flaxman, S.R.; Price, H.; Jonas, J.B.; Keeffe, J.; Leasher, J.; Naidoo, K.; Pesudovs, K.; Resnikoff, S.; et al. Global prevalence of vision impairment and blindness: Magnitude and temporal trends, 1990–2010. *Ophthalmology* **2013**, *120*, 2377–2384. [[CrossRef](#)] [[PubMed](#)]

2. Eckert, K.A.; Carter, M.J.; Lansingh, V.C.; Wilson, D.A.; Furtado, J.M.; Frick, K.D.; Resnikoff, S. A Simple Method for Estimating the Economic Cost of Productivity Loss Due to Blindness and Moderate to Severe Visual Impairment. *Ophthalmic Epidemiol.* **2015**, *22*, 349–355. [[CrossRef](#)] [[PubMed](#)]
3. Ramrattan, R.S.; Wolfs, R.C.; Panda-Jonas, S.; Jonas, J.B.; Bakker, D.; Pols, H.A.; Hofman, A.; de Jong, P.T. Prevalence and causes of visual field loss in the elderly and associations with impairment in daily functioning: The Rotterdam Study. *Arch. Ophthalmol.* **2001**, *119*, 1788–1794. [[CrossRef](#)] [[PubMed](#)]
4. Köberlein, J.; Beifus, K.; Schaffert, C.; Finger, R.P. The economic burden of visual impairment and blindness: A systematic review. *BMJ Open* **2013**, *3*, e003471. [[CrossRef](#)]
5. WHO. Draft action plan for the prevention of avoidable blindness and visual impairment 2014–2019. In *Universal Eye Health: A Global Action Plan 2014–2019*; World Health Organization: Geneva, Switzerland, 2013.
6. Bourne, R.R.A.; Flaxman, S.R.; Braithwaite, T.; Cicinelli, M.V.; Das, A.; Jonas, J.B.; Keeffe, J.; Kempen, J.H.; Leasher, J.; Limburg, H.; et al. Magnitude, temporal trends, and projections of the global prevalence of blindness and distance and near vision impairment: A systematic review and meta-analysis. *Lancet. Glob. Health* **2017**, *5*, e888–e897. [[CrossRef](#)]
7. Williams, D.L. Oxidative stress and the eye. *Vet. Clin. N. Am. Small Anim. Pract.* **2008**, *38*, 179–192. [[CrossRef](#)] [[PubMed](#)]
8. Domènech, E.B.; Marfany, G. The Relevance of Oxidative Stress in the Pathogenesis and Therapy of Retinal Dystrophies. *Antioxidants* **2020**, *9*, 347. [[CrossRef](#)] [[PubMed](#)]
9. Datta, S.; Cano, M.; Ebrahimi, K.; Wang, L.; Handa, J.T. The impact of oxidative stress and inflammation on RPE degeneration in non-neovascular AMD. *Prog. Retin. Eye Res.* **2017**, *60*, 201–218. [[CrossRef](#)]
10. Bungau, S.; Abdel-Daim, M.M.; Tit, D.M.; Ghanem, E.; Sato, S.; Maruyama-Inoue, M.; Yamane, S.; Kadonosono, K. Health Benefits of Polyphenols and Carotenoids in Age-Related Eye Diseases. *Oxidative Med. Cell. Longev.* **2019**, *2019*, 9783429. [[CrossRef](#)]
11. Abdel-Daim, M.M.; El-Tawil, O.S.; Bungau, S.G.; Atanasov, A.G. Applications of Antioxidants in Metabolic Disorders and Degenerative Diseases: Mechanistic Approach. *Oxidative Med. Cell. Longev.* **2019**, *2019*, 4179676. [[CrossRef](#)]
12. Kowluru, R.A.; Chan, P.S. Oxidative stress and diabetic retinopathy. *Exp. Diabetes Res.* **2007**, *2007*, 43603. [[CrossRef](#)]
13. Cabrera, M.P.; Chihuaif, R.H. Antioxidants and the integrity of ocular tissues. *Vet. Med. Int.* **2011**, *2011*, 905153. [[CrossRef](#)] [[PubMed](#)]
14. Mitra, R.N.; Conley, S.M.; Naash, M.I. Therapeutic Approach of Nanotechnology for Oxidative Stress Induced Ocular Neurodegenerative Diseases. *Adv. Exp. Med. Biol.* **2016**, *854*, 463–469. [[CrossRef](#)] [[PubMed](#)]
15. Barreiro, E. Role of Protein Carbonylation in Skeletal Muscle Mass Loss Associated with Chronic Conditions. *Proteomes* **2016**, *4*, 18. [[CrossRef](#)]
16. Jahngen-Hodge, J.; Cyr, D.; Laxman, E.; Taylor, A. Ubiquitin and ubiquitin conjugates in human lens. *Exp. Eye Res.* **1992**, *55*, 897–902. [[CrossRef](#)]
17. Dudek, E.J.; Shang, F.; Valverde, P.; Liu, Q.; Hobbs, M.; Taylor, A. Selectivity of the ubiquitin pathway for oxidatively modified proteins: Relevance to protein precipitation diseases. *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.* **2005**, *19*, 1707–1709. [[CrossRef](#)] [[PubMed](#)]
18. Obin, M.; Shang, F.; Gong, X.; Handelman, G.; Blumberg, J.; Taylor, A. Redox regulation of ubiquitin-conjugating enzymes: Mechanistic insights using the thiol-specific oxidant diamide. *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.* **1998**, *12*, 561–569. [[CrossRef](#)]
19. Jahngen-Hodge, J.; Obin, M.S.; Gong, X.; Shang, F.; Nowell, T.R., Jr.; Gong, J.; Abasi, H.; Blumberg, J.; Taylor, A. Regulation of ubiquitin-conjugating enzymes by glutathione following oxidative stress. *J. Biol. Chem.* **1997**, *272*, 28218–28226. [[CrossRef](#)]
20. Trpkovic, A.; Resanovic, I.; Stanimirovic, J.; Radak, D.; Mousa, S.A.; Cenic-Milosevic, D.; Jevremovic, D.; Isenovic, E.R. Oxidized low-density lipoprotein as a biomarker of cardiovascular diseases. *Crit. Rev. Clin. Lab. Sci.* **2015**, *52*, 70–85. [[CrossRef](#)]
21. Jacob, K.D.; Noren Hooten, N.; Trzeciak, A.R.; Evans, M.K. Markers of oxidant stress that are clinically relevant in aging and age-related disease. *Mech. Ageing Dev.* **2013**, *134*, 139–157. [[CrossRef](#)]
22. Chen, J.; Patil, S.; Seal, S.; McGinnis, J.F. Rare earth nanoparticles prevent retinal degeneration induced by intracellular peroxides. *Nat. Nanotechnol.* **2006**, *1*, 142–150. [[CrossRef](#)]

23. Martínez-Fernández de la Cámara, C.; Salom, D.; Sequedo, M.D.; Hervás, D.; Marín-Lambies, C.; Aller, E.; Jaijo, T.; Díaz-Llopis, M.; Millán, J.M.; Rodrigo, R. Altered antioxidant-oxidant status in the aqueous humor and peripheral blood of patients with retinitis pigmentosa. *PLoS ONE* **2013**, *8*, e74223. [\[CrossRef\]](#)
24. Liu, Q.; Ju, W.K.; Crowston, J.G.; Xie, F.; Perry, G.; Smith, M.A.; Lindsey, J.D.; Weinreb, R.N. Oxidative stress is an early event in hydrostatic pressure induced retinal ganglion cell damage. *Investig. Ophthalmol. Vis. Sci.* **2007**, *48*, 4580–4589. [\[CrossRef\]](#)
25. Crabb, J.W.; Miyagi, M.; Gu, X.; Shadrach, K.; West, K.A.; Sakaguchi, H.; Kamei, M.; Hasan, A.; Yan, L.; Rayborn, M.E.; et al. Drusen proteome analysis: An approach to the etiology of age-related macular degeneration. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 14682–14687. [\[CrossRef\]](#)
26. Serebryany, E.; Yu, S.; Trauger, S.A.; Budnik, B.; Shakhnovich, E.I. Dynamic disulfide exchange in a crystallin protein in the human eye lens promotes cataract-associated aggregation. *J. Biol. Chem.* **2018**, *293*, 17997–18009. [\[CrossRef\]](#)
27. Weikel, K.A.; Chiu, C.J.; Taylor, A. Nutritional modulation of age-related macular degeneration. *Mol. Asp. Med.* **2012**, *33*, 318–375. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Saccà, S.C.; Corazza, P.; Gandolfi, S.; Ferrari, D.; Sukkar, S.; Iorio, E.L.; Traverso, C.E. Substances of Interest That Support Glaucoma Therapy. *Nutrients* **2019**, *11*, 239. [\[CrossRef\]](#)
29. Perez, C.I.; Singh, K.; Lin, S. Relationship of lifestyle, exercise, and nutrition with glaucoma. *Curr. Opin. Ophthalmol.* **2019**, *30*, 82–88. [\[CrossRef\]](#) [\[PubMed\]](#)
30. Weikel, K.A.; Garber, C.; Baburins, A.; Taylor, A. Nutritional modulation of cataract. *Nutr. Rev.* **2014**, *72*, 30–47. [\[CrossRef\]](#)
31. Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: The Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. *JAMA* **2013**, *309*, 2005–2015. [\[CrossRef\]](#)
32. Thornalley, P.J.; Langborg, A.; Minhas, H.S. Formation of glyoxal, methylglyoxal and 3-deoxyglucosone in the glycation of proteins by glucose. *Biochem. J.* **1999**, *344*, 109–116.
