

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. 1A). 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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Received for publication 31 October 2005 and accepted in revised form 19 February 2006.

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.

DOI: 10.2337/dc05-2096

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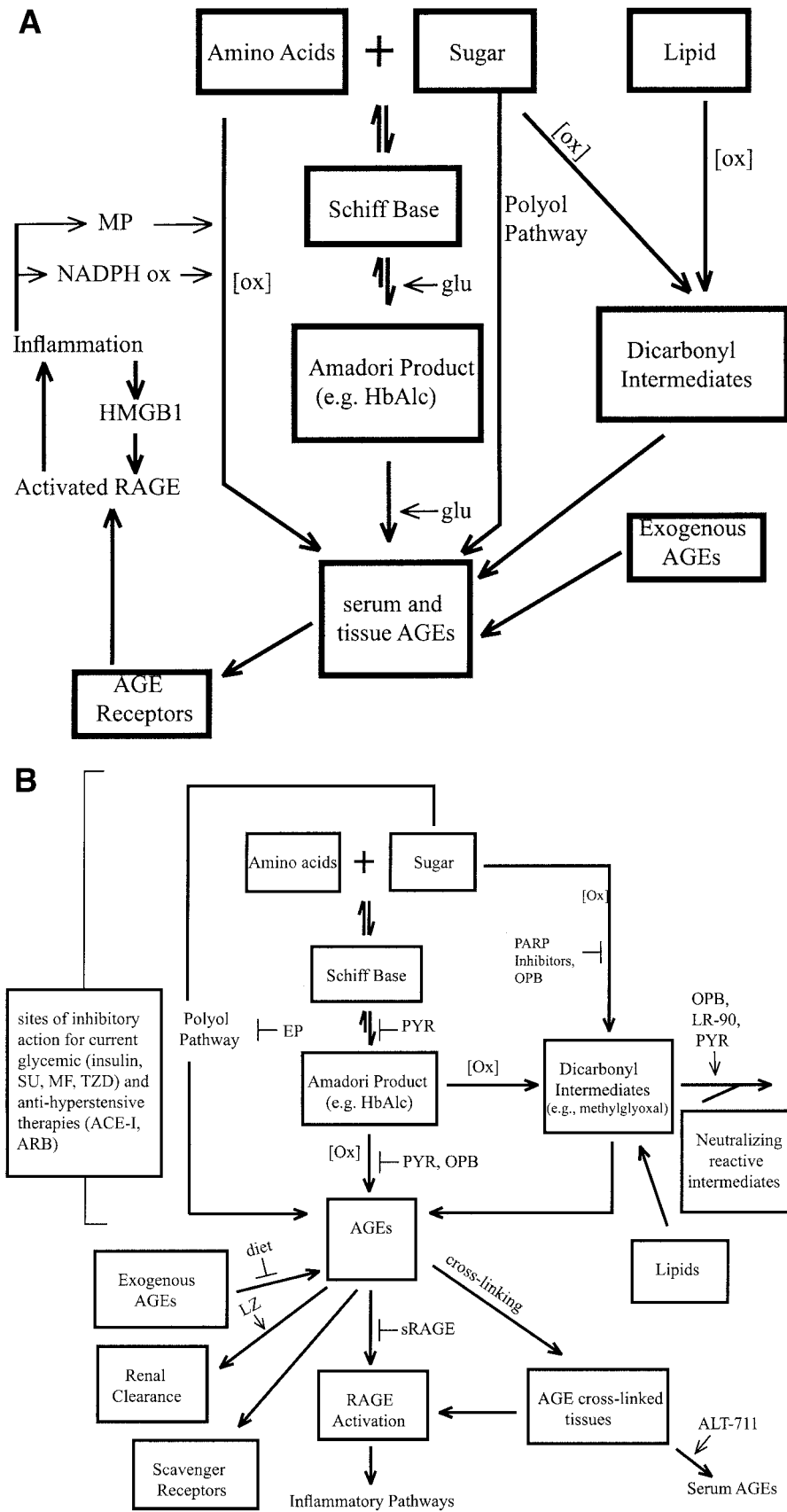


Figure 1—A: Mechanisms of AGE formation. MP, myeloperoxidase. B: AGE therapeutic agents' mechanisms of action. ACE-I, ACE inhibitor; ALT-711, alagebrium chloride; diet, low-AGE diet; EP, palrestat; LR-90, 4-4'-(2 chlorophenylureido phenoxyisobutyric acid); LZ, lysozyme; MF, metformin; OPB, OPB 9195; 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 pyrrolidine, N^{ϵ} -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 temper-

ature, 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- κ B (NF- κ B) 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- κ B 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- κ B, 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 corre-

lated 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- κ B 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. 1B). 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 glycooxidation 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 diabe-

tes (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'-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- κ B (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-phenylthiazolium bromide.

References

1. Brownlee M: Biochemistry and molecular cell biology of diabetic complications. *Nature* 414:813–820, 2001
2. Beckman JA, Creager MA, Libby P: Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. *JAMA* 287:2570–2581, 2002
3. Giardino I, Edelstein D, Brownlee M: Nonenzymatic glycosylation in vitro and in bovine endothelial cells alters basic fibroblast growth factor activity: a model for intracellular glycosylation in diabetes. *J Clin Invest* 94:110–117, 1994
4. Ahmed N: Advanced glycation endproducts: role in pathology of diabetic complications. *Diabetes Res Clin Pract* 67:3–21, 2005
5. Goldberg T, Cai W, Peppia M, Dardaine V, Baliga BS, Uribarri J, Vlassara H: Advanced glycoxidation end products in commonly consumed foods. *J Am Diet Assoc* 104:1287–1291, 2004
6. Koschinsky T, He CJ, Mitsuhashi T, Bucala R, Liu C, Buenting C, Heitmann K, Vlassara H: Orally absorbed reactive glycation products (glycotoxins): an environmental risk factor in diabetic nephropathy. *Proc Natl Acad Sci U S A* 94:6474–6479, 1997
7. Uribarri J, Peppia M, Cai W, Goldberg T, Lu M, Baliga S, Vassalotti JA, Vlassara H: Dietary glycotoxins correlate with circulating advanced glycation end product levels in renal failure patients. *Am J Kidney Dis* 42:532–538, 2003
8. Uribarri J, Cai W, Sandu O, Peppia M, Goldberg T, Vlassara H: Diet-derived advanced glycation end products are major contributors to the body's AGE pool and induce inflammation in healthy subjects. *Ann N Y Acad Sci* 1043:461–466, 2005
9. Vlassara H, Uribarri J: Glycoxidation and diabetic complications: modern lessons and a warning? *Rev Endocr Metab Disord* 5:181–188, 2004
10. Cerami C, Founds H, Nicholl I, Mitsuhashi T, Giordano D, Vanpatten S, Lee A, Al Abed Y, Vlassara H, Bucala R, Cerami A: Tobacco smoke is a source of toxic reactive glycation products. *Proc Natl Acad Sci U S A* 94:13915–13920, 1997
11. Cai W, Gao QD, Zhu L, Peppia M, He C, Vlassara H: Oxidative stress-inducing carbonyl compounds from common foods: novel mediators of cellular dysfunction. *Mol Med* 8:337–346, 2002
12. Cai W, He JC, Zhu L, Peppia M, Lu C, Uribarri J, Vlassara H: High levels of dietary advanced glycation end products transform low-density lipoprotein into a

