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The role of advanced glycation end products in aging and metabolic diseases: bridging association and causality

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Abstract

Accumulation of advanced glycation end products (AGEs) on nucleotides, lipids and peptides/proteins are an inevitable component of the aging process in all eukaryotic organisms, including humans. To date, a substantial body of evidence shows that AGEs and their functionally compromised adducts are linked to and perhaps responsible for changes seen during aging and for the development of many age-related morbidities. However, much remains to be learned about the biology of AGE formation, causal nature of these associations and whether new interventions might be developed that will prevent or reduce the negative impact of AGEs-related damage. To facilitate achieving these latter ends, we show how invertebrate models, notably *Drosophila melanogaster* and *Caenorhabditis elegans,* can be used to explore AGE-related pathways in depth and to identify and assess drugs that will mitigate against the detrimental effects of AGE-adduct development.

Graphical Abstract

Chaudhuri et al. discuss mechanistic evidence for the role of glycolytic byproducts that lead to accumulation of Advanced Glycation End products (AGEs) in the onset of age-related diseases. They outline how model organisms can unveil these mechanisms that will help develop better therapeutics to overcome diabetic pathologies and neurodegenerative diseases.

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Declaration of Interests

The authors declare no competing interests.

Introduction

A heterogeneous group of molecules collectively called advanced glycation end products (AGEs), are produced in the classical Maillard reaction, discovered at the beginning of the 20th century (Maillard, 1912). More than three decades ago, Monnier and Cerami proposed a Maillard theory of aging postulating that slow and continuous accumulation of AGEs was a causal factor in aging (Bjorksten, 1968; Monnier, 1989; Monnier et al., 1988; Sell and Monnier, 1989). Furthermore, they proposed that the protracted buildup of these compounds may alter the structure and function of proteins, thus affecting several of the hallmarks of aging(Gugliucci and Menini, 2017; López-Otín et al., 2013). This process may also contribute to the pathology of metabolic diseases, such as diabetes and atherosclerosis, as well as oxidative stress and inflammation associated with neurodegenerative diseases of aging. Support for this hypothesis includes an age-dependent increase in browning (Maillard reaction), fluorescence, cross-linking, and insolubility, and accrual of AGEs in collagens and lens crystallins (Monnier et al., 1984; Monnier VM, Stevens VJ, 1981). Despite this accumulating evidence, debate continues over whether AGEs are causal or just a consequence of aging and age-related diseases (Gugliucci, 2017).

The link between age-related diseases and AGEs has been difficult to unravel for several reasons: **(1)** the variety of sources for AGEs, **(2)** the gradual build-up of AGEs, which can take decades to be detected in humans, **(3)** lack of accessible and sensitive methods to quantify specific AGEs, **(4)** a growing number of targets of AGEs, and **(5)** a lack of models that recapitulate the pathologies resulting from the accumulation of AGEs. These factors have complicated efforts to model causation by connecting an AGE to one specific target and a specific disease relevant to aging.

In this review, we first focus on the chemistry of AGEs, how they accumulate through *in situ* synthesis or via ingestion of food, the naturally occurring mechanisms that reduce their formation, and their metabolism at the whole-body level. We then explore the causative mechanistic links that impact aging and metabolic diseases via accumulation of AGEs. We suggest considerations for developing model systems to study the impact of AGEs. Finally, we illustrate how modeling of AGEs using model organisms, notably worms and flies, can be used to identify ‘anti-AGE’ drugs and examine their relevance to age-related diseases, including diabetic complications and neurodegeneration.

The Maillard reaction: initiators, propagators, and chemistry

AGEs are produced by glycation in cells or in long-lived extracellular proteins. Protein glycation is a complex series of sequential reactions collectively called the Maillard reaction (Fig. 1), present in all tissues and fluids where significant concentration of glucose, fructose, or more reactive dicarbonyls react with proteins (Stevens et al. 1977, Sell et al. 1991, Monnier et al. 1992). When glucose mediates the reaction, initially the Amadori adduct fructosyl-lysine is formed. In hemoglobin, this adduct is called HbA1c, which revolutionized the diagnosis and follow-up of diabetic patients (Rahbar et al., 1969). However, HbA1c only assesses glucose derived products. There is an urgent need to develop markers of AGEs based on downstream products of glucose metabolism like α-dicarbonyl compounds (α-

DCs) that are significantly more reactive than glucose and examine their link with various age-related pathologies.

Chronic hyperglycemia results in several metabolic and biochemical perturbations, including elevation of a series of highly reactive α-DCs, such as methylglyoxal (MGO), glyoxal (GO), and 3-deoxyglucosone (3DG) (Henning et al., 2014; Singh et al., 2014). The α-DCs are unavoidable byproducts of anaerobic glycolysis and to a smaller degree lipid peroxidation (Lange et al., 2012; Rabbani and Thornalley, 2014; Thornalley et al., 2003), which react indiscriminately with proteins, lipids, and DNA to yield AGEs (Peppa and Vlassara, 2005; Thornalley et al., 2003). The production of MGO, a key precursor of the AGEs, occurs spontaneously from the triose phosphate isomers glyceraldehyde-3 phosphate and dihydroxyacetone phosphate during glycolysis (Kiefer et al., 2014; Sousa Silva et al., 2013) and is primarily due to β-elimination of a phosphate group from the enediolate phosphate intermediate. The study of the role of α-DCs like MGO in AGEs has lagged due to their unstable nature which makes them hard to detect in standard metabolomics studies and because they are non-enzymatic byproducts of metabolic reactions.

Important sources of AGEs include hydroimidazolones derived from arginine residues modified by GO, MGO, and 3-DG, which we will discuss extensively in this review. Other crucial AGEs compounds are Nε-carboxymethyl-lysine (CML), Nε-carboxyethyl-lysine (CEL), pentosidine, pyrraline and glucosepane (Nemet et al., 2011; Sveen et al., 2015). As discussed in detail below, the catabolism of cellular AGEs yields a new pool of second generation, highly reactive AGE intermediates (peptides and free adducts) named glycotoxins by some authors (Koschinsky et al., 1997; Uribarri et al., 2003a, 2003b), which pass through the bloodstream to compound cellular damage. Some of these serum AGE-small adducts react with new proteins, (e.g., LDL, collagen) perpetuating and propagating oxidative modifications and/or producing new AGE crosslinks *in vitro* and *in vivo* (Gugliucci and Bendayan, 1996; Makita et al., 1994;Vlassara et al., 1992).

AGE formation renders irreversible damage to the biological macromolecules, altering their structural and functional integrity (Singh et al., 2014). Kinetics of AGE formation through a reaction between MGO and most susceptible amino acids such as arginine, lysine, and cysteine have been studied *in vitro* by incubating bovine serum albumin (BSA) and MGO under physiological conditions (Lo et al., 1994). These studies indicate the irreversible formation of MGO adducts mainly on arginine and lysine. As shown in Figure 1,the adducts primarily result from a reaction between MGO and Nα-acetylarginine on the peptide backbone leading to an irreversible imidazolone derivative or hydroimidazolone (MG-H1) through autoxidation of an intermediate 1,5-dihydroimidazolone (Lo et al., 1994). Also, modification of the lysine residues triggered by MGO results in Nε-carboxyethyl-lysine (CEL) (Rabbani and Thornalley, 2015a).

In addition to amino acids, specific nucleotides are also susceptible to modification by MGO. DNA-AGEs such as N^2(1-carboxyethyl)-2'-deoxyguanosine (CEdG) has been described as a potential biomarker of chronic hyperglycemia in mice. CEdG levels are significantly elevated in urine collected from hyperglycemic $Lepr^{db/db}$ mice compared to normoglycemic control mice (Jaramillo et al., 2017). Of note, many of the same compounds

are produced in baked and roasted foods (Delgado-Andrade and Fogliano, 2018). Administration of dietary AGEs in mice suggests that AGEs consumed through dry heat cooked food (Cai et al., 2012; Goldberg et al., 2004) could potentially enhance the risk for age-related diseases (Goldberg et al., 2004), as depicted further below in Figure 4, via activation of the receptor of AGE (RAGE). The biochemistry of AGE formation and its downstream effects are complex and remain to be fully understood. Nevertheless, it is crucial to understand the dicarbonyl detoxification mechanisms that are potential therapeutic targets for AGE-mediated diseases as well as the pathways for AG Es disposal after they are generated.

The α-DC detoxification network

In the first decade of glycation research, the emphasis was on extracellular glycation of collagens, crystallins and other long-lived proteins. These proteins are correlated with aging and have shown to be important in the pathogenesis of diabetic complications. Since then, more attention has been devoted to intracellular glycation, especially by methylglyoxal, which is between 2 and 3 orders of magnitude more reactive than glucose. The importance of methylglyoxal is underscored by the existence of several conserved enzymes involved in its detoxification.

