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REVIEWS

Review

Clinical/Translational Aspects of Advanced Glycation End-Products

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Advanced glycation end-products (AGEs) have been implicated in chronic hyperglycemia and age-related diseases. Endogenous AGEs produced by humans generate oxidative stress and activation of inflammatory signaling pathways via AGE-specific receptors. The present review summarizes current knowledge on the pathogenic role of AGEs in chronic noncommunicable diseases. Although correlations exist between glycation and the pathogenesis of these diseases, uncertainties remain in light of recurrent intervention failures of apparently promising animal models to be translated into clinically useful anti-AGE strategies. Future intervention of AGEs or their receptors should embrace more carefully executed clinical trials. Nevertheless, suppressing symptoms via lifetime drug application is unlikely to eliminate the burden of chronic diseases unless deep-rooted lifestyle issues that cause these diseases are simultaneously addressed.

Highlights

Glycation of biomacromolecules derived from exogenous/endogenous sources results in life-long accumulation of advanced glycation end-products (AGEs) in the body.

Molecular mechanisms of the interaction of AGEs with their receptors are fairly well defined from animal experiments. Recent studies highlight the role of AGEs as damage-associated molecular patterns (DAMPs) which trigger innate immunity defenses and sterile inflammation.

Stimulation of intracellular signaling pathways by AGEs produce damage that is implicated in the progression of chronic diseases in various organs.

Despite the efforts of laborious clinical trials, only metformin has successfully completed a Phase IV trial in intercepting the AGE–RAGE axis.

Recurrent failures in clinical translation of apparently promising intervention strategies derived from animal models challenge the clinical validity of these models.

Advanced Glycation Products and Human Health

Over the past couple of decades, advanced glycation end products (AGEs) have received considerable attention as one of the many mechanisms proposed for aging; the progressive accumulation of damage to an organism over time leading to disease and death [1]. AGEs are a heterogeneous group of molecules that were initially discovered at the beginning of the 20th century in the classic Maillard reaction. With the discovery of glycated-hemoglobin in diabetic patients, it became apparent that **glycation** (see Glossary), a nonenzymatic reaction between reducing sugars such as glucose, and proteins, lipids, or nucleic acids, also occurs under physiological and pathological conditions [2].

AGEs have generated interest in the biochemical and medical fields because of their biological effects on humans, particularly in relation to aging [3]. Most of the AGEs are detrimental to human health by disrupting our body's hormonal functions [4] and participating in age-related, noncommunicable, chronic inflammatory diseases [5]. For example, accumulation of methylglyoxal, a highly reactive glucose metabolite and a major AGE precursor, may cause hemodynamic, inflammatory, metabolic, and structural changes in the kidneys that contribute to the manifestation of diabetic chronic kidney disease via downregulation of renal glyoxalase-1 and increases in methylglyoxal-derived hydroimidazolone (MG-H1) formation [6,7]. Nevertheless, there is continuous debate on whether AGEs are the causal factor or just a consequence of age-related diseases [8,9]. The present review is targeted toward association of the AGE accumulation with different human disorders, molecular mechanisms of the association, and recent developments in therapeutic strategies that antagonize the reaction products of glycation.

Exogenous and Endogenous Glycation Reactions

The chemistry of food glycation is complex (Figure 1A). The reaction starts with condensation of the carbonyl group of reducing sugars and the amino group of proteins into unstable Schiff base intermediates that are reorganized as more stable Amadori products. Early-stage glycation products undergo a complex series of pH-dependent reactions, producing intermediate-stage glycation products, which yield AGEs such as furanones, pyranones, and melaniodins on further biochemical interactions. Although dietary intake of AGEs may increase their serum level [10], a definitive relationship between food-derived AGEs and their accumulation in the body has not been substantiated. For example, the contribution of dietary α-dicarbonyls to the formation of endogenous AGEs appears to be negligible; dietary methylglyoxal is rapidly degraded in the gastrointestinal tract and is unlikely to reach the circulation [11].

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Although there are reviews summarizing that dietary AGEs cause oxidative stress and inflammation and are involved in the development of chronic diseases [12], it is important to clarify that many studies in the field of dietary AGE research were conducted using unsubstantiated and contradictory analytic methods [13]. Using liquid chromatography analysis, AGEs derived from food and beverages were found to be absorbed as AGE-free adducts and AGE-rich peptides, with the latter degraded efficiently after absorption [14]. Hence, AGEs absorbed from dietary sources appear to have low toxicity in humans with normal renal clearance function.

There are important differences between exogenous and endogenous AGEs that are formed systemically throughout life via nonenzymatic glycation of endogenous proteins and lipids [15]. These endogenous reactions are exacerbated during aging, oxidative stress, hyperglycemia, hypertension, and chronic renal diseases. Considering that physiological conditions in human tissues, such as concentration and type of carbohydrates, pH value, and temperature are different from situations *in vitro*, the chemical reactions of *in vivo* glycation are not exactly same (Figure 1B) [5]. Many endogenous AGEs have been identified from human plasma and hemodialysates [16] as well as other tissues/organs [17]. Factors associated with AGE accumulation in the body are summarized in Figure 2.