- potent redox-sensitive mitogen-activated protein kinase stimulant in diabetic patients. *Circulation* 110:285–291, 2004
13. Lin RY, Reis ED, Dore AT, Lu M, Ghods N, Fallon JT, Fisher EA, Vlassara H: Lowering of dietary advanced glycation endproducts (AGE) reduces neointimal formation after arterial injury in genetically hypercholesterolemic mice. *Atherosclerosis* 163:303–311, 2002
 14. Lin RY, Choudhury RP, Cai W, Lu M, Fallon JT, Fisher EA, Vlassara H: Dietary glycotoxins promote diabetic atherosclerosis in apolipoprotein E-deficient mice. *Atherosclerosis* 168:213–220, 2003
 15. Zheng F, He C, Cai W, Hattori M, Steffes M, Vlassara H: Prevention of diabetic nephropathy in mice by a diet low in glycoxidation products. *Diabetes Metab Res Rev* 18:224–237, 2002
 16. Vlassara H: Advanced glycation in health and disease: role of the modern environment. *Ann N Y Acad Sci* 1043:452–460, 2005
 17. Kilhovd BK, Berg TJ, Birkeland KI, Thorsby P, Hanssen KF: Serum levels of advanced glycation end products are increased in patients with type 2 diabetes and coronary heart disease. *Diabetes Care* 22:1543–1548, 1999
 18. Basta G, Schmidt AM, De Caterina R: Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes. *Cardiovasc Res* 63:582–592, 2004
 19. Aronson D, Rayfield EJ: How hyperglycemia promotes atherosclerosis: molecular mechanisms. *Cardiovasc Diabetol* 1:1, 2002
 20. Al Abed Y, Mitsuhashi T, Li H, Lawson JA, FitzGerald GA, Founds H, Donnelly T, Cerami A, Ulrich P, Bucala R: Inhibition of advanced glycation endproduct formation by acetaldehyde: role in the cardioprotective effect of ethanol. *Proc Natl Acad Sci U S A* 96:2385–2390, 1999
 21. McCance DR, Dyer DG, Dunn JA, Bailie KE, Thorpe SR, Baynes JW, Lyons TJ: Maillard reaction products and their relation to complications in insulin-dependent diabetes mellitus. *J Clin Invest* 91:2470–2478, 1993
 22. Sebekova K, Faist V, Hofmann T, Schinzel R, Heidland A: Effects of a diet rich in advanced glycation end products in the rat remnant kidney model. *Am J Kidney Dis* 41 (Suppl. 1):S48–S51, 2003
 23. Sugiyama S, Miyata T, Horie K, Iida Y, Tsuyuki M, Tanaka H, Maeda K: Advanced glycation end-products in diabetic nephropathy. *Nephrol Dial Transplant* 11 (Suppl. 5):91–94, 1996
 24. Monnier VM, Sell DR, Nagaraj RH, Miyata S, Grandhee S, Odetti P, Ibrahim SA: Maillard reaction-mediated molecular damage to extracellular matrix and other tissue proteins in diabetes, aging, and uremia. *Diabetes* 41 (Suppl. 2):36–41, 1992
 25. Skolnik EY, Yang Z, Makita Z, Radoff S, Kirstein M, Vlassara H: Human and rat mesangial cell receptors for glucose-modified proteins: potential role in kidney tissue remodelling and diabetic nephropathy. *J Exp Med* 174:931–939, 1991
 26. Vlassara H, Striker LJ, Teichberg S, Fuh H, Li YM, Steffes M: Advanced glycation end products induce glomerular sclerosis and albuminuria in normal rats. *Proc Natl Acad Sci U S A* 91:11704–11708, 1994
 27. Makita Z, Bucala R, Rayfield EJ, Friedman EA, Kaufman AM, Korbet SM, Barth RH, Winston JA, Fuh H, Manogue KR: Reactive glycosylation endproducts in diabetic uraemia and treatment of renal failure. *Lancet* 343:1519–1522, 1994
 28. Berg TJ, Bangstad HJ, Torjesen PA, Osterby R, Bucala R, Hanssen KF: Advanced glycation end products in serum predict changes in the kidney morphology of patients with insulin-dependent diabetes mellitus. *Metabolism* 46:661–665, 1997
 29. Shimoiike T, Inoguchi T, Umeda F, Nawata H, Kawano K, Ochi H: The meaning of serum levels of advanced glycosylation end products in diabetic nephropathy. *Metabolism* 49:1030–1035, 2000
 30. Bucala R, Vlassara H: Advanced glycosylation end products in diabetic renal and vascular disease. *Am J Kidney Dis* 26:875–888, 1995
 31. Chen AS, Taguchi T, Sugiyama M, Wakasugi Y, Kamei A, Wang MW, Miwa I: Pyridoxal-aminoguanidine adduct is more effective than aminoguanidine in preventing neuropathy and cataract in diabetic rats. *Horm Metab Res* 36:183–187, 2004
 32. Wada R, Yagihashi S: Role of advanced glycation end products and their receptors in development of diabetic neuropathy. *Ann N Y Acad Sci* 1043:598–604, 2005
 33. Murata T, Nagai R, Ishibashi T, Inomuta H, Ikeda K, Horiuchi S: The relationship between accumulation of advanced glycation end products and expression of vascular endothelial growth factor in human diabetic retinas. *Diabetologia* 40:764–769, 1997
 34. Chibber R, Molinatti PA, Rosatto N, Lambourne B, Kohner EM: Toxic action of advanced glycation end products on cultured retinal capillary pericytes and endothelial cells: relevance to diabetic retinopathy. *Diabetologia* 40:156–164, 1997
 35. Yamagishi S, Amano S, Inagaki Y, Okamoto T, Koga K, Sasaki N, Yamamoto H, Takeuchi M, Makita Z: Advanced glycation end products-induced apoptosis and overexpression of vascular endothelial growth factor in bovine retinal pericytes. *Biochem Biophys Res Commun* 290:973–978, 2002
 36. Nakamura N, Hasegawa G, Obayashi H, Yamazaki M, Ogata M, Nakano K, Yoshikawa T, Watanabe A, Kinoshita S, Fujinami A, Ohta M, Imamura Y, Ikeda T: Increased concentration of pentosidine, an advanced glycation end product, and interleukin-6 in the vitreous of patients with proliferative diabetic retinopathy. *Diabetes Res Clin Pract* 61:93–101, 2003
 37. Boehm BO, Schilling S, Rosinger S, Lang GE, Lang GK, Kientsch-Engel R, Stahl P: Elevated serum levels of N(epsilon)-carboxymethyl-lysine, an advanced glycation end product, are associated with proliferative diabetic retinopathy and macular oedema. *Diabetologia* 47:1376–1379, 2004
 38. Fosmark DS, Torjesen PA, Kilhovd BK, Berg TJ, Sandvik L, Hanssen KF, Agardh CD, Agardh E: Increased serum levels of the specific advanced glycation end product methylglyoxal-derived hydroimidazolone are associated with retinopathy in patients with type 2 diabetes mellitus. *Metabolism* 55:232–236, 2006
 39. Forbes JM, Yee LT, Thallas V, Lassila M, Candido R, Jandeleit-Dahm KA, Thomas MC, Burns WC, Deemer EK, Thorpe SM, Cooper ME, Allen TJ: Advanced glycation end product interventions reduce diabetes-accelerated atherosclerosis. *Diabetes* 53:1813–1823, 2004
 40. Wendt T, Harja E, Bucciarelli L, Qu W, Lu Y, Rong LL, Jenkins DG, Stein G, Schmidt AM, Yan SF: RAGE modulates vascular inflammation and atherosclerosis in a murine model of type 2 diabetes. *Atherosclerosis* 185:70–77, 2005
 41. Bierhaus A, Haslbeck KM, Humpert PM, Liliensiek B, Dehmer T, Morcos M, Sayed AA, Andrassy M, Schiekofler S, Schneider JG, Schulz JB, Heuss D, Neundorfer B, Dierl S, Huber J, Tritschler H, Schmidt AM, Schwaninger M, Haering HU, Schleicher E, Kasper M, Stern DM, Arnold B, Nawroth PP: Loss of pain perception in diabetes is dependent on a receptor of the immunoglobulin superfamily. *J Clin Invest* 114:1741–1751, 2004
 42. Hammes HP, Du X, Edelstein D, Taguchi T, Matsumura T, Ju Q, Lin J, Bierhaus A, Nawroth P, Hannak D, Neumaier M, Bergfeld R, Giardino I, Brownlee M: Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. *Nat Med* 9:294–299, 2003
 43. Stracke H, Hammes HP, Werkmann D, Mavrikis K, Bitsch I, Netzel M, Geyer J, Kopcke W, Sauerland C, Bretzel RG, Federlin KF: Efficacy of benfotiamine versus thiamine on function and glycation products of peripheral nerves in di-

