Diabetes and Advanced Glycoxidation End Products

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The morbidity caused by diabetes has traditionally been classified into macro- and microvascular complications. Although macrovascular complications have received greater attention, microvascular complications are unique to diabetes, and hyperglycemia contributes to their development. Numerous hyperglycemia-related mechanisms are hypothesized to mediate micro- and macrovascular complications. These include the aldose reductase–mediated polyol pathway, the hexosamine pathway, protein kinase C activation, generation of reactive oxidant stress, poly(ADP ribose) polymerase (PARP) activation, and accumulation of advanced glycoxidation (also termed advanced glycation or glycosylation) end products (AGEs) (1,2). AGEs are particularly important, as they form intra- and extracellularly (3,4), are imported from food (5–9) and tobacco smoke (10), and can be deleterious, independent of hyperglycemia (9,11–16). They are implicated in the development of macrovascular disease (13,14,17–20), nephropathy (21–30), neuropathy (31,32), and retinopathy (21,33–38). The remediation of AGEs has also been shown to improve diabetic micro- and macrovascular disease (39–44). AGEs thus offer an important target for prevention of diabetic morbidity. The focus of this review will be on the origin of AGEs, their mechanism of injury, and therapeutic options under development.

FORMATION OF AGEs — AGEs are nonenzymatically formed by reducing glucose, lipids, and/or certain amino acids on proteins, lipids, and nucleic acids (Fig. 1*A*). For example, glucose and a free amino group form reversible intermediates of a Schiff base and an Amadori product (e.g., HbA_{1c}) before a series of reactions that irreversibly generate an AGE (45,46). This process was first identified in 1912 and is known as the Maillard or "browning" reaction due to the associated yellow-brown color change (45,47,48). When formed endogenously, this reaction is driven forward by hyperglycemia (4,49).

Alternate mechanisms of AGE formation include the "carbonyl stress" pathway, where oxidation of sugars and/or lipids create dicarbonyl intermediate compounds that use highly reactive carbonyl groups to bind amino acids and form AGEs (50,51) (Fig. 1). Non–glucose-dependent AGE pathways involve neutrophils, monocytes, and macrophages, which, upon inflammatory stimulation, produce myeloperoxidase and NADPH oxidase enzymes that induce AGE formation by oxidizing amino acids (52,53). Once bound by AGEs, receptors for AGE (RAGE) associated with reactive oxygen species (ROS) generation promote more AGEs via the NADPH oxidase pathway (54,55). Monocytes, macrophages, and dendritic cells also secrete the nuclear protein amphoterin (also termed high-mobility group box 1 [HMGB1]) (56–58), and HMGB1 can bind and activate RAGE and thus induce further inflammation (59–61). Another mechanism of AGE formation is the aldose reductase–mediated polyol pathway. Glucose entering the polyol pathway may directly form AGEs via 3-deoxyglucosone AGE intermediates, but this reaction also causes depletion of NADPH and glutathione, and the resultant oxidative stress indirectly increases formation of AGEs (62).

Given their varied mechanisms of formation, it is not surprising that AGEs are a heterogeneous group of compounds. Many AGEs fluoresce under ultraviolet light, and some are capable of intra- and intermolecular cross-linking, but not all share those properties (54,63). Once formed, certain cross-linking AGEs form stable cross-link structures with other proteins in the body, including structural proteins (e.g., collagen), intracellular proteins, membrane phospholipids, DNA, and lipoproteins (e.g., LDL cholesterol), and also bind to AGE receptors (64–67).

ENDOGENOUS SOURCES OF AGEs IN DIABETIC SUBJECTS

— People with diabetes have higher levels of AGEs than nondiabetic subjects because hyperglycemia and oxidative stress both contribute to their accumulation. Studies have shown 20–30% higher AGE levels in people with uncomplicated diabetes (68,69) and 40–100% higher levels in subjects with type 2 diabetes complicated by coronary artery disease or microalbuminuria (17,70). Multivariate analyses in subjects with diabetes have identified renal function, age, urinary albumin-to-creatinine ratio, systolic blood pressure, and anemia as independent predictors of AGE levels (70,71). Renal impairment decreases clearance of AGEs in both diabetic and nondiabetic populations (51). Subjects with end-stage renal disease have shown significant elevations in circulating AGEs compared with healthy control subjects (by 5- to 100-fold) (46,72,73). Renal transplant has been shown to normalize AGE levels in subjects with end-stage renal disease ($n = 2$) (73). These observations indicate that AGE turnover is more dynamic than