Glyoxalases such as Glyoxalase I *(GLO1)* and *DJ1/PARK7* are an evolutionarily conserved group of enzymes involved in the detoxification of reactive α-DCs including methylglyoxal and glyoxal that eventually get converted into lactic or glycolic acid as depicted in Figure 2. Studies using knockdown of these enzymes in mice, cell culture, and worms show that these genes play a critical role in avoiding pathologies resulting from the accumulation of α-DC-mediated modification of amino acids or nucleotides (Giacco et al., 2014; Lee et al., 2012; Richarme et al., 2017). The tissue-specificity and sub-cellular localization of the different glyoxalase enzymes are not well-understood. The glyoxalase system can be either glutathione GSH-dependent (e.g., Glo1) or GSH-independent (e.g., Dj-1) (Lee et al., 2012; Thornalley, 1990). In recent years, a number of human cohort studies have provided evidence that polymorphisms associated with genes that detoxify α-DCs are linked to certain age-related diseases. Polymorphisms associated with the glyoxalase Glo1 gene have been associated with the development of nephropathy and retinopathy in Type 2 diabetics (Gale et al., 2004; Kalousová et al., 2008; Wu et al., 2011). Genotyping of whole blood lysates obtained from Type 1 and Type 2 diabetics revealed a marked decrease in the activity of glyoxalase 1 that was predicted to be associated to three SNPs (Peculis et al., 2013). In addition to glyoxalase I, variations within the Dj-1 glyoxalase have also been associated with age-related diseases such as Parkinson's Disease (PD). A study on 294 PD patients in Southern Italy demonstrated the association of sporadic PD with 5 SNPs within the 3535 bp region extending from the promoter to intron 2 of the Dj-1 gene (De Marco et al., 2010).

Recent studies in *Escherichia coli,* show that the protein *DJ1* possesses deglycase activity that restores proteins and amino acids from glycation events induced by methylglyoxal or glyoxal (Richarme et al., 2015). The deglycase activity of *DJ1* was not supported in a follow-up study due to a potential artifact arising from TRIS buffer (Pfaff et al., 2017). More recent studies using deglycase-depleted cells reinforce *DJ1* as a bona fide deglycase

(Richarme and Dairou, 2017). The late stages of the Maillard reaction which form AGEs are considered to be irreversible. However, if the early steps of AGE formation are reversible, then it will provide another step for potential intervention.

Other detoxifying enzymes fo r dicarbonyls and early glycation adducts

Another class of enzymes that play a critical role in the detoxification of the reactive α-DCs are the evolutionarily conserved NADPH-dependent aldo-keto reductases (AKRs) (Vander Jagt et al., 1992). AGE-mediated atherosclerotic lesion formation was enhanced in the absence of AKRs (Baba et al., 2009). Another mechanism of protection against glycation mediated damage has been shown via AGE sequestration by lysozymes resulting in improved renal excretion in mice (Zheng et al., 2001). As stated earlier, due to its stability in the closed ring conformation, glucose is not as strong a glycating agent as MGO. However, its effects are important in erythrocytes and other cells that mainly use GLUT I (non-insulin-dependent) transporter. As shown in Figure 1, the early Schiff base formed between glucose and lysine in proteins stabilizes into a fructosamine. Until the last decade, the fate of fructosamines in mammalian cells was only considered to be their spontaneous conversion into AGEs, as shown in Figure 1. A mammalian fructosamine-3-kinase (FN-3-K), which phosphorylates fructoselysine (FL) residues on glycated proteins to FL-3-phosphate has been isolated and cloned (Delpierre et al., 2004). This unveiled an unsuspected intracellular metabolism of glucose adducts. FN-3-K phosphorylates both low-molecular-mass and protein-bound fructosamines with high affinity for the third carbon of their deoxyfructose moiety, producing fructosamine 3-phosphates. The latter are unstable and spontaneously decompose into inorganic phosphate and 3-deoxyglucosone, regenerating the unglycated amine (Gugliucci, 2005). The presence of proteins related to fructosamine 3-kinase in many prokaryotic and eukaryotic genomes implies that this ‘deglycation’ process is not restricted to erythrocytes nor to just mammals (Collard et al., 2004; Dunmore et al., 2018; Van Schaftingen et al., 2007; Szwergold et al., 2011).

A schematic describing some of the known steps in the formation and detoxification of AGEs is shown in Figure 2. Potentially, several regulators of glyoxalases and alpha keto-reductases in addition to other enzymes that can detoxify AGEs exist and remain to be discovered. Therefore, in addition to our existing body of knowledge on the detoxification pathways, there is a need for studies using model systems that are amenable to large genetic screens, such as worms, flies, and yeast, to determine additional genes and their regulators in the α-dicarbonyl detoxification network. These studies are likely to be pivotal in providing new drug targets that limit the accumulation of AGEs that build up with age.

Systemic metabolism and handling o f AGEs, the key role o f the kidney

AGEs are produced endogenously but are also consumed through the diet especially through dry heat cooked food. Human serum contains partially hydrolyzed AGE peptides and free AGE adducts (Bucala et al., 1994; Gugliucci and Bendayan, 1996). These are increased in diabetes and more so in end-stage renal failure even in the absence of diabetes. In earlier studies, we presented the renal fate of AGEs (Gugliucci and Bendayan 1995, 1996) and have shown that AGE peptides are filtered and then reabsorbed in the proximal tubules followed by excretion of free AGE adducts, as depicted in Figure 3. To determine this fate, AGE-BSA

and AGE-peptides were injected in rats, and AGE-products in renal tissue of rats were monitored by colloidal gold post-embedding immunoelectron microscopy (Gugliucci and Bendayan 1995, 1996). We showed that the endo-lysosomal apparatus of the proximal convoluted tubule plays a role in the disposal of AGE-peptides. AGE-peptide clearance in humans and rats is lower than the creatinine clearance which suggests that not all of circulating AGE-peptides are secreted in the urine; reabsorption also occurs. No mammalian enzyme is known to mediate the catabolism of AGE moieties after the lysosomal hydrolysis of peptide bonds. We suggest the existence of a secretory process of resulting AGE-amino acids (free adducts) into urine which could explain the presence of AGE-adducts such as pentosidine in the urine of diabetic patients. These pathways, which depend on glomerular filtration and tubular function, are severely compromised in chronic and end-stage renal failure (Thornalley, 2005a, 2006). Due to this fact, AGE adducts are one of the classical ‘middle toxins’ associated with renal failure (Vlassara, 1994). With the arrival of sensitive LC-MS/MS techniques, a more comprehensive representation of the role of the kidney in AGE control emerged, including the fate of small peptides and free adducts (Agalou et al., 2005; Rabbani and Thornalley, 2009; Rabbani et al., 2007; Thornalley, 2005b; Thornalley and Rabbani, 2009). Key AGEs have been described and shown in Figure 1.

The influence of AGE build up during aging and its im pact on aging

There is significant evidence for the buildup of AGEs with aging in multiple species. Contributions from the Baynes, Thorpe and Monnier laboratories over the past three decades have been key in this area (Monnier and Taniguchi, 2016). The characterization of several cross-links present in collagen and their association with arterial disease has underscored their importance in diabetic complications as well as in aging (Baynes, 2001; Baynes et al., 1989; Dyer et al., 1991; Thorpe and Baynes, 2003). Based on a population-based study on adults aged 65 and older, high plasma carboxymethyl-lysine (CML) levels were found to be positively correlated with an increased risk of mortality in older adults due to all-cause mortality or cardiovascular disease (CVD) (Semba et al., 2009). Interestingly, the association of CML with increased mortality risk (all-cause or CVD) was found to be independent of diabetes mellitus (Semba et al., 2009). Thus, AGEs may not just be a biomarker but also a potential driver of aging.

As depicted in Figure 1, AGEs are a heterogeneous group of compounds that include more than 20 different products. These products that have been described further potentially mediate a wide variety of pathological effects (Monnier et al., 2014, 2015; Piperi et al., 2012; Sveen et al., 2015). Evidence of their accumulation in crystallins, collagens and basement membranes has accumulated over the years. Several studies have addressed the buildup of AGEs in long-lived proteins (Hammes et al., 1999; Sell et al., 1992; Vashishth, 2009). One human study that quantified autofluorescence of eye lens as an indicator of AGE accumulation with age (Cahn et al., 2014). The buildup of different AGEs (e.g., pentosidine) in the skin and increased crosslinking of collagen due to AGEs has been observed in the skin biopsies of diabetic patients (Monnier et al., 2005; Sell et al., 1992). Pentosidine, a pentose-mediated protein cross-linking is present in multiple types of human tissues including plasma proteins and red blood cells (Sell and Monnier, 1989). Similar work has underscored the importance of AGEs in bone health with age (Yamagishi, 2011). In particular, the

extracellular matrix is an important compartment for the buildup of AGEs due to the presence of long-lived proteins like collagen (Singh et al., 2014). Accumulation of AGEs has also been suggested to contribute to the accumulation of lipofuscin with age (Nowotny et al., 2014).

