



Guest Editorial

Intervention against the Maillard reaction in vivo

Received 21 July 2003, and in revised form 1 August 2003

Abstract

The field of Maillard/glycation reactions in vivo has grown enormously during the past 20 years, going from 25 to 500 publications per year. It is now well recognized that many of the “advanced” products form oxidatively or anaerobically and can have deleterious effects on macromolecular and biological function. The feasibility of developing pharmacological agents with beneficial in vivo properties, based on in vitro inhibition of glycation, has been surprisingly successful. This Editorial sets the stage for a series of articles by experts in the field, who have made key contributions to our understanding of the Maillard reaction in vivo. © 2003 Elsevier Inc. All rights reserved.

Keywords: Glycation; Oxidation; Diabetes; Aging; End stage renal disease; Inflammation

The Maillard reaction, originally described by Maillard [1], can best be appreciated during food preparation. The non-enzymatic chemical reaction that occurs between reducing sugars and amino groups leads to the formation of reaction products, which are flavorful and aromatic. However, a heating process which is too long results in the formation of unpleasant tasting, often polymerized, molecules (melanoidins), which are UV active, fluorescent, and often crosslinked. In the years that followed Maillard’s discovery, much research has contributed to our fundamental understanding of Maillard chemistry and its nutritional repercussions [2–4]. While “exogenous” forms of the Maillard reaction may have important biological and even genotoxic properties when ingested [5], the purpose of this Highlight Section of the Archives of Biochemistry and Biophysics is to review selected conceptual efforts to intervene against the “endogenous” Maillard reaction, i.e., the formation of Maillard reaction products in vivo, better known as the “glycation” reaction.

The chemistry and biology of the Maillard reaction and its “advanced glycation endproducts” (AGEs)¹ in

aging and disease have been reviewed many times over the years [6–8]. While several reviews have addressed selected aspects of glycation and advanced glycation, there has been relatively little effort to summarize current intervention strategies against the damaging effects of the Maillard reaction in vivo. Progress in understanding the role of carbonyl and oxidant stress in vivo requires accurate and highly specific determinations of the chemical nature of the molecular changes, as well as the cellular response to these changes. Enormous progress has been achieved in the last 15 years. However, to understand the biologic consequences of specific chemical modifications, both biologists and chemists require targeted inhibitors.

Initially, research focused on the search of Maillard reaction inhibitors, such as aminoguanidine [9]. However, it is now well recognized that, while blocking the Maillard reaction in experimental animals (most commonly in models of diabetes) has therapeutic benefit, conversely, several agents used to treat diabetes actually reduce the formation of AGEs.

In this spirit, we have assembled in this issue a series of papers from research groups that have made seminal contributions to the pharmacological inhibition of the Maillard reaction in vivo. Additionally, this lead paper provides an overview of the chemistry of the Maillard reaction in vivo and a conceptual framework as a basis for the rational design of pharmacological agents that can interfere with the process. We also provide a summary of various agents that have been found to have anti-AGE properties in vivo.

¹ *Abbreviations used:* AGEs, advanced glycation endproducts; DR, dietary restriction; CML, carboxymethyl lysine; DCCT, diabetes control and complications trial; UKPDS, United Kingdom Prospective Diabetes Study; MCOs, metal-catalyzed oxidation reactions; 3-DG, 3-deoxyglucosone; GOL, glyoxal lysine amide; CEL, carboxyethyl lysine; OPD, *o*-phenylenediamine; SOD, superoxide dismutase; PTB, phenacylthiazolium bromide; SSAO, semicarbazide sensitive amine oxidase; AIIR, angiotensin II receptor; ACE, angiotensin-converting enzyme.

Strategical approaches for inhibition of the Maillard reaction in vivo

Control of carbonyl stress at the food intake level

The carbonyl stress that stems from the Maillard reaction can be inhibited at several “hierarchical” levels that are tentatively summarized in Fig. 1. An outline of this concept was provided earlier [10]. First, and foremost, considerable evidence suggests that decreasing food intake will decrease not only the overall carbonyl stress, defined by the sum of carbonyls originating from carbohydrates and lipids, but also overall oxidant stress. Obviously, there is an intimate relationship between the Maillard reaction in foods, the goal of which is to make food palatable, to stimulate appetite and growth. In that regard, the discovery of fire, and the processing of raw foods at high temperature, is thought to have played an important role in evolution by allowing humans to extract large quantities of calories from the otherwise toxic seeds of angiosperms [11].

Serendipitous evidence for a role of Maillard products in controlling body weight and glycemia comes from a recent study by Vlassara and co-workers [5]. Diabetic subjects, who received low AGE content diets for a period of 6 weeks, had a statistically significant drop in body weight. The diet also had an impact on mean glycemia, which resulted in a 20% increase and a similar decrease in the high and low AGE groups, respectively. Thus, a glycaemic gap of 40% between the two groups was present at the end of the study. While various mechanisms might explain the difference in body weight and glycemia, the data strongly suggest that AGE content in foods does impact body metabolism, either through psychosocial, organoleptic [12] or biochemical pathways. In this regard, one way to decrease the overall food intake and carbonyl stress would be to remove the flavor from cooked foods. This, however, is unlikely to be accepted as an intervention therapy, let alone as a way of life.