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Abbreviations: AGE, advanced glycoxidation end product; ARB, angiotensin-II receptor blocker; CML, N^{ε}–carboxymethyllysine; HMGB1, high-mobility group box 1; NF-κB, nuclear factor-κB; PARP, poly(ADP ribose) polymerase; RAGE, receptors for AGE; ROS, reactive oxygen species.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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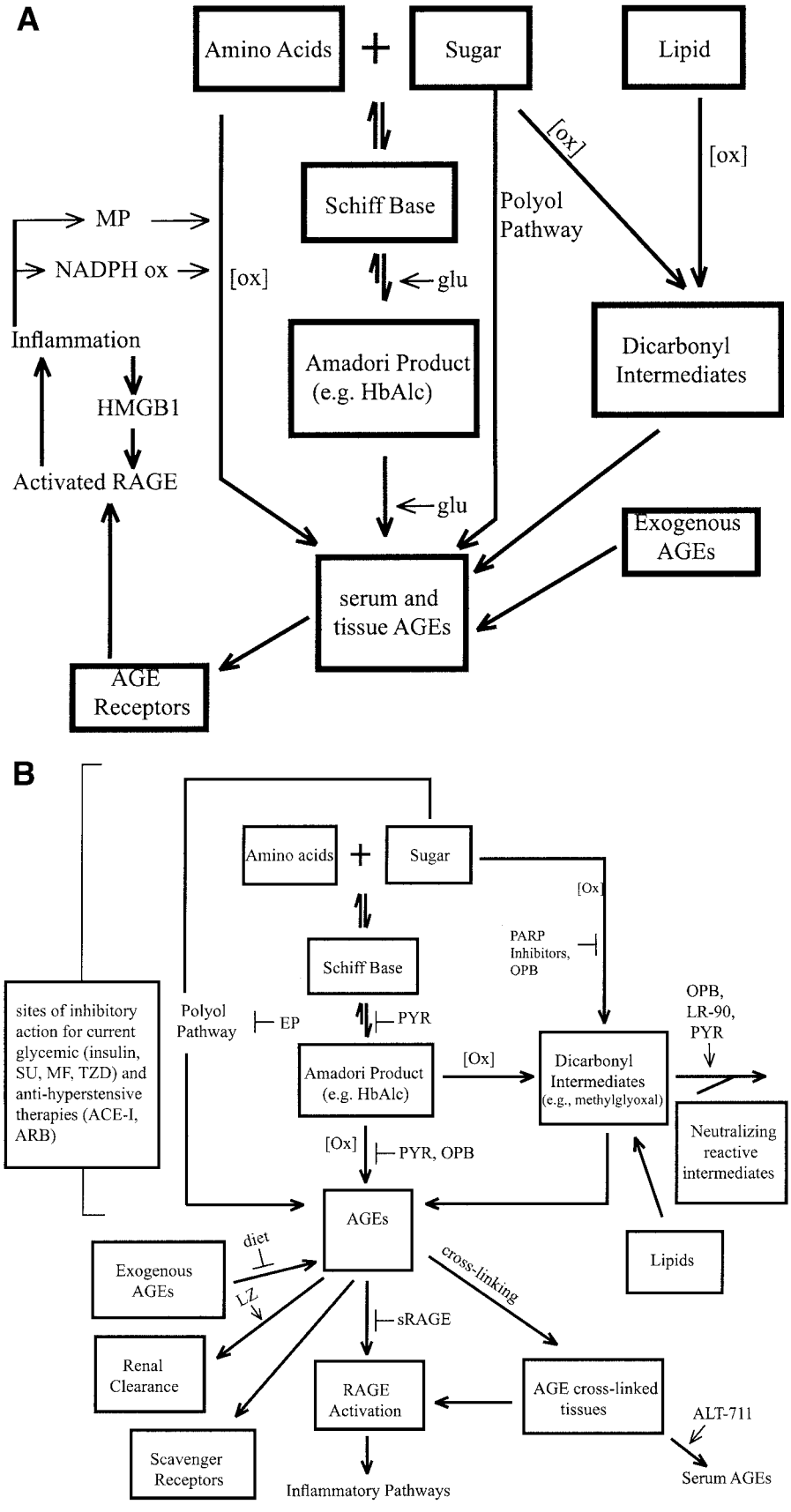

Figure 1—A: *Mechanisms of AGE formation. MP, myeloperoxidase.* B: *AGE therapeutic agents' mechanisms of action. ACE-I, ACE inhibitor; ALT-711, alagebrium chloride; diet, low-AGE diet; EP, epalrestat; LR-90, 4-4′-(2 chlorophenylureido phenoxyisobutyric acid); LZ, lysozyme; MF, metformin; OPB, OPB 9195; PYR, pyridoxamine; sRAGE, soluble RAGE; SU, sulfonylurea; TZD, thiazolidinedione.*

previously appreciated and that endogenous AGEs are determined by AGE production (endogenous glycemia and oxidative stress) as well as renal AGE excretion.