The above evidence although suggestive is merely correlative. Evidence for a direct and causal relationship of AGEs with lifespan comes from studies in *C. elegans.* Overexpression of the glyoxalase GLOD-4 in worms extends lifespan and also inhibits dicarbonyl-mediated modification of mitochondrial proteins by AGEs (Morcos et al., 2008). Loss of function of GLOD-4, on the other hand, shortens lifespan which is exacerbated under high glucose conditions in *C. elegans* (Chaudhuri et al., 2016). Interestingly, a recent study has shown that low concentrations of MGO, formed via inactivation of the enzyme glycine-C-acetyltransferase (GCAT) involved in threonine catabolism, can promote lifespan through proteohormesis (Ravichandran et al., 2018). Reduction of age-related accumulation of AGEs by the FDA-approved (for the treatment of tuberculosis (TB)) drug Rifampicin also enhances worm lifespan through activation of DAF-16/FOXO (Golegaonkar et al., 2015). The additional evidence underlying the role of AGEs in determining lifespan comes from studies correlating the levels of AGEs in different species. Previous results from the Monnier group have shown that the accumulation of pentosidine in skin samples varies at a rate inversely related to maximum lifespan. This has been studied across eight mammalian species (Sell et al., 1996). Thus, there is significant evidence on the effect of AGEs in the aging process and for their causal influence on rates of organismal aging. We further discuss the evidence for the role of AGEs in age-associated diseases below.

AGEs and their link with age-related diseases in humans

Prolonged hyperglycemia in diabetes leads to a number of pathologies collectively termed diabetic complications. These include neuropathy, cardiomyopathy, nephropathy and retinopathy (Singh et al., 2014). One of the most compelling explanations for the molecular basis of diabetic complications is the buildup of glucose catabolism-derived reactive by products, the α-DCs (Henning et al., 2014). Notably, accumulation of MGO greater than 600 nM in the plasma distinguishes diabetics with pain and no pain in humans (Bierhaus et al., 2012). Variations in susceptibility to diabetic complications under similar glycemic levels could potentially be due to differences in detoxification of these α-DCs and associated AGEs. Notably, the active involvement of AGEs has also been implicated in age-related neurodegenerative diseases (Castellani et al., 1996). A key question in the field is whether AGEs are passive bystanders or if they actively contribute to cellular damage. Direct role for AGEs has been shown in mice by administering synthetic AGEs (derived from MGO treatment of BSA) that enhanced the accumulation of triglycerides and premature development of insulin resistance due to the reduction in anti-AGE receptor 1 (AGER1) and sirtuin 1 (SIRT1) in various tissues (Cai et al., 2012). Diet-derived AGEs has also been shown to exacerbate conditions of diabetic complications resulting in accumulation of pro-inflammatory factors such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) in the serum of streptozotocin-induced diabetic mice (LV et al., 2016). AGEs also lead to significant vascular complications and damage to organs such as the heart and kidney (LV et

al., 2016). These studies provide evidence behind the direct involvement of α-DCs such as MGO in mediating a variety of age-related disease complications.

Pathways underlying AGE-mediated effects on aging and age-related diseases

The RAGE pathway and oxidative stress: a key mechanism by which AGEs participate in the pathogenicity o f chronic diseases

AGEs likely contribute to the age-related increase in inflammation or 'inflammaging' (Franceschi and Campisi, 2014). They are ligands to several pro- and anti-inflammatory cellular receptors. A key pro-inflammatory receptor is the receptor for advanced glycation end-products (RAGE), a multi-ligand protein discovered and isolated from bovine lung (Coughlan et al., 2007; Miyata et al., 1996; Rodríguez-Ayala et al., 2005; Schmidt and Stern, 2000; Schmidt et al., 1996; Tanji et al., 2000; Yamagishi, 2011). RAGE belongs to the immunoglobulin superfamily of receptors and coordinates intracellular RAGE signaling (Daffu et al., 2013). As depicted in Figure 4, besides AGE adducts, RAGE binds a wide array of ligands including the leukocyte integrin Mac-1, S100 / calgranulins, high mobility group box 1 protein (HMGB1), modified LDL, DNA, RNA and amyloid fibrils. These ligands bear similar structural features: multiple β-sheets. RAGE identifies its ligands through them (Ramasamy et al., 2011;Yan et al., 2010a). Upon ligand binding, RAGE starts a signaling cascade with activation of nuclear factor-kB (NF-kB), oxidative stress and inflammation as shown in Figure 4. RAGE signals via phosphatidylinositol-3 kinase (PI-3K), Ki-Ras and the MAPKs, Erk1, and Erk2 (Ramasamy et al., 2011;Yan et al., 2010a). These pathways orchestrate the translocation of NF-kB from the cytoplasm to the nucleus stimulating inflammation and tissue injury operated by the RAGE-dependent expression of pro-inflammatory mediators such as monocyte chemoattractant protein-1 (MCP-1) and vascular cell adhesion molecule-1 (VCAM-1). Through these pathways, RAGE activity is associated with diabetic microvascular complications including nephropathy, retinopathy, and neuropathy (Ramasamy et al., 2011;Yan et al., 2010a). Besides these cascades leading to inflammation, in endothelial (glomerular) and mesangial cells, RAGE activation increases reactive oxygen species (ROS). As we illustrate in Figure 4, a protagonist role is taken by reduced nicotinamide adenine dinucleotide phosphate, NAD(P)H oxidase, which in analogy to what happens in neutrophils, is a significant player in oxidative stress and dysfunction (D'Agati and Schmidt, 2010). The extracellular domain of RAGE is present in the bloodstream and may play a role in cardiovascular disease and chronic diseases (Figure 3). The C-terminal truncated form of RAGE mRNA lacks the encoding sequences for the transmembrane and intracytoplasmic domains (Forbes et al., 2005; Kalousová et al., 2007; Nakashima et al., 2010). The truncated version (endogenous secretory RAGE: esRAGE) is released and found in the circulation in humans. This esRAGE cancels the effects of AGEs on cells in culture (Bowman and Schmidt, 2013) and overexpression of esRAGE in mice reverses diabetic vascular dysfunction. Hence, esRAGE may play a decoy function: a feedback mechanism has been proposed by which esRAGE prevents RAGE signaling. Another form of soluble RAGE (sRAGE) (Figure 4) stems from proteolytic cleavage of the native RAGE expressed on the cell surface, both may act as decoys protecting from excessive ligand binding to RAGE and its consequent inflammatory cascades (Leonardis et

al., 2012; Raposeiras-Roubín et al., 2010; Vazzana et al., 2009; Yan et al., 2010b). Further, the protective effect of esRAGE against dopaminergic neuronal death through inhibition of AGE-albumin build-up suggests the direct link between AGE-RAGE interaction and neurodegeneration (Bayarsaikhan et al., 2016).

Is AMPK a key target for damage by MGO that impairs metabolism?

Another mechanism by which MGO stress may be implicated in metabolic disease and especially in the metabolic syndrome (MetS) is by its putative action against AMPK. AMPK induces a cascade of events within cells in response to the fluxes and availability of metabolites. The role of AMPK in regulating cellular energy status (by sensing low energy using [AMP] as its signal) and activating catabolic pathways while inhibiting anabolic routes, places this enzyme at a central control point in maintaining energy homeostasis. Once activated, AMPK-mediated downstream phosphorylation events switch cells from active ATP consumption to active ATP production. Thus, it increases glucose transport, glycolysis, beta-oxidation, and inhibits lipogenesis and cholesterol biosynthesis. The mammalian AMPK is a trimeric enzyme composed of a catalytic α subunit and non-catalytic β and γ subunits. The N-terminal half of the α subunits contains a typical serine/threonine kinase catalytic domain. Direct AMP-binding studies have shown that AMP is bound to the γ subunits by a pair of so-called Bateman's domains. Three arginine residues are the binding site for AMP (the binding is electrostatic and not the AMP-Mg++ complex, but free AMP is sensed), making this allosteric site vulnerable to carbonyl attack, especially by MGO.

A very small rise in AMP levels can induce a dramatic increase in the activity of AMPK which suggests that blocking of the AMP allosteric sites, even minimally, can have amplified reduction in AMPK activation. We proposed that an increased flux of MGO could achieve this effect. This increased flux may be produced by fructose surges (excess sugar in the diet, especially liquid), hyperglycemia, as well as an overflow of the glycolytic pathway caused by inactivation of glyceraldehyde-3-P dehydrogenase (GAPDH), in turn induced by its direct oxidation by reactive oxygen species (ROS) or indirectly by their activation of polyadenorybosyl polymerase (PARP) as proposed by Michael Brownlee (Brownlee, 2005). Inactivation of AMPK would favor lipogenesis, insulin resistance, and hyperglycemia, all hallmarks of MetS and diabetes (Gugliucci, 2009, 2016, 2017).