33. Rabbani, N.; Thornalley, P.J. Dicarbonyl stress in cell and tissue dysfunction contributing to ageing and disease. *Biochem. Biophys. Res. Commun.* **2015**, *458*, 221–226. [\[CrossRef\]](#)
34. Rabbani, N.; Thornalley, P.J. Dicarbonyl proteome and genome damage in metabolic and vascular disease. *Biochem. Soc. Trans.* **2014**, *42*, 425–432. [\[CrossRef\]](#)
35. Bejarano, E.; Taylor, A. Too sweet: Problems of protein glycation in the eye. *Exp. Eye Res.* **2019**, *178*, 255–262. [\[CrossRef\]](#)
36. Kandarakis, S.A.; Piperi, C.; Topouzis, F.; Papavassiliou, A.G. Emerging role of advanced glycation-end products (AGEs) in the pathobiology of eye diseases. *Prog. Retin. Eye Res.* **2014**, *42*, 85–102. [\[CrossRef\]](#)
37. Lapolla, A.; Flamini, R.; Dalla Vedova, A.; Senesi, A.; Reitano, R.; Fedele, D.; Basso, E.; Seraglia, R.; Traldi, P. Glyoxal and methylglyoxal levels in diabetic patients: Quantitative determination by a new GC/MS method. *Clin. Chem. Lab. Med.* **2003**, *41*, 1166–1173. [\[CrossRef\]](#)
38. Allaman, I.; Bélanger, M.; Magistretti, P.J. Methylglyoxal, the dark side of glycolysis. *Front. Neurosci.* **2015**, *9*, 23. [\[CrossRef\]](#)
39. Cepas, V.; Collino, M.; Mayo, J.C.; Sainz, R.M. Redox Signaling and Advanced Glycation Endproducts (AGEs) in Diet-Related Diseases. *Antioxidants* **2020**, *9*, 142. [\[CrossRef\]](#)
40. Miyata, S.; Monnier, V. Immunohistochemical detection of advanced glycosylation end products in diabetic tissues using monoclonal antibody to pyrraline. *J. Clin. Investig.* **1992**, *89*, 1102–1112. [\[CrossRef\]](#)
41. Sell, D.R.; Nagaraj, R.H.; Grandhee, S.K.; Odetti, P.; Lapolla, A.; Fogarty, J.; Monnier, V.M. Pentosidine: A molecular marker for the cumulative damage to proteins in diabetes, aging, and uremia. *Diabetes Metab. Rev.* **1991**, *7*, 239–251. [\[CrossRef\]](#)
42. Schleicher, E.D.; Wagner, E.; Nerlich, A.G. Increased accumulation of the glycooxidation product N(epsilon)-(carboxymethyl)lysine in human tissues in diabetes and aging. *J. Clin. Investig.* **1997**, *99*, 457–468. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Stitt, A.W.; Moore, J.E.; Sharkey, J.A.; Murphy, G.; Simpson, D.A.; Bucala, R.; Vlassara, H.; Archer, D.B. Advanced glycation end products in vitreous: Structural and functional implications for diabetic vitreopathy. *Investig. Ophthalmol. Vis. Sci.* **1998**, *39*, 2517–2523.
44. Dyer, D.G.; Blackledge, J.A.; Katz, B.M.; Hull, C.J.; Adkisson, H.D.; Thorpe, S.R.; Lyons, T.J.; Baynes, J.W. The Maillard reaction in vivo. *Z. Fur Ernahr.* **1991**, *30*, 29–45. [\[CrossRef\]](#)

45. Pescosolido, N.; Barbato, A.; Giannotti, R.; Komaiha, C.; Lenarduzzi, F. Age-related changes in the kinetics of human lenses: Prevention of the cataract. *Int. J. Ophthalmol.* **2016**, *9*, 1506–1517. [[CrossRef](#)] [[PubMed](#)]
46. Tessier, F.; Obrenovich, M.; Monnier, V.M. Structure and mechanism of formation of human lens fluorophore LM-1. Relationship to vesperlysine A and the advanced Maillard reaction in aging, diabetes, and cataractogenesis. *J. Biol. Chem.* **1999**, *274*, 20796–20804. [[CrossRef](#)]
47. Xu, J.; Chen, L.J.; Yu, J.; Wang, H.J.; Zhang, F.; Liu, Q.; Wu, J. Involvement of Advanced Glycation End Products in the Pathogenesis of Diabetic Retinopathy. *Cell. Physiol. Biochem. Int. J. Exp. Cell. Physiol. Biochem. Pharmacol.* **2018**, *48*, 705–717. [[CrossRef](#)]
48. Okamoto, T.; Yamagishi, S.; Inagaki, Y.; Amano, S.; Koga, K.; Abe, R.; Takeuchi, M.; Ohno, S.; Yoshimura, A.; Makita, Z. Angiogenesis induced by advanced glycation end products and its prevention by cerivastatin. *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.* **2002**, *16*, 1928–1930. [[CrossRef](#)]
49. Baynes, J.W.; Thorpe, S.R. Role of oxidative stress in diabetic complications: A new perspective on an old paradigm. *Diabetes* **1999**, *48*, 1–9. [[CrossRef](#)]
50. Frey, T.; Antonetti, D.A. Alterations to the blood-retinal barrier in diabetes: Cytokines and reactive oxygen species. *Antioxid Redox Signal* **2011**, *15*, 1271–1284. [[CrossRef](#)]
51. Klaassen, I.; Van Noorden, C.J.; Schlingemann, R.O. Molecular basis of the inner blood-retinal barrier and its breakdown in diabetic macular edema and other pathological conditions. *Prog. Retin. Eye Res.* **2013**, *34*, 19–48. [[CrossRef](#)]
52. Nojima, H.; Watanabe, H.; Yamane, K.; Kitahara, Y.; Sekikawa, K.; Yamamoto, H.; Yokoyama, A.; Inamizu, T.; Asahara, T.; Kohno, N. Effect of aerobic exercise training on oxidative stress in patients with type 2 diabetes mellitus. *Metab. Clin. Exp.* **2008**, *57*, 170–176. [[CrossRef](#)] [[PubMed](#)]
53. Rodiño-Janeiro, B.K.; González-Peteiro, M.; Uceda-Somoza, R.; González-Juanatey, J.R.; Alvarez, E. Glycated albumin, a precursor of advanced glycation end-products, up-regulates NADPH oxidase and enhances oxidative stress in human endothelial cells: Molecular correlate of diabetic vasculopathy. *Diabetes Metab. Res. Rev.* **2010**, *26*, 550–558. [[CrossRef](#)] [[PubMed](#)]
54. Cai, W.; Ramdas, M.; Zhu, L.; Chen, X.; Striker, G.E.; Vlassara, H. Oral advanced glycation endproducts (AGEs) promote insulin resistance and diabetes by depleting the antioxidant defenses AGE receptor-1 and sirtuin 1. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 15888–15893. [[CrossRef](#)] [[PubMed](#)]
55. Kowluru, R.A.; Kanwar, M. Effects of curcumin on retinal oxidative stress and inflammation in diabetes. *Nutr. Metab.* **2007**, *4*, 8. [[CrossRef](#)] [[PubMed](#)]
56. Rodríguez, M.L.; Pérez, S.; Mena-Mollá, S.; Desco, M.C.; Ortega, Á.L. Oxidative Stress and Microvascular Alterations in Diabetic Retinopathy: Future Therapies. *Oxidative Med. Cell. Longev.* **2019**, *2019*, 4940825. [[CrossRef](#)]
57. Tang, J.; Zhu, X.W.; Lust, W.D.; Kern, T.S. Retina accumulates more glucose than does the embryologically similar cerebral cortex in diabetic rats. *Diabetologia* **2000**, *43*, 1417–1423. [[CrossRef](#)]
58. Thorens, B.; Mueckler, M. Glucose transporters in the 21st Century. *Am. J. Physiol. Endocrinol. Metab.* **2010**, *298*, E141–E145. [[CrossRef](#)]
59. Sugasawa, K.; Deguchi, J.; Okami, T.; Yamamoto, A.; Omori, K.; Uyama, M.; Tashiro, Y. Immunocytochemical analyses of distributions of Na, K-ATPase and GLUT1, insulin and transferrin receptors in the developing retinal pigment epithelial cells. *Cell Struct. Funct.* **1994**, *19*, 21–28. [[CrossRef](#)]
60. You, Z.-P.; Zhang, Y.-L.; Shi, K.; Shi, L.; Zhang, Y.-Z.; Zhou, Y.; Wang, C.-y. Suppression of diabetic retinopathy with GLUT1 siRNA. *Sci. Rep.* **2017**, *7*, 7437. [[CrossRef](#)]
61. Ulas, M.; Orhan, C.; Tuzcu, M.; Ozercan, I.H.; Sahin, N.; Gencoglu, H.; Komorowski, J.R.; Sahin, K. Anti-diabetic potential of chromium histidinate in diabetic retinopathy rats. *BMC Complement. Altern. Med.* **2015**, *15*, 16. [[CrossRef](#)]
62. Badr, G.A.; Tang, J.; Ismail-Beigi, F.; Kern, T.S. Diabetes downregulates GLUT1 expression in the retina and its microvessels but not in the cerebral cortex or its microvessels. *Diabetes* **2000**, *49*, 1016–1021. [[CrossRef](#)] [[PubMed](#)]
63. Rajah, T.T.; Olson, A.L.; Grammas, P. Differential glucose uptake in retina- and brain-derived endothelial cells. *Microvasc. Res.* **2001**, *62*, 236–242. [[CrossRef](#)]
64. Nass, N.; Bartling, B.; Navarrete Santos, A.; Scheubel, R.J.; Börgermann, J.; Silber, R.E.; Simm, A. Advanced glycation end products, diabetes and ageing. *Z. Fur. Gerontol. Und Geriatr.* **2007**, *40*, 349–356. [[CrossRef](#)] [[PubMed](#)]

65. Stitt, A.W.; Li, Y.M.; Gardiner, T.A.; Bucala, R.; Archer, D.B.; Vlassara, H. Advanced glycation end products (AGEs) co-localize with AGE receptors in the retinal vasculature of diabetic and of AGE-infused rats. *Am. J. Pathol.* **1997**, *150*, 523–531.