EXOGENOUS SOURCES OF AGEs: DIETARY GLYCOTOXINS

— As indicated above, hyperglycemia, renal insufficiency, and aging are prooxidant states that contribute to the endogenous levels of AGEs. Importantly, diet is an underappreciated source of AGE toxicity (9). Dietary AGEs include reactive AGE precursors (e.g., 1- or 3-deoxyglucosone, methylglyoxal, and pentosidine) and non–cross-linking AGEs, such as pyrraline, $N^{\varepsilon-}$carboxymethyllysine (CML), carboxyethyllysine, and their derivatives (5,63,74–76). Diet-derived AGEs are similar to native AGEs with respect to prooxidant and proinflammatory properties (9,11). Amino lipids from dietary fats (e.g., 4-hydroxynonenal, CML, and their analogs) are also major targets for lipid peroxidation (77,78). Thus, ingested glycoxidation and lipoxidation products can accelerate free radical generation and oxidative and carbonyl stress (79). Autoxidation of glucose is also accompanied by generation of ROS such as superoxide radicals (80).

In human subjects with or without diabetes, a single high-AGE meal leads to significant elevations in serum AGEs compared with a normal meal (6). An estimated 10% of AGEs ingested are absorbed into the body's circulation, and two-thirds of those absorbed are retained (6). The intestinal epithelium absorbs early derivatives (i.e., Amadori products) as well as intermediate and late AGEs (81). AGE-modified mono-, di-, or tripeptides can be readily transported across the intestinal wall, carrying one or more AGE. The nature of most AGE derivatives involved in this traffic has not been determined, but a number of these have been reported (5,81). The presence in most foods of two well-characterized, structurally distinct AGE derivatives (i.e., methylglyoxal and CML) has enabled studies in animals and humans that have confirmed their substantial toxic role in multiple target systems (9,11–15,22,82–85).

AGE content in ~250 human foods has been quantified for comparative purposes (5). High-temperature cooking (e.g., broiling, grilling, frying, roasting) significantly increases AGE levels (5), while cooking foods under lower temperature, for shorter times, and with higher water content (e.g., boiling, steaming) allows smaller AGE increases (5,8). Both protein- and lipid-linked AGE levels are highest in meat and animal food products (based on estimates of CML by enzyme-linked immunosorbent assay) (5). High dietary AGE intake is associated with atherosclerosis (13,14), nephropathy (15,22), and impaired wound healing (82) in diabetic animal models. For instance, while diabetic animals fed standard diets developed expected vascular or renal tissue injury, age-matched diabetic cohorts fed a low-AGE diet remained largely free of pathology despite untreated chronic hyperglycemia (15). More interestingly, reduced intake of dietary AGE is shown to prevent type 1 and type 2 diabetes and insulin resistance in experimental settings (83,84). In diabetic individuals, increased dietary AGE intake has also been shown to be associated with high serum AGE, increased inflammatory markers such as C-reactive protein (74), and impaired endothelial function (86). Thus, diet is a significant source of AGEs that may contribute to the inflammatory state of diabetes.

AGE receptors

It is important to understand the relationship of AGEs to their receptors because as a group, these receptors occupy both positive and negative roles in the actions and fate of AGEs. In their positive role, some receptors normally aid in clearing AGEs from the circulation and may help to mitigate the prooxidant effects of AGEs. In contrast, RAGE and other receptors appear to activate a stress response leading to inflammation and cellular dysfunction. The complexities of this system are still not fully understood, but this review will elaborate on what is currently known.