The impact of AGEs on diabetes-associated complications

Peripheral neuropathy

Some of the strongest evidence of the role of AGEs in diabetic complications comes from mice studies in peripheral neuropathy. As an example, we depict a summary of those pathways in Figure 5. To understand the important role of glycation in diabetic neuropathy one should consider that the neural deficit is the consequence of a metabolic shift at three levels: the endothelium of the vasa nervorum, the sensory neuron (dorsal root ganglia) axon and the Schwann cell (glia) (Münch et al., 2012). Figure 5 illustrates some characteristics of this triple hit, which bear common pathways in all cells as well as specific differences in others. Hyperglycemia leads to polyol and fructose accumulation (fructose is 7 times more

reactive than glucose), NADPH and GSH depletion and oxidative/nitrosative stress. This damages DNA which induces the repair enzyme poly (ADP-ribose) polymerase (PARP) activity. PARP inactivates GAPDH, blocking the second phase of glycolysis, leading to MGO accumulation and more glycation and damage. Protein kinase C beta (PKC β) is activated in the vasa nervorum as a result of these metabolic shifts, and inflammatory damage to the endothelium ensues, leading to increased permeability and cell death. Conversely, protein kinase C alpha (PKC α) is inactivated in neuronal axons, which induces dysfunction. Glycation of the extracellular matrix molecules impairs nerve regeneration, compounding the problem. Dorsal root neuron RAGE is increased, and so are the NF-kB cascade responses. When protracted these changes are damaging to neurons, which do not reproduce and therefore accumulate the damage. Lastly, MAPK pathways are activated by the same metabolic shifts leading to COX 2 activation with potent pro-inflammatory components (Münch et al., 2012). In support of these general pathways, variation in Glo1 abundance among mice strains has been shown to lead to a differential response of sensory neurons to dicarbonyl stress (Jack et al., 2011). In another study in mice, it was found that post-translational modifications in the voltage-gated sodium channel Na(v)1.8 induced by plasma methylglyoxal, results in hyperalgesia (Bierhaus et al., 2012). This study was crucial in delineating a novel therapeutic target for dicarbonyl-mediated diabetic neuropathy. In addition, and consistent with the latter, the dicarbonyl sensitive neuronal receptor *TRPA1* (Transient Receptor Potential cation channel A1), was shown to mediate pain in Trpa1 null mice and the agonist, methylglyoxal, to activate the receptor in mammalian cell culture (Andersson et al., 2013). More recently, hyperpolarization-activated cyclic nucleotide-gated 2 (HCN2) ion channels in mice have also been identified to be a potential therapeutic target for neuropathy, a condition that develops progressively with age in diabetic patients (Tsantoulas et al., 2017). In conclusion, AG Es participate in the triple hit at the basis of diabetic neuropathy by acting on vasa nervorum, neurons and glia.

Diabetic nephropathy

In addition to diabetic neuropathy, the role of α-DC-derived AGEs has also been implicated in diabetic nephropathy (Rabbani and Thornalley, 2018a). Knockdown of the α-DC detoxification gene glyoxalase I (Glo1) in non-diabetic mice recapitulates diabetic nephropathy symptoms without hyperglycemia (Giacco et al., 2014). Some features of AGE-mediated diabetic nephropathy, namely myofibroblast-based fibrosis, has also been modeled in a rat proximal tubule cell line by inducing trans-differentiation of epithelial cells into myofibroblasts using labeled AGE (^{125}I-AGE-BSA). AGE-RAGE signaling elicits this response by stimulating the production of TGF-β and other cytokines which, in turn, trigger transdifferentiation (Oldfield et al., 2001). Endothelial damage and early renal dysfunction also seem to result from AGE accumulation. Thus, the appearance of this phenotype was inhibited in streptozotocin-treated Glo1 overexpressing rats (Brouwers et al., 2014). Similarly, overexpression of Glo1 completely prevented hyperglycemia-induced renal pathology, possibly by lowering induced oxidative stress mediated by α-DC induced glomerular protein modifications (Giacco et al., 2014).

Diabetic macroangiopathy

The detoxification of α-DCs, a precursor of AGE, also underlies the prevention of diabetes-mediated micro and macro vascular damage that may contribute to atherosclerosis and stroke (Brownlee, 2001, 2005). In support of the role of AGEs in vascular derangement, intravenous(IV) administration of AGEs in healthy rats significantly triggered the expression of vascular endothelial growth factor (VEGF) and inhibited pigment epithelium-derived factor (PEDF) levels that lead to diabetic retinopathy due to enhanced vascular permeability (Yamagishi et al., 2006). AGEs also induce diabetic macroangiopathy primarily by interacting with the endothelial cells lining the walls of the blood vessels. The latter activity results in a pro-inflammatory cascade followed by vascular dysfunction (Basta et al., 2004). Along similar lines but involving a different endpoint, it was earlier reported that both *in vitro* and *in vivo* administration of aminoguanidine (a nucleophilic hydrazine compound, that prevents AGE crosslinking) inhibits AGE formation and subsequent age-related increase in collagen cross-linking in the wall of the arteries (Brownlee et al., 1986).

Dietary AGEs and obesity

In addition to AGE accumulation via the endogenous route, exogenous sources such as diet also contribute significantly to a number of AGE-related pathologies. Thus, in mice, dietary AGEs supplemented with high fat (high AGE-high fat or HAGE-HF) resulted in hepatosteatosis and steatohepatitis, both characteristics of Non-alcoholic Fatty Liver Disease or NAFLD (Sayej et al., 2016). In addition to gaining weight, treated animals also showed secretion of various inflammatory cytokines from the adipose tissue (Sayej et al., 2016). The latter finding is consistent with the effects of, standard western diet, a major source of AGEs as a causative factor behind chronic inflammation and oxidative stress leading to insulin resistance (IR) (Gugliucci, 2017; Uribarri et al., 2015; Vlassara and Uribarri, 2014).

Modeling the influence of AGEs in neurodegenerative diseases

Parkinson's Disease (PD)

In addition to diabetic complications, AGEs are also known to contribute to age-related neurodegeneration (reviewed in (Münch et al., 2012)). For example, a diet with high glycemic index, when fed to mice, has been shown to cause a significant increase in the formation of AGEs in the brain, primarily in the substantia nigra (Uchiki et al., 2012). In addition, glycation mediated AGE formation has been reported at the periphery of Lewy bodies in PD patients (Castellani et al., 1996). Lewy bodies are aggregations of intracytoplasmic inclusions in the subcortical neurons in PD patients (Trojanowski et al., 1998). In cases of incidental Lewy body disease, AGEs appear in newly formed Lewy bodies further suggesting that AGEs may play a critical role in triggering Lewy body formation in pre-PD individuals (Münch et al., 2000).

Further evidence of the role of AGEs in neurodegenerative diseases comes from the findings that *DJ1*, glutathione-independent glyoxalase, involved in the detoxification of AGEs, is associated with familial, early-onset and sporadic forms of PD (Lee et al., 2012). This protective role of *DJ-1* against dicarbonyls was modeled using mouse embryonic fibroblast and human SH-SY5Y cell models for dopaminergic neurons (Lee et al., 2012). In addition,

studies using cell culture and *C. elegans* suggest that the glyoxalase function of DJ-1 is critical for neuroprotection against toxic oxaldehydes (Lee et al., 2012). Further, using human and mice primary cells, *DJ-1* has also been identified as a stabilizer of Nrf2 under conditions of toxicity related stress (Clements et al., 2006; Im et al., 2012; Xue et al., 2012). Thus, the loss of *DJ-1* function may lead to increased levels of methylglyoxal and AGEs, making it an attractive therapeutic target for diseases associated with oxidative stress (Ariga et al., 2013). At the mechanistic level, the relationship between AGEs and PD could also be due to the ability of AGEs to cross-link alpha-synuclein, as has been shown using *in vitro* studies (Shaikh and Nicholson, 2008). A recent study has shown that glycation of α-synuclein, a protein whose aggregation is the hallmark of PD, enhanced the aggregation and reduced clearance of toxic oligomers. *In vitro* and *in vivo* studies using flies and mice showed that enhanced glycation is toxic while glycation inhibitors reduce aggregation and enhance clearance of α-synuclein, and rescue behavioral phenotypes (Vicente Miranda et al., 2017). Furthermore, these studies also explain the significantly increased risk of PD amongst diabetics.

Alzheimer's Disease (AD)

In addition to PD, AGEs have also been associated with AD. Previous studies suggest that the glycation of such AD-associated proteins such as Aβ and tau play a critical role in the pathogenesis of this disorder. For example, plaques extracted from AD brains show a 3-fold increase in AGE content compared to age-matched healthy individuals (Vitek et al., 1994). Also, AGEs have been suggested to stabilize and promote the formation of aggregated forms of Aβ and tau (Chen et al., 2006; Ledesma et al., 1994; Woltjer et al., 2003). More specifically like PD, glycation is considered to be responsible for the increase in the cross-linking of Aβ and tau proteins and the subsequent formation of stable oligomeric forms. Additional direct evidence favoring an AGE-AD connection comes from, *in vitro* studies using neuroblastoma cell lines and *in vivo* experiments involving mice. These experiments show that glycation (AGE formation) also upregulates the expression of the amyloid precursor protein (APP), an enhancement that increases Aβ peptides levels (Ko et al., 2010) that can in turn trigger phosphorylation of tau proteins as characterized in rat septal cholinergic neurons (Zheng et al., 2002). In addition to phosphorylating tau proteins, an important hallmark of the disease, AGEs also co-localize with other markers of neurodegeneration, such as nNOS (a nitrooxidative stress marker) and caspase-3 (an apoptotic cell-death marker) (Lüth et al., 2005). The connection between glycated protein levels in the cerebrospinal fluid (CSF) of AD patients (Ahmed et al., 2005; Li et al., 2013) further reinforces the importance of glycated Aβ as a potential mechanism for disease progression. It has been shown consistently that there is a significant buildup of AGEs in serum and CSF of patients with AD compared to controls (Ahmed et al., 2005). Thus, it seems reasonable to postulate that the age-dependent decrease in glyoxalase levels and a concomitant increase in AGE buildup, contributes, at least in part, to the increased incidence of Alzheimer's and other neurodegenerative diseases (Kuhla et al., 2007). These studies may also help explain mechanisms underlying the epidemiological evidence that suggest link between type 2 diabetes and AD (Li et al., 2015).