66. Chen, M.; Curtis, T.M.; Stitt, A.W. Advanced glycation end products and diabetic retinopathy. *Curr. Med. Chem.* **2013**, *20*, 3234–3240. [[CrossRef](#)]
67. McVicar, C.M.; Ward, M.; Colhoun, L.M.; Guduric-Fuchs, J.; Bierhaus, A.; Fleming, T.; Schlotterer, A.; Kolibabka, M.; Hammes, H.P.; Chen, M.; et al. Role of the receptor for advanced glycation endproducts (RAGE) in retinal vasodegenerative pathology during diabetes in mice. *Diabetologia* **2015**, *58*, 1129–1137. [[CrossRef](#)]
68. Genuth, S.; Sun, W.; Cleary, P.; Sell, D.R.; Dahms, W.; Malone, J.; Sivitz, W.; Monnier, V.M. Glycation and carboxymethyllysine levels in skin collagen predict the risk of future 10-year progression of diabetic retinopathy and nephropathy in the diabetes control and complications trial and epidemiology of diabetes interventions and complications participants with type 1 diabetes. *Diabetes* **2005**, *54*, 3103–3111. [[CrossRef](#)]
69. Genuth, S.; Sun, W.; Cleary, P.; Gao, X.; Sell, D.R.; Lachin, J.; Monnier, V.M. Skin advanced glycation end products glucosepane and methylglyoxal hydroimidazolone are independently associated with long-term microvascular complication progression of type 1 diabetes. *Diabetes* **2015**, *64*, 266–278. [[CrossRef](#)] [[PubMed](#)]
70. Yamagishi, S.; Nakamura, K.; Matsui, T.; Inagaki, Y.; Takenaka, K.; Jinnouchi, Y.; Yoshida, Y.; Matsuura, T.; Narama, I.; Motomiya, Y.; et al. Pigment epithelium-derived factor inhibits advanced glycation end product-induced retinal vascular hyperpermeability by blocking reactive oxygen species-mediated vascular endothelial growth factor expression. *J. Biol. Chem.* **2006**, *281*, 20213–20220. [[CrossRef](#)]
71. Borg, D.J.; Forbes, J.M. Targeting advanced glycation with pharmaceutical agents: Where are we now? *Glycoconj. J.* **2016**, *33*, 653–670. [[CrossRef](#)]
72. Gardiner, T.A.; Anderson, H.R.; Stitt, A.W. Inhibition of advanced glycation end-products protects against retinal capillary basement membrane expansion during long-term diabetes. *J. Pathol.* **2003**, *201*, 328–333. [[CrossRef](#)] [[PubMed](#)]
73. Hammes, H.P.; Martin, S.; Federlin, K.; Geisen, K.; Brownlee, M. Aminoguanidine treatment inhibits the development of experimental diabetic retinopathy. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 11555–11558. [[CrossRef](#)] [[PubMed](#)]
74. Hammes, H.P.; 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. [[CrossRef](#)] [[PubMed](#)]
75. Hammes, H.P.; Strödter, D.; Weiss, A.; Bretzel, R.G.; Federlin, K.; Brownlee, M. Secondary intervention with aminoguanidine retards the progression of diabetic retinopathy in the rat model. *Diabetologia* **1995**, *38*, 656–660. [[CrossRef](#)]
76. Freedman, B.I.; Wuerth, J.P.; Cartwright, K.; Bain, R.P.; Dippe, S.; Hershon, K.; Mooradian, A.D.; Spinowitz, B.S. Design and baseline characteristics for the aminoguanidine Clinical Trial in Overt Type 2 Diabetic Nephropathy (ACTION II). *Control. Clin. Trials* **1999**, *20*, 493–510. [[CrossRef](#)]
77. Bolton, W.K.; Cattran, D.C.; Williams, M.E.; Adler, S.G.; Appel, G.B.; Cartwright, K.; Foiles, P.G.; Freedman, B.I.; Raskin, P.; Ratner, R.E.; et al. Randomized trial of an inhibitor of formation of advanced glycation end products in diabetic nephropathy. *Am. J. Nephrol.* **2004**, *24*, 32–40. [[CrossRef](#)]
78. Nowotny, K.; Jung, T.; Höhn, A.; Weber, D.; Grune, T. Advanced glycation end products and oxidative stress in type 2 diabetes mellitus. *Biomolecules* **2015**, *5*, 194–222. [[CrossRef](#)]
79. Berger, J.; Shephard, D.; Morrow, F.; Sadowski, J.; Haire, T.; Taylor, A. Reduced and total ascorbate in guinea pig eye tissues in response to dietary intake. *Curr. Eye Res.* **1988**, *7*, 681–686. [[CrossRef](#)]
80. Berger, J.; Shepard, D.; Morrow, F.; Taylor, A. Relationship between dietary intake and tissue levels of reduced and total vitamin C in the nonscorbutic guinea pig. *J. Nutr.* **1989**, *119*, 734–740. [[CrossRef](#)]
81. Taylor, A.; Jacques, P.F.; Nowell, T.; Perrone, G.; Blumberg, J.; Handelman, G.; Jozwiak, B.; Nadler, D. Vitamin C in human and guinea pig aqueous, lens and plasma in relation to intake. *Curr. Eye Res.* **1997**, *16*, 857–864. [[CrossRef](#)]
82. Yeum, K.J.; Shang, F.M.; Schalch, W.M.; Russell, R.M.; Taylor, A. Fat-soluble nutrient concentrations in different layers of human cataractous lens. *Curr. Eye Res.* **1999**, *19*, 502–505. [[CrossRef](#)]
83. Sajithlal, G.B.; 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. [[CrossRef](#)]

84. Li, X.; Zheng, T.; Sang, S.; Lv, L. Quercetin inhibits advanced glycation end product formation by trapping methylglyoxal and glyoxal. *J. Agric. Food Chem.* **2014**, *62*, 12152–12158. [[CrossRef](#)] [[PubMed](#)]
85. Sampath, C.; Rashid, M.R.; 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. Pharm.* **2017**, *87*, 73–81. [[CrossRef](#)]
86. 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. [[CrossRef](#)]
87. Navarro, M.; Morales, F.J.; Ramos, S. Olive leaf extract concentrated in hydroxytyrosol attenuates protein carbonylation and the formation of advanced glycation end products in a hepatic cell line (HepG2). *Food Funct.* **2017**, *8*, 944–953. [[CrossRef](#)]
88. 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. [[CrossRef](#)]
89. Basta, G.; Lazzarini, G.; Del Turco, S.; Ratto, G.M.; Schmidt, A.M.; De Caterina, R. At least 2 distinct pathways generating reactive oxygen species mediate vascular cell adhesion molecule-1 induction by advanced glycation end products. *Arterioscler. Thromb. Vasc. Biol.* **2005**, *25*, 1401–1407. [[CrossRef](#)]
90. Nam, M.H.; Son, W.R.; Lee, Y.S.; Lee, K.W. Glycolaldehyde-derived advanced glycation end products (glycol-AGEs)-induced vascular smooth muscle cell dysfunction is regulated by the AGES-receptor (RAGE) axis in endothelium. *Cell Commun. Adhes.* **2015**, *22*, 67–78. [[CrossRef](#)]
91. Dobi, A.; Bravo, S.B.; Veeren, B.; Paradela-Dobarro, B.; Álvarez, E.; Meilhac, O.; Viranaicken, W.; Baret, P.; Devin, A.; Rondeau, P. Advanced glycation end-products disrupt human endothelial cells redox homeostasis: New insights into reactive oxygen species production. *Free Radic. Res.* **2019**, *53*, 150–169. [[CrossRef](#)]