Overwhelming evidence suggests that dietary restriction (DR) in various species leads to both decreased carbonyl stress and modification of proteins in various tissues, as well as longevity. Rodents receiving 30–50% fewer calories than control animals generally have an equal decrease in body weight and decrease in skin collagen Amadori products, carboxymethyl lysine (CML), and pentosidine [13–15]. In dietary restricted C57BL mice, glycated collagen, CML, and pentosidine accumulation in skin were inversely related to maximal life span [15]. Hens treated with dietary restriction had decreased collagen pentosidine and improved indices of tendon collagen stiffness [16].

In contrast to the rodents, the effects of dietary restriction on glycation are less pronounced in primates. A recent study involving our laboratory showed decreased skin collagen glycation in chronically dietary restricted monkeys. However, no significant effect was observed on glycoxidation markers, such as pentosidine and CML [17]. Thus, in the non-diabetic primate, glycoxidative processes are not responsive to DR for unknown tissue-specific or systemic reasons. In support of the former possibility, earlier studies revealed that glycoxidation products were elevated in skin from individuals with Type 1 but not Type 2 diabetes [18]. In contrast, Amadori products and pentosidine were elevated in glomerular basement membranes from type 2 subjects, suggesting tissue specific sensitivity to carbonyl stress. Thus, it is possible that carbonyl stress in primates treated by dietary restriction is lower than in controls, but only in tissues more critical for survival. It will be important to test this hypothesis, since recent data unequivocally demonstrate that Rhesus monkeys treated by dietary restriction live longer, and have lower insulin levels and glycemia [19].

Taken together, these studies strongly suggest that decreasing food intake is a powerful intervention to combat overall carbonyl stress. However, systematic studies on the relationship between food intake, body

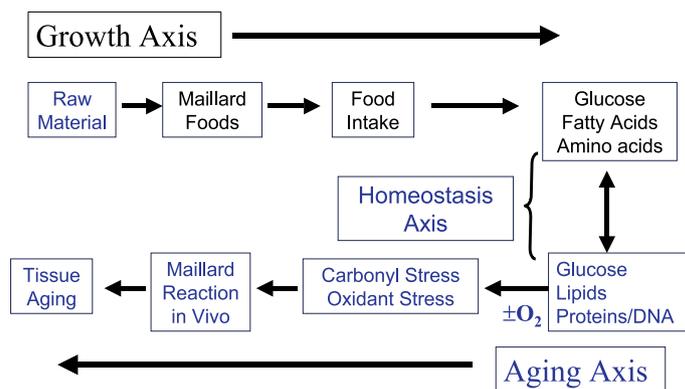


Fig. 1. The exogenous form of the Maillard reaction is needed for growth and maintenance of tissue homeostasis. However, together with lipids and oxygen, the endogenous Maillard reaction driven by glucose as the chief source of carbohydrates and electrons catalyzes tissue modifications in aging, diabetes, atherosclerosis and chronic diseases with an inflammatory component.

weight, and tissue-specific carbonyl stress in primates are needed to better understand the role of the latter on the endogenous Maillard reaction. Moreover, specific nutritional studies are needed on the role of the Maillard reaction in controlling appetite, food preference, and induction of “glycotoxicity,” in order to better understand the extrinsic role of the Maillard reaction in tissue-aging and longevity.

Control of carbonyl stress at the glycemic level

The relationship between glycemia and the Maillard reaction in vivo is summarized in Fig. 2. According to this scheme, lowering glycemia would most likely result in an overall decrease in the modification rate of extracellular proteins. This has been clearly documented by us and coworkers in the DCCT study in which diabetic individuals underwent long-term intensive control of glycemia, which resulted in lower levels of glycation and glycoxidation markers, when compared to conventionally treated patients [20]. Particular measurements included Amadori products, CML, pentosidine, fluorescence at 370/440 nm, and pepsin insoluble collagen, all of which were improved.

Similarly, one would expect a major impact of glycemic control on the formation of cellular oxoaldehydes, such as methylglyoxal, and cellular AGEs. Such results would be predicted in response to decreasing glycemia in diabetes. However, one cannot automatically assume that intracellular AGE formation, in non-diabetic subjects, would decrease in response to decreasing glycemia. Indeed, there may be compensatory mechanisms for maintaining intracellular glycemia within a narrow range, whereby rate-controlling enzymes of the glycolytic pathway play a major role in dictating up- and downstream concentrations of reactive carbonyls resulting from glycolysis.

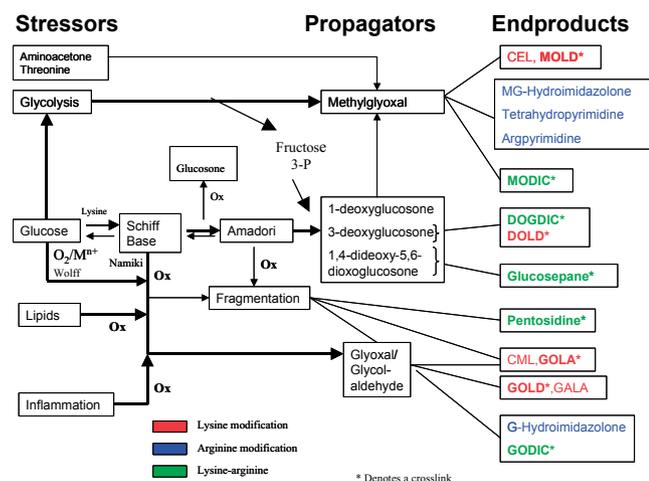


Fig. 2. Selected chemical pathways and advanced glycation endproducts of relevance to the Maillard reaction in vivo.