Beneficial AGE receptors that enhance clearance of AGEs include AGE-R1 and lysozyme (87–92). AGE-R1 is active in AGE-specific ligand binding and degradation (88). Low expression of AGE-R1 in the kidneys of nonobese diabetic mice was associated with high tissue AGE levels and with kidney disease. Also, human circulating mononuclear cells from diabetic subjects with severe diabetes complications showed low expression of AGE-R1 and high serum AGE (90). These studies suggested that this molecule may be suppressed or saturated in the presence of high-AGE–induced oxidant stress. Overexpression of AGE-R1 confirmed enhanced endocytosis and degradation of AGE but also revealed an inhibitory action on AGE- and RAGE-induced mitogen-activated protein kinase phosphorylation and nuclear factor-κB (NF-κB) activity (87). This suggested that AGE-R1 may mitigate AGE-induced oxidative species and related cellular toxicity. Subsequent studies confirmed that AGE-R1 suppresses intracellular oxidative species via the epidermal growth factor receptor and Shc/Grb2/Ras pathways (93). This molecule, therefore, may exert a protective function against AGE- and RAGE-promoted cellular activation. However, AGE-R1 may be suppressed or downregulated in circumstances of sustained AGE-induced oxidant stress when RAGE is upregulated and AGE-R1–to–RAGE ratio is negative (i.e., aging or severely complicated diabetes). An inverse AGE-R1–to–RAGE ratio may thus be consistent with improved AGE and oxidative species homeostasis.

A lesser-known soluble receptor important in the "detoxification" of AGE is lysozyme (91). Lysozyme is a member of the human immune defense system and exhibits high AGE-binding affinity, recognizing at least two structurally distinct AGEs, CML and methylglyoxal derivatives. The lysozyme AGE-binding site overlaps with the domain of the bactericidal activity of this family of proteins (91). Lysozyme binding to AGE enhances AGE removal and clearance, and early studies showed that lysozyme could deplete diabetic or uremic sera of AGEs (92). Additional studies in diabetic mice demonstrated that lysozyme administration decreases circulating AGE levels and enhances the renal excretion of AGEs (94).

The roles of AGE-R2, AGE-R3, and the scavenger receptors (class A, type II [e.g., MSR-AII] and class B, type I [e.g., SR-B1, CD36]) are less well defined. Studies using AGE-R3 knockout mice exhibited accelerated AGE-induced glomerular injury (95), while other mouse studies indicate that scavenger receptors may be involved in AGE degradation. Yet some scavenger receptors may promote proinflammatory effects via NF-κB upregulation (CD36) (96) and dyslipidemic effects via impairment of reverse cholesterol transport to the liver (SR-B1) (97).

The best-studied proinflammatory AGE receptor thus far is RAGE, a member of the immunoglobulin superfamily of cell surface molecules (98,99). It binds AGEs and also recognizes S100/calgranulins (e.g., S100A12 , also termed extracellular newly identified RAGE bind-

ing protein, and S100B), HMGB1, and amyloid-β peptide (59–61,100,101). Once a ligand is recognized, RAGE promotes multiple signaling pathways that generate ROS. These pathways include p21ras, extracellular signal–regulated kinase-1 and -2, mitogen-activated protein kinases, and cdc42/rac (102). Of note, ROS generation is also enhanced by AGEs, independent of RAGE, in part due to antioxidant depletion (49,103,104). ROS, via activation of the redox-sensitive transcription factor NF-κB, upregulates many inflammatory and "response-to-injury" genes, including those governing RAGE expression (100,102). These events lead to endothelial dysfunction due to increased vasoconstriction and inflammation and decreased vasodilation (100,105,106). RAGE upregulation is thought to contribute to a synergistic cycle, first hypothesized by Basta et al. (105), wherein S100/calgranulins and HMGB1, together with ROS and inflammatory cytokines, further activate RAGE and attract more activated macrophages. This was hypothesized to help sustain the AGE-induced inflammatory stress response and may play a key role in the pathogenesis of micro- and macrovascular disease, as discussed in the next two sections.

ROLE OF AGEs IN PROMOTING MICROVASCULAR DISEASE

— Vascular dysfunction, including basement membrane thickening, increased vascular permeability and prothrombotic state, and decreased blood flow, is a ubiquitous trait of microvascular disease of the retina, nephron, and peripheral nerve (49,107). AGEs play a role in causing these abnormalities and the attendant microvascular disease (21–30,33–38). CML is a common AGE that has been localized to retinal blood vessels of human type 2 diabetic subjects and found to correlate to the degree of retinopathy present (33). AGE accumulation (based on skin biopsy measurements) correlates with the occurrence of retinopathy and microalbuminuria, independent of age or duration of type 1 diabetes (21). In a murine model, AGEs worsened diabetic neuropathy by reducing sensory motor conduction velocity and decreasing blood flow to peripheral nerves. These changes were prevented by concomitant use of an aminoguanidine-antioxidant AGE-lowering therapy (31). The serum AGE levels of CML and fructosyllysine strongly correlated with early nephropathy based on microalbuminuria (21).