Molecular Mechanisms

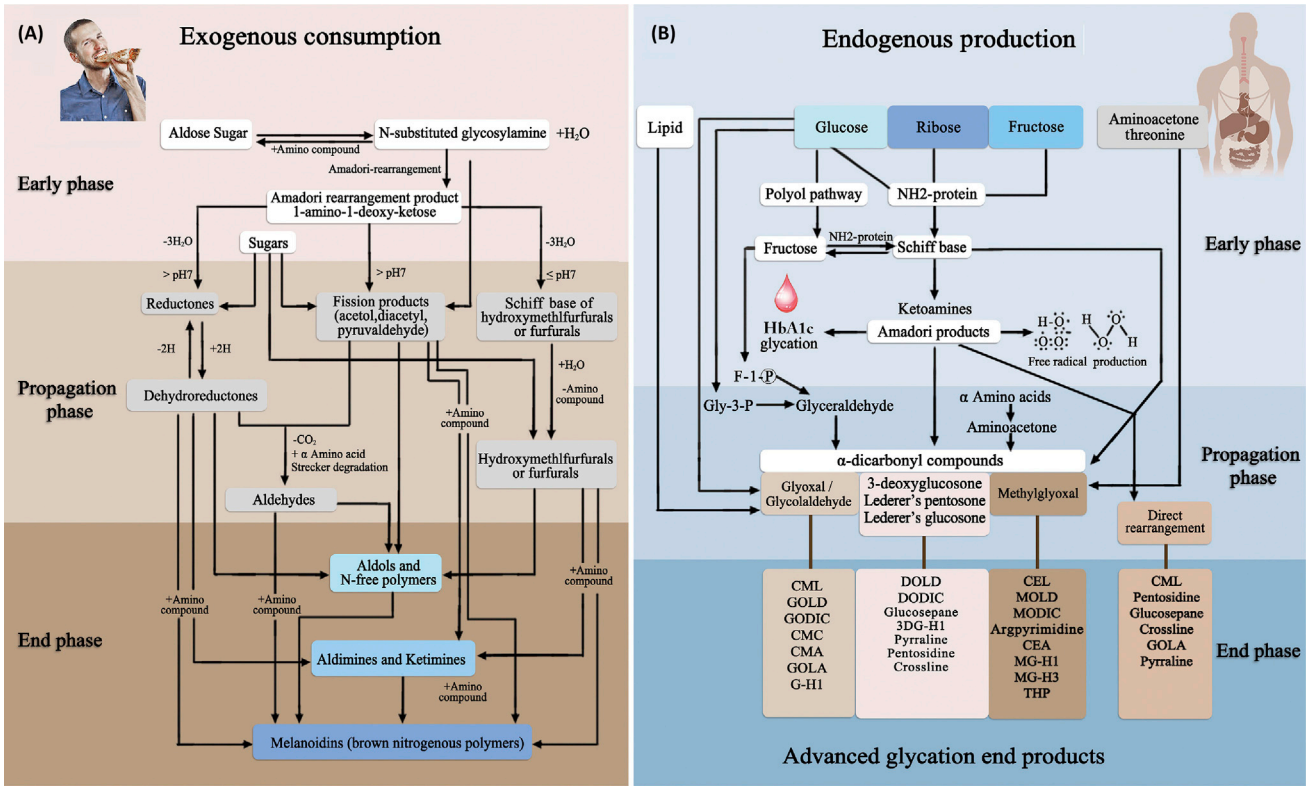
Accumulation of AGEs occurs in the presence of high levels of systemic glucose and fructose, which increase the glycation rate of long-lived proteins. Through exogenous consumption and endogenous production, AGEs participate in diabetes- and age-related human disorders via two important mechanisms. The first involves covalent crosslinking of serum proteins, enzymes, lipids, DNA, extracellular matrix (ECM) proteins, and biochemical alteration of their biological functions. Glycation of DNA produces immunogenic DNA-AGEs in diabetes [18], neurodegenerative diseases [19], and cancer [20]. Crosslinking of long-lifetime ECM proteins by AGEs causes deterioration in their physical and biomechanical properties during aging and diabetes. Glycation of type I collagen interferes with normal collagen crosslinking [21] and alters the molecular arrangement of tropocollagen that prevents intrafibrillar molecular sliding in stiffened tendons [22]. Glycation causes post-translational crosslinking of elastin that decreases viscoelasticity and results in stiffening of the skin and vascular systems [23]. Glycation of lens α-crystallin induces crosslinking of the water-soluble protein, and accounts for reduction in lens transparency and increases in light scattering in diabetic and age-associated cataracts [24].

The second mechanism involves interaction of AGEs with their cell surface receptors. The receptor for AGE (RAGE) is the most-studied AGE–receptor interaction, commonly referred to as the AGE–RAGE axis [25]; RAGE is a pattern-recognizing, multiligand receptor with different isoforms expressed in the lung, heart, kidney, brain, skeletal muscles, and in different types of cells including endothelial cells, macrophages/monocytes, neutrophils, and lymphocytes [26]. Because of the structural specificity of the RAGE V domain [27], not all AGEs possess the same affinity for RAGE. For example, methylglyoxal-derived AGEs have strong affinity for RAGE [28]. Conversely, free, nonpeptide-bound glycated amino acids do not invoke the RAGE response [29] while *N*-carboxymethyl-lysine (CML)-modified proteins are unable to bind to RAGE to activate inflammatory signaling pathways [30]. Studies on the AGE–RAGE axis have often been performed with strongly crosslinked proteins (due to extreme processing conditions) that may resemble nutrition proteins. However, what was often excluded from those studies was the influence of digestion and transport into the body. Because of their high molecular weight, it is unlikely that crosslinked food proteins can be transferred from food into circulation in an intact form and reach RAGE.

Apart from AGEs, RAGE also regulate the effects of other extracellular ligands such as extracellular high mobility group box (HMGB)1 and the S100 family of calcium-binding proteins (**alarmins**), as well as β-amyloid peptides, which are implicated in Alzheimer's disease [31]. Although many AGEs have been identified in humans, AGE–RAGE signaling is mostly investigated using AGE-modified albumin, CML, *N*-carboxyethyl-lysine (CEL), and pentosidine. Prior to their interactions with membrane surface RAGEs, exogenous and endogenous AGEs or their precursors are partially detoxicated

Glossary

Alarmins: endogenous, constitutively expressed, chemotactic, and immune-activating proteins/peptides that are released as a result of degranulation, cell injury, or death, or in response to immune induction.
Aptamer: short, single-stranded DNA or RNA that selectively bind to a specific target, including proteins, peptides, carbohydrates, small molecules, toxins and live cells. They assume a variety of shapes due to their tendency to form helices and single-stranded loops. Aptamers bind targets with high selectivity and specificity, as determined by their tertiary structures. Target recognition and binding involve 3D, shape-dependent interactions as well as hydrophobic interactions, base-stacking, and intercalation.
Glycation: (nonenzymatic glycosylation) is the result of the covalent bonding of a reducing sugar molecule to a protein or lipid molecule (e.g., triacylglycerol), without the controlling action of an enzyme. The process may occur either inside the body (endogenous glycation) or outside the body (exogenous glycation). Glycation impairs the functioning of biomolecules, and does not require the expenditure of adenosine triphosphate (ATP, the energy storing molecule).
Pericytes: (Rouget cells or mural cells) are multifunctional cells that wrap around the endothelial cells that line the capillaries and venules throughout the body.
Retinal leukostasis: increased leukocyte adhesion to the retinal capillaries in diabetic patients together with reduced retinal blood flow. Retinal leukostasis may contribute to the formation of nonperfused capillaries, which is believed to be major contributor for the progression of diabetic retinopathy.
Web of causation: an inter-relationship of multiple factors that contribute to the occurrence of a disease.



Trends in Endocrinology & Metabolism

Figure 1. Exogenous and Endogenous Glycation Reactions.

(A) Glycation reaction products (Maillard reaction) produced during high-temperature food processing are consumed as part of our daily diet. (B) Endogenous glycation is produced by complex nonenzymatic reactions between glucose, ribose, fructose, amino acids, and lipids. Abbreviations: 3DG-H1, 3-deoxyglucosone-derived hydroimidazolone 1; CEA, N^{ε}-carboxyethyl-arginine; CEL, N^{ε}-carboxyethyl-lysine; CMA, N^{ε}-carboxymethyl-arginine; CMC, carboxymethyl cysteine; CML, N^{ε}-carboxymethyl-lysine; DODIC, 3-deoxyglucosone-derived imidazolium crosslink; DOLD, 3-deoxyglucosone-derived lysine dimer; G-H1, glyoxal-derived hydroimidazolone 1; GODIC, glyoxal-derived imidazolium crosslink; GOLA, N^{ε}-[2-[(5-amino-5-carboxypentyl) amino]-2-oxoethyl]-lysine; GOLD, glyoxal-derived lysine dimer; HbA1c, glycated hemoglobin; MG-H1, methylglyoxal-derived hydroimidazolone 1; MG-H3, methylglyoxal-derived hydroimidazolone 3; MODIC, methylglyoxal-derived imidazolium crosslink; MOLD, methylglyoxal-derived lysine dimer; THP, tetrahydropyrimidine.