92. Wang, X.L.; Yu, T.; Yan, Q.C.; Wang, W.; Meng, N.; Li, X.J.; Luo, Y.H. AGEs Promote Oxidative Stress and Induce Apoptosis in Retinal Pigmented Epithelium Cells RAGE-dependently. *J. Mol. Neurosci.* **2015**, *56*, 449–460. [[CrossRef](#)] [[PubMed](#)]
93. Cai, W.; Torreggiani, M.; Zhu, L.; Chen, X.; He, J.C.; Striker, G.E.; Vlassara, H. AGER1 regulates endothelial cell NADPH oxidase-dependent oxidant stress via PKC-delta: Implications for vascular disease. *Am. J. Physiol. Cell Physiol.* **2010**, *298*, C624–C634. [[CrossRef](#)]
94. Vlassara, H.; Cai, W.; Goodman, S.; Pyzik, R.; Yong, A.; Chen, X.; Zhu, L.; Neade, T.; Beeri, M.; Silverman, J.M.; et al. Protection against loss of innate defenses in adulthood by low advanced glycation end products (AGE) intake: Role of the antiinflammatory AGE receptor-1. *J. Clin. Endocrinol. Metab.* **2009**, *94*, 4483–4491. [[CrossRef](#)] [[PubMed](#)]
95. Uribarri, J.; Cai, W.; Ramdas, M.; Goodman, S.; Pyzik, R.; Chen, X.; Zhu, L.; Striker, G.E.; Vlassara, H. Restriction of advanced glycation end products improves insulin resistance in human type 2 diabetes: Potential role of AGER1 and SIRT1. *Diabetes Care* **2011**, *34*, 1610–1616. [[CrossRef](#)] [[PubMed](#)]
96. Taylor, A. Mechanistically linking age-related diseases and dietary carbohydrate via autophagy and the ubiquitin proteolytic systems. *Autophagy* **2012**, *8*, 1404–1406. [[CrossRef](#)]
97. Rowan, S.; Bejarano, E.; Taylor, A. Mechanistic targeting of advanced glycation end-products in age-related diseases. *Biochim. Et Biophys. Acta. Mol. Basis Dis.* **2018**, *1864*, 3631–3643. [[CrossRef](#)]
98. Antognelli, C.; Talesa, V.N. Glyoxalases in Urological Malignancies. *Int. J. Mol. Sci.* **2018**, *19*, 415. [[CrossRef](#)]
99. Thornalley, P.J. The glyoxalase system: New developments towards functional characterization of a metabolic pathway fundamental to biological life. *Biochem. J.* **1990**, *269*, 1–11. [[CrossRef](#)]
100. Abordo, E.A.; Minhas, H.S.; Thornalley, P.J. Accumulation of alpha-oxoaldehydes during oxidative stress: A role in cytotoxicity. *Biochem. Pharmacol.* **1999**, *58*, 641–648. [[CrossRef](#)]
101. Clelland, J.D.; Thornalley, P.J. S-2-hydroxyacylglutathione-derivatives: Enzymatic preparation, purification and characterisation. *J. Chem. Soc. Perkin Trans. 1* **1991**, 3009–3015. [[CrossRef](#)]
102. Shinohara, M.; Thornalley, P.J.; Giardino, I.; Beisswenger, P.; Thorpe, S.R.; Onorato, J.; Brownlee, M. Overexpression of glyoxalase-I in bovine endothelial cells inhibits intracellular advanced glycation endproduct formation and prevents hyperglycemia-induced increases in macromolecular endocytosis. *J. Clin. Investig.* **1998**, *101*, 1142–1147. [[CrossRef](#)] [[PubMed](#)]
103. McCann, V.J.; Davis, R.E.; Welborn, T.A.; Constable, I.J.; Beale, D.G. Glyoxalase phenotypes in patients with diabetes mellitus. *Aust. N. Z. J. Med.* **1981**, *11*, 380–382. [[CrossRef](#)]

104. Kalousová, M.; Germanová, A.; Jáchymová, M.; Mestek, O.; Tesar, V.; Zima, T. A419C (E111A) polymorphism of the glyoxalase I gene and vascular complications in chronic hemodialysis patients. *Ann. N. Y. Acad. Sci.* **2008**, *1126*, 268–271. [[CrossRef](#)] [[PubMed](#)]
105. Ranganathan, S.; Ciaccio, P.J.; Walsh, E.S.; Tew, K.D. Genomic sequence of human glyoxalase-I: Analysis of promoter activity and its regulation. *Gene* **1999**, *240*, 149–155. [[CrossRef](#)]
106. Conboy, C.M.; Spyrou, C.; Thorne, N.P.; Wade, E.J.; Barbosa-Morais, N.L.; Wilson, M.D.; Bhattacharjee, A.; Young, R.A.; Tavaré, S.; Lees, J.A.; et al. Cell cycle genes are the evolutionarily conserved targets of the E2F4 transcription factor. *PLoS ONE* **2007**, *2*, e1061. [[CrossRef](#)] [[PubMed](#)]
107. Orso, F.; Corà, D.; Ubezio, B.; Provero, P.; Caselle, M.; Taverna, D. Identification of functional TFAP2A and SP1 binding sites in new TFAP2A-modulated genes. *Bmc Genom.* **2010**, *11*, 355. [[CrossRef](#)] [[PubMed](#)]
108. Xue, M.; Rabbani, N.; Momiji, H.; Imbasi, P.; Anwar, M.M.; Kitteringham, N.; Park, B.K.; Souma, T.; Moriguchi, T.; Yamamoto, M.; et al. Transcriptional control of glyoxalase 1 by Nrf2 provides a stress-responsive defence against dicarbonyl glycation. *Biochem. J.* **2012**, *443*, 213–222. [[CrossRef](#)]
109. Kold-Christensen, R.; Johannsen, M. Methylglyoxal Metabolism and Aging-Related Disease: Moving from Correlation toward Causation. *Trends Endocrinol. Metab.* **2020**, *31*, 81–92. [[CrossRef](#)]
110. Li, D.; Ferrari, M.; Ellis, E.M. Human aldo-keto reductase AKR7A2 protects against the cytotoxicity and mutagenicity of reactive aldehydes and lowers intracellular reactive oxygen species in hamster V79-4 cells. *Chem. Biol. Interact.* **2012**, *195*, 25–34. [[CrossRef](#)]
111. Li, D.; Ellis, E.M. Aldo-keto reductase 7A5 (AKR7A5) attenuates oxidative stress and reactive aldehyde toxicity in V79-4 cells. *Toxicol. Vitro. Int. J. Publ. Assoc. Bibra* **2014**, *28*, 707–714. [[CrossRef](#)]
112. Baba, S.P.; Barski, O.A.; Ahmed, Y.; O’Toole, T.E.; Conklin, D.J.; Bhatnagar, A.; Srivastava, S. Reductive metabolism of AGE precursors: A metabolic route for preventing AGE accumulation in cardiovascular tissue. *Diabetes* **2009**, *58*, 2486–2497. [[CrossRef](#)] [[PubMed](#)]
113. Vander Jagt, D.L.; Hunsaker, L.A. Methylglyoxal metabolism and diabetic complications: Roles of aldose reductase, glyoxalase-I, betaine aldehyde dehydrogenase and 2-oxoaldehyde dehydrogenase. *Chem. Biol. Interact.* **2003**, *143-144*, 341–351. [[CrossRef](#)]
114. Morgenstern, J.; Fleming, T.; Schumacher, D.; Eckstein, V.; Freichel, M.; Herzig, S.; Nawroth, P. Loss of Glyoxalase 1 Induces Compensatory Mechanism to Achieve Dicarbonyl Detoxification in Mammalian Schwann Cells. *J. Biol. Chem.* **2017**, *292*, 3224–3238. [[CrossRef](#)]
115. Lodd, E.; Wiggerhauser, L.M.; Morgenstern, J.; Fleming, T.H.; Poschet, G.; Büttner, M.; Tabler, C.T.; Wohlfart, D.P.; Nawroth, P.P.; Kroll, J. The combination of loss of glyoxalase1 and obesity results in hyperglycemia. *JCI Insight* **2019**, *4*. [[CrossRef](#)] [[PubMed](#)]
116. Schumacher, D.; Morgenstern, J.; Oguchi, Y.; Volk, N.; Kopf, S.; Groener, J.B.; Nawroth, P.P.; Fleming, T.; Freichel, M. Compensatory mechanisms for methylglyoxal detoxification in experimental & clinical diabetes. *Mol. Metab.* **2018**, *18*, 143–152. [[CrossRef](#)]