ROLE OF AGEs IN PROMOTING MACROVASCULAR DISEASE

— Elevated serum AGEs are associated with increased coronary artery disease in type 2 diabetic subjects (17). AGEs may be associated with atherosclerosis in a number of ways, including increased endothelial dysfunction, elevated vascular LDL, increased plaque destabilization, neointimal proliferation (108), and inhibited vascular repair after injury.

By generating oxidative stress, AGEs promote vasoconstriction, inflammation, and prothrombotic gene expression, which result in endothelial dysfunction (2). Activation of NF-κB and activator protein-1 transcription factors by AGEs leads to increased expression of endothelin-1, adhesion molecules, inflammatory cytokines, and plasmin activator inhibitor 1 (2,109–112). In conjunction with protein kinase C activation and oxidative stress, AGEs decrease both prostacyclin and nitric oxide (2,113–116), resulting in vasoconstriction. AGEs' induction of angiotensin II and endothelin further contributes to vasoconstriction and leads to proinflammatory and mitogenic effects on vascular smooth muscle cells (112,117–120).

Inflammation and endothelial dysfunction provide fertile ground for a stepwise progression to atheroma in diabetic subjects (18,19). AGE-mediated atherosclerotic mechanisms include quenching nitric oxide (121), cross-linking collagen's resistance to vascular remodeling (122), and impairing LDL removal (both by trapping LDL in the subendothelium [123] and by decreasing LDL receptor recognition of AGE-modified LDL [124,125]). AGE binding to LDL apolipoprotein B impairs its hepatic receptor-mediated uptake and removal (125). Conversely, the glycated apolipoprotein B induces increased retention of LDL in the aortic wall and increased recognition by macrophages (126,127). Accordingly, there is increased localization of AGE-LDL in vessels and increased production of foam cells via macrophage recognition and ingestion (126,127). In this way, glycated LDL propagates atheroma formation more so than "naked" LDL (19).

Neointimal formation (vascular smooth muscle cell proliferation) after balloon injury is suppressed by AGE blockade (108). Whether this is a direct effect on vascular smooth muscle cells or a result of inhibiting inflammation and endothelial dysfunction is still an area of active investigation (108,128).

AGEs AND ARTERIAL STIFFNESS

— Diabetic subjects (both type 1 and type 2 diabetes) have increased arterial stiffness, as measured by diastolic dysfunction (129), increased pulse-wave velocity, and decreased arterial compliance (130–139). There is also a correlation between increased arterial stiffness and impaired glucose tolerance (133,139–141).

AGEs play a likely role in the altered stiffness of the vessel wall as AGE cross-linked vascular collagen and elastin impair arterial elasticity. Arterial stiffness is determined by both the material properties of the vessel wall (130,142) and the vasoreactivity governed by endothelial function (137,138,143,144). Certain therapeutic agents have shown promise in decreasing arterial stiffness (145,146).

THERAPEUTIC OPTIONS AGAINST AGEs

— AGEs clearly contribute to the progression of micro- and macrovascular complications of diabetes and therefore present a promising target for therapeutic interventions. These therapies act through diverse pathways, including decreasing AGE absorption, inhibiting the production of Amadori products, preventing Amadori product progression to AGEs, decreasing oxidative stress, binding and detoxifying dicarbonyl intermediates, and interrupting biochemical pathways that impact on AGE levels (Fig. 1*B*). These agents include investigational medications, Food and Drug Administration–approved medications with recognized benefits in diabetes (e.g., ACE inhibitors, angiotensin-II receptor blockers [ARBs], metformin, pioglitazone), and dietary therapies (Table 1). There are no Food and Drug Administration–approved agents for the specific indication of AGE modification to date, though some such medications are in clinical and preclinical testing.

AGENTS THAT PREVENT AGE FORMATION

— Aminoguanidine, which interferes with AGE production, has been shown to improve nephropathy (147,148), retinopathy (149,150), and vessel elasticity (122) when administered to diabetic rats. While increased incidence of glomerulonephritis has been seen with higher-dose amino-