- abetic rats. *Exp Clin Endocrinol Diabetes* 109:330–336, 2001
44. Li F, Drel VR, Szabo C, Stevens MJ, Obrosova IG: Low-dose poly(ADP-ribose) polymerase inhibitor-containing combination therapies reverse early peripheral diabetic neuropathy. *Diabetes* 54:1514–1522, 2005
 45. John WG, Lamb EJ: The Maillard or browning reaction in diabetes. *Eye* 7:230–237, 1993
 46. Raj DS, Choudhury D, Welbourne TC, Levi M: Advanced glycation end products: a Nephrologist's perspective. *Am J Kidney Dis* 35:365–380, 2000
 47. Maillard LC: Action des acides amines sur les sucres: formation des malanodines par voie methodique. *Compte-Rendu de l'Académie des Sciences* 154:66–68, 1912
 48. Frye EB, Degenhardt TP, Thorpe SR, Baynes JW: Role of the Maillard reaction in aging of tissue proteins: advanced glycation end product-dependent increase in imidazolium cross-links in human lens proteins. *J Biol Chem* 273:18714–18719, 1998
 49. Singh R, Barden A, Mori T, Beilin L: Advanced glycation end-products: a review. *Diabetologia* 44:129–146, 2001
 50. Miyata T, Ueda Y, Yamada Y, Izuhara Y, Wada T, Jadoul M, Saito A, Kurokawa K, van Ypersele DS: Accumulation of carbonyls accelerates the formation of pentosidine, an advanced glycation end product: carbonyl stress in uremia. *J Am Soc Nephrol* 9:2349–2356, 1998
 51. Miyata T, van Ypersele dS, Kurokawa K, Baynes JW: Alterations in nonenzymatic biochemistry in uremia: origin and significance of “carbonyl stress” in long-term uremic complications. *Kidney Int* 55:389–399, 1999
 52. Anderson MM, Requena JR, Crowley JR, Thorpe SR, Heinecke JW: The myeloperoxidase system of human phagocytes generates N^ε-(carboxymethyl)lysine on proteins: a mechanism for producing advanced glycation end products at sites of inflammation. *J Clin Invest* 104:103–113, 1999
 53. Anderson MM, Heinecke JW: Production of N^ε-(carboxymethyl)lysine is impaired in mice deficient in NADPH oxidase: a role for phagocyte-derived oxidants in the formation of advanced glycation end products during inflammation. *Diabetes* 52:2137–2143, 2003
 54. Ramasamy R, Yan SF, Schmidt AM: The RAGE axis and endothelial dysfunction: maladaptive roles in the diabetic vasculature and beyond. *Trends Cardiovasc Med* 15:237–243, 2005
 55. Wautier MP, Chappey O, Corda S, Stern DM, Schmidt AM, Wautier JL: Activation of NADPH oxidase by AGE links oxidant stress to altered gene expression via RAGE. *Am J Physiol Endocrinol Metab* 280:E685–E694, 2001
 56. Wang H, Vishnubhakat JM, Bloom O, Zhang M, Umbrellino M, Sama A, Tracey KJ: Proinflammatory cytokines (tumor necrosis factor and interleukin 1) stimulate release of high mobility group protein-1 by pituitary cells. *Surgery* 126:389–392, 1999
 57. Gardella S, Andrei C, Ferrera D, Lotti LV, Torrisi MR, Bianchi ME, Rubartelli A: The nuclear protein HMGB1 is secreted by monocytes via a non-classical, vesicle-mediated secretory pathway. *EMBO Rep* 3:995–1001, 2002
 58. Dumitriu IE, Baruah P, Valentinis B, Voll RE, Herrmann M, Nawroth PP, Arnold B, Bianchi ME, Manfredi AA, Rovere-Querini P: Release of high mobility group box 1 by dendritic cells controls T cell activation via the receptor for advanced glycation end products. *J Immunol* 174:7506–7515, 2005
 59. Huttunen HJ, Fages C, Rauvala H: Receptor for advanced glycation end products (RAGE)-mediated neurite outgrowth and activation of NF-kappaB require the cytoplasmic domain of the receptor but different downstream signaling pathways. *J Biol Chem* 274:19919–19924, 1999
 60. Taguchi A, Blood DC, del Toro G, Canet A, Lee DC, Qu W, Tanji N, Lu Y, Lalla E, Fu C, Hofmann MA, Kislinger T, Ingram M, Lu A, Tanaka H, Hori O, Ogawa S, Stern DM, Schmidt AM: Blockade of RAGE-amphoterin signalling suppresses tumour growth and metastases. *Nature* 405:354–360, 2000
 61. Hori O, Brett J, Slattery T, Cao R, Zhang J, Chen JX, Nagashima M, Lundh ER, Vijay S, Nitecki D: The receptor for advanced glycation end products (RAGE) is a cellular binding site for amphotericin: mediation of neurite outgrowth and co-expression of rage and amphotericin in the developing nervous system. *J Biol Chem* 270:25752–25761, 1995
 62. Kaneko M, Bucciarelli L, Hwang YC, Lee L, Yan SF, Schmidt AM, Ramasamy R: Aldose reductase and AGE-RAGE pathways: key players in myocardial ischemic injury. *Ann NY Acad Sci* 1043:702–709, 2005
 63. Wautier JL, Schmidt AM: Protein glycation: a firm link to endothelial cell dysfunction. *Circ Res* 95:233–238, 2004
 64. Peppas M, Uribarri J, Vlassara H: Advanced glycoxidation: a new risk factor for cardiovascular disease? *Cardiovasc Toxicol* 2:275–287, 2002
 65. Peppas M, Uribarri J, Vlassara H: The role of advanced glycation end products in the development of atherosclerosis. *Curr Diab Rep* 4:31–36, 2004
 66. Eble AS, Thorpe SR, Baynes JW: Nonenzymatic glycosylation and glucose-dependent cross-linking of protein. *J Biol Chem* 258:9406–9412, 1983
 67. Vlassara H: The AGE: E-receptor in the pathogenesis of diabetic complications. *Diabetes Metab Res Rev* 17:436–443, 2001
 68. Tan KC, Chow WS, Tam S, Bucala R, Betteridge J: Association between acute-phase reactants and advanced glycation end products in type 2 diabetes. *Diabetes Care* 27:223–228, 2004
 69. Berg TJ, Clausen JT, Torjesen PA, Dahl-Jorgensen K, Bangstad HJ, Hanssen KF: The advanced glycation end product N^ε-(carboxymethyl)lysine is increased in serum from children and adolescents with type 1 diabetes. *Diabetes Care* 21:1997–2002, 1998
 70. Sharp PS, Rainbow S, Mukherjee S: Serum levels of low molecular weight advanced glycation end products in diabetic subjects. *Diabet Med* 20:575–579, 2003
 71. Thomas MC, Tsalamandris C, MacIsaac R, Medley T, Kingwell B, Cooper ME, Jerums G: Low-molecular-weight AGEs are associated with GFR and anemia in patients with type 2 diabetes. *Kidney Int* 66:1167–1172, 2004
 72. Papanastasiou P, Grass L, Rodela H, Patrikarea A, Oreopoulos D, Diamandis EP: Immunological quantification of advanced glycosylation end-products in the serum of patients on hemodialysis or CAPD. *Kidney Int* 46:216–222, 1994
 73. Makita Z, Radoff S, Rayfield EJ, Yang Z, Skolnik E, Delaney V, Friedman EA, Cerami A, Vlassara H: Advanced glycosylation end products in patients with diabetic nephropathy. *N Engl J Med* 325:836–842, 1991
 74. Vlassara H, Cai W, Crandall J, Goldberg T, Oberstein R, Dardaine V, Peppas M, Rayfield EJ: Inflammatory mediators are induced by dietary glycotoxins, a major risk factor for diabetic angiopathy. *Proc Natl Acad Sci U S A* 99:15596–15601, 2002
 75. Forster A, Kuhne Y, Henle T: Studies on absorption and elimination of dietary maillard reaction products. *Ann N Y Acad Sci* 1043:474–481, 2005
 76. Henle T: AGEs in foods: do they play a role in uremia? *Kidney Int Suppl* 63: S145–S147, 2003
 77. Fu MX, Requena JR, Jenkins AJ, Lyons TJ, Baynes JW, Thorpe SR: The advanced glycation end product, N^ε-(carboxymethyl)lysine, is a product of both lipid peroxidation and glycoxidation reactions. *J Biol Chem* 271:9982–9986, 1996
 78. Bucala R, Makita Z, Koschinsky T, Cerami A, Vlassara H: Lipid advanced glycosylation: pathway for lipid oxidation in vivo. *Proc Natl Acad Sci U S A* 90:6434–6438, 1993
 79. Miyata T, Ishikawa N, van Ypersele dS: Carbonyl stress and diabetic complications. *Clin Chem Lab Med* 41:1150–1158, 2003
 80. Wolff SP, Dean RT: Glucose autoxidation