117. Fujii, E.; Iwase, H.; Ishii-Karakasa, I.; Yajima, Y.; Hotta, K. The presence of 2-keto-3-deoxygluconic acid and oxoaldehyde dehydrogenase activity in human erythrocytes. *Biochem. Biophys. Res. Commun.* **1995**, *210*, 852–857. [[CrossRef](#)] [[PubMed](#)]
118. Collard, F.; Vertommen, D.; Fortpied, J.; Duester, G.; Van Schaftingen, E. Identification of 3-deoxyglucosone dehydrogenase as aldehyde dehydrogenase 1A1 (retinaldehyde dehydrogenase 1). *Biochimie* **2007**, *89*, 369–373. [[CrossRef](#)]
119. McDowell, R.E.; McGahon, M.K.; Augustine, J.; Chen, M.; McGeown, J.G.; Curtis, T.M. Diabetes Impairs the Aldehyde Detoxifying Capacity of the Retina. *Investig. Ophthalmol. Vis. Sci.* **2016**, *57*, 4762–4771. [[CrossRef](#)] [[PubMed](#)]
120. Bonifati, V.; Rizzu, P.; van Baren, M.J.; Schaap, O.; Breedveld, G.J.; Krieger, E.; Dekker, M.C.; Squitieri, F.; Ibanez, P.; Joosse, M.; et al. Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. *Science* **2003**, *299*, 256–259. [[CrossRef](#)]
121. Lev, N.; Roncevic, D.; Ickowicz, D.; Melamed, E.; Offen, D. Role of DJ-1 in Parkinson’s disease. *J. Mol. Neurosci.* **2006**, *29*, 215–225. [[CrossRef](#)]
122. Lee, J.Y.; Song, J.; Kwon, K.; Jang, S.; Kim, C.; Baek, K.; Kim, J.; Park, C. Human DJ-1 and its homologs are novel glyoxalases. *Hum. Mol. Genet.* **2012**, *21*, 3215–3225. [[CrossRef](#)]

123. Richarme, G.; Mihoub, M.; Dairou, J.; Bui, L.C.; Leger, T.; Lamouri, A. Parkinsonism-associated protein DJ-1/Park7 is a major protein deglycase that repairs methylglyoxal- and glyoxal-glycated cysteine, arginine, and lysine residues. *J. Biol. Chem.* **2015**, *290*, 1885–1897. [[CrossRef](#)] [[PubMed](#)]
124. Pfaff, D.H.; Fleming, T.; Nawroth, P.; Teleman, A.A. Evidence Against a Role for the Parkinsonism-associated Protein DJ-1 in Methylglyoxal Detoxification. *J. Biol. Chem.* **2017**, *292*, 685–690. [[CrossRef](#)]
125. Richarme, G.; Liu, C.; Mihoub, M.; Abdallah, J.; Leger, T.; Joly, N.; Liebart, J.C.; Jurkunas, U.V.; Nadal, M.; Bouloc, P.; et al. Guanine glycation repair by DJ-1/Park7 and its bacterial homologs. *Science* **2017**, *357*, 208–211. [[CrossRef](#)]
126. Galligan, J.J.; Wepy, J.A.; Streeter, M.D.; Kingsley, P.J.; Mitchener, M.M.; Wauchope, O.R.; Beavers, W.N.; Rose, K.L.; Wang, T.; Spiegel, D.A.; et al. Methylglyoxal-derived posttranslational arginine modifications are abundant histone marks. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 9228–9233. [[CrossRef](#)]
127. Zheng, Q.; Omans, N.D.; Leicher, R.; Osunsade, A.; Agustinus, A.S.; Finkin-Groner, E.; D'Ambrosio, H.; Liu, B.; Chandralapaty, S.; Liu, S.; et al. Reversible histone glycation is associated with disease-related changes in chromatin architecture. *Nat. Commun.* **2019**, *10*, 1289. [[CrossRef](#)]
128. Kalapos, M.P. On the mammalian acetone metabolism: From chemistry to clinical implications. *Biochim. Biophys. Acta* **2003**, *1621*, 122–139. [[CrossRef](#)]
129. Salomón, T.; Sibbersen, C.; Hansen, J.; Britz, D.; Svart, M.V.; Voss, T.S.; Møller, N.; Gregersen, N.; Jørgensen, K.A.; Palmfeldt, J.; et al. Ketone Body Acetoacetate Buffers Methylglyoxal via a Non-enzymatic Conversion during Diabetic and Dietary Ketosis. *Cell Chem. Biol.* **2017**, *24*, 935–943.e937. [[CrossRef](#)]
130. Barnosky, A.R.; Hoddy, K.K.; Unterman, T.G.; Varady, K.A. Intermittent fasting vs daily calorie restriction for type 2 diabetes prevention: A review of human findings. *Transl. Res. J. Lab. Clin. Med.* **2014**, *164*, 302–311. [[CrossRef](#)] [[PubMed](#)]
131. Takahashi, A.; Takabatake, Y.; Kimura, T.; Maejima, I.; Namba, T.; Yamamoto, T.; Matsuda, J.; Minami, S.; Kaimori, J.Y.; Matsui, I.; et al. Autophagy Inhibits the Accumulation of Advanced Glycation End Products by Promoting Lysosomal Biogenesis and Function in the Kidney Proximal Tubules. *Diabetes* **2017**, *66*, 1359–1372. [[CrossRef](#)]
132. Uchiki, T.; Weikel, K.A.; Jiao, W.; Shang, F.; Caceres, A.; Pawlak, D.; Handa, J.T.; Brownlee, M.; Nagaraj, R.; Taylor, A. Glycation-altered proteolysis as a pathobiologic mechanism that links dietary glycemic index, aging, and age-related disease (in nondiabetics). *Aging Cell* **2012**, *11*, 1–13. [[CrossRef](#)] [[PubMed](#)]
133. Zhang, Y.; Cross, S.D.; Stanton, J.B.; Marmorstein, A.D.; Le, Y.Z.; Marmorstein, L.Y. Early AMD-like defects in the RPE and retinal degeneration in aged mice with RPE-specific deletion of Atg5 or Atg7. *Mol. Vis.* **2017**, *23*, 228–241. [[PubMed](#)]
134. Chai, P.; Ni, H.; Zhang, H.; Fan, X. The Evolving Functions of Autophagy in Ocular Health: A Double-edged Sword. *Int. J. Biol. Sci.* **2016**, *12*, 1332–1340. [[CrossRef](#)] [[PubMed](#)]
135. Rosa, M.D.; Distefano, G.; Gagliano, C.; Rusciano, D.; Malaguarnera, L. Autophagy in Diabetic Retinopathy. *Curr. Neuropharmacol.* **2016**, *14*, 810–825. [[CrossRef](#)]
136. Grimm, S.; Ernst, L.; Grötzinger, N.; Höhn, A.; Breusing, N.; Reinheckel, T.; Grune, T. Cathepsin D is one of the major enzymes involved in intracellular degradation of AGE-modified proteins. *Free Radic. Res.* **2010**, *44*, 1013–1026. [[CrossRef](#)]
137. Bulteau, A.L.; Verbeke, P.; Petropoulos, I.; Chaffotte, A.F.; Friguet, B. Proteasome inhibition in glyoxal-treated fibroblasts and resistance of glycated glucose-6-phosphate dehydrogenase to 20 S proteasome degradation in vitro. *J. Biol. Chem.* **2001**, *276*, 45662–45668. [[CrossRef](#)]
138. Raupbach, J.; Ott, C.; Koenig, J.; Grune, T. Proteasomal degradation of glycated proteins depends on substrate unfolding: Preferred degradation of moderately modified myoglobin. *Free Radic. Biol. Med.* **2020**, *152*, 516–524. [[CrossRef](#)]
139. Gavilán, E.; Pintado, C.; Gavilan, M.P.; Daza, P.; Sánchez-Aguayo, I.; Castaño, A.; Ruano, D. Age-related dysfunctions of the autophagy lysosomal pathway in hippocampal pyramidal neurons under proteasome stress. *Neurobiol. Aging* **2015**, *36*, 1953–1963. [[CrossRef](#)]
140. Kwon, Y.T.; Ciechanover, A. The Ubiquitin Code in the Ubiquitin-Proteasome System and Autophagy. *Trends Biochem. Sci.* **2017**, *42*, 873–886. [[CrossRef](#)]
141. Cuervo, A.M. Autophagy and aging: Keeping that old broom working. *Trends Genet.* **2008**, *24*, 604–612. [[CrossRef](#)]