Table 1—*Therapeutic agents targeting AGEs: human and animal data*

Category of therapy: human studies	Most advanced stage of trials as relates to AGEs	Trial results	Safety concerns
Prevent AGE formation			
Therapeutic entity			
Aminoguanidine	Human, phase III	↓ nephropathy, ↓ retinopathy	↑ glomerulonephritis, ↓ vitamin B6, ↓ iNOS
Benfotiamine	Human, phase II	↓ neuropathy	None reported
AR inhibitors (epalrestat, zopolrestat)	Human, phase II	↓ AGE levels, ↓ neuropathy, ↑ esophageal motility	None reported
AGE cross-link disrupter			
Therapeutic entity			
ALT-711 (alagebrium chloride)	Human, phase III	↓ arterial stiffness, ↓ pulse pressure, breaks cross-links formed by AGEs, ↑ diastolic heart function	None reported
Antihypertensive			
Therapeutic entity			
ARB	Human, phase III	↓ macrophages in carotid artery plaque	↓ GFR, rare angioedema
ACE inhibitor	Human, phase II	↓ RAGE levels	↓ GFR, rare angioedema
Dietary factors			
Therapeutic entity			
Low-AGE diet	Human data, stage N/A	↓ AGE levels, ↓ C-reactive protein	None reported
Prevent AGE formation			
Therapeutic entity			
ALT-946	Animal (diabetic rats)	↓ nephropathy better than aminoguanidine	None reported
LR-90	Animal (diabetic rats)	↓ nephropathy, ↓ oxidative stress	↑ weight gain
OPB 9195	Animal (diabetic rats)	↓ stenosis after vessel injury, ↓ nephropathy	↓ vitamin B6
PARP inhibitors	Animal (diabetic rats)	↓ endothelial dysfunction, ↓ diastolic dysfunction, ↓ neuropathy	None reported
Pyridoxamine	Animal (diabetic rats)	↓ nephropathy, ↓ cholesterol, ↓ weight	None reported
AGE cross-link disrupter			
Therapeutic entity			
PTB	Animal (diabetic rats)	↓ AGEs	None reported
AGE binder			
Therapeutic entity			
Soluble RAGE	Animal (diabetic mice)	↓ stenosis after vessel injury, ↓ neuropathy	None reported
Lysozyme	Animal (diabetic and apolipoprotein E–null mice)	↓ AGEs, ↓ nephropathy, ↓ atherosclerosis	None reported
Antioxidants			
Therapeutic entity			
Green tea	Animal (diabetic rats)	↑ AGEs, ↑ AGE cross-links	None reported
Vitamins E and C	Animal (diabetic rats)	↑ AGEs, ↑ AGE cross-links	↑ CV morbidity from vitamin E >400 IU
Oral hypoglycemic agents			
Therapeutic entity			
Metformin	Animal (diabetic rats)	↓ AGEs, ↓ AGE cross-links	Lactic acidosis
Pioglitazone	In vitro	↓ AGEs, ↓ AGE cross-links	↑ hepatitis, ↑ CHF if susceptible

AR, aldose reductase; CHF, congestive heart failure; CV, cardiovascular; GFR, glomerular filtration rate; iNOS, inducible nitric oxide synthase; PTB, *N*-phenacylthiazolium bromide; TZD, thiazolidinedione.

guanidine in human phase III trials, the lower dose was equally effective at ameliorating proteinuria ($P < 0.001$) and preventing retinopathy progression ($P = 0.03$) and was free of serious side effects (151). However, aminoguanidine's binding of pyridoxal may lead to vitamin B6 deficiency and associated neurotoxicity (152). Aminoguanidine's toxicity has halted further studies, but its positive impact on proteinuria and vascular elasticity provide proof of concept and have encouraged continued development of other AGE-targeted therapies.

Pyridoxamine is one of three vitamin B6 natural forms. It retarded AGE formation and inhibited diabetic nephropathy equally to aminoguanidine and lowered cholesterol levels more than aminoguanidine while inducing mild weight loss in both nondiabetic and diabetic rats (153). No human trials with pyridoxamine have been published.

Other vitamin B6 analogs have shown less promise. A pyridoxal-aminoguanidine adduct inhibited cataract formation and diabetic neuropathy in a rat model better than aminoguanidine alone (31). However, a study on a combination of pyridoxine (600 mg daily) and folic acid (15 mg daily) administration to type 2 diabetic human subjects did not improve preexisting markers of endothelial dysfunction (e.g., plasmin activator inhibitor 1 and fibrinogen) (154).

The peroxisome proliferator–activated receptor agonist OPB 9195 has inhibitory actions on glycoxidation and lipoxidation reactions, thereby decreasing formation of AGEs and dicarbonyl intermediates. This compound is also hypothesized to scavenge dicarbonyl intermediates (155). In animal models, OPB 9195 reduced progression of nephropathy (49), lowered blood pressure (156), reduced oxidative stress (156), and impaired carotid artery intimal proliferation following balloon damage to the endothelium (155). No human data with this agent have been published, as it has shown similar pyridoxal-trapping toxicity to aminoguanidine (157).