Potential factors underlying AGE-mediated effects on neurodegeneration

A. Inflammation—Inflammation downstream of AGE production has been implicated as the key mechanism contributing to diseases. For instance, AGE-albumin in the extracellular space of human microglial cells triggers the generation of multi-ligand receptors for AGE or RAGE on cell membranes (Bayarsaikhan et al., 2016). The RAGE interaction with the secreted AGE-albumin complexes has been shown to mediate an apoptotic cascade leading to the death of human dopaminergic neurons. Elevated AGE-albumin accumulation from activated microglia has been suggested as potential biomarkers for neurodegenerative diseases (Bayarsaikhan et al., 2016). Activation of macrophages leads to an exacerbated release of AGE-albumin complexes, a major AGE derivative that drives shared pathways resulting in the progression of neurodegeneration (Byun et al., 2017). Upregulation of RAGE-mediated signaling has also been shown in the 6-hydroxydopamine (6-OHDA) induced rat model for neurotoxicity (Serratos et al., 2016).

B. Cellular stress and decreased proteostasis—Other than AGE-RAGE interaction enhancing inflammation, one of the key mechanisms that lead to AGE-mediated neurodegeneration is the generation of cellular stress and decline in proteostasis with age. This stems from the damage caused by AGEs to proteins and its subsequent crosslinking and accumulation in aggregates. Accumulation of AGEs potentially through such cross-linking events results in sustained cellular stress eventually leading to neuronal cell death (Guerrero et al., 2012). Such increased cellular stress can also happen due to activation of RAGE-like receptors (Hipkiss et al., 2013; Peppa and Vlassara, 2005).

C. Glycation o f neurotransmitters—Moreover MGO, besides proteins, may selectively modify dopamine. The reaction leads to toxic metabolites like 1-acetyl-6.7-dihydroxy-1,2,3,4,-tetrahydroisoquinoline (ADTIQ) (Deng et al., 2012). This active metabolite is present in human brain tissue, including the substantia nigra. ADTIQ levels are increased in PD patients (Deng et al., 2012), and the structure of ADTIQ resembles that of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) elucidated using hyperglycemic cell and rat models (Song et al., 2014). Interestingly, in a separate study, ADTIQ has been shown as an endogenous neurotoxin in SH-SY5Y neuroblastoma cells, and the levels of both MGO and ADTIQ are increased in the striatum of a streptozotocin-induced diabetic rat model (Xie et al., 2015).

Despite our mechanistic understanding of the AGE-mediated effects in the pathology of age-related diseases, designing viable therapeutic strategies can be a challenge without modeling the disease in experimental organisms and without a better understanding of the pathways targeted by AGES and those involved in AGE formation and detoxification.

Using invertebrate models to study the impact of AGEs

To better understand the role of AGEs in modulating age-related disease, there is an urgent need for suitable models to study the biological mechanisms of AGE formation, detoxification, and link to the disease pathology in a short time-frame. This is a major bottleneck in our current understanding of the biochemistry of AGE formation and its consequences, and hence rapid drug development to combat the associated pathologies. To

that end, there has been a considerable effort to model AGE accumulation in a variety of model organisms ranging from invertebrates to vertebrates in order to gain a mechanistic insight (Table 1). These studies also highlight the importance of model organisms in designing and delineating putative therapeutic targets that could have otherwise been extremely challenging to determine. A good model to study long-term AGE-related complications should have the following characteristics: **(1)** Recapitulate, at least some of the pathologies associated with α-DC and AGE buildup, **(2)** Recapitulate the build-up of AGEs in an age-dependent manner in a short time frame and **(3)** Be amenable to high-throughput genetic and drug screens.

Vertebrate model organisms such as rodents are a natural first choice to model AGE-mediated pathologies, due to the relatively close link to human disease biology and a track record as a model for studying disease pathology in diabetes (King, 2012). However, a rodent's relatively long life span and associated cost of maintenance pose limitations to conduct rapid genetic or drug screens or to serve as a model that recapitulates the effects of aging while studying AGE-mediated pathologies. Using *C. elegans,* it has been shown that *glod-4,* an orthologue of mammalian Glo1 glyoxalase, plays a critical role in regulating worm lifespan (Morcos et al., 2008). This study enabled the understanding of the long-term effects of dicarbonyl-mediated damage of mitochondrial proteins via generation of enhanced mitochondrial ROS (reactive oxygen species) that was rescued by the glyoxalase overexpressing animals (Morcos et al., 2008). Also, *C. elegans* displayed endogenous accumulation of dicarbonyls and AGEs as the glyoxalase-1 activity declined with age (Morcos et al., 2008). In our recent study, we have utilized the *C. elegans* mutant for *glod-4* as a model to study diabetic complications. The *C. elegans glod-4* mutant accumulates toxic α-DCs and exhibits several diabetes-related pathologies akin to the mouse model. These include accumulation of α-DCs, hyperesthesia (or hyper sensitivity to touch), neuronal damage and early mortality, in a glucose-dependent fashion (Chaudhuri et al., 2016). The short-lived *glod-4* mutant has also been shown to accumulate a broad variety of AGE-modified proteins (Golegaonkar et al., 2015). Furthermore, the powerful genetic tools available to study *C. elegans* led to the identification of a conserved and critical role for TRPA-1, in sensing MGO and also in SKN-1/Nrf2 activation to detoxify α-DCs. In addition, we utilized the worm to undertake a phenotypic drug screen for neuropathy by screening for compounds that reduce the increased hypersensitivity to touch in *glod-4* mutants. This screen has identified several novel pharmacological leads, including Podocarpic and Lipoic acid as activators of *TRPA1* that ameliorate the deleterious consequences of MGO accumulation in *C. elegans* (Chaudhuri et al., 2016). The relevance of a worm model to study diabetic complications is underscored by the facts that Nrf2 has been previously shown to play an important role in ameliorating diabetic pathologies in mice (Jiang et al., 2010; Li et al., 2012; Zheng et al., 2011),and that lipoic acid is used clinically for diabetic nephropathy (McIlduff and Rutkove, 2011; Ziegler et al., 1999).

Other studies have also utilized *C. elegans* or *D. melanogaster* to study MGO and AGE stress.*C. elegans* has been modeled to study glucose toxicity due to AGE-mediated modification of mitochondrial proteins (Schlotterer et al., 2009). A reduction of AGE-mediated glycation by the Rifampicin has been shown to extend *C. elegans* lifespan through

the activation of DAF-16/FOXO signaling (Golegaonkar et al., 2015). A study in fruit flies demonstrated that the mutant for the glycolytic enzyme, triose phosphate isomerase (TPI) leads to increased neurodegeneration (Gnerer et al., 2006). TPI is a key isomerase that converts dihydroxyacetone phosphate (DHAP) to glyceraldehyde-3-phosphate (GAP), and in its absence, DHAP non enzymatically generates increased concentrations of MGO and thus AGEs (Gnerer et al., 2006). In another study using flies, a mutant for the fatty acid synthase *(FASN)* gene was used to understand the interplay between dietary sugar, MGO, and lipogenesis (Garrido et al., 2015). Their study demonstrates the requirement for fatty acid synthesis to prevent the potentially detrimental intracellular build-up of MGO-derived AGEs (Garrido et al., 2015). In addition to invertebrate animal models such as worm and flies, another model system frequently utilized to characterize glycation mediated protein damage is *S. cerevisiae* (yeast). In a recent study, mutants that enhance MGO production were used to understand glycation-mediated protein misfolding associated with huntingtin protein in yeast, flies and human cells (Vicente Miranda et al., 2016). The key finding in the study was that glycation enhanced the aggregation and inhibited clearance of HTT suggesting a direct role of protein glycation in neurodegeneration (Vicente Miranda et al., 2016). In a recent study utilizing flies and mice, it was also shown that accumulation of toxic oligomers due to age-related glycation of α-synuclein resulted in disruption of synaptic transmission in the neurons (Vicente Miranda et al., 2017).

The vertebrate, invertebrate, and cell culture models suggest the direct involvement of AGEs in mediating a broad spectrum of disease pathologies and also the feasibility to gain a mechanistic understanding of these processes. In particular, the use of short-lived animal models has allowed one to monitor the progression of pathology and its link with AGEs. Overall, the evidence linking AGEs with diabetic complications and neurodegenerative diseases is emerging, but the mechanisms by which they influence disease progression and genetic networks involved in modulating AGEs remain poorly understood. Greater utilization of expedient genetic models should help reveal the mechanisms in place to reduce the accumulation of AGEs.