- tion and protein modification: the potential role of 'autoxidative glycosylation' in diabetes. *Biochem J* 245:243–250, 1987
81. Finot PA: Historical perspective of the Maillard reaction in food science. *Ann N Y Acad Sci* 1043:1–8, 2005
 82. Peppas M, Brem H, Ehrlich P, Zhang JG, Cai W, Li Z, Croitoru A, Thung S, Vlassara H: Adverse effects of dietary glycotoxins on wound healing in genetically diabetic mice. *Diabetes* 52:2805–2813, 2003
 83. Hofmann SM, Dong HJ, Li Z, Cai W, Altomonte J, Thung SN, Zeng F, Fisher EA, Vlassara H: Improved insulin sensitivity is associated with restricted intake of dietary glycoxidation products in the db/db mouse. *Diabetes* 51:2082–2089, 2002
 84. Peppas M, He C, Hattori M, McEvoy R, Zheng F, Vlassara H: Fetal or neonatal low-glycotoxin environment prevents autoimmune diabetes in NOD mice. *Diabetes* 52:1441–1448, 2003
 85. Uribarri J, Peppas M, Cai W, Goldberg T, Lu M, He C, Vlassara H: Restriction of dietary glycotoxins reduces excessive advanced glycation end products in renal failure patients. *J Am Soc Nephrol* 14:728–731, 2003
 86. Stirban A, Sander D, Buenting CE: Food advanced glycation endproducts (AGE) acutely impair endothelial function in patients with diabetes mellitus (Abstract). *Diabetes* 52 (Suppl. 1):A19, 2003
 87. Lu C, He JC, Cai W, Liu H, Zhu L, Vlassara H: Advanced glycation endproduct (AGE) receptor 1 is a negative regulator of the inflammatory response to AGE in mesangial cells. *Proc Natl Acad Sci U S A* 101:11767–11772, 2004
 88. Li YM, Mitsuhashi T, Wojciechowicz D, Shimizu N, Li J, Stitt A, He C, Banerjee D, Vlassara H: Molecular identity and cellular distribution of advanced glycation endproduct receptors: relationship of p60 to OST-48 and p90 to 80K-H membrane proteins. *Proc Natl Acad Sci U S A* 93:11047–11052, 1996
 89. He CJ, Zheng F, Stitt A, Striker L, Hattori M, Vlassara H: Differential expression of renal AGE-receptor genes in NOD mice: possible role in nonobese diabetic renal disease. *Kidney Int* 58:1931–1940, 2000
 90. He CJ, Koschinsky T, Buenting C, Vlassara H: Presence of diabetic complications in type 1 diabetic patients correlates with low expression of mononuclear cell AGE-receptor-1 and elevated serum AGE. *Mol Med* 7:159–168, 2001
 91. Li YM, Tan AX, Vlassara H: Antibacterial activity of lysozyme and lactoferrin is inhibited by binding of advanced glycation-modified proteins to a conserved motif. *Nat Med* 1:1057–1061, 1995
 92. Mitsuhashi T, Li YM, Fishbane S, Vlassara H: Depletion of reactive advanced glycation endproducts from diabetic uremic sera using a lysozyme-linked matrix. *J Clin Invest* 100:847–854, 1997
 93. Cai W, He JC, Zhu L, Lu C, Vlassara H: AGE-receptor-1 suppresses cell reactive oxygen species and inhibits activation induced by AGE via the EGFR signaling pathway. *Proc Natl Acad Sci U S A*. In press
 94. Zheng F, Cai W, Mitsuhashi T, Vlassara H: Lysozyme enhances renal excretion of advanced glycation endproducts in vivo and suppresses adverse age-mediated cellular effects in vitro: a potential AGE sequestration therapy for diabetic nephropathy? *Mol Med* 7:737–747, 2001
 95. Iacobini C, Menini S, Oddi G, Ricci C, Amadio L, Pricci F, Olivieri A, Sorcini M, Di Mario U, Pesce C, Pugliese G: Galectin-3/AGE-receptor 3 knockout mice show accelerated AGE-induced glomerular injury: evidence for a protective role of galectin-3 as an AGE receptor. *FASEB J* 18:1773–1775, 2004
 96. Miyazaki A, Nakayama H, Horiuchi S: Scavenger receptors that recognize advanced glycation end products. *Trends Cardiovasc Med* 12:258–262, 2002
 97. Ohgami N, Nagai R, Miyazaki A, Ikegami M, Arai H, Horiuchi S, Nakayama H: Scavenger receptor class B type I-mediated reverse cholesterol transport is inhibited by advanced glycation end products. *J Biol Chem* 276:13348–13355, 2001
 98. Schmidt AM, Vianna M, Gerlach M, Brett J, Ryan J, Kao J, Esposito C, Hegarty H, Hurler W, Clauss M: Isolation and characterization of two binding proteins for advanced glycosylation end products from bovine lung which are present on the endothelial cell surface. *J Biol Chem* 267:14987–14997, 1992
 99. Neeper M, Schmidt AM, Brett J, Yan SD, Wang F, Pan YC, Elliston K, Stern D, Shaw A: Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins. *J Biol Chem* 267:14998–15004, 1992
 100. Yan SF, Ramasamy R, Naka Y, Schmidt AM: Glycation, inflammation, and RAGE: a scaffold for the macrovascular complications of diabetes and beyond. *Circ Res* 93:1159–1169, 2003
 101. Hofmann MA, Drury S, Fu C, Qu W, Taguchi A, Lu Y, Avila C, Kambham N, Bierhaus A, Nawroth P, Neurath MF, Slatery T, Beach D, McClary J, Nagashima M, Morser J, Stern D, Schmidt AM: RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. *Cell* 97:889–901, 1999
 102. Basta G, Lazzarini G, Del Turco S, Ratto GM, Schmidt AM, De Caterina R: At least 2 distinct pathways generating reactive oxygen species mediate vascular cell adhesion molecule-1 induction by advanced glycation end products. *Arterioscler Thromb Vasc Biol* 25:1401–1407, 2005
 103. Bierhaus A, Chevion S, Chevion M, Hofmann M, Quehenberger P, Illmer T, Luther T, Berentshtein E, Tritschler H, Muller M, Wahl P, Ziegler R, Nawroth PP: Advanced glycation end product-induced activation of NF- κ B is suppressed by α -lipoic acid in cultured endothelial cells. *Diabetes* 46:1481–1490, 1997
 104. Neumann A, Schinzel R, Palm D, Riederer P, Munch G: High molecular weight hyaluronic acid inhibits advanced glycation endproduct-induced NF-kappaB activation and cytokine expression. *FEBS Lett* 453:283–287, 1999
 105. Basta G, Lazzarini G, Massaro M, Simioncini T, Tanganelli P, Fu C, Kislinger T, Stern DM, Schmidt AM, De Caterina R: Advanced glycation end products activate endothelium through signal-transduction receptor RAGE: a mechanism for amplification of inflammatory responses. *Circulation* 105:816–822, 2002
 106. Rojas A, Morales MA: Advanced glycation and endothelial functions: a link towards vascular complications in diabetes. *Life Sci* 76:715–730, 2004
 107. Chappey O, Dosquet C, Wautier MP, Wautier JL: Advanced glycation end products, oxidant stress and vascular lesions. *Eur J Clin Invest* 27:97–108, 1997
 108. Zhou Z, Wang K, Penn MS, Marso SP, Lauer MA, Forudi F, Zhou X, Qu W, Lu Y, Stern DM, Schmidt AM, Lincoff AM, Topol EJ: Receptor for AGE (RAGE) mediates neointimal formation in response to arterial injury. *Circulation* 107:2238–2243, 2003
 109. Schmidt AM, Stern D: Atherosclerosis and diabetes: the RAGE connection. *Curr Atheroscler Rep* 2:430–436, 2000
 110. Rosen P, Nawroth PP, King G, Moller W, Tritschler HJ, Packer L: The role of oxidative stress in the onset and progression of diabetes and its complications: a summary of a Congress Series sponsored by UNESCO-MCBN, the American Diabetes Association and the German Diabetes Society. *Diabetes Metab Res Rev* 17:189–212, 2001
 111. Zeiher AM, Fisslthaler B, Schray-Utz B, Busse R: Nitric oxide modulates the expression of monocyte chemoattractant protein 1 in cultured human endothelial cells. *Circ Res* 76:980–986, 1995
 112. Quehenberger P, Bierhaus A, Fasching P, Muellner C, Klevesath M, Hong M, Stier G, Sattler M, Schleicher E, Speiser W, Nawroth PP: Endothelin 1 transcription is controlled by nuclear factor- κ B in AGE-stimulated cultured endothelial cells. *Diabetes* 49:1561–1570, 2000
 113. Williams SB, Cusco JA, Roddy MA, Johnstone MT, Creager MA: Impaired nitric oxide-mediated vasodilation in patients with non-insulin-dependent di-