ALT-946 therapy for 12 weeks has been shown to reduce renal AGEs by histologic analysis and to decrease albuminuria by 250% compared with aminoguanidine therapy in diabetic hypertensive rats (158). An additional study showed that ALT-946 therapy in a rat model reduced albuminuria both when used at the onset of diabetes and when initiated after 16 weeks of diabetes (159). No human data with this agent have been published.

AGENTS THAT DISRUPT AGE CROSS-LINKS — A promising line of AGE therapy investigates agents that disrupt the cross-links that bind AGEs to human tissue. ALT-711 (alagebrium chloride) is capable of cleaving AGE cross-links, thus allowing endogenous AGE removal from vessel walls (160). A randomized, placebo-controlled trial in 93 hypertensive subjects age >50 years showed significant reduction in pulse pressure and arterial stiffness in ALT-711–treated subjects compared with placebo (161). A 16-week open-label trial of ALT-711 in 23 humans with systolic hypertension and moderately severe diastolic heart failure (22% with diabetes) decreased left ventricular mass, improved left ventricular filling, and improved patient ratings of quality of life (162). In diabetic rats, ALT-711 has been shown to decrease levels of AGEs (163,164), RAGE expression (163,164), diabetic nephropathy (163), myocardial stiffness (164), and has attenuated atherosclerosis and decreased cholesterol and systolic blood pressure in diabetic hyperlipidemic mice (39).

Another AGE cross-link breaker is *N*-phenacylthiazolium bromide. In diabetic rat models, this agent has been shown to decrease AGEs (165,166) but has not decreased nephropathy as measured by proteinuria (166,167). No human studies with this agent are available.

SOLUBLE AGE-BINDING PEPTIDES — Soluble RAGE is thought to bind to RAGE ligands (e.g., AGEs, β-amyloid, S100/calgranulins, HMGB1), thus preventing RAGE activation and the attendant cellular dysfunction. Soluble RAGE was able to significantly attenuate arterial restenosis in apolipoprotein E–deficient mice after femoral artery intimal injury (168). This agent also inhibited atherosclerosis progression in diabetic apolipoprotein E–deficient mice independent of glucose and cholesterol levels (40). Soluble RAGE therapy for 3 weeks also restored pain perception in neuropathic diabetic mice to levels of controls ($P < 0.005$) (41). Work in humans with this agent has not been published.

Once lysozyme was found to bind and improve AGE removal (91), its potential therapeutic value was evident. It was initially thought that lysozyme could be developed as an AGE-binding matrix useful in the depletion of AGE from diabetic or uremic sera (92). Additional studies in diabetic mice demonstrated that lysozyme administration decreases circulating AGE levels and enhances renal AGE excretion (94). These studies also showed that lysozyme could suppress adverse AGE-mediated cellular activation in vitro and could prevent diabetic nephropathy in vivo (94). Lysozyme appears to confer resistance to AGE-induced oxidative species, which thus allows lysozyme to block cellular apoptosis in vitro, to reduce mortality in vivo (169), and to reduce atherosclerosis in apolipoprotein E knockout mice (170). Lysozyme could be developed into a therapeutic target for human use, but no studies in humans have been published to date.

OTHER AGENTS THAT REMEDIATE AGEs — Benfotiamine is a highly bioavailable thiamine prodrug (171) currently available in the U.S. as a dietary supplement. Benfotiamine (42,43,172) and high-dose thiamine (43,172,173) have both been shown to reduce AGE formation. Both compounds also decrease hexosamine levels, inhibit protein kinase C activation, and decrease oxidative stress, thus impacting four different mediators of diabetic vascular disease (42). Benfotiamine has improved neuropathy in an open-label trial (174) and in a 40-patient placebo-controlled trial (175). In experimental animals, benfotiamine improved nephropathy (172) and retinopathy (42). In a rat model, benfotiamine therapy improved neuropathy (measured by nerve conduction velocity) better than high-dose thiamine both at onset and after 2 months of diabetes induction (43).

PARP has been shown to inhibit glyceraldehyde-3-phosphate dehydrogenase, resulting in increased AGE formation through the dicarbonyl intermediate pathway (176). PARP inhibitors have improved endothelial function (177,178), diabetic neuropathy (44), and diastolic function (177) compared with control diabetic rats. No human data with these agents have been published.