Glyoxalases as a target for therapy

Thus given the evidence implicating AGEs in diabetic complications, detoxification of AGEs represents an orthogonal approach to the treatment of diabetes in addition to lowering glucose. Given the success of aminoguanidine (also known as Pimagedine) in animal models, a human clinical trial was conducted to prevent diabetic kidney disease (Freedman et al., 1999). Unfortunately, the trial was discontinued due to side effects (Thornalley, 2003), which has led to reduced enthusiasm in the field that lowering AGEs would be a successful strategy in treating age-related diseases. However, there is need to devise novel approaches to lower AGEs by harnessing the endogenous defenses in place, which are likely to prevent AGE formation and potentially pose fewer side effects. In a recent promising clinical trial, a combination therapy of trans-Resveratrol (tRes) and Hesperetin (HESP) helped improve glycemic control and vascular inflammation in healthy overweight and obese individuals via induction of Glo1 (Rabbani and Thornalley, 2018b). This suggests that inhibition of MGO accumulation via induction of glyoxalases could be a potential therapeutic strategy for glycation-mediated diseases.

Glyoxalase expression and activity are shown to be regulated by the nuclear factor erythroid 2 related factor 2 (Nrf2) (Xue et al., 2012), a basic leucine zipper (bZIP) protein that also regulates the expression of other antioxidant proteins (Rabbani and Thornalley, 2015b). This is due to the presence of stress-response-related regulatory elements such as AREs (antioxidant-response elements) on the 5' flanking region of the Glo1 gene (Xue et al., 2012). Urine samples from 10 week old $Nrf2^{-/-}$ mice compared with age-matched controls showed significant accumulation of AGEs such as MGdG and MG-H1 as well as macromolecular damage (Xue et al., 2012). Similar results were described by our group using *C. elegans* (Chaudhuri et al., 2016). Furthermore, we found that the Nrf2 mediated regulation of both the glutathione-dependent and independent glyoxalases (*GLO1* and *DJ1*) is mediated by the activation of the cation channel *TRPA1,* an upstream sensor for α-DCs (Chaudhuri et al., 2016). Sensing of dicarbonyls by *TRPA1* is relayed to the transcription factor SKN-1/Nrf via MAP kinase-mediated signaling and ultimately enhances the expression of Glo1 and Dj1 to initiate the detoxification program (Chaudhuri et al., 2016). Chronic activation *in vitro* in the vascular endothelial cells of diabetic rats of the nuclear factor-kappaB (NF-kappaB), downstream of AGE activation of RAGE, has also been shown to regulate glyoxalases (Bierhaus et al., 2001). Thus given the evidence for Nrf2 in detoxifying AGEs across species, it represents an excellent target to mitigate AGEs and associated diseases.

Conclusions

We have a limited understanding of the causal effects of both dietary and endogenous AGEs on aging and age-related diseases. More importantly, many aspects of AGE accumulation including how they are formed, sensed and detoxified remains poorly understood. Regulatory mechanisms that underlie these pathways are potential targets for human diseases. There is a significant need to develop therapeutics that lower AGEs and can serve as an orthogonal approach to treat various age-related human metabolic diseases, like metabolic syndrome, diabetic complications as well as neurodegenerative diseases. Vertebrate and invertebrate model systems with a genetically impaired glyoxalase system have been instrumental in assessing the extent of α-DC and AGE-mediated disease pathologies and mechanisms. Invertebrate model organisms have the advantage to recapitulate AGE-mediated pathologies over a short time frame which otherwise takes years to build up in humans. The worm, fly and yeast models with their relatively short lifespans and easy-to-use genetic tools, could help answer some of the complex mechanistic questions associated with diseases such as obesity, neurodegeneration and diabetic complications in a much more rapid manner. The combination of AGE-mediated stress with existing human disease models is likely to better mimic the age-related disease pathology in humans. They could also be used as a tool for high-throughput drug screening platform to identify novel pharmaceutical leads for these diseases. A key limitation to using certain invertebrate models could be trying to model AGE-related disease complications associated with organs, such as the bone, the heart, and the vascular system. The initial discoveries of disease mechanisms or mode of action for drug molecules from high-throughput screens made possible in invertebrates could be further validated in vertebrate model systems in pre-clinical trials. Importantly, despite certain drawbacks from a physiological point of view,

utilizing the strength of invertebrate model systems in gaining mechanistic insights and performing high-throughput small molecule and genetic screens could potentially fuel major discoveries. The use of invertebrate models allows the examination of these complex diseases through the lens of aging, which is normally harder to recapitulate in traditional pre-clinical models.

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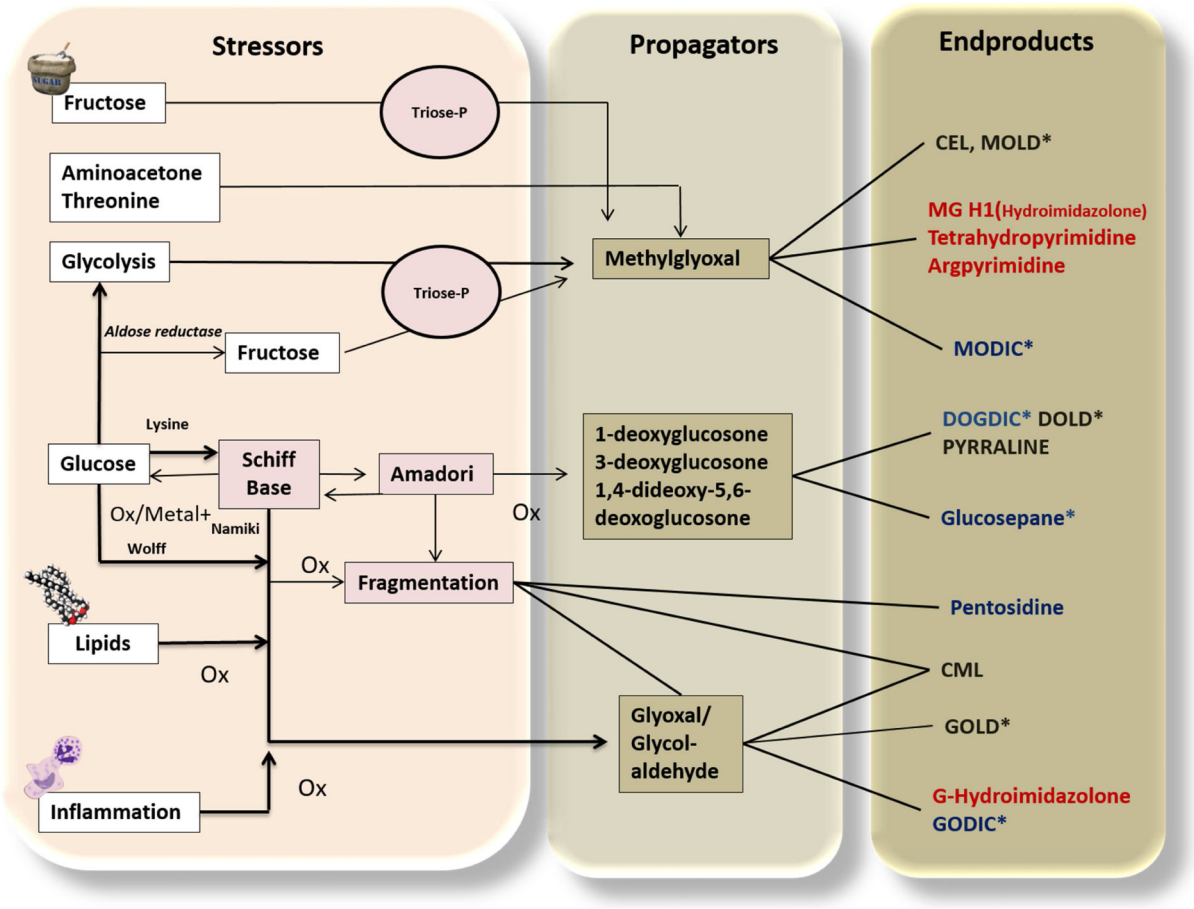

Figure 1. The Maillard reaction or glycation.

Main pathways and main known advanced glycation end products (AGEs) relevant to human pathology. In the left column, we represent the main initiators or stressors, propagators in the center (very potent alpha dicarbonyls, of which methylglyoxal is the most important and reactive) and end products in the right column. Among the endproducts: black represents lysine modifications; red indicates arginine adducts and blue denotes lysine-arginine modifications. CEL: carboxy ethyl lysine; CML: carboxy methyl lysine; MG-H1: methylglyoxal hydroimidazolone; MODIC: methylglyoxal dimer imidazolone crosslink; MOLD: Methylglyoxal lysine dimer; GODIC:glyoxal-derived imidazolium cross-link; DOGDIC, 3-deoxyglucosone-derived imidazolium cross-link; GOLD: glyoxal lysine dimer; DOLD: deoxyglucosone lysine dimer. * indicates crosslinked products. Modified from: (Monnier et al., 2005).