It is beyond the scope of this review to address pharmacological and other strategies to decrease glycemia. The reader is referred to the vast literature on hypoglycemic agents in relationship to the preventive treatment of diabetic complications. However, the goal of euglycemia is very difficult to achieve, in spite of the examples set by large-scale studies such as Diabetes Control and Complications Trial (DCCT) and United Kingdom Prospective Diabetes Study (UKPDS). For this reason, therapies that are specifically targeted at preventing micro- and macrovascular disease in the presence of suboptimal control of glycemia are needed.

Control of carbonyl stress by acting on the Maillard reaction itself

The strategies described above have the advantage of acting on all forms of metabolic stresses, i.e., those stemming from carbohydrates and lipids, as well as oxidant stress. Although there is no consistent evidence for impaired antioxidant defense in aging, specific strategies are needed to combat stochastic damage to tissue molecules for at least three reasons: (1) molecular damage increases with age, regardless of any attempt to decrease food intake, because glucose, lipids, and O₂ are vital to survival; (2) euglycemia cannot be achieved in most diabetic individuals; and (3) enzymatic detoxification of carbonyls and free radicals is overwhelmed in diabetes [7].

The successful intervention against the Maillard reaction in vivo hinges in part on precise understanding of its chemistry. For this reason, we provide an update of the major pathways involved in the generation of reactive carbonyl species derived from glucose in Fig. 2. Purely oxidative processes and lipoxidation reactions are considered only to the extent that their products can form common intermediates and end products of the Maillard reaction, such as glycolaldehyde/glyoxal. Indeed, the paper by Metz et al. [21] in this series points to a critical role for lipoxidation reactions in diabetic complications and the role of pyridoxamine in trapping them.

Nutritional sources of carbohydrates, such as fructose, galactose, and arabinose, are not considered here, although it is recognized that they could be important contributors to in vivo AGE formation in diseases with impaired renal function. Similarly, the role of ascorbic acid and its ascorbylation chemistry, as a key player in glycation and browning of crystallins in the aging human lens, is not reviewed here.

Overview of the Maillard reaction

The Maillard reaction in vivo is pragmatically divided into three kinetic compartments consisting of “stressors,” i.e., the sources of carbonyl agents that can drive the reaction, “propagators,” i.e., the reactive carbonyl

agents that arise from the precursor “stressors,” and the “endproducts” that mark the molecular aging process resulting from the Maillard reaction (Fig. 2). This conceptual distinction is quite helpful in defining the various steps where one should interfere against the reaction.

Inhibition of glycooxidation: the roles of redox active metals and chelators in AGE formation and diabetic complications

In the initial stage of the Maillard reaction, where glycation per se occurs, glucose reacts with an amine to make a labile Schiff base that rearranges to the Amadori product. The Schiff base is highly prone to oxidation and free radical generation, which leads to the formation of oxoaldehydes, glyoxal and methylglyoxal, i.e., the so-called Namiki pathway of the Maillard reaction [22]. This form of “glycooxidation” can also result from metal-catalyzed glucose autoxidation, which leads to the formation of glyoxal and arabinose [23]. D-Glucosone rapidly decomposes into ribulose and is therefore not observed in vivo [23]. In this process, H₂O₂ is released. This, so-called, “Wolff” pathway of the Maillard reaction is an important in vitro process and is observed during long-term incubation of proteins with reducing sugars in phosphate buffer. It is attributed to the presence of Cu²⁺ and Fe³⁺, which contaminate all phosphate based buffers [24].

Glucose autoxidation, per se, is unlikely to be of major relevance for AGE formation during normal aging or in uncomplicated diabetes. However, the Amadori product and ascorbic acid are much more prone to autoxidation than glucose in the presence of catalytic metals. Because of this “pecking order” of biological redox reactions [25], ascorbic acid and Amadori products of glucose are much more reducing than glucose itself and, therefore, more likely to contribute to carbonyl stress. Interestingly, the redox chemistry of ascorbic acid and the Amadori products is virtually identical, resulting in the formation of dehydroascorbic acid and glucosone, respectively. However, formation of the latter is also thought to be artificially enhanced by nucleophilic trapping agents, such as *o*-phenylenediamine [26]. Diseases in which the Wolff and Namiki pathways might be important are renal diseases, whereby oxidative processes are intense [27], in the aging lens, and in atherosclerosis, in which catalytic metals have been found [28,29]. However, more data are needed to assess the true in vivo significance of these pathways.