- abetes mellitus. *J Am Coll Cardiol* 27: 567–574, 1996
114. Johnstone MT, Creager SJ, Scales KM, Cusco JA, Lee BK, Creager MA: Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation* 88:2510–2516, 1993
 115. Milstein S, Guttenplan JB: Near quantitative production of molecular nitrogen from metabolism of dimethylnitrosamine. *Biochem Biophys Res Commun* 87: 337–342, 1979
 116. Inoguchi T, Li P, Umeda F, Yu HY, Kakimoto M, Imamura M, Aoki T, Etoh T, Hashimoto T, Naruse M, Sano H, Utsumi H, Nawata H: High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C–dependent activation of NAD(P)H oxidase in cultured vascular cells. *Diabetes* 49:1939–1945, 2000
 117. Hopfner RL, Gopalakrishnan V: Endothelin: emerging role in diabetic vascular complications. *Diabetologia* 42:1383–1394, 1999
 118. Achmad TH, Winterscheidt A, Lindemann C, Rao GS: Oxidized low density lipoprotein acts on endothelial cells in culture to enhance endothelin secretion and monocyte migration. *Methods Find Exp Clin Pharmacol* 19:153–159, 1997
 119. Xie H, Bevan JA: Oxidized low-density lipoprotein enhances myogenic tone in the rabbit posterior cerebral artery through the release of endothelin-1. *Stroke* 30:2423–2429, 1999
 120. Christlieb AR, Janka HU, Kraus B, Gleason RE, Icasas-Cabral EA, Aiello LM, Cabral BV, Solano A: Vascular reactivity to angiotensin II and to norepinephrine in diabetic subjects. *Diabetes* 25:268–274, 1976
 121. Bucala R, Tracey KJ, Cerami A: Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilatation in experimental diabetes. *J Clin Invest* 87: 432–438, 1991
 122. Brownlee M, Vlassara H, Kooney A, Ulrich P, Cerami A: Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking. *Science* 232: 1629–1632, 1986
 123. Brownlee M, Vlassara H, Cerami A: Non-enzymatic glycosylation products on collagen covalently trap low-density lipoprotein. *Diabetes* 34:938–941, 1985
 124. Bucala R, Makita Z, Vega G, Grundy S, Koschinsky T, Cerami A, Vlassara H: Modification of low density lipoprotein by advanced glycation end products contributes to the dyslipidemia of diabetes and renal insufficiency. *Proc Natl Acad Sci U S A* 91:9441–9445, 1994
 125. Steinbrecher UP, Witztum JL: Glycosylation of low-density lipoproteins to an extent comparable to that seen in diabetes slows their catabolism. *Diabetes* 33: 130–134, 1984
 126. Klein RL, Laimins M, Lopes-Virella MF: Isolation, characterization, and metabolism of the glycosylated and nonglycosylated subfractions of low-density lipoproteins isolated from type I diabetic patients and nondiabetic subjects. *Diabetes* 44:1093–1098, 1995
 127. Sobenin IA, Tertov VV, Koschinsky T, Bunting CE, Slavina ES, Dedov II, Orekhov AN: Modified low density lipoprotein from diabetic patients causes cholesterol accumulation in human intimal aortic cells. *Atherosclerosis* 100:41–54, 1993
 128. Ballinger ML, Thomas MC, Nigro J, Ivey ME, Dilley RJ, Little PJ: Glycosylated and carboxy-methylated proteins do not directly activate human vascular smooth muscle cells. *Kidney Int* 68:2756–2765, 2005
 129. Berg TJ, Snorgaard O, Faber J, Torjesen PA, Hildebrandt P, Mehlsen J, Hanssen KF: Serum levels of advanced glycation end products are associated with left ventricular diastolic function in patients with type 1 diabetes. *Diabetes Care* 22: 1186–1190, 1999
 130. Oxlund H, Rasmussen LM, Andreassen TT, Heickendorff L: Increased aortic stiffness in patients with type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 32:748–752, 1989
 131. Megnien JL, Simon A, Valensi P, Flaud P, Merli I, Levenson J: Comparative effects of diabetes mellitus and hypertension on physical properties of human large arteries. *J Am Coll Cardiol* 20:1562–1568, 1992
 132. Airaksinen KE, Salmela PI, Linnaluoto MK, Ikaheimo MJ, Ahola K, Ryhanen LJ: Diminished arterial elasticity in diabetes: association with fluorescent advanced glycosylation end products in collagen. *Cardiovasc Res* 27:942–945, 1993
 133. Salomaa V, Riley W, Kark JD, Nardo C, Folsom AR: Non-insulin-dependent diabetes mellitus and fasting glucose and insulin concentrations are associated with arterial stiffness indexes: the ARIC Study: Atherosclerosis Risk in Communities Study. *Circulation* 91:1432–1443, 1995
 134. Hu J, Wallensten M, Gennser G: Increased stiffness of the aorta in children and adolescents with insulin-dependent diabetes mellitus. *Ultrasound Med Biol* 22:537–543, 1996
 135. Berry KL, Skyrme-Jones RA, Cameron JD, O'Brien RC, Meredith IT: Systemic arterial compliance is reduced in young patients with IDDM. *Am J Physiol* 276: H1839–H1845, 1999
 136. Taniwaki H, Kawagishi T, Emoto M, Shoji T, Kanda H, Maekawa K, Nishizawa Y, Morii H: Correlation between the intima-media thickness of the carotid artery and aortic pulse-wave velocity in patients with type 2 diabetes: vessel wall properties in type 2 diabetes. *Diabetes Care* 22:1851–1857, 1999
 137. Giannattasio C, Failla M, Piperno A, Grappiolo A, Gamba P, Paleari F, Mancina G: Early impairment of large artery structure and function in type 1 diabetes mellitus. *Diabetologia* 42:987–994, 1999
 138. Romney JS, Lewanczuk RZ: Vascular compliance is reduced in the early stages of type 1 diabetes. *Diabetes Care* 24: 2102–2106, 2001
 139. Schram MT, Henry RM, van Dijk RA, Kostense PJ, Dekker JM, Nijpels G, Heine RJ, Bouter LM, Westerhof N, Stehouwer CD: Increased central artery stiffness in impaired glucose metabolism and type 2 diabetes: the Hoorn Study. *Hypertension* 43:176–181, 2004
 140. van Popele NM, Westendorp IC, Bots ML, Reneman RS, Hoeks AP, Hofman A, Grobbee DE, Witteman JC: Variables of the insulin resistance syndrome are associated with reduced arterial distensibility in healthy non-diabetic middle-aged women. *Diabetologia* 43:665–672, 2000
 141. Scuteri A, Najjar SS, Muller DC, Andres R, Hougaku H, Metter EJ, Lakatta EG: Metabolic syndrome amplifies the age-associated increases in vascular thickness and stiffness. *J Am Coll Cardiol* 43: 1388–1395, 2004
 142. Reddy GK: AGE-related cross-linking of collagen is associated with aortic wall matrix stiffness in the pathogenesis of drug-induced diabetes in rats. *Microvasc Res* 68:132–142, 2004
 143. Jadhav UM, Kadam NN: Non-invasive assessment of arterial stiffness by pulse-wave velocity correlates with endothelial dysfunction. *Indian Heart J* 57:226–232, 2005
 144. Ziemann SJ, Melenovsky V, Kass DA: Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler Thromb Vasc Biol* 25:932–943, 2005
 145. Huijberts MS, Wolffenbuttel BH, Boudier HA, Crijns FR, Kruseman AC, Poitevin P, Levy BI: Aminoguanidine treatment increases elasticity and decreases fluid filtration of large arteries from diabetic rats. *J Clin Invest* 92:1407–1411, 1993
 146. Wolffenbuttel BH, Boulanger CM, Crijns FR, Huijberts MS, Poitevin P, Swennen GN, Vasani S, Egan JJ, Ulrich P, Cerami A, Levy BI: Breakers of advanced glycation end products restore large artery properties in experimental diabetes. *Proc Natl Acad Sci U S A* 95:4630–4634, 1998
 147. Souliis T, Cooper ME, Sastra S, Thallas V, Panagiotopoulos S, Bjerrum OJ, Jerums G: Relative contributions of advanced glycation and nitric oxide synthase