by glyoxalase-1 [32], or by decoys soluble RAGE (sRAGE) and endogenous secretory RAGE (esRAGE), which are proteolytically cleaved and spliced versions, respectively, of full-length RAGEs that lack cytoplasmic domains for intracellular signaling [33]. Apart from esRAGE, there are other forms of soluble RAGE isoforms such as cRAGE (proteolytic cleavage), DN-RAGE (dominant negative) and ΔN-RAGE (Δ-RAGE isoform without domain V) that are either cleaved from the complete transmembrane RAGE or spliced from the *RAGE* gene [34]. These soluble isoforms have the alleged function of binding with AGEs and other RAGE ligands and prevent the latter from interacting with the complete RAGE receptor, thereby protecting cells from damage by activation of the complete RAGE receptor. The types of AGEs, RAGE isoforms/shedding and signaling are also likely to be disease specific and linked to the specific pathological features in a mechanistic fashion; the way the AGE–RAGE axis is to be targeted is likely to be disease specific [23]. Other AGE–receptor (AGER) complexes such as AGE-R1/OST-48 and AGE-R3/galectin-3 as well as some members of scavenger receptors (including MSR-AII, MSR-BI, and CD36) also contribute to detoxification by internalizing AGEs to be degraded [35].

The signaling events involved in the AGE–RAGE axis are complex because of the diversity of RAGE ligands and their effects in different cell types (Figure 3). A major end-result of AGE–RAGE signaling is

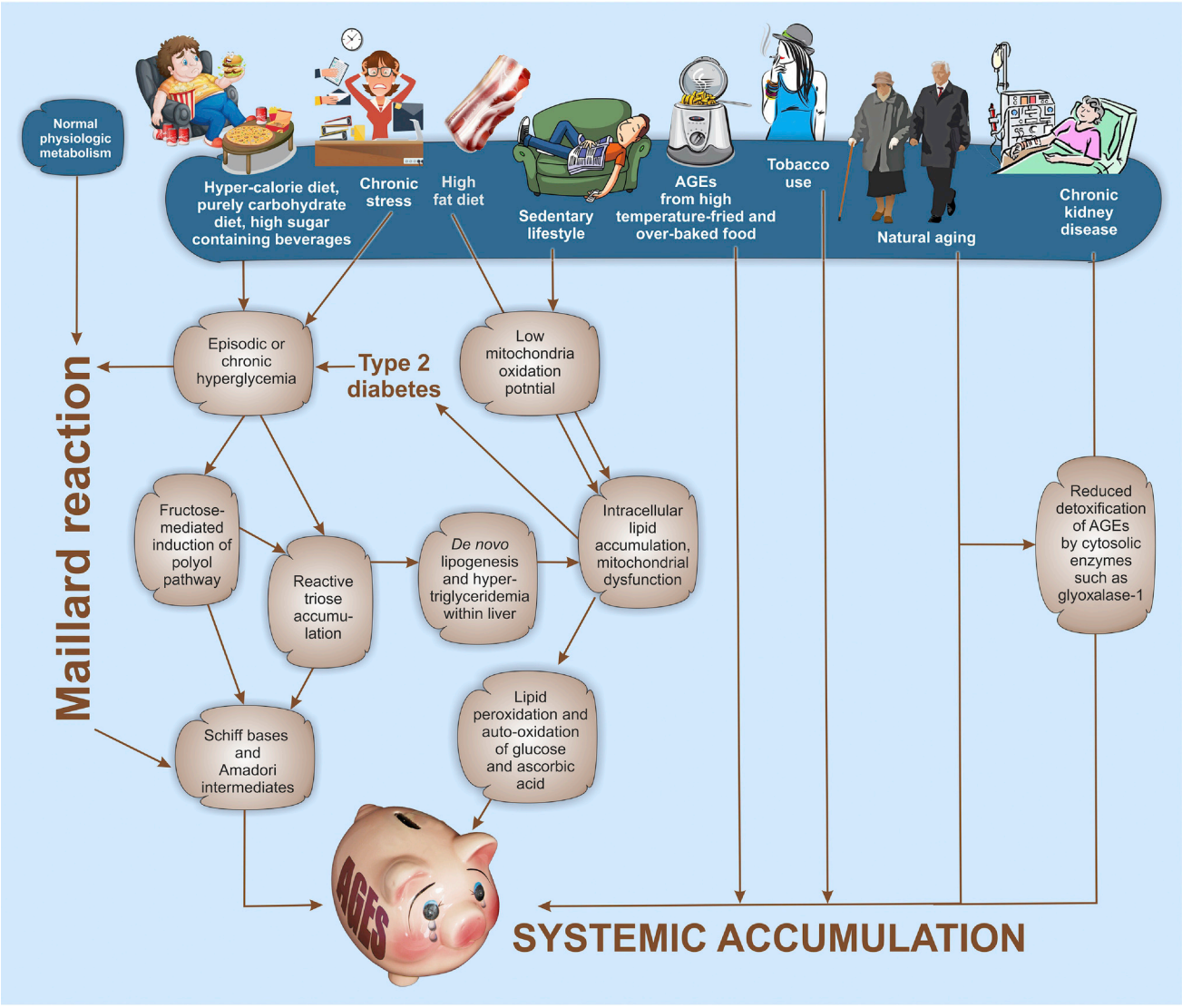


Figure 2. Factors Associated with Accumulation of Advanced Glycation End-Products (AGEs) in Humans.
Primary factors include chronic hyperglycemia, aging, dietary patterns, impairment of AGE detoxification mechanisms, and unhealthy lifestyle.

the generation of oxidative stress. AGEs induce intracellular reactive oxygen species (ROS) production through mitochondrial dysfunction, activation of NADPH oxidases, or the redox crosstalk between these two ROS-producing mechanisms [36]. These observed effects often depend on the way in which the AGEs are produced. Generation of ROS further induces endoplasmic reticulum stress through stimulation of the unfolded protein response, leading to CCAAT/enhancer binding proteins homologous protein (CHOP)-induced apoptosis [37].