Under conditions of accelerated metal-catalyzed glycooxidation, or in the presence of an electron acceptor like nitroblue tetrazolium, the Amadori product generates superoxide and H₂O₂ [30,31]. The latter can form hydroxyl radicals in the presence of metals and generate CML from Amadori products [32]. A similar reaction is catalyzed by peroxyxynitrite [33]. Of importance is gly-

cooxidation, which favors lipid peroxidation [34]. Both of these processes are precursors of carboxymethyl lysine (CML), which itself can bind redox-active metals and, thus, perpetuate the vicious cycle [35].

A key question is to what extent oxygen radicals are derived from metal-catalyzed oxidation reactions (MCO). Further, what roles do glycation products play in their in vivo formation? Indirect evidence for MCO, in experimental diabetes, comes from studies by Cameron [36], who showed that treatment with metal chelators suppressed the development of nerve conduction and blood flow defects in streptozotocin-diabetic rats. Indeed, when CML-rich tendons were implanted into and retrieved one month later from the peritoneal cavity of diabetic rats, their content in redox active Cu²⁺ was highly increased [37]. Furthermore, several of the currently known AGE inhibitors were screened for and discovered using an assay that favored glycooxidation. Typically, researchers incubated albumin with glucose or ribose in phosphate buffer, which had not been chelated to remove metal, and used CML-specific immunoassays or generic fluorescence to screen for the effects of inhibitors. However, Price et al. [38] noted many AGE and other post-Amadori inhibitors, thought to act as trapping agents, were also able to chelate the transition metals in the buffer. Examples include aminoguanidine, OPB-9195, pyridoxamine, ALT-711 (phenacylthiazolium bromide), and NNC39-0028 (2,3-diaminophenazine). In addition to these compounds, Rahbar and co-workers [39], in this issue, present a number of anti-AGE compounds, which have chelation properties. The most potent one, LR-90, also has beneficial effects on the progression of diabetic nephropathy.

As pointed out by Metz and colleagues [21], who provide an update on pyridoxamine in this issue, most AGE inhibitors have more than one biological property, which makes it very difficult to approach their precise mechanism of action. One could add to the list of potential metal chelators C-peptide and sRAGE. Insulin C-peptide has been found efficacious at preventing albumin permeation and decreased motor nerve conduction velocity in diabetic rats [40]. The activity was linked to sequences rich in glycine which likely chelate Zn²⁺, a divalent metal homologous to Cu²⁺. The intriguing possibility that sRAGE may act as a metal chelator is an exciting area to study.

In further support for a link between metal-catalyzed oxidation and diabetic nephropathy is the finding that redox-active transition metals are increased in diabetic rat kidney [41]. However, we found no difference in the quantities of metal-catalyzed degradation products of ascorbate in urine obtained from STZ treated or normal rats [42]. Thus, it is not clear how metal-catalyzed oxidation might mediate the pathogenesis of diabetic complications, because the compartments in which accelerated MCO occurs appear to contribute little to total

oxidation, as reflected by the presence of ascorbate oxidation products in the urine [42].

The Amadori product as a pharmacological target of the Maillard reaction

The Amadori product is quantitatively the single most important glycation product in extracellular proteins, lens, and red blood cells. In contrast to AGEs, which accumulate exponentially with age in collagen and lens [43,44], levels of the Amadori product do not change markedly with age, except in growing mice [45]. Thus, the Amadori product is in a steady state between formation, reversal, and degradation, and its levels are significantly decreased by long-term control of glycemia [20]. Under anaerobic conditions, the Amadori product is a precursor of 3-deoxyglucosone, 1-deoxyglucosone and, most importantly the lysine-linked 1,4-dideoxy-5,6-dioxoglucosone, which is the direct precursor of glucosepane crosslinks [46]. The structure of these reactive carbonyl compounds, together with those of the other dioxo compounds (methylglyoxal and glycolaldehyde), is shown in Fig. 3.

It should be noted that 3-deoxyglucosone (3-DG) can also originate from fructose via the sorbitol pathway [47]. Its biological role has been reviewed in detail [48]. The difficult synthesis of 1-deoxyglucosone has been achieved [49], but its *in vivo* presence has not been demonstrated yet. Perhaps this is due, in part, to its high reactivity and lability. Most, if not all di-oxo compounds, can form both adducts and crosslinks with lysine and arginine residues.

As shown in Fig. 2, the Amadori product can give rise to a number of AGEs, including pentosidine, CML, pyrroline, glyoxal lysine amide (GOLA), crosslines (not shown), glucosepane [50–56], and indirectly to all other AGE products derived either from methylglyoxal or glyoxal/glycolaldehyde. However, it is important to note

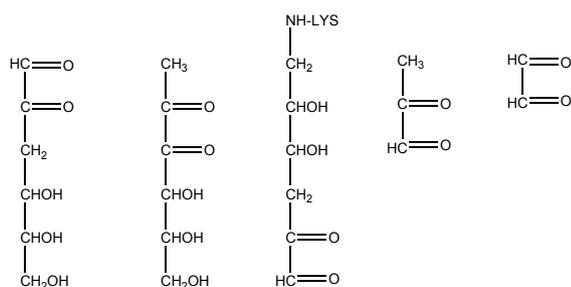


Fig. 3. The structure of the reactive dicarbonyl agents that can form during the Maillard reaction. In solution, hydration of these compounds impacts on their reactivity with nucleophiles. *In vivo*, methylglyoxal is thought to be primarily contributed by triose phosphates, while glyoxal can originate from multiple sources including glycooxidation, lipoxidation, ascorbic acid, and oxidation of serine by myeloperoxidase. Compounds are from left to right: deoxyglucosone, 1-deoxyglucosone, 1,4-dideoxyglucosone, methylglyoxal, glyoxal.