142. Shang, F.; Taylor, A. Ubiquitin Conjugates: A Sensitive Marker of Oxidative Stress. In *Biomarkers for Antioxidant Defense and Oxidative Damage: Principles and Practical Applications*; Wiley: Hoboken, NJ, USA, 2010; pp. 219–228. [[CrossRef](#)]
143. Shang, F.; Taylor, A. Roles for the ubiquitin-proteasome pathway in protein quality control and signaling in the retina: Implications in the pathogenesis of age-related macular degeneration. *Mol. Asp. Med.* **2012**, *33*, 446–466. [[CrossRef](#)] [[PubMed](#)]
144. Itoh, K.; Ishii, T.; Wakabayashi, N.; Yamamoto, M. Regulatory mechanisms of cellular response to oxidative stress. *Free Radic. Res.* **1999**, *31*, 319–324. [[CrossRef](#)] [[PubMed](#)]
145. Kobayashi, M.; Itoh, K.; Suzuki, T.; Osanai, H.; Nishikawa, K.; Katoh, Y.; Takagi, Y.; Yamamoto, M. Identification of the interactive interface and phylogenetic conservation of the Nrf2-Keap1 system. *Genes Cells Devoted Mol. Cell. Mech.* **2002**, *7*, 807–820. [[CrossRef](#)]
146. Katoh, Y.; Itoh, K.; Yoshida, E.; Miyagishi, M.; Fukamizu, A.; Yamamoto, M. Two domains of Nrf2 cooperatively bind CBP, a CREB binding protein, and synergistically activate transcription. *Genes Cells Devoted Mol. Cell. Mech.* **2001**, *6*, 857–868. [[CrossRef](#)]
147. Cullinan, S.B.; Gordan, J.D.; Jin, J.; Harper, J.W.; Diehl, J.A. The Keap1-BTB protein is an adaptor that bridges Nrf2 to a Cul3-based E3 ligase: Oxidative stress sensing by a Cul3-Keap1 ligase. *Mol. Cell. Biol.* **2004**, *24*, 8477–8486. [[CrossRef](#)]
148. Nguyen, T.; Sherratt, P.J.; Nioi, P.; Yang, C.S.; Pickett, C.B. Nrf2 controls constitutive and inducible expression of ARE-driven genes through a dynamic pathway involving nucleocytoplasmic shuttling by Keap1. *J. Biol. Chem.* **2005**, *280*, 32485–32492. [[CrossRef](#)]
149. Antognelli, C.; Trapani, E.; Delle Monache, S.; Perrelli, A.; Daga, M.; Pizzimenti, S.; Barrera, G.; Cassoni, P.; Angelucci, A.; Trabalzini, L.; et al. KRIT1 loss-of-function induces a chronic Nrf2-mediated adaptive homeostasis that sensitizes cells to oxidative stress: Implication for Cerebral Cavernous Malformation disease. *Free Radic. Biol. Med.* **2018**, *115*, 202–218. [[CrossRef](#)]
150. Antognelli, C.; Trapani, E.; Delle Monache, S.; Perrelli, A.; Fornelli, C.; Retta, F.; Cassoni, P.; Talesa, V.N.; Retta, S.F. Data in support of sustained upregulation of adaptive redox homeostasis mechanisms caused by KRIT1 loss-of-function. *Data Brief* **2018**, *16*, 929–938. [[CrossRef](#)]
151. Gambelunghe, A.; Giovagnoli, S.; Di Michele, A.; Boncompagni, S.; Dell’Omo, M.; Leopold, K.; Iavicoli, I.; Talesa, V.N.; Antognelli, C. Redox-Sensitive Glyoxalase 1 Up-Regulation Is Crucial for Protecting Human Lung Cells from Gold Nanoparticles Toxicity. *Antioxidants* **2020**, *9*, 697. [[CrossRef](#)]
152. James, D.; Devaraj, S.; Bellur, P.; Lakkanna, S.; Vicini, J.; Boddupalli, S. Novel concepts of broccoli sulforaphanes and disease: Induction of phase II antioxidant and detoxification enzymes by enhanced-glucoraphanin broccoli. *Nutr. Rev.* **2012**, *70*, 654–665. [[CrossRef](#)]
153. Angeloni, C.; Malaguti, M.; Rizzo, B.; Barbalace, M.C.; Fabbri, D.; Hrelia, S. Neuroprotective effect of sulforaphane against methylglyoxal cytotoxicity. *Chem. Res. Toxicol.* **2015**, *28*, 1234–1245. [[CrossRef](#)]
154. Alfarano, M.; Pastore, D.; Fogliano, V.; Schalkwijk, C.G.; Oliviero, T. The Effect of Sulforaphane on Glyoxalase I Expression and Activity in Peripheral Blood Mononuclear Cells. *Nutrients* **2018**, *10*, 1773. [[CrossRef](#)] [[PubMed](#)]
155. Pereira, A.; Fernandes, R.; Crisóstomo, J.; Seica, R.M.; Sena, C.M. The Sulforaphane and pyridoxamine supplementation normalize endothelial dysfunction associated with type 2 diabetes. *Sci. Rep.* **2017**, *7*, 14357. [[CrossRef](#)]
156. Nishimoto, S.; Koike, S.; Inoue, N.; Suzuki, T.; Ogasawara, Y. Activation of Nrf2 attenuates carbonyl stress induced by methylglyoxal in human neuroblastoma cells: Increase in GSH levels is a critical event for the detoxification mechanism. *Biochem. Biophys. Res. Commun.* **2017**, *483*, 874–879. [[CrossRef](#)]
157. Liu, G.H.; Qu, J.; Shen, X. NF-kappaB/p65 antagonizes Nrf2-ARE pathway by depriving CBP from Nrf2 and facilitating recruitment of HDAC3 to MafK. *Biochim. Biophys. Acta* **2008**, *1783*, 713–727. [[CrossRef](#)] [[PubMed](#)]
158. Antognelli, C.; Gambelunghe, A.; Muzi, G.; Talesa, V.N. Peroxynitrite-mediated glyoxalase I epigenetic inhibition drives apoptosis in airway epithelial cells exposed to crystalline silica via a novel mechanism involving argpyrimidine-modified Hsp70, JNK, and NF-κB. *Free Radic. Biol. Med.* **2015**, *84*, 128–141. [[CrossRef](#)] [[PubMed](#)]
159. Zhang, H.; Li, H.; Xi, H.S.; Li, S. HIF1α is required for survival maintenance of chronic myeloid leukemia stem cells. *Blood* **2012**, *119*, 2595–2607. [[CrossRef](#)]

160. Rauh, D.; Fischer, F.; Gertz, M.; Lakshminarasimhan, M.; Bergbrede, T.; Aladini, F.; Kambach, C.; Becker, C.F.; Zerweck, J.; Schutkowski, M.; et al. An acetylome peptide microarray reveals specificities and deacetylation substrates for all human sirtuin isoforms. *Nat. Commun.* **2013**, *4*, 2327. [[CrossRef](#)]
161. Lundby, A.; Lage, K.; Weinert, B.T.; Bekker-Jensen, D.B.; Secher, A.; Skovgaard, T.; Kelstrup, C.D.; Dmytriiev, A.; Choudhary, C.; Lundby, C.; et al. Proteomic analysis of lysine acetylation sites in rat tissues reveals organ specificity and subcellular patterns. *Cell Rep.* **2012**, *2*, 419–431. [[CrossRef](#)]
162. Reiniger, N.; Lau, K.; McCalla, D.; Eby, B.; Cheng, B.; Lu, Y.; Qu, W.; Quadri, N.; Ananthakrishnan, R.; Furmanský, M.; et al. Deletion of the receptor for advanced glycation end products reduces glomerulosclerosis and preserves renal function in the diabetic OVE26 mouse. *Diabetes* **2010**, *59*, 2043–2054. [[CrossRef](#)]
163. Irshad, Z.; Xue, M.; Ashour, A.; Larkin, J.R.; Thornalley, P.J.; Rabbani, N. Activation of the unfolded protein response in high glucose treated endothelial cells is mediated by methylglyoxal. *Sci. Rep.* **2019**, *9*, 7889. [[CrossRef](#)] [[PubMed](#)]
164. Rowan, S.; Jiang, S.; Chang, M.L.; Volkin, J.; Cassalman, C.; Smith, K.M.; Streeter, M.D.; Spiegel, D.A.; Moreira-Neto, C.; Rabbani, N.; et al. A low glycemic diet protects disease-prone Nrf2-deficient mice against age-related macular degeneration. *Free Radic. Biol. Med.* **2020**, *150*, 75–86. [[CrossRef](#)] [[PubMed](#)]