It is prudent to mention that RAGE can be constitutively or inducibly expressed in different cells, depending on the cell type and developmental stage. Unlike most cell types in which constitutively expressed RAGE during embryonic development subsides following differentiation into adult cells, RAGE is constitutively expressed in alveolar epithelial cells of the lung and is a critical regulator of lung physiology. Rapid downregulation of RAGE occurs during lung tumorigenesis, interrupting

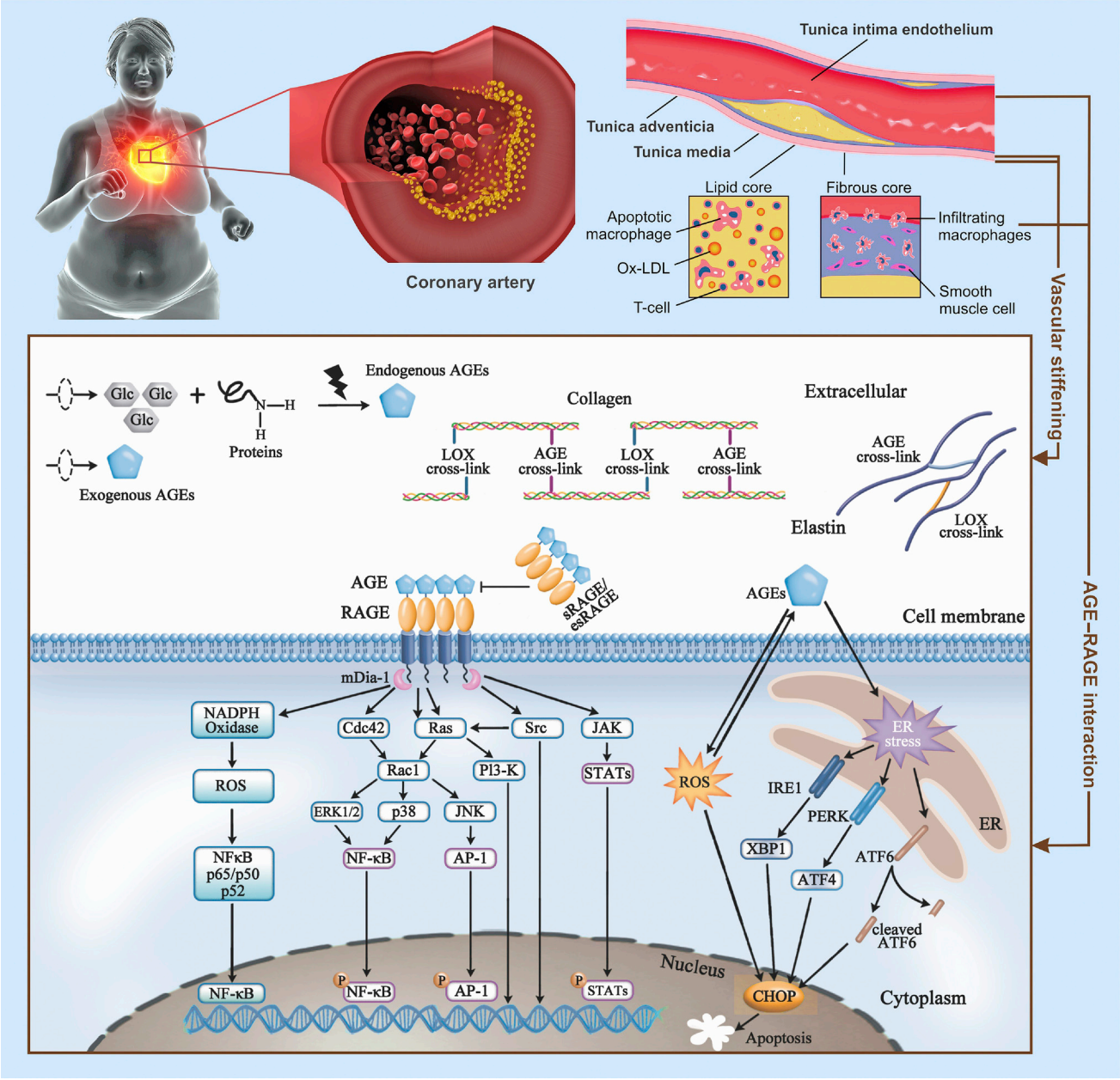


Figure 3. Molecular Mechanisms Linking Advanced Glycation End-Products (AGEs) to Human Disorders.
Atherosclerosis of coronary blood vessels is shown as an example. The first mechanism involves glycation crosslinking. Unlike the enzymatic lysyl oxidase (LOX)-derived crosslinking, glycation crosslinking is nonenzymatic. Glycation causes post-translational crosslinking of collagen and elastin that decreases viscoelasticity and results in stiffening of the skin and vascular systems [23]. The second mechanism involves interaction of AGEs with their transmembrane cell surface receptors. Interaction of AGE with the extracellular domains of the receptor for AGEs (RAGE) activates multiple intracellular downstream signaling pathways that, in turn, activate transcription factors such as nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB), activator protein-1 (AP-1), and signal transducer and activator of transcription (STAT) in different cell types [24]. AGE-RAGE-mediated pathogenesis may be counteracted by decoys of soluble RAGE (sRAGE) and endogenous secretory RAGE (esRAGE) [25]. In addition, AGEs not only directly induce

(Figure legend at the bottom of the next page.)

with cell–cell and cell–substrate communication that is vital for canal cell induction, progression, and migration [38]. This downregulation is strikingly different from other cells in which RAGE is inducibly expressed in large quantities during inflammation. In addition, RAGE is highly expressed on immune cells such as neutrophils, T and B lymphocytes, monocytes, macrophages, and dendritic cells, and plays important roles in perpetuating the immune response; accordingly, interference of RAGE expression may have deleterious consequences [39].

Apart from interacting with RAGE, some AGEs may also cointeract with Toll-like receptors (TLRs) [40] by acting as damage-associated molecular patterns (DAMPs) [41] (Figure 4). A discussion of the molecular mechanisms involved in glycation cannot be complete without distinguishing between short- and long-lived proteins. For short-lived proteins with rapid turnover, glycation produces early glycation products such as Amadori products only because time does not permit AGE formation. Because AGEs are not produced for short-lived proteins and other biomolecules, the RAGE-dependent pathway is not involved. Short-lived extracellular glycation products are also unlikely to act as DAMPs in the RAGE-independent pathway because they may be repaired by transglycation, which separates the carbohydrate portion from the glycated protein and returns the protein to its original function, or by frutoamine-3-kinase that catalyzes the repair of glycation intermediates [23]. In this context, it should also be emphasized that in addition to the chronic state of low-grade inflammation and oxidative stress, there are disease flare-ups with bursts of additionally enhanced levels of inflammation and oxidative stress.

From Glycation to Diseases

Glycation damage has been associated with, or implicated as a causative factor in multiple inflammatory pathways that contribute to the development of age-related, noncommunicable chronic diseases in various organs [23]. There is increasing evidence to support the validity of AGE/sRAGE ratio as a universal biomarker/risk marker for these disease entities [42]. For example, using liquid chromatography–mass spectrometry for determining CML and CEL, fluorescence for pentosidine, colorimetry for fructosamine, and enzyme-linked immunosorbent assay for identifying sRAGE, the AGE/sRAGE ratio was found to be four times higher in adults with nonalcoholic fatty liver disease, compared with controls [43].

To date, the strongest evidence linking *in vivo* AGE accumulation and chronic diseases may be found in diabetes [44]. Chronic hyperglycemia generates oxidative stress via AGE formation, the polyol pathway, the protein kinase C pathway, and the hexosamine biosynthetic pathway, ultimately resulting in alteration of gene expressions that damage pancreatic β cells and induce insulin resistance [45]. Diabetic macrovascular complications such as coronary heart disease, peripheral arterial disease, and stroke share the central pathological mechanism of atherosclerosis. AGEs contribute to these complications via their accumulation in atherosclerotic plagues, participation in arterial stiffening and calcification, activation of platelet aggregation, reduction of fibrinolysis, and clot dissolution [25].