that, except for glucosepane, whose structure cannot be explained without Amadori products as precursors [57], no AGE product is solely derived from glucose. Thus, studies aimed at inhibiting the consequences of increased glycation by glucose itself should include glucosepane as a marker. Interestingly, glucosepane is currently the single most important AGE crosslink in old human collagen and human lenses [56]. Except for the lens, no data on cellular levels are available.

Should one attempt to inhibit or reverse the formation of Amadori products? Several studies, with drugs effective against diabetic complications in the rat, revealed that neither glycosylated hemoglobin nor tissue Amadori products were decreased by treatment [58,59]. This would lead one to the conclusion that Amadori products do not play a role in the pathogenesis of diabetic complications. In recent years, however, multiple studies by Cohen and co-workers, reviewed in this issue [60], have shown that diabetic mice injected with an antibody to glycosylated albumin, or treated with the drug EXO-225 [61], which inhibits albumin glycation, had a lower progression rate of diabetic nephropathy. Binding proteins for short-term glycosylated proteins studies have been described in arteries and macrophages, which might mediate abnormal signaling [62,63]. Interestingly, diclofenac (EXO-225) is also an anti-inflammatory agent. Thus, if found efficacious against diabetic complications in humans, the precise mechanism of action of diclofenac will be difficult to unravel.

Another answer to this question is found in the example of glucosepane, the AGE crosslink whose formation likely imparts increased stiffness to arteries, joints, and the lens in diabetes. Because glucosepane may play a major role in inhibiting turnover of the extracellular matrix, there is a strong rationale for specific testing of the role of Amadori vs other AGE products in diabetic complications and aging. Thus, there is an urgent need to develop an animal model in which the biological role of Amadori products can be independently tested from glycemia.

Our laboratory has approached the question of *in vivo* deglycation by searching in soil organisms for “amadoriase” enzymes that might be used in transgenic animal models of diabetes. These studies are reviewed in this issue [64]. Amadoriases were first described by Horiuchi in *Corynebacterium* and *Aspergillus* sp. and belong to the class of fructosyl amino acid oxidases, i.e., FAD enzymes that deglycate fructosyl-amines, while generating glucosone and the original amine [65,66]. Unfortunately, these enzymes, while quite active against glycosylated low molecular substrates, are inactive against glycosylated proteins. Thus, protein engineering will be needed to overcome steric inhibition. However, while this work was in progress, Szwegold et al. [67] found a fructosamine 3-kinase that was able to deglycate proteins upon phosphorylation

of the Amadori product. The enzyme was cloned [68,69] and found to deglycate hemoglobin [70], though not at the N-terminus of the β -chain. The exact biological significance of this enzyme is still elusive. Obviously, this ATP dependent enzyme, while able to deglycate intracellular protein, would not be of use for deglycation of extracellular proteins, such as albumin and collagen. Further research in this exciting area of the Maillard reaction is warranted.

Inhibition of the Maillard reaction at the post-Amadori step

Trapping of dicarbonyl compounds

Aminoguanidine. Both glyoxal and methylglyoxal (MG) can originate from fragmented Amadori products, or Schiff base intermediates, during the glycation process. However, the major source of methylglyoxal formation is thought to originate from glycolysis, whereby spontaneous dephosphorylation of glyceraldehyde 3-P and dihydroxyacetone phosphate at the triose isomerase step results in MG formation [71]. In addition, ascorbic acid, threonine, and aminoacetone are MG precursors. Recently, infusion of the latter into rats was found to stimulate argpyrimidine formation in aortic smooth muscle cells via oxidation of aminoacetone into methylglyoxal and H_2O_2 by semicarbazide oxidase [72]. Thus, some of the biological effects attributed to AGEs may in fact be due to H_2O_2 . Glyoxal/glycolaldehyde is unique in that it can also originate from oxidized free fatty acids and metabolic pathways, in addition to ascorbic acid and carbohydrates [73]. It is responsible for the formation of ubiquitous CML, which is also a potent ligand for the RAGE receptor [74].

Methylglyoxal and glyoxal can react with lysine residues to form carboxyethyl lysine (CEL) and CML, respectively, while all three oxoaldehydes can make the analogous di-lysyl crosslinks MOLD, GOLD, and DOLD, respectively [75]. However, the reactivity of these carbonyl compounds, as well as those of the other α,β -dicarbonyl compounds, is tilted toward the guanidino group of arginine, and comparatively high levels of corresponding dehydroimidazolones are formed upon reaction with arginine residues [76]. All compounds are also reactive with *o*-phenylenediamine-like reagents to form quinoxalines [26,77] and all three oxoaldehydes are a rich source of AGE adducts and crosslinks. Thus, a question, why is *o*-phenylenediamine (OPD) not being evaluated as an anti-AGE drug? In fact, its oxidation product (2,3-diaminophenazine NNC39-0028) has been investigated and found to improve collagen solubility in diabetic rats, without affecting diabetes-induced pathophysiology such as the increase in urinary albumin excretions rate or albumin clearance [78]. OPD, itself, has genotoxic properties [79], which explains the low enthusiasm for pursuing its anti-AGE properties on a broader scale.