Aldose reductase inhibitors have been shown to decrease AGE formation (179–181) by inhibiting the first and rate-limiting step in the polyol pathway. Epalrestat, an aldose reductase inhibitor, also reduced production of the dicarbonyl intermediate 3-deoxyglucosone (179). Aldose reductase inhibitors have

been shown to improve nerve conduction velocity (182) and to improve esophageal motility (183,184) in people with diabetic neuropathy. In a murine model, the aldose reductase inhibitor zopolrestat suppressed AGE-induced increases in vascular adhesion molecules and chemotactic factors for monocytes (185).

LR-90 [4-4′-(2 chlorophenylureido phenoxyisobutyric acid)] inhibits AGE production by scavenging dicarbonyl intermediates and by chelating transition metals that catalyze the production of AGEs. In diabetic rat studies, it has been shown to reduce AGE formation, nephropathy, and oxidative stress (186). No human data with this agent have been published. Numerous compounds have been found to have some AGE inhibitory activity in vitro, including pentoxifylline, D-penicillamine, desferoxamine, diclofenac, and inositol (187).

CURRENTLY AVAILABLE ANTI-AGE THERAPIES — By minimizing hyperglycemia, oral hypoglycemic agents decrease the formation of AGEs, but some have other AGE-preventive mechanisms as well. Namely, metformin and pioglitazone have been shown in vitro to prevent AGE formation (188).

ACE inhibitors (temocaprilat) and ARBs (olmesartan, candesartan, irbesartan, losartan, telmisartan, and valsartan) were effective in vitro at decreasing AGE formation (157). Studies in humans have shown decreased vascular inflammation with irbesartan (189) and decreased RAGE levels with perindopril (190). Perindopril has also inhibited atherosclerosis in mice (191). However, after 12 weeks of ramipril therapy in mice, there was no significant impact on RAGE levels or expression of the proinflammatory transcription factor, NF-κB (192). In humans, the exact mechanism by which ACE inhibitors and ARBs effect AGEs and their effect is uncertain. Studies in humans with antioxidants have shown mixed benefit (193–196). These studies are summarized in Table 1.

DIETARY AGE RESTRICTION — Dietary AGE intake is a significant determinant of circulating and tissue AGE levels, as well as of diabetic injury (6,9,11–15,22,82–85). A low-AGE diet (approximately fivefold lower AGE versus regular diet) in diabetic subjects for 6 weeks in a general clinical research center setting decreased serum AGE levels and inflammatory markers such as C-reactive protein (CRP) (74). In a nondiabetic peritoneal dialysis population, similar reductions in AGEs and C-reactive protein were associated with a low-AGE diet for 4 weeks (approximately threefold lower AGE intake versus control was achieved by instructing patients how to prepare their meals without frying, roasting, or broiling) (85). A low-AGE diet prevented intimal proliferation after arterial balloon injury in a nondiabetic, apolipoprotein E knock-out, hyperlipidemic mouse model (13) and inhibited aortic root atheroma development by 50% within 2 months of diabetes in the same mouse model (14). These studies show that dietary restriction of AGEs can significantly reduce vascular inflammation and atherosclerosis.

CONCLUSIONS — AGEs are ubiquitous substances, the formation of which is accelerated in diabetic subjects and contributes to tissue ROS and inflammation, resulting in micro- and macrovascular complications. Therapeutic options to reduce their morbidity would be tremendously useful. Currently available agents for treatment of diabetes and hypertension decrease AGEs and may in fact provide benefit through AGE reduction. Decreasing the AGE content of the diet is effective, feasible, and not discordant with the current dietary recommendations of the American Diabetes Association and American Heart Association. Some potential therapies include ALT-711, ALT-946, aldose reductase inhibitors, lysozyme, LR-90, trientine, pyridoxamine, PARP inhibitors, and soluble RAGE. In human trials, aldose reductase inhibitors have improved neuropathy and esophageal motility and ALT-711 has reduced arterial stiffness and improved some measurements of diastolic heart failure. The other agents remain in earlier stages of research and development. These therapies target AGEs by differing methods, offering hope that even if there is no magic bullet against them there are at least several arrows in our quiver.

NOTE ADDED IN PROOF — The authors were recently made aware of animal studies showing remediation of AGEs (197) and decreased diabetic neuropathy (198) by the copper chelator trientine. One human study (199) performed with trientine reported improved left ventricular mass, but AGE levels were not measured in that study. Work from Price et al. (200) showed that copper chelation may be one of the significant mechanisms of action of pyridoxamine and *N*-phenycylthiazolium bromide.

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