There is increasing evidence of the association of AGEs with diabetic microvascular complications. Progressive deterioration of small arterioles results in retinopathy, nephropathy, and neuropathy. Diabetic retinopathy is the major cause of acquired blindness. Under hyperglycemic conditions, damage to the vascular and neural components of the retina may be caused by AGE crosslinking of ECM proteins that increases vascular stiffness that alters vascular structure and function. In addition, AGE–RAGE interaction induces **pericyte** apoptosis, vascular inflammation, **retinal leukostasis**, angiogenesis, and breakdown of the blood–retina barrier [46]. Diabetic nephropathy is one of the most critical consequences of AGE-induced damage because the kidney is the major clearance site of AGEs. The AGE–RAGE axis contributes to the pathogenesis of diabetic nephropathy by

endoplasmic reticulum (ER) stress, but also induce oxidative stress via reactive oxygen species (ROS). Abbreviations: ATF, activating transcription factor; CHOP, CCAAT/enhancer binding proteins homologous protein; ERK1/2, extracellular signal-regulated kinase 1/2; JAK, Janus kinase; JNK, c-Jun N-terminal kinase; IRE1, serine/threonine-protein kinase/endoribonuclease; NADPH, dihydronicotinamide-adenine dinucleotide phosphate; PERK, protein kinase ribonucleic acid-like endoplasmic reticulum kinase; PI3-K, phosphoinositide 3-kinase; XBP1, X-box binding protein-1.

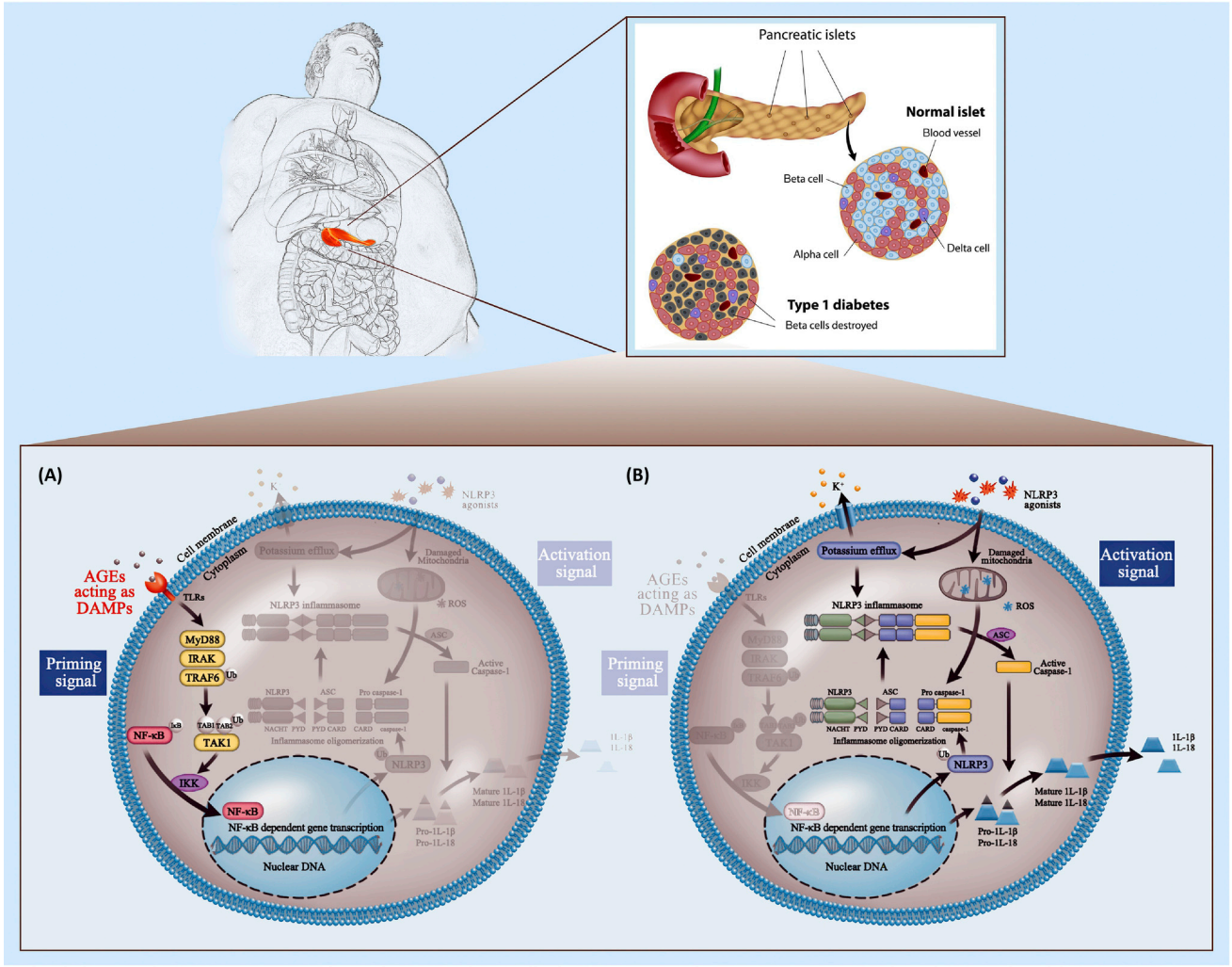


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Figure 4. Putative Involvement of Advanced Glycation End-Products (AGEs) in the Activation of Nucleotide-Binding Domain, Leucine-Rich Repeat Family, Pyrin Domain-Containing Protein-3 (NLRP3) Inflammasome Complexes That Promote Pancreatic Islet Damage.

(A) Apart from interacting with the receptor for AGEs (RAGE), some AGEs may also co-interact with Toll-like receptors (TLRs) [40] by acting as damage-associated molecular patterns (DAMPs) [30], amplifying priming signals for the transcription of nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB)-dependent genes such as NLRP3, pro-interleukin (IL)-1β and pro-IL-18 [66]. (B) Activation signals created by oxidative stress derived from mitochondrial damage trigger assembly of cytosolic NLRP3 inflammasome complexes in cells involved in innate immunity, resulting in sterile inflammation and chronic tissue destruction via caspase-1 catalyzed release of IL-1β and IL-18 [67,68]. Extracellular efflux of potassium ions is required for the formation of inflammasomes. Abbreviations: ASC, apoptosis-associated speck-like protein containing caspase recruitment domain-containing protein; CARD, caspase-recruitment domain-containing protein; IKK, IκB kinase; IRAK, interleukin-1 receptor-associated kinase; MYD88, myeloid differentiation primary response 88; NACHT, [NAIP (neuronal apoptosis inhibitory protein), CIITA (MHC class II transcription activator), HET-E (incompatibility locus protein from *Podospora anserina*) and TP1 (telomerase-associated protein)] domain; PYD, pyrin domain; ROS, reactive oxygen species; TAK1, transforming growth factor-β-activated kinase 1; TNFR, tumor necrosis factor receptor associated factors; Ub, ubiquitin.