- inhibition to aminoguanidine-mediated renoprotection in diabetic rats. *Diabetologia* 40:1141–1151, 1997
148. Soulis-Liparota T, Cooper M, Papazoglou D, Clarke B, Jerums G: Retardation by aminoguanidine of development of albuminuria, mesangial expansion, and tissue fluorescence in streptozocin-induced diabetic rat. *Diabetes* 40:1328–1334, 1991
 149. Hammes HP, Brownlee M, Edelstein D, Saleck M, Martin S, Federlin K: Aminoguanidine inhibits the development of accelerated diabetic retinopathy in the spontaneous hypertensive rat. *Diabetologia* 37:32–35, 1994
 150. Hammes HP, Strodtter D, Weiss A, Bretzel RG, Federlin K, Brownlee M: Secondary intervention with aminoguanidine retards the progression of diabetic retinopathy in the rat model. *Diabetologia* 38:656–660, 1995
 151. Bolton WK, Cattran DC, Williams ME, Adler SG, Appel GB, Cartwright K, Foiles PG, Freedman BI, Raskin P, Ratner RE, Spinowitz BS, Whittier FC, Wuerth JP: Randomized trial of an inhibitor of formation of advanced glycation end products in diabetic nephropathy. *Am J Nephrol* 24:32–40, 2004
 152. Jakus V, Rietbrock N: Advanced glycation end-products and the progress of diabetic vascular complications. *Physiol Res* 53:131–142, 2004
 153. Degenhardt TP, Alderson NL, Arrington DD, Beattie RJ, Basgen JM, Steffes MW, Thorpe SR, Baynes JW: Pyridoxamine inhibits early renal disease and dyslipidemia in the streptozotocin-diabetic rat. *Kidney Int* 61:939–950, 2002
 154. Baliga BS, Reynolds T, Fink LM, Fonseca VA: Hyperhomocysteinemia in type 2 diabetes mellitus: cardiovascular risk factors and effect of treatment with folic acid and pyridoxine. *Endocr Pract* 6:435–441, 2000
 155. Miyata T, Ishikawa S, Asahi K, Inagi R, Suzuki D, Horie K, Tatsumi K, Kurokawa K: 2-Isopropylidenehydrazono-4-oxo-thiazolidin-5-ylacetanilide (OPB-9195) treatment inhibits the development of intimal thickening after balloon injury of rat carotid artery: role of glycoxidation and lipoxidation reactions in vascular tissue damage. *FEBS Lett* 445:202–206, 1999
 156. Mizutani K, Ikeda K, Tsuda K, Yamori Y: Inhibitor for advanced glycation end products formation attenuates hypertension and oxidative damage in genetic hypertensive rats. *J Hypertens* 20:1607–1614, 2002
 157. Miyata T, van Ypersele dS, Ueda Y, Ichimori K, Inagi R, Onogi H, Ishikawa N, Nangaku M, Kurokawa K: Angiotensin II receptor antagonists and angiotensin-converting enzyme inhibitors lower in vitro the formation of advanced glycation end products: biochemical mechanisms. *J Am Soc Nephrol* 13:2478–2487, 2002
 158. Wilkinson-Berka JL, Kelly DJ, Koerner SM, Jaworski K, Davis B, Thallas V, Cooper ME: ALT-946 and aminoguanidine, inhibitors of advanced glycation, improve severe nephropathy in the diabetic transgenic (mREN-2)27 rat. *Diabetes* 51:3283–3289, 2002
 159. Forbes JM, Soulis T, Thallas V, Panagiotopoulos S, Long DM, Vasas S, Wagle D, Jerums G, Cooper ME: Renoprotective effects of a novel inhibitor of advanced glycation. *Diabetologia* 44:108–114, 2001
 160. Vasas S, Foiles P, Founds H: Therapeutic potential of breakers of advanced glycation end product-protein crosslinks. *Arch Biochem Biophys* 419:89–96, 2003
 161. Kass DA, Shapiro EP, Kawaguchi M, Capriotti AR, Scuteri A, deGroof RC, Lakatta EG: Improved arterial compliance by a novel advanced glycation end-product crosslink breaker. *Circulation* 104:1464–1470, 2001
 162. Little WC, Zile MR, Kitzman DW, Huddle WG, O'Brien TX, deGroof RC: The effect of alagebrium chloride (ALT-711), a novel glucose cross-link breaker, in the treatment of elderly patients with diastolic heart failure. *J Card Fail* 11:191–195, 2005