Because of its central role in the formation of both intra- and extracellular AGEs, as well as their biological ability to modulate cell signaling [181], the targeting of oxoaldehydes should be a high priority in the design of anti-AGE therapy. In this regard, it is not surprising that the supernucleophile aminoguanidine was the prototypic anti-AGE compound, with profound biological effects on experimental diabetes [9]. The reactivity of this agent with oxoaldehydes to form triazines and its biological efficacy are reviewed by Thornalley in this series [80]. Paradoxically, however, there is only scanty information on the *in vivo* formation from aminoguanidine triazines in the literature [81]. Furthermore, aminoguanidine is also an inhibitor of nitric oxide synthase [82] and semicarbazide sensitive amine oxidase [83]. Both of these compounds have been implicated in the pathogenesis of diabetic complications. Although the beneficial effects of aminoguanidine against diabetic complications have been widely confirmed in the diabetic rat model, clinical trials while improving several indices of nephropathy, retinopathy, and lowering hyperlipidemia failed due to the drug's its inability to reach its major target of increasing creatinine doubling time [106].

Pyridoxamine (Amadorin). This class of "post-Amadori" inhibitors was originally discovered based on their ability to inhibit CML formation in a model system, consisting of ribonuclease A incubated with high concentrations of ribose, which is notorious for its very high activity as an AGE and crosslink precursor. A detailed account of the biological and biochemical properties of pyridoxamine, the first-generation post-Amadori inhibitor of its class, is provided by Baynes and colleagues in this series [21]. Pyridoxamine is able to prevent complications in the diabetic rat with higher efficacy than aminoguanidine and is able to trap lipid peroxidation products and dicarbonyl compounds. The authors propose that hyperlipidemia might play a major role in the pathogenesis of diabetic complications based on the finding that PM has a hypolipidemic effect and that PM is a potent scavenger of lipid peroxidation products. However, pyridoxamine has also been found to have transition-metal chelating properties, a property shared by many other AGE inhibitors [38]. To make matters more complicated, pyridoxamine, like aminoguanidine, is also an inhibitor of semicarbazide-sensitive amine oxidase, an enzyme implicated in diabetic complications [84]. Pyridoxamine is currently in clinical trial.

Control of carbonyl stress by trapping AGEs and blocking AGE receptors

Completely different approaches, from those discussed above, are those based on (i) trapping of AGE products on circulating proteins before these have a chance to act on an AGE receptor, (ii) inhibition of

AGE-ligand-receptor interaction, and (iii) inhibition of signal transduction mediated by AGE-receptor engagement. These approaches hinge on a deeper understanding of the structure–function relationship between AGE-ligand and their receptor.

The prototype “drug” for the trapping of AGE-ligands is the recombinant soluble RAGE receptor (sRAGE), which is reviewed in this issue by Schmidt and coll. [85]. Considerable data have accumulated, which suggest that “AGEs”, i.e., proteins that were incubated for prolonged time with glucose, are either taken up by macrophage scavenger type receptors [86] or can engage the RAGE receptor. In latter case, a signaling cascade is triggered in endothelial cells, resulting in the activation of the RAS-ERK1/2-NF- κ B pathway [86]. Multiple cells bearing the RAGE, such as endothelial cells, retinal pericytes, macrophages, mesangial cells, renal podocytes, etc., have been found to respond to AGE-ligands in vitro. While other receptors, such as the AGE-R1(OST-48), AGE-R2(80 K-H, p60), and AGE-R3 (Gal-3) receptors, have been described in various cells [87], their pathogenetic role in diabetic complications is less well established. Of interest, however, is the fact that galectin-3-deficient mice develop accelerated glomerulopathy associated with increased AGE deposition. The galectin-3-deficient genotype was associated with reduced expression of receptors implicated in AGE removal (macrophage scavenger receptor A and AGE-R1) and increased expression of receptors mediating cell activation (RAGE and AGE-R2).

RAGE clearly emerges as an important mediator of proinflammatory stimuli that can be triggered, not only by AGE-ligands, but also by S100/calgranulins, amyloid- β , and other fibrillar proteins. Thus, RAGE appears to be a “pattern”-recognition receptor of broad biological significance. Indeed, infusion of sRAGE into experimental animals has been found to help decrease the progression rate of diabetic nephropathy, atherosclerosis, metastasis, and inflammation. Yet, the precise mechanism by which sRAGE exerts its effects is still unclear. Does it really act by trapping RAGE ligands? Does it act by preventing RAGE receptor dimerization? Does it act as a metal chelator? If the animal studies are confirmed in the human, the development of low-molecular weight competitive inhibitors as drugs will be potentially extremely useful to combat diabetic complications, Alzheimer’s disease, cancer, and many other diseases in which the inflammatory component plays a major role. However, the recent discovery of a secretory splice variant of RAGE suggests the existence of an endogenous trapping system against AGE products and ligands of RAGE [88]. If so, administration of RAGE inhibitors may adversely impact on this system.