inducing glomerular hypertrophy, **podocyte** damage, basement membrane thickening, mesangial ECM expansion, and proteinuria, ultimately resulting in end-stage renal disease [47]. Diabetic neuropathy is characterized by the loss of peripheral sensation, which, when combined with impaired vascular function, results in cardiac autonomic neuropathy and nonhealing ulcers. AGEs are involved in the pathogenesis of neuropathy via modification of axonal cytoskeletal proteins, resulting in axonal

degeneration and breakdown. Dysfunction of endothelial **pericytes** by AGE accumulation causes basement membrane hypertrophy in nerve blood vessels and subsequent deterioration of the blood–nerve barrier. In addition, AGE-modified myelin is susceptible to phagocytosis by macrophages and results in segmental demyelination of peripheral nerves [48]. Hyperglycemia also induces ECM glycation and activates the AGE–RAGE axis that encourages tumor cell proliferation and tumor-promoting inflammation in the cancer microenvironment [49].

AGEs have also been implicated as a causative factor in other life threatening nondiabetes-related chronic diseases, including hypertension, cardiovascular diseases, chronic kidney diseases, pulmonary diseases such as chronic obstructive pulmonary disease (COPD), neurological diseases such as Alzheimer's disease, liver diseases, and cancer. The presence of AGEs has been demonstrated in cancers involving the larynx, breast, colon, pancreas, and prostate, and primary acute myeloid leukemia.

Although there is increasing evidence linking AGE accumulation in organs to their dysfunction in chronic diseases, it must be stressed that a correlation does not imply a direct cause-and-effect relationship. While it is fair to say that AGEs or the AGE–RAGE axis appears to be a driving force in the pathogenesis of these chronic diseases, other factors may also contribute to disease progression, as suggested by the multiple-hits hypothesis that was original proposed for the pathogenesis of nonalcoholic fatty liver disease. This is exemplified by the different pathways that are capable of generating oxidative stress, and the variability of DAMP ligands that can interact with RAGE or TLRs. In addition, the use of non-human experimental models to evaluate exposure to AGEs generates results that remain questionable (Table S1 in the supplemental information online), and cannot be translated into applicable therapeutic strategies in human clinical trials.

Modulation of the AGE–RAGE Axis

Modulation of the AGE–RAGE axis offers attractive opportunities for scientists and clinicians to design antiglycation strategies against the progression of chronic diseases. There are eight strategies currently used for modulating the AGE–RAGE axis (Box 1).

Although easier said than done, the most affordable way of modulating the AGE–RAGE axis is to minimize glycation through self-implemented, sustained lifestyle changes. Although cooking with moist heat, at lower temperatures, and for shorter periods of time all reduce AGE formation, heat-treated proteins are resistant to enzymatic degradation, which negatively influences their absorption [50]. This is because the ingested high-molecular-weight AGEs have to be degraded by gastrointestinal proteases prior to the liberation of partially absorbable low-molecular-weight degraded products. In addition, oral bioavailability secondary to poor absorption from the gastrointestinal tract, is low (10%) because AGE crosslinks are resistant to enzymatic/chemical hydrolysis [51]. Increase in physical exercise and smoking cessation have also been suggested as nonpharmological intervention strategies (Box 1).

Pharmacological strategies targeting the interception of the AGE–RAGE axis include: (i) inhibiting the formation or effect of reactive precursors and/or AGEs (AGE inhibitors); (ii) breaking protein crosslinks (AGE crosslink breakers); (iii) RAGE antagonists; and (iv) antioxidative stress medications. The AGE inhibitors limit AGE or AGE crosslink formation, but fail to act on the permanent crosslinks already created due to life-long exposure of long-lived proteins. Clinical results are mixed and some drugs such as aminoguanidine induce unwanted adverse effects that have resulted in their cessation from further human clinical trials. Nevertheless, newer entities are being investigated in Phase I trials (Table 1). The AGE crosslink breakers purportedly revert AGE-induced crosslinking of long-lived proteins. Although the concept of AGE crosslink breakers is promising, their real effects are unlikely to be the result of cleavage or reversal of existing proteins. In reality, they may have more direct effects on AGE formation, such as antioxidation and chelation, and their reaction mechanism with α-dicarbonyl intermediates [52]. Despite some positive results reported for the AGE crosslink breakers such as alagebrium, no strong evidence of beneficial effects were demonstrated in humans.

Box 1. Therapeutic Strategies for Modulating the AGE–RAGE Axis

(i) **Lifestyle changes**

Clinical trial shows that low AGE content in high unsaturated fatty acid-rich diet reduces serum AGEs and RAGE mRNA in patients with metabolic syndrome [53].

Dietary intervention and physical activity reduce circulating AGEs in cancer survivors and have the potential to decrease recurrence incidence and mortality [54].

(ii) **AGE inhibitors**

The AGE-inhibiting capability of aminoguanidine was only demonstrated in animal studies [55] but not in clinical trials [56].

Recent human clinical trials show that AGE inhibitors such as quercetin [57] and L-carnosine [58] reduce serum AGEs and/or their precursors.

(iii) **AGE crosslink breakers**

Agents that split AGE crosslinks in tissue proteins include thiazolium derivatives such as alagebrium and *N*-phenacylthiazolium bromide, and pyridinium derivatives such as TRC4149 and TRC4186. Their effects were demonstrated in animal models of diabetes but not in clinical studies [56].

(iv) **RAGE antagonists**

RAGE antagonists include anti-RAGE antibodies, sRAGE, and RAGE inhibitors (e.g., azeliragon and TTP4000) [56]. The first two categories have only demonstrated effects in animal models.

Clinical trial for azeliragon, a RAGE inhibitor for treating patients with Alzheimer's disease, was discontinued in 2018 after the drug failed to demonstrate significant cognitive or functional improvements in a Phase III trial (https://www.genengnews.com/news/vtv-halts-trials-of-alzheimers-candidate-azeliragon-after-phase-iii-failure/).

New small-molecule RAGE inhibitors designed to bind either to the extracellular or intracellular domain of RAGE have never progressed beyond computer docking, cell-line, or animal disease model studies [59].

(v) **Enhancers of glyoxalase-1 expression**
Concomitant prescription of trans-resveratrol and hesperetin improve metabolic and vascular health in obese individuals [60].

(vi) **Drugs clinically approved for other purposes**
Clinical trials supported AGE-inhibiting and/or RAGE-antagonizing effects of some antidiabetic and antihypertensive drugs [56].
A clinical trial showed that atorvastatin reduces AGEs and angiopoietin-like 2, and may have cardioprotective effects [61].

(vii) **Phytochemicals**
Clinical evidence shows that myoinositol hexaphosphate reduces AGEs and glycated hemoglobin (HbA1c) in patients with type 2 diabetes [62].