 163. Forbes JM, Thallas V, Thomas MC, Founds HW, Burns WC, Jerums G, Cooper ME: The breakdown of preexisting advanced glycation end products is associated with reduced renal fibrosis in experimental diabetes. *FASEB J* 17:1762–1764, 2003
 164. Candido R, Forbes JM, Thomas MC, Thallas V, Dean RG, Burns WC, Tikellis C, Ritchie RH, Twigg SM, Cooper ME, Burrell LM: A breaker of advanced glycation end products attenuates diabetes-induced myocardial structural changes. *Circ Res* 92:785–792, 2003
 165. Cooper ME, Thallas V, Forbes J, Scalbert E, Sastra S, Darby I, Soulis T: The cross-link breaker, N-phenacylthiazolium bromide prevents vascular advanced glycation end-product accumulation. *Diabetologia* 43:660–664, 2000
 166. Schwedler SB, Verbeke P, Bakala H, Weiss MF, Vilar J, Depreux P, Fourmaintraux E, Striker LJ, Striker GE: N-phenacylthiazolium bromide decreases renal and increases urinary advanced glycation end products excretion without ameliorating diabetic nephropathy in C57BL/6 mice. *Diabetes Obes Metab* 3:230–239, 2001
 167. Oturai PS, Christensen M, Rolin B, Pedersen KE, Mortensen SB, Boel E: Effects of advanced glycation end-product inhibition and cross-link breakage in diabetic rats. *Metabolism* 49:996–1000, 2000
 168. Sakaguchi T, Yan SF, Yan SD, Belov D, Rong LL, Sousa M, Andrassy M, Marso SP, Duda S, Arnold B, Liliensiek B, Nawroth PP, Stern DM, Schmidt AM, Naka Y: Central role of RAGE-dependent neointimal expansion in arterial restenosis. *J Clin Invest* 111:959–972, 2003
 169. Liu H, Zheng F, Cao Q, Ren B, Zhu L, Striker G, Vlassara H: Amelioration of oxidant stress by the defensin lysozyme. *Am J Physiol Endocrinol Metab* 290:E824–E832, 2006
 170. Liu H, Zheng F, Zhu L, Uribarri J, Tunstead JR, Ren B, Badimon J, Striker GE, Vlassara H: The immune defense protein lysozyme ameliorates acute vascular injury and atherosclerosis in hyperlipidemic mice. *Am J Pathol*, 2005. In press
 171. Loew D: Pharmacokinetics of thiamine derivatives especially of benfotiamine. *Int J Clin Pharmacol Ther* 34:47–50, 1996
 172. Babaei-Jadidi R, Karachalias N, Ahmed N, Battah S, Thornalley PJ: Prevention of incipient diabetic nephropathy by high-dose thiamine and benfotiamine. *Diabetes* 52:2110–2120, 2003
 173. La Selva M, Beltramo E, Pagnozzi F, Bena E, Molinatti PA, Molinatti GM, Porta M: Thiamine corrects delayed replication and decreases production of lactate and advanced glycation end-products in bovine retinal and human umbilical vein endothelial cells cultured under high glucose conditions. *Diabetologia* 39:1263–1268, 1996
 174. Winkler G, Pal B, Nagybeganyi E, Ory I, Porochnavec M, Kempler P: Effectiveness of different benfotiamine dosage regimens in the treatment of painful diabetic neuropathy. *Arzneimittelforschung* 49:220–224, 1999
 175. Haupt E, Ledermann H, Kopcke W: Benfotiamine in the treatment of diabetic polyneuropathy: a three-week randomized, controlled pilot study (BEDIP study). *Int J Clin Pharmacol Ther* 43:71–77, 2005
 176. Du X, Matsumura T, Edelstein D, Rossetti L, Zsengeller Z, Szabo C, Brownlee M: Inhibition of GAPDH activity by poly(ADP-ribose) polymerase activates three major pathways of hyperglycemic damage in endothelial cells. *J Clin Invest* 112:1049–1057, 2003
 177. Pacher P, Liaudet L, Soriano FG, Mabley JG, Szabo E, Szabo C: The role of poly(ADP-ribose) polymerase activation in the development of myocardial and endothelial dysfunction in diabetes. *Diabetes* 51:514–521, 2002
 178. Soriano FG, Pacher P, Mabley J, Liaudet L, Szabo C: Rapid reversal of the diabetic endothelial dysfunction by pharmacological inhibition of poly(ADP-ribose) polymerase. *Circ Res* 89:684–691, 2001
 179. Hamada Y, Nakamura J, Naruse K, Komori T, Kato K, Kasuya Y, Nagai R,