Another endogenous AGE trapping system is lysozyme. Vlassara and colleagues [89] made the original observation that the enzymatic and bactericidal activity

of lysozyme was inhibited by AGE-modified proteins that was associated with a single AGE-binding domain. Subsequent studies revealed that a lysozyme affinity column was able to remove AGEs from the serum of uremic patients, and that the bound fraction exhibited crosslinking activity that was preventable by aminoguanidine [90]. When lysozyme was injected into diabetic rodents, it decreased immunoreactive serum AGEs, enhanced urinary AGE excretion, and decreased albuminuria [91]. It also stimulated the uptake and degradation of 125 I-labeled AGE-BSA and labeled serum AGE by macrophages, while suppressing AGE-induced TNF- α and IGF-I production. In mesangial cells, lysozyme suppressed the AGE-induced PDGF- β , α 1 type IV collagen, and tenascin mRNA levels, and restored the AGE-suppressed expression and activity of MMP-9. Thus, lysozyme appears to accelerate renal AGE clearance and to suppress macrophage and mesangial cell-specific gene activation in vitro while improving diabetes-related albuminuria. Lysozyme thus appears to be useful against diabetic renal damage by sequestering AGEs. These provocative studies suggest it might be possible to generate animal models of AGE overload by knocking out lysozyme. Vice versa, transgenic animals expressing high serum levels of lysozyme might be protected against the toxicity of end stage renal disease.

Control of carbonyl stress by acting on oxidant stress

The beneficial effect of metal chelators in preventing or delaying diabetic complications in the experimental animal suggests free radical stress might be involved in diabetic complications. In the Fenton reaction, redox-active transition metals reduce hydrogen peroxide to the highly damaging hydroxyl radicals. In diabetes, the two major sources of H₂O₂ appear to be NADH oxidase and mitochondria. The former was found to be increased in retina of diabetic rats [92], and the latter has been hypothesized to form the basis of the “unifying theory of diabetic complications” [93].

According to this hypothesis, the increased flux from glucose to pyruvate and NADH into mitochondria, possibly combined with an additive increase in electron entry at complex II, leads to increased mitochondrial electron leakage and reduction of oxygen into superoxide anion [94]. This would result in oxidant damage to glyceraldehyde 3-phosphate dehydrogenase and activation of deleterious pathways upstream of this enzyme, i.e., methylglyoxal derived AGE formation, activation of protein kinase C, activation of the hexosamine pathway leading to increased gene transcription such as PAI-1, and increased aldose reductase activity. Superoxide formation would also increase DNA damage and NADH consumption due to increased poly-ADP-ribosyl polymerase activity [94]. This novel paradigm

suggests trapping of excess superoxide with, e.g., superoxide dismutase (SOD) mimetics should prevent metabolic dysfunction. In this regard, alternative approaches such as using high doses of vitamin B1 or its analog benfotiamine to divert excess carbohydrates toward the reductive pentose pathway have been found highly efficacious at preventing microvascular disease in diabetic rats [95,96].

The mechanism of action appears to involve activation of transketolase and not oxidation, although thiamine itself has been reported to have anti-oxidant properties [97]. Of interest is the fact that thiamine was highly efficacious at decreasing a large number of AGE and oxidation products in the kidney and plasma of the diabetic rats [96]. This raises the question of the precise mechanism of action of the vitamin. Several clinical studies with thiamine on diabetic neuropathy have been published suggesting beneficial effects of the vitamin at high dose. A German patent by Woerwag [98] describes the utilization of benfotiamine, a lipophilic derivative of thiamine with improved tissue uptake, for the treatment of diabetic retinopathy and nephropathy. Large-scale clinical trials will be needed to validate its effects against microvascular complications in the human.

Reversing of covalent bonds with AGE breakers

The enolization of the Amadori product and formation of dicarbonyl compounds along its backbone lead to the hypothesis that certain chemical compounds like phenacylthiazolium bromide (PTB, ALT-711) might be able to cleave AGE-dicarbonyls and prevent, if not reverse, crosslinks of similar structure [99]. Indeed, feeding this drug to STZ-diabetic rats was efficacious at preventing and reversing biochemical abnormalities suggestive of crosslinking. Subsequent studies in dogs, monkeys, and humans that are reviewed in this issue by Vasan et al. [100] have shown positive effects on several endpoints of vascular and cardiac stiffness. However, the mechanism of action of AGE “breakers” such as PTB is unclear since most AGE crosslinks (MOLD, GOLD, glucosepane, glycinamide, MODI, GODIC, DODIC, etc.) would not be cleaved by PTB or analogous compounds. While this special issue of the Archives was under preparation, the clinical results of phase II trials with ALT-711 (PTB) were completed, which revealed no efficacy of the drug on parameters such as hypertension [101].