(viii) **Experimental molecular approaches**
Agents that modulate miRNAs that are upregulated (e.g., miR-21 and miR-192) or downregulated (miR-190a) during methylgloxyal/AGE–RAGE signaling have beneficial effects on diabetes and/or renal dysfunction in animal models [63].
Experimental studies have demonstrated the therapeutic potential of a DNA-aptamer [64] and a RAGE-aptamer against the AGE–RAGE axis in diabetes-associated complications and cancer.

Among the three classes of RAGE antagonists (Box 1), only the RAGE inhibitors have proceeded to the clinical development stage. According to the amyloid cascade hypothesis, there is potential interaction of β-amyloid proteins with RAGE receptors. Two RAGE inhibitors, TTP4000 and azeliragon, classified as antidimentives, were designed for improving the cognitive function of patients with mild Alzheimer's disease [56]. TTP4000 inactivates RAGE by causing the ligand to bind with itself instead of the receptor, while azeliragon blocks the ligand from binding to RAGE. A Phase I clinical trial for TTP4000 was completed in 2013 but results have not yet been released. Azeliragon, which has been in clinical

Table 1. Randomized Clinical Trials (2016–2019) Targeting the AGE–RAGE Axis[a]

Interventions	Treatment duration	Study design	Phase of clinical trial	Subject characteristics	Mechanism of action	Primary outcomes	Refs
Atorvastatin initial dose: 40 mg; maintenance dose: 10 mg/day	30 days	RCT	II	Acute myocardial infarction (*n* = 190)	AGE inhibitor	Atorvastatin reduced ANGPTL2 and AGEs, and may have cardioprotective effects	[61]
Epicatechin (100 mg/day), quercetin 3-glucoside (160 mg/day)	4 weeks	Double-blind RCT	I	Apparently healthy (pre) hypertensive adults (*n* = 37)	AGE inhibitor	Quercetin but not epicatechin reduced serum methylglyoxal	[57]
Trans-resveratrol (90 mg/day) + hesperetin (120 mg/day)	8 weeks	Randomized double-blind cross-over trial	I	Overweight and obese health subjects (*n* = 29)	Anti-oxidative stress via gloxyalase-1 induction	Increased expression and activity of glyoxalase-1, decreased plasma methylglyoxal, total body methylglyoxal-protein glycation, increase oral insulin sensitivity and arterial dilatation	[60]
Hydroxytyrosol (7.5 mg/day) and vitamin E (10 mg/day)	4 months	Double-blind RCT	I	Children with biopsy-proven NAFLD (*n* = 80)	AGE inhibitor, antioxidant effects	Hydroxytyrosol and vitamin E reduced AGEs, carbonylated proteins, and S-nitrosylated proteins	[69]
ω-3 fatty acid supplements (1000 mg/day)	12 weeks	Double-blind RCT	I	Diabetic nephropathy (*n* = 60)	AGE and RAGE inhibitor	ω-3 fatty acid reduced serum AGEs and RAGEs	[70]
Coenzyme Q10 supplements (100 mg/day)	12 weeks	Double-blind RCT	I	Diabetic nephropathy (*n* = 50)	AGE inhibitor	Coenzyme Q10 reduced serum insulin levels, and plasma MDA and AGEs	[71]
Myo-inositol hexaphosphate (IP6; 380 mg of calcium-magnesium IP6 three times daily)	12 weeks	Randomized cross-over trial	I	T2DM (*n* = 35)	AGE inhibitor	Myoinositol hexaphosphate reduced AGEs and HbA1c	[62]
L-carnosine (1000 mg/day)	12 weeks	Double-blind RCT	I	T2DM (*n* = 54)	AGE inhibitor, antioxidant effect	L-Carnosine reduced fasting glucose, serum levels of triglycerides, AGEs and TNF-α	[58]

(Continued on next page)

Table 1. *Continued*

Interventions	Treatment duration	Study design	Phase of clinical trial	Subject characteristics	Mechanism of action	Primary outcomes	Refs
High saturated fatty acid-rich diet; high-unsaturated fatty acid-rich diet; low-fat, high-complex carbohydrate-rich diet supplemented with long-chain n-3 polyunsaturated fatty acid; low-fat, high-complex carbohydrate-rich diet supplemented with placebo	12 weeks	RCT	I	Metabolic syndrome (*n*=75)	AGE and RAGE inhibitor	High-unsaturated fatty acid-rich diet reduced serum AGEs and RAGE mRNA, and increased AGER1 and glyoxalase-1 mRNA, compared with the other diets. Low-fat, high-complex carbohydrate-rich n-3 diet reduced serum AGEs and increased AGER1 mRNA, compared with high-saturated fatty acid-rich diet and low-fat, high-complex carbohydrate-rich	[53]

[a]Abbreviations: AGER1, advanced glycation end product receptor-1; ANGPTL2, angiopoietin-like protein 2; CFU, colony-forming unit; HbA1c, glycated hemoglobin; MDA, malondialdehyde; NAFLD, nonalcoholic fatty liver disease; RCT, randomized controlled trial; T2DM, type 2 diabetes mellitus; TNF-α, tumor necrosis factor-α.

trials since 2005, was thought to be a promising candidate when the Phase III STEADFAST study was announced. Like many other therapeutics for Alzheimer's disease that failed to meet prespecified clinical endpoints in Phase III clinical trials, azeliragon missed both co-primary efficacy endpoints in the Phase IIIa and IIIb studies. Patients with mild Alzheimer's disease taking azeliragon did not demonstrate improvement in cognitive or functional outcomes compared with the placebo candidates.

Enzymatic detoxication of α-dicarbonyls, especially methylgloxyal, by glyoxalase-1 reduces oxidative stress. A combination of trans-resveratrol and hesperetin increases glyoxalase-1 expression and improves metabolic and vascular health in overweight and obese human subjects in a recent Phase I clinical trial [60]. This is achieved via activation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2), which regulates basal and inducible glyoxalase-1 expression.

Scientists have also re-examined drugs that have been clinically approved for other purposes, such as antidiabetic, antihypertensive, statins, and dietary supplements for their potential in modulating the AGE–RAGE axis. Metformin, an insulin-sensitizing antidiabetic drug, was found to reduce AGE deposition by preventing DNA methylation, thereby ameliorating fundamental aging factors that underlie multiple age-related conditions. The drug has successfully completed Phase IV of the Metformin in Longevity Study (MILES) clinical trial in May 2018 (NCT02432287). Nevertheless, the crossover design could have resulted in a carryover effect, and that certain genes that were differentially expressed might not have been detected because of the small sample size. Another Phase II trial on the effect of metformin on patients with mild cognitive impairment due to Alzheimer's disease was completed in September 2017 (NCT01965756). The results suggested that metformin was associated with trends that suggested improvement in learning/memory and attention.