165. Stratmann, B.; Goldstein, B.; Thornalley, P.J.; Rabbani, N.; Tschoepe, D. Intracellular Accumulation of Methylglyoxal by Glyoxalase 1 Knock Down Alters Collagen Homeostasis in L6 Myoblasts. *Int. J. Mol. Sci.* **2017**, *18*, 480. [[CrossRef](#)]
166. Nigro, C.; Leone, A.; Fiory, F.; Prevezano, I.; Nicolò, A.; Mirra, P.; Beguinot, F.; Miele, C. Dicarbonyl Stress at the Crossroads of Healthy and Unhealthy Aging. *Cells* **2019**, *8*. [[CrossRef](#)] [[PubMed](#)]
167. Giacco, F.; Du, X.; D'Agati, V.D.; Milne, R.; Sui, G.; Geoffrion, M.; Brownlee, M. Knockdown of glyoxalase 1 mimics diabetic nephropathy in nondiabetic mice. *Diabetes* **2014**, *63*, 291–299. [[CrossRef](#)] [[PubMed](#)]
168. Jang, S.; Kwon, D.M.; Kwon, K.; Park, C. Generation and characterization of mouse knockout for glyoxalase 1. *Biochem. Biophys. Res. Commun.* **2017**, *490*, 460–465. [[CrossRef](#)] [[PubMed](#)]
169. Shafie, A.; Xue, M.; Barker, G.; Zehnder, D.; Thornalley, P.J.; Rabbani, N. Reappraisal of putative glyoxalase 1-deficient mouse and dicarbonyl stress on embryonic stem cells in vitro. *Biochem. J.* **2016**, *473*, 4255–4270. [[CrossRef](#)]
170. Moraru, A.; Wiederstein, J.; Pfaff, D.; Fleming, T.; Miller, A.K.; Nawroth, P.; Teleman, A.A. Elevated Levels of the Reactive Metabolite Methylglyoxal Recapitulate Progression of Type 2 Diabetes. *Cell Metab.* **2018**, *27*, 926–934. [[CrossRef](#)]
171. Morcos, M.; Du, X.; Pfisterer, F.; Hutter, H.; Sayed, A.A.; Thornalley, P.; Ahmed, N.; Baynes, J.; Thorpe, S.; Kukudov, G.; et al. Glyoxalase-1 prevents mitochondrial protein modification and enhances lifespan in *Caenorhabditis elegans*. *Aging Cell* **2008**, *7*, 260–269. [[CrossRef](#)]
172. Distler, M.G.; Plant, L.D.; Sokoloff, G.; Hawk, A.J.; Aneas, I.; Wuenschell, G.E.; Termini, J.; Meredith, S.C.; Nobrega, M.A.; Palmer, A.A. Glyoxalase 1 increases anxiety by reducing GABA_A receptor agonist methylglyoxal. *J. Clin. Investig.* **2012**, *122*, 2306–2315. [[CrossRef](#)]
173. Brouwers, O.; Niessen, P.M.; Ferreira, I.; Miyata, T.; Scheffer, P.G.; Teerlink, T.; Schrauwen, P.; Brownlee, M.; Stehouwer, C.D.; Schalkwijk, C.G. Overexpression of glyoxalase-I reduces hyperglycemia-induced levels of advanced glycation end products and oxidative stress in diabetic rats. *J. Biol. Chem.* **2011**, *286*, 1374–1380. [[CrossRef](#)] [[PubMed](#)]
174. Berner, A.K.; Brouwers, O.; Pringle, R.; Klaassen, I.; Colhoun, L.; McVicar, C.; Brockbank, S.; Curry, J.W.; Miyata, T.; Brownlee, M.; et al. Protection against methylglyoxal-derived AGEs by regulation of glyoxalase 1 prevents retinal neuroglial and vasodegenerative pathology. *Diabetologia* **2012**, *55*, 845–854. [[CrossRef](#)] [[PubMed](#)]
175. Brouwers, O.; Niessen, P.M.; Miyata, T.; Østergaard, J.A.; Flyvbjerg, A.; Peutz-Kootstra, C.J.; Sieber, J.; Mundel, P.H.; Brownlee, M.; Janssen, B.J.; et al. Glyoxalase-1 overexpression reduces endothelial dysfunction and attenuates early renal impairment in a rat model of diabetes. *Diabetologia* **2014**, *57*, 224–235. [[CrossRef](#)]
176. Qi, W.; Keenan, H.A.; Li, Q.; Ishikado, A.; Kannt, A.; Sadowski, T.; Yorek, M.A.; Wu, I.H.; Lockhart, S.; Coppey, L.J.; et al. Pyruvate kinase M2 activation may protect against the progression of diabetic glomerular pathology and mitochondrial dysfunction. *Nat. Med.* **2017**, *23*, 753–762. [[CrossRef](#)]
177. Yao, D.; Brownlee, M. Hyperglycemia-induced reactive oxygen species increase expression of the receptor for advanced glycation end products (RAGE) and RAGE ligands. *Diabetes* **2010**, *59*, 249–255. [[CrossRef](#)]

178. Dobler, D.; Ahmed, N.; Song, L.; Eboigbodin, K.E.; Thornalley, P.J. Increased dicarbonyl metabolism in endothelial cells in hyperglycemia induces anoikis and impairs angiogenesis by RGD and GFOGER motif modification. *Diabetes* **2006**, *55*, 1961–1969. [[CrossRef](#)] [[PubMed](#)]
179. Miller, A.G.; Smith, D.G.; Bhat, M.; Nagaraj, R.H. Glyoxalase I is critical for human retinal capillary pericyte survival under hyperglycemic conditions. *J. Biol. Chem.* **2006**, *281*, 11864–11871. [[CrossRef](#)]
180. Barati, M.T.; Merchant, M.L.; Kain, A.B.; Jevans, A.W.; McLeish, K.R.; Klein, J.B. Proteomic analysis defines altered cellular redox pathways and advanced glycation end-product metabolism in glomeruli of db/db diabetic mice. *Am. J. Physiol. Ren. Physiol.* **2007**, *293*, F1157–F1165. [[CrossRef](#)]
181. Palsamy, P.; Subramanian, S. Resveratrol protects diabetic kidney by attenuating hyperglycemia-mediated oxidative stress and renal inflammatory cytokines via Nrf2-Keap1 signaling. *Biochim. Et Biophys. Acta* **2011**, *1812*, 719–731. [[CrossRef](#)]
182. Miller, A.G.; Tan, G.; Binger, K.J.; Pickering, R.J.; Thomas, M.C.; Nagaraj, R.H.; Cooper, M.E.; Wilkinson-Berka, J.L. Candesartan attenuates diabetic retinal vascular pathology by restoring glyoxalase-I function. *Diabetes* **2010**, *59*, 3208–3215. [[CrossRef](#)]
183. Phillips, S.A.; Mirrlees, D.; Thornalley, P.J. Modification of the glyoxalase system in streptozotocin-induced diabetic rats. Effect of the aldose reductase inhibitor Statil. *Biochem. Pharmacol.* **1993**, *46*, 805–811. [[CrossRef](#)]
184. Bierhaus, A.; Fleming, T.; Stoyanov, S.; Leffler, A.; Babes, A.; Neacsu, C.; Sauer, S.K.; Eberhardt, M.; Schönöler, M.; Lasitschka, F.; et al. Methylglyoxal modification of Nav1.8 facilitates nociceptive neuron firing and causes hyperalgesia in diabetic neuropathy. *Nat. Med.* **2012**, *18*, 926–933. [[CrossRef](#)]
185. Atkins, T.W.; Thornalley, P.J. Erythrocyte glyoxalase activity in genetically obese (ob/ob) and streptozotocin diabetic mice. *Diabetes Res.* **1989**, *11*, 125–129. [[PubMed](#)]
186. McLellan, A.C.; Thornalley, P.J. Glyoxalase activity in human red blood cells fractionated by age. *Mech. Ageing Dev.* **1989**, *48*, 63–71. [[CrossRef](#)]
187. López-Otín, C.; Blasco, M.A.; Partridge, L.; Serrano, M.; Kroemer, G. The hallmarks of aging. *Cell* **2013**, *153*, 1194–1217. [[CrossRef](#)]
188. Schalkwijk, C.G.; Stehouwer, C.D.A. Methylglyoxal, a Highly Reactive Dicarbonyl Compound, in Diabetes, Its Vascular Complications, and Other Age-Related Diseases. *Physiol. Rev.* **2020**, *100*, 407–461. [[CrossRef](#)] [[PubMed](#)]