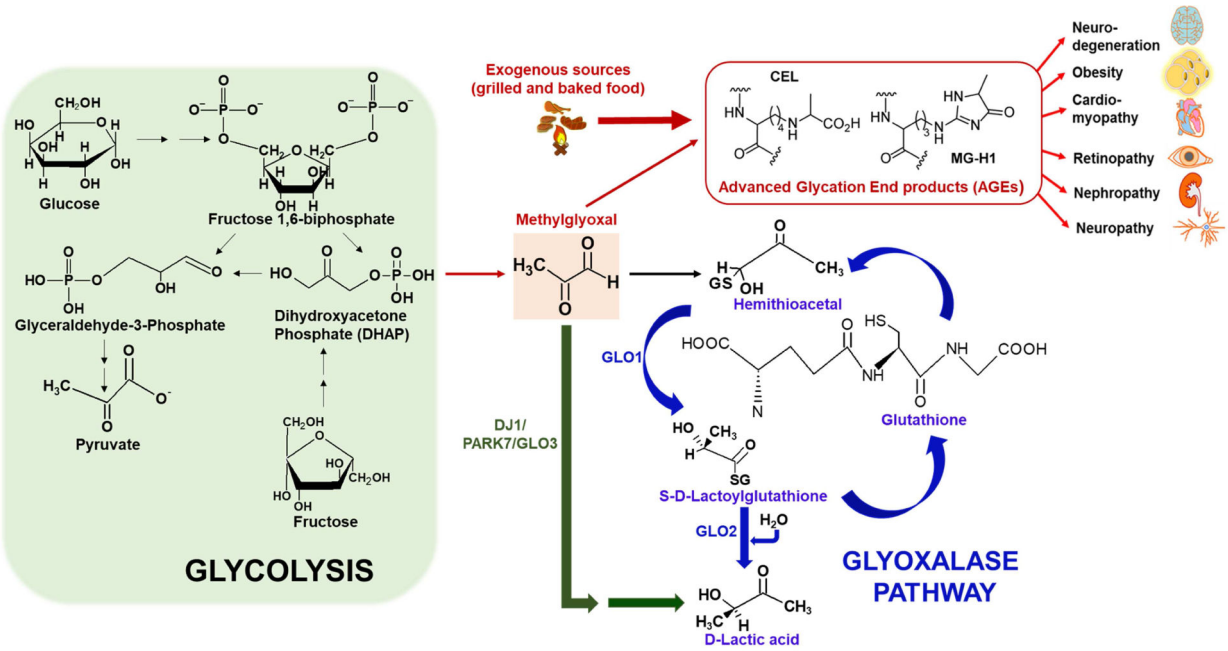

Figure 2. Form ation of AGEs and the glyoxalase system.

The figure illustrates the formation and detoxification of MGO, a glycolytic byproduct that is formed either from glucose or fructose. Endogenously derived glycolytic byproducts (e.g., Methylglyoxal or MGO) or their AGE derivatives (e.g., Nε-carboxyethyl-lysine or CEL and Methylglyoxal-derived hydroimidazolone or MG-H1) lead to a variety of diseases affecting different organs (such as brain, heart, eyes, kidney, lungs) that complicate with age. In addition to endogenous sources such as glucose or fructose derived dihydroxyacetone phosphate (DHAP) (formed during glycolysis), exogenous dietary sources such as dry heat-cooked food predominantly produces AGEs (large red arrow). Detoxification of MGO occurs via glutathione (GSH)-dependent glyoxalase pathway mediated by two mitochondrial enzymes glyoxalase 1 *(GLO1)* and glyoxalase 2 *(GLO2)* that eventually converts MGO to lactic acid. Alternatively, MGO can also be converted to lactic acid in a single step mediated by a GSH-independent cytosolic enzyme glyoxalase 3 *(GLO3),* a mammalianorthologue of the protein *DJ1/PARK7.*

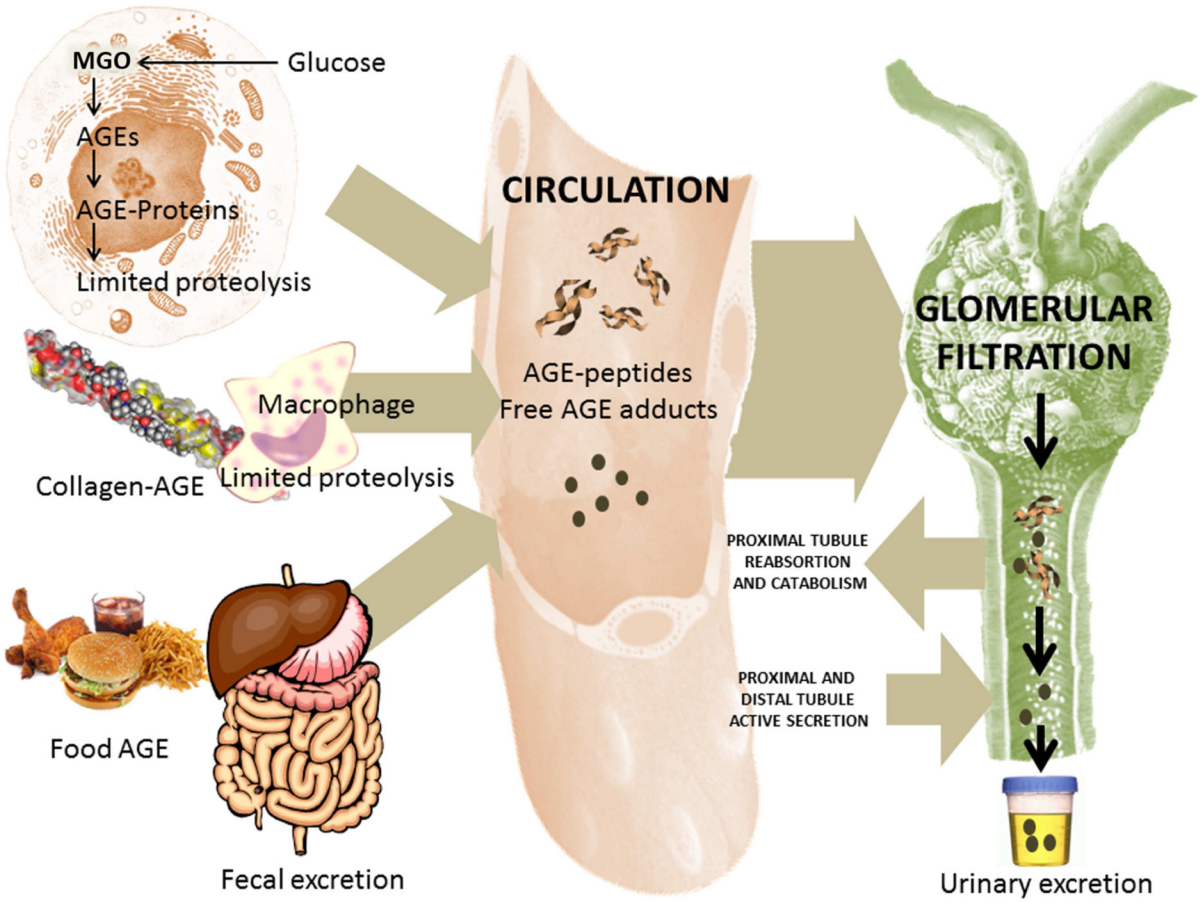

Figure 3. AGE m etabolism.

The diagram represents schematically the production of endogenous AGEs (intra and extracellular) and the intake from certain foods (left), their presence in the circulation (center) and their elimination/catabolism mainly by the kidneys (right). AGEs can be produced intracellularly by multiple pathways as shown in Figure 1. Of special relevance are MGO-generated AGEs. Other AGEs are generated on collagens and other proteins in the ECM as we AGE and more rapidly during diabetes. Partial proteolysis by macrophages, or in cells by ubiquitin-mediated proteasome pathways produce partially digested AGE-peptides as well as free adducts. Some AGEs are thought to also enter the bloodstream from foods. These adducts are very reactive and can damage proteins as they transit in the bloodstream. Renal function is critical for their elimination, as end-stage renal failure patients have very high concentrations of these molecules, which can be lowered by dialysis. These AGEs are filtered and reabsorbed in part in the proximal tube to be detoxified to some extent and then secreted distally for urinary excretion.