Not every antidiabetic drug was as successful, however. The Phase III TOMMORROW secondary Alzheimer's disease prevention trial (NCT01931566) examined the effectiveness of low-dose pioglitazone in delaying the onset of mild cognitive impairment due to Alzheimer's disease in cognitively normal subjects who are at high-risk of developing the disease. The clinical trial was halted in early 2018 after a futility analysis revealed only a 15% chance of success (https://www.alzforum.org/news/research-news/theres-no-tomorrow-tommorrow). Antihypertensive agents, including angiotensin receptor blockers, angiotensin-converting enzyme inhibitors, and calcium channel blockers, showed modulatory effects on the AGE–RAGE axis; however, their mechanisms and efficacy are still

inconclusive. Only atorvastatin, a lipid-lowering β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) reductase inhibitor, has recently completed a Phase II clinical trial and was found to exert a potential cardioprotective effect by reducing AGEs and angiopoietin-like protein 2. Other dietary supplements have only completed Phase I clinical trials (Table 1).

Phytochemicals (e.g., quercetin, sulforaphane, iridoids, curcumin, phytate, and rice bran) and molecular approaches (e.g., miRNAs, DNA-**aptamer**, and RAGE-aptamer) have also been reported for their potentials in modulating the AGE–RAGE axis (Box 1). However, only quercetin, myoinositol hexaphosphate, and rice bran have completed Phase I clinical trials recently.

In examining these recent clinical trials, it must be remembered that the probability of success (POS) for drugs moving from Phase I to II was 66.4%, while the POS for drugs moving from Phase II to III was 58.3%. This means that approximately two out of every five drugs that reach confirmatory Phase II trials still fail to receive approval for the indication being investigated [65]. In the context of modulating the AGE–RAGE axis, potential factors that influence the success of clinical trials may be broadly classified into:

(i) Disease factors

- Web of causation. Chronic noncommunicable diseases are usually multifactorial in nature with risk factors interacting between the host and environment in various pathways. This is exemplified by the multiple risk factors involved in Alzheimer's disease, and that therapeutics that target holistically amyloid proteins may generate trial data that lack clinical efficacy.
- Correlation versus causality. Although AGEs are associated with chronic metabolic diseases, they may not be the agents that cause those diseases. For example, alarmins also act as ligands for RAGE apart from AGEs. Likewise, although AGEs are involved in the modulation of inflammasome signaling, a host of other DAMPs also function as ligands for TLRs.
- Multiple hits. Sequential or simultaneous interaction of more than one risk factor (multiple hits) has been suggested in the pathogenesis of chronic renal disease, nonalcoholic fatty liver disease, COPD, Parkinson's disease, Alzheimer's disease, and cancer. Targeting one risk factor at one destined time point may not produce the anticipated clinical efficacy.

(ii) Human factors

- Lost in translation. Differences between the human genome and those of rodent or nematode disease models may lead to setbacks in the design of therapeutics for metabolic diseases that have their roots in innate immunity. Translational failure may be due, in part, to methodological flaws in animal studies, resulting in systematic bias and erroneous conclusions regarding clinical efficacy. Failure may also result because of critical disparities, usually disease specific, between the animal models and trials testing the therapeutic regime. Humanized animal models are seldom used in the study of AGE-related therapeutics.
- Gene polymorphisms. Polymorphisms within the ligand-binding regions of the *RAGE* gene may affect the expression and function of RAGE. Such a factor is seldom considered in subject recruitment in clinical trials.

(iii) Trial design factors

- Unexpected failure where short-term benefits identified in Phase II trials are not associated with long-term benefits during larger and lengthier Phase III trials.
- Use of biomarkers in Phase II trials that do not reliably predict clinical outcomes or translate into effective product performance in Phase III trials.
- A therapeutic agent's anticipated mechanism of action does not translate into clinical benefits. Other untested or unknown mechanisms of action may prevail.
- Prescribing therapy at time points when the window of opportunity has elapsed.

Outstanding Questions

How do AGEs participate in cellular metabolic memory? Epidemiological studies suggested that chronic hyperglycemia can epigenetically modify gene expression profiles in human cells, that are sustained even after cessation of therapeutic intervention. This phenomenon of metabolic memory contributes subsequently to the development of diabetic complications such as vasculopathies and cancer. Understanding these mechanisms may open new vistas in therapeutic intervention of various chronic disorders.

How do AGEs act as cellular stress signals that modulate innate immunity and trigger sterile inflammation? The contribution of DAMPs on NLRP3 inflammasome activation has mostly been studied using alarmins such as the HMGB1 and S100 proteins. It is only recently that AGEs are identified in NLRP3 inflammasome activation that promotes pancreatic islet damage, diabetes-associated complications and osteoarthritis. Appreciation of the roles of AGEs as ligands for TLRs may lead to development of antagonists of AGE–TLR interaction as prophylactic/therapeutic agents.

Would simultaneous application of *N*-acetylcysteine, an oxidative stress detoxification agent, with anti-AGE/RAGE therapeutics improve the clinical efficiency of the test agents in future clinical trials?

Would careful appraisal of genetic polymorphism of the *RAGE* gene during subject recruitment improve the clinical outcomes of trials on therapeutic invention of the adverse effects of glycation?

Would biomarker-stratified trials designed to enable sequential evaluation across biomarker-defined subgroups increase the robustness of Phase III trials, such as those that investigate modulation of the AGE–RAGE axis on the progress of neurodegenerative diseases, and enhance their chance of meeting prespecified clinical endpoints?

Concluding Remarks and Future Perspectives

Research conducted on exogenous and endogenous glycation has been nothing short of phenomenal since glycation of food substances was articulated. Nevertheless, exciting questions remain to be addressed, particularly regarding the therapeutic aspects against glycation (see Outstanding Questions). A simple Ovid Medline search using strings related to AGEs/RAGE yielded an exorbitant quantity of studies, of which controlled human clinical studies remain the minority. Invertebrate/vertebrate models are useful for studying fundamental cellular processes such as oxidative stress, intracellular signaling, and apoptosis. These models also provide high throughput vigor in the selection of potential candidates for the treatment of AGE-implicated chronic diseases. The limitations of these models in preclinical studies for predicting the effectiveness of treatment strategies in clinical trials have been cannoned recently because of recurrent intervention failures of apparently promising animal models to be translated into clinically useful modalities. Nevertheless, animal models are necessary to bridge the gap between the test tube and the human condition in the foreseeable future. Ongoing technical advances, particularly with gene transfer techniques and the use of human–mouse transgenic animals, may help close the gap; however, difficulties are inherent.

Embracing the human element in controlled studies should be accentuated in future AGE–RAGE intervention. However, it must be remembered that there is no therapeutic panacea that can completely eliminate the burden of chronic diseases. The AGE–RAGE axis provides the fundamental cue in understanding the development of these diseases. Unlike acute infectious diseases, AGE accumulation and chronic diseases take years to develop. As candidly expressed by the Centers for Disease Control and Prevention and the World Health Organization, chronic diseases are lifestyle diseases that are preventable and reversible. Unless dietary and lifestyle issues are simultaneously addressed, adoption of a medical paradigm that is deep-rooted in suppressing symptoms via the lifetime use of a wonder drug is unlikely to be successful in addressing the root cause of the problem.

Supplemental Information

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