189. Xue, M.; Rabbani, N.; Thornalley, P.J. Glyoxalase in ageing. *Semin. Cell Dev. Biol.* **2011**, *22*, 293–301. [[CrossRef](#)]
190. Rabbani, N.; Xue, M.; Thornalley, P.J. Methylglyoxal-induced dicarbonyl stress in aging and disease: First steps towards glyoxalase 1-based treatments. *Clin. Sci.* **2016**, *130*, 1677–1696. [[CrossRef](#)]
191. Sharma-Luthra, R.; Kale, R.K. Age related changes in the activity of the glyoxalase system. *Mech. Ageing Dev.* **1994**, *73*, 39–45. [[CrossRef](#)]
192. Amicarelli, F.; Di Ilio, C.; Masciocco, L.; Bonfigli, A.; Zarivi, O.; D’Andrea, M.R.; Di Giulio, C.; Miranda, M. Aging and detoxifying enzymes responses to hypoxic or hyperoxic treatment. *Mech. Ageing Dev.* **1997**, *97*, 215–226. [[CrossRef](#)]
193. Kirk, J.E. The glyoxalase I activity of arterial tissue in individuals of various ages. *J. Gerontol.* **1960**, *15*, 139–141. [[CrossRef](#)]
194. Haik, G.M., Jr.; Lo, T.W.; Thornalley, P.J. Methylglyoxal concentration and glyoxalase activities in the human lens. *Exp. Eye Res.* **1994**, *59*, 497–500. [[CrossRef](#)] [[PubMed](#)]
195. Mailankot, M.; Padmanabha, S.; Pasupuleti, N.; Major, D.; Howell, S.; Nagaraj, R.H. Glyoxalase I activity and immunoreactivity in the aging human lens. *Biogerontology* **2009**, *10*, 711–720. [[CrossRef](#)] [[PubMed](#)]
196. Kuhla, B.; Boeck, K.; Lüth, H.J.; Schmidt, A.; Weigle, B.; Schmitz, M.; Ogunlade, V.; Münch, G.; Arendt, T. Age-dependent changes of glyoxalase I expression in human brain. *Neurobiol. Aging* **2006**, *27*, 815–822. [[CrossRef](#)] [[PubMed](#)]
197. Wu, J.C.; Li, X.H.; Peng, Y.D.; Wang, J.B.; Tang, J.F.; Wang, Y.F. Association of two glyoxalase I gene polymorphisms with nephropathy and retinopathy in Type 2 diabetes. *J. Endocrinol. Investig.* **2011**, *34*, e343–e348. [[CrossRef](#)]
198. Rasul, A.; Rashid, A.; Waheed, P.; Khan, S.A. Expression analysis of glyoxalase I gene among patients of diabetic retinopathy. *Pak. J. Med. Sci.* **2018**, *34*, 139–143. [[CrossRef](#)] [[PubMed](#)]
199. Zaidi, A.; Waheed, P.; Rashid, A.; Khan, S.A. Gene Expression of Glyoxalase II in Diabetic Retinopathy. *J. Coll. Physicians Surg. Pak.* **2018**, *28*, 523–526. [[CrossRef](#)]

200. Sachdeva, R.; Schlotterer, A.; Schumacher, D.; Matka, C.; Mathar, I.; Dietrich, N.; Medert, R.; Kriebs, U.; Lin, J.; Nawroth, P.; et al. TRPC proteins contribute to development of diabetic retinopathy and regulate glyoxalase 1 activity and methylglyoxal accumulation. *Mol. Metab.* **2018**, *9*, 156–167. [[CrossRef](#)]
201. Arai, M.; Nihonmatsu-Kikuchi, N.; Itokawa, M.; Rabbani, N.; Thornalley, P.J. Measurement of glyoxalase activities. *Biochem. Soc. Trans.* **2014**, *42*, 491–494. [[CrossRef](#)]
202. Peters, A.S.; Wortmann, M.; Fleming, T.H.; Nawroth, P.P.; Bruckner, T.; Böckler, D.; Hakimi, M. Effect of metformin treatment in patients with type 2 diabetes with respect to glyoxalase 1 activity in atherosclerotic lesions. *Vasa. Z. Fur Gefasskrankh.* **2019**, *48*, 186–192. [[CrossRef](#)]
203. Cheng, A.S.; Cheng, Y.H.; Chiou, C.H.; Chang, T.L. Resveratrol upregulates Nrf2 expression to attenuate methylglyoxal-induced insulin resistance in Hep G2 cells. *J. Agric. Food Chem.* **2012**, *60*, 9180–9187. [[CrossRef](#)] [[PubMed](#)]
204. Maher, P.; Dargusch, R.; Ehren, J.L.; Okada, S.; Sharma, K.; Schubert, D. Fisetin lowers methylglyoxal dependent protein glycation and limits the complications of diabetes. *PLoS ONE* **2011**, *6*, e21226. [[CrossRef](#)] [[PubMed](#)]
205. Liu, Y.W.; Zhu, X.; Zhang, L.; Lu, Q.; Wang, J.Y.; Zhang, F.; Guo, H.; Yin, J.L.; Yin, X.X. Up-regulation of glyoxalase 1 by mangiferin prevents diabetic nephropathy progression in streptozotocin-induced diabetic rats. *Eur. J. Pharmacol.* **2013**, *721*, 355–364. [[CrossRef](#)]
206. Liu, Y.W.; Cheng, Y.Q.; Liu, X.L.; Hao, Y.C.; Li, Y.; Zhu, X.; Zhang, F.; Yin, X.X. Mangiferin Upregulates Glyoxalase 1 Through Activation of Nrf2/ARE Signaling in Central Neurons Cultured with High Glucose. *Mol. Neurobiol.* **2017**, *54*, 4060–4070. [[CrossRef](#)] [[PubMed](#)]
207. Suantawee, T.; Thilavech, T.; Cheng, H.; Adisakwattana, S. Cyanidin Attenuates Methylglyoxal-Induced Oxidative Stress and Apoptosis in INS-1 Pancreatic β -Cells by Increasing Glyoxalase-1 Activity. *Nutrients* **2020**, *12*, 1319. [[CrossRef](#)]
208. Xue, M.; Weickert, M.O.; Qureshi, S.; Kandala, N.B.; Anwar, A.; Waldron, M.; Shafie, A.; Messenger, D.; Fowler, M.; Jenkins, G.; et al. Improved Glycemic Control and Vascular Function in Overweight and Obese Subjects by Glyoxalase 1 Inducer Formulation. *Diabetes* **2016**, *65*, 2282–2294. [[CrossRef](#)]
209. Angeloni, C.; Turrioni, S.; Bianchi, L.; Fabbri, D.; Motori, E.; Malaguti, M.; Leoncini, E.; Maraldi, T.; Bini, L.; Brigidi, P.; et al. Novel targets of sulforaphane in primary cardiomyocytes identified by proteomic analysis. *PLoS ONE* **2013**, *8*, e83283. [[CrossRef](#)]
210. Maeda, S.; Matsui, T.; Ojima, A.; Takeuchi, M.; Yamagishi, S. Sulforaphane inhibits advanced glycation end product-induced pericyte damage by reducing expression of receptor for advanced glycation end products. *Nutr. Res.* **2014**, *34*, 807–813. [[CrossRef](#)]
211. Lv, J.; Bao, S.; Liu, T.; Wei, L.; Wang, D.; Ye, W.; Wang, N.; Song, S.; Li, J.; Chudhary, M.; et al. Sulforaphane delays diabetes-induced retinal photoreceptor cell degeneration. *Cell Tissue Res.* **2020**. [[CrossRef](#)]
212. Oh, S.; Ahn, H.; Park, H.; Lee, J.I.; Park, K.Y.; Hwang, D.; Lee, S.; Son, K.H.; Byun, K. The attenuating effects of pyridoxamine on adipocyte hypertrophy and inflammation differ by adipocyte location. *J. Nutr. Biochem.* **2019**, *72*, 108173. [[CrossRef](#)]
213. Stitt, A.; Gardiner, T.A.; Alderson, N.L.; Canning, P.; Frizzell, N.; Duffy, N.; Boyle, C.; Januszewski, A.S.; Chachich, M.; Baynes, J.W.; et al. The AGE inhibitor pyridoxamine inhibits development of retinopathy in experimental diabetes. *Diabetes* **2002**, *51*, 2826–2832. [[CrossRef](#)] [[PubMed](#)]
214. He, Y.; Zhou, C.; Huang, M.; Tang, C.; Liu, X.; Yue, Y.; Diao, Q.; Zheng, Z.; Liu, D. Glyoxalase system: A systematic review of its biological activity, related-diseases, screening methods and small molecule regulators. *Biomed Pharm.* **2020**, *131*, 110663. [[CrossRef](#)] [[PubMed](#)]

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