- Horiuchi S, Hotta N: Epalrestat, an aldose reductase inhibitor, reduces the levels of Nε-(carboxymethyl)lysine protein adducts and their precursors in erythrocytes from diabetic patients. *Diabetes Care* 23:1539–1544, 2000
180. Nakamura N, Yamazaki K, Satoh A, Urakaze M, Kobayashi M, Yamabe H, Osawa H, Shirato K, Sugawara T, Nakamura M, Tamura M, Okumura K: Effects of eparlestat on plasma levels of advanced glycation end products in patients with type 2 diabetes. *In Vivo* 17:177–180, 2003
181. Suarez G, Rajaram R, Bhuyan KC, Oronsky AL, Goidl JA: Administration of an aldose reductase inhibitor induces a decrease of collagen fluorescence in diabetic rats. *J Clin Invest* 82:624–627, 1988
182. Brown MJ, Bird SJ, Watling S, Kaleta H, Hayes L, Eckert S, Foyt HL: Natural progression of diabetic peripheral neuropathy in the Zenarestat study population. *Diabetes Care* 27:1153–1159, 2004
183. Kinekawa F, Kubo F, Matsuda K, Fujita Y, Kobayashi M, Funakoshi F, Uchida N, Watanabe S, Tomita T, Uchida Y, Kuriyama S: Effect of an aldose reductase inhibitor on esophageal dysfunction in diabetic patients. *Hepato gastroenterology* 52:471–474, 2005
184. Okamoto H, Nomura M, Nakaya Y, Uehara K, Saito K, Kimura M, Chikamori K, Ito S: Effects of epalrestat, an aldose reductase inhibitor, on diabetic neuropathy and gastroparesis. *Intern Med* 42:655–664, 2003
185. Dan Q, Wong R, Chung SK, Chung SS, Lam KS: Interaction between the polyol pathway and non-enzymatic glycation on aortic smooth muscle cell migration and monocyte adhesion. *Life Sci* 76:445–459, 2004
186. Figarola JL, Scott S, Loera S, Tessler C, Chu P, Weiss L, Hardy J, Rahbar S: LR-90 a new advanced glycation end-product inhibitor prevents progression of diabetic nephropathy in streptozotocin-diabetic rats. *Diabetologia* 46:1140–1152, 2003
187. Rahbar S, Figarola JL: Novel inhibitors of advanced glycation endproducts. *Arch Biochem Biophys* 419:63–79, 2003
188. Rahbar S, Natarajan R, Yerneni K, Scott S, Gonzales N, Nadler JL: Evidence that pioglitazone, metformin and pentoxifylline are inhibitors of glycation. *Clin Chim Acta* 301:65–77, 2000
189. Cipollone F, Fazia M, Iezzi A, Pini B, Cuccurullo C, Zucchelli M, De Cesare D, Uchino S, Spigonardo F, De Luca M, Muraro R, Bei R, Bucci M, Cuccurullo F, Mezzetti A: Blockade of the angiotensin II type 1 receptor stabilizes atherosclerotic plaques in humans by inhibiting prostaglandin E2-dependent matrix metalloproteinase activity. *Circulation* 109:1482–1488, 2004
190. Forbes JM, Thorpe SR, Thallas-Bonke V, Pete J, Thomas MC, Deemer ER, Bassal S, El Osta A, Long DM, Panagiotopoulos S, Jerums G, Osicka TM, Cooper ME: Modulation of soluble receptor for advanced glycation end products by angiotensin-converting enzyme-1 inhibition in diabetic nephropathy. *J Am Soc Nephrol* 16:2363–2372, 2005
191. Candido R, Jandeleit-Dahm KA, Cao Z, Nesteroff SP, Burns WC, Twigg SM, Dillely RJ, Cooper ME, Allen TJ: Prevention of accelerated atherosclerosis by angiotensin-converting enzyme inhibition in diabetic apolipoprotein E-deficient mice. *Circulation* 106:246–253, 2002
192. Forbes JM, Cooper ME, Thallas V, Burns WC, Thomas MC, Brammar GC, Lee F, Grant SL, Burrell LA, Jerums G, Osicka TM: Reduction of the accumulation of advanced glycation end products by ACE inhibition in experimental diabetic nephropathy. *Diabetes* 51:3274–3282, 2002
193. Ziegler D, Hanefeld M, Ruhnau KJ, Meissner HP, Lobisch M, Schutte K, Gries FA: Treatment of symptomatic diabetic peripheral neuropathy with the anti-oxidant alpha-lipoic acid: a 3-week multicentre randomized controlled trial (ALADIN Study). *Diabetologia* 38:1425–1433, 1995
194. Ziegler D, Hanefeld M, Ruhnau KJ, Hasche H, Lobisch M, Schutte K, Kerum G, Malessa R: Treatment of symptomatic diabetic polyneuropathy with the antioxidant α-lipoic acid: a 7-month multicenter randomized controlled trial (ALADIN III Study): ALADIN III Study Group: Alpha-Lipoic Acid in Diabetic Neuropathy. *Diabetes Care* 22:1296–1301, 1999
195. Mustata GT, Rosca M, Biemel KM, Reihl O, Smith MA, Viswanathan A, Strauch C, Du Y, Tang J, Kern TS, Lederer MO, Brownlee M, Weiss MF, Monnier VM: Paradoxical effects of green tea (*Camellia sinensis*) and antioxidant vitamins in diabetic rats: improved retinopathy and renal mitochondrial defects but deterioration of collagen matrix glycoxylation and cross-linking. *Diabetes* 54:517–526, 2005
196. Culbertson SM, Vassilenko EI, Morrison LD, Ingold KU: Paradoxical impact of antioxidants on post-Amadori glycoxylation: counterintuitive increase in the yields of pentosidine and Nε-pentoxymethyllysine using a novel multifunctional pyridoxamine derivative. *J Biol Chem* 278:38384–38394, 2003
197. Hamada Y, Nakashima E, Naruse K, Nakae M, Naiki M, Fujisawa H, Oiso Y, Hotta N, Nakamura J: A copper chelating agent suppresses carbonyl stress in diabetic rat lenses. *J Diabetes Complications* 19:328–334, 2005
198. Cameron NE, Cotter MA: Neurovascular dysfunction in diabetic rats: potential contribution of autoxidation and free radicals examined using transition metal chelating agents. *J Clin Invest* 96:1159–1163, 1995
199. Cooper GJ, Phillips AR, Choong SY, Leonard BL, Crossman DJ, Brunton DH, Saafi L, Dissanayake AM, Cowan BR, Young AA, Occleshaw CJ, Chan YK, Leahy FE, Keogh GF, Gamble GD, Allen GR, Pope AJ, Boyd PD, Poppitt SD, Borg TK, Doughty RN, Baker JR: Regeneration of the heart in diabetes by selective copper chelation. *Diabetes* 53:2501–2508, 2004
200. Price DL, Rhett PM, Thorpe SR, Baynes JW: Chelating activity of advanced glycation end-product inhibitors. *J Biol Chem* 276:48967–48972, 2001