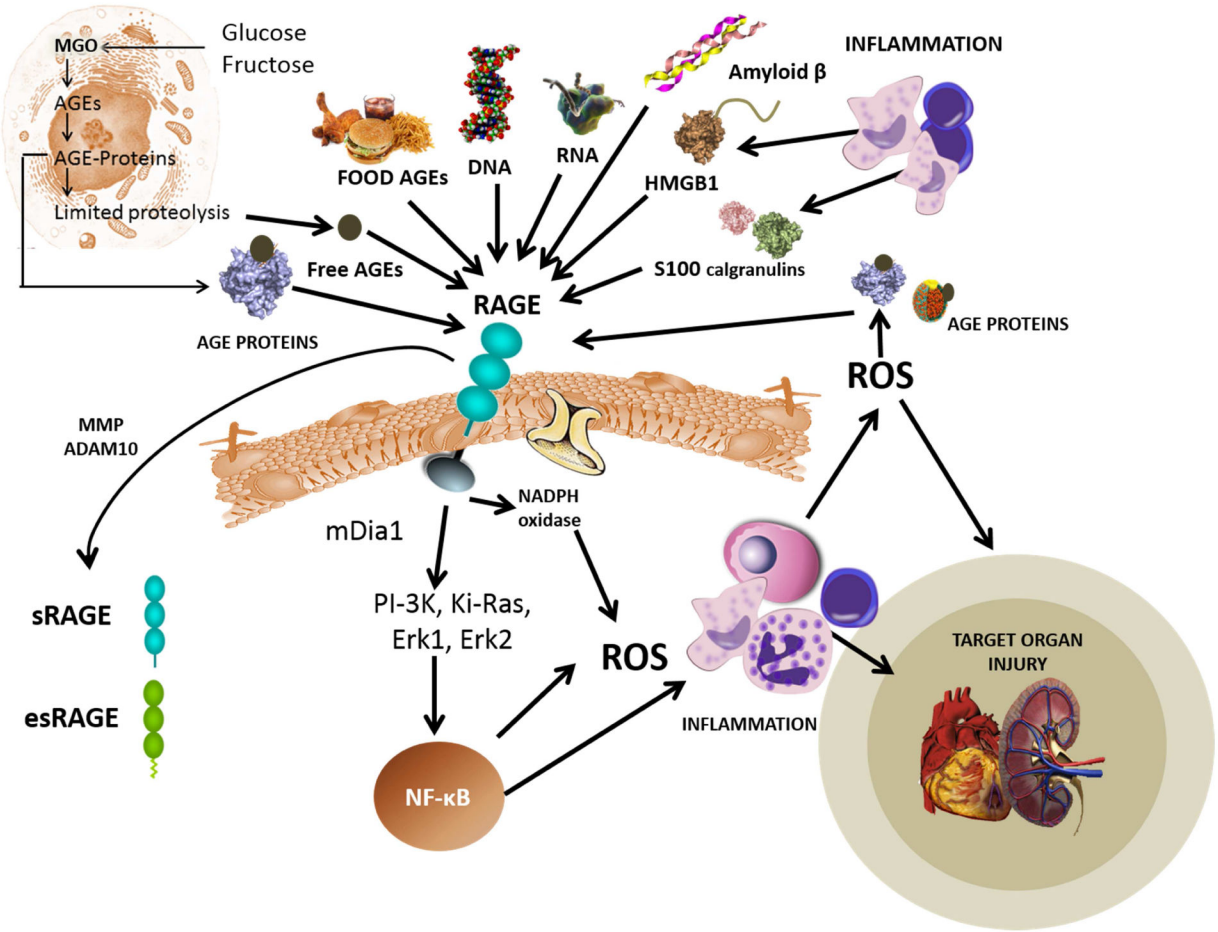

Figure 4. The receptor for advanced glycation endp ro ducts (RAGE) is akey pathway for inflammatory complication sinaging and chronic disease.

As shown in the top part of the figure, RAGE is a pattern recognition receptor that participates in primary immunity and has a variety of ligands. Important for our topic are the AGE, AGE-proteins, AGE-peptides and adducts from primary AGE catabolism and dietary AGEs. RAGE activation leads to a signaling cascade that produces NF-kB coordinated inflammatory responses that can lead to target tissue injury. Soluble forms of the receptor (esRAGE and sRAGE) are released to the circulation. Some authors suggest they serve to modulate the response acting as decoy ligands. They are useful as biomarkers of the whole-body AGE-RAGE axis.

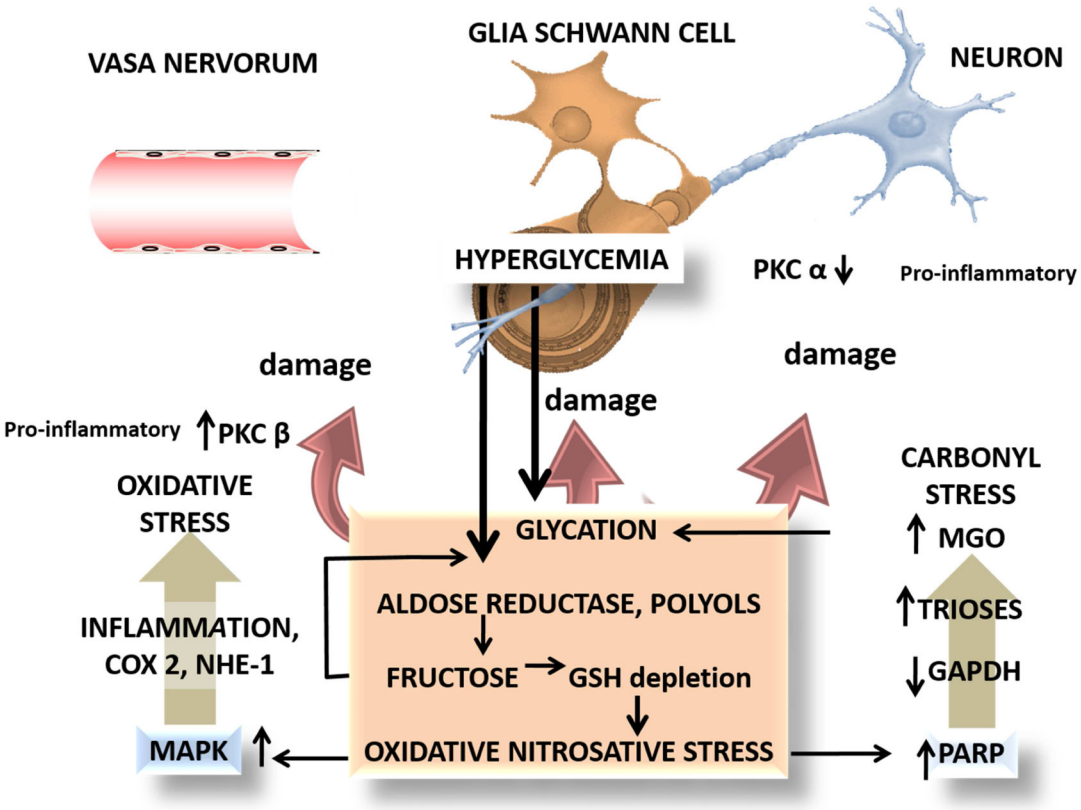

Figure 5: Glycation and diabetic neuropathy: an example of pathways and targets where AGEs, MGO, and carbonyl stress play an important role.

Evidence shows that a triple hit may be operating to produce the final damage: vasa vasorum, the Schwann cell and the neuron axon all three are targets for the damage. Hyperglycemia produces AGEs via the mechanisms shown here and in detail in Figure 1. The interplay with oxidative stress (depletion of glutathione) activates MAPK and its inflammatory cascades which via PKC beta produces vascular damage. Oxidative stress on DNA induces the repair enzyme PARP which has the drawback of inactivating GAPDH, therefore blocking glycolysis at the triose level with the upstream consequence of further MGO accumulation, compounding the problem (Brownlee's hypothesis, see text). Final damage occurs to the neuronal axon, neuronal mitochondria, and the glial Schwann cells.

Table 1.

Summary of model organisms, phenotypes and genes / proteins / pathways related to α-DC or AGE-mediated pathologies that exacerbate with age

Model Organism	Phenotypes	Genes / Proteins / Pathways	Citations
Mice / Rats	Neuropathy / Nephropathy / Neurodegeneration / Cardiomyopathy / Retinopathy / Vasculopathy	*TGF-β*[a] / *VCAM1*[b] / *RAGE*[c,d] / *VEGF*[e] / Na_v *1.8*[f] / *GLO1*[g] / *TRPA1*[h] / *HCN2*[i] / *DJ-1*[j] / *NF-kB*[k]	(Oldfield et al., 2001)[a]; (Schmidt et al., 1995)[b]; (Bayarsaikhan et al., 2016)[c]; (Ma et al., 2009)[d]; (Yamagishi et al., 2006)[e]; (Bierhaus et al., 2012)[f]; (Jack et al., 2011)[g]; (Andersson et al., 2013)[h]; (Tsantoulas et al., 2017)[i]; (Lee et al., 2012)[j]; (Zhou et al., 2016)[k]
Fly	Neurodegeneration / Paralysis / Fat accumulation / Lifespan	Glo1[∫a, ∫b] / FASN[∫a] / Tpi[∫b] / wstd[∫b]	(Garrido et al., 2015)[∫a]; (Gnerer et al., 2006)[∫b]
Worm	Neurodegeneration / Hyperesthesia / Lifespan	TRPA-1[ǂa] / Nrf[ǂa] / p38 MAPK[ǂa] / DJ-1 related (djr-1.1 & djr-1.2)[ǂa, ǂb, ǂc, ǂd, ǂe] / GLOD-4[ǂa, ǂb, ǂc, ǂf, ǂg] / DAF-18[ǂc] / JNK-1[ǂc] / DAF-16[ǂc, ǂf]	(Chaudhuri et al., 2016)[ǂa]; (Chen et al., 2015)[ǂb];(Golegaonkar et al., 2015)[ǂc]; (Lee et al., 2012)[ǂd]; (Lee et al., 2013, 2012)[ǂe];(Mendler et al., 2014)[ǂf]; (Morcos et al., 2008)[ǂg]
Yeast	Proteotoxicity / Proteostasis	Glo1[ǂ∫a, ǂ∫b , ǂ∫c] / Tpi[ǂ∫a] / Hsp26p[ǂ∫b] / Hsp104 [ǂ∫c] / ALKR [ǂ∫c] / Sln1 [ǂ∫c] / Hog1[ǂ∫c]	(Vicente Miranda et al., 2016)[ǂ∫a]; (Gomes et al., 2008)[ǂ∫b]; (Zemva et al., 2017)[ǂ∫c]