Drugs against diabetic complications that also act as AGE inhibitors

Finally, a number of drugs based on non-glycation theories of diabetic complications have been developed, which also inhibit AGE formation in vitro, in vivo, or both. Examples of these drugs include aldose reductase inhibitors, which inhibit pentosidine formation in the

lens [102] and other tissues and ACE inhibitors (Table 1). Activation of the aldose reductase pathway in diabetes may lead to increased 3-deoxyglucosone formation from fructose or fructose 3-phosphate and, thus, increased glycation. However, pentosidine was elevated in galactosemic rat lenses. A more likely pathway could involve increased ascorbic acid degradation in the galactosemic lens due to glutathione depletion.

As described above, thiamine (vitamin B1) inhibits plasma and renal AGE formation in vivo [96]. Its proposed mechanism of action involves diversion of triose glycolytic intermediates toward the reductive pentose pathway. Thus, most AGE products that are derived from methylglyoxal are decreased. However, markers of oxidation, such as free thiols, are also improved. It is, yet, unknown whether thiamine is an AGE inhibitor in vitro, but, given its known antioxidant properties, it would not be surprising if it would also inhibit glycooxidation. It is also possible that its thiazolidine structure participates in the breaking of dicarbonyl AGE precursors.

Conclusions

Several conclusions emerge from this review, which are relevant for the design of AGE inhibitors and the treatment of age-, and disease-related conditions, where the Maillard reaction is accelerated. First, and foremost, from a purely chemical viewpoint, AGE inhibitors do not act predictably on all aspects of sugar mediated modifications of proteins, and the results vary according to the in vitro model system used for screening. In their recent work, Culbertson and colleagues [103] have categorized various agents into chelators, free radical traps, antioxidants, carbonyl traps, and multifunctional inhibitors, and examined their effects on pentosidine, CML, and crosslink formation in presence or absence of metals. They also examined the effects of the inhibitors during the continuous glycation reaction or on post-Amadori products. The results are complex and seemingly paradoxical, and vary for ribose or glucose mediated glycation. A striking finding, however, is that several inhibitors enhanced pentosidine formation from ribose and poorly inhibited CML formation from ribated protein. For example, the free radical trap and antioxidant Trolox enhanced pentosidine both during ribation and from ribated protein, failed to suppress CML formation, but totally suppressed crosslinking. Conversely, pyridoxamine inhibited CML formation during ribation, but only in the presence of metals, and was less effective against crosslinking from ribated protein. Yet, different results were obtained with glucose as the glycating agent. Thus, it is clear that inhibition of one pathway may favor another pathway, and, expectedly, no single AGE inhibitor will act on all pathways. The key question is which pathway should be preferentially

Table 1

AGE and glycation inhibitors. The drugs below have been categorized according to their original pharmacological application. They may be listed multiple times depending on their established or anticipated mechanism(s) of action. Most drugs listed below have been found to be efficacious in one or several endpoints of diabetic complications in the rat or human.

Generic name	Brand name	Properties	References
1. Drugs specifically developed as AGE inhibitors			
Aminoguanidine	Pimagedine	Prototype nucleophilic compound with multiple biochemical and biological anti-AGE effects in vitro and anti-diabetic properties in animals and humans	[80,105]
Pyridoxamine	Pyridorin	Mixed clinical results, trials abandoned in 1998	[106]
		Antilipidemic, ALE/AGE trapping	[21,107,108]
Hydrazono-oxo-thiazolidine derivative/OPB-9195		Prevents db nephropathy in db	[109]
		Prevents db nephropathy in Zucker rats	[59]
		Inhibits increase in albuminuria, plasma creatinine, hyperlipidemia, and plasma lactate/pyruvate ratio in diabetic rats and decreases the AGE/ALEs, carboxymethyl lysine and carboxyethyl lysine, crosslinking, and fluorescence but not pentosidine in skin collagen of diabetic rats	[109]
		Prevents db retinopathy in rat	[110]
		Prevents glucose-induced DNA damage but not intracellular ROS formation in EC	[111]
		Currently in clinical trial	
		Glycoxidation and lipoxidation inhibitor	[112,113]
		Inhibits plasma and renal AGE formation, albuminuria, and glomerulosclerosis in OLETF-rat	[114]
		Pentosidine and precursors are lowered in uremia	[115]
		Glomerular CML db rat	[116]
2,3-Diaminophenazine/ NNC39-0028		Inhibits glyoxal-mediated protein phosphorylation in thymocytes and fibroblasts	[117]
		Inhibits intimal thickening following balloon injury	[112]
		Suppresses the renal expression of TGF-beta, VEGF, and type IV collagen mRNAs and proteins in OLETF rat	[118]
		Improves MNCV, reduces serum and sciatic nerve AGEs, and DNA damage in db rats; improves advanced nephropathy in RAGE transgenic mice	[119]
		Reduces blood pressure and oxidative damage in SHRSP rat	[120]
		OPB-9195 traps RCOs, chelates transition metals, and scavenges mildly carbon-centered and hydroxyl radicals	[121,122]
		Attenuation of vascular hypertrophy and inhibition of mesenteric and renal AGE formation in db rats	[123]
		Collagen crosslink inhibitor, no effect on vascular dysfunction in db rats	[78]
		Improves renal AGEs and various parameters better than AG	[124]
2. AGE breakers and drugs with potential AGE breaking activity			
<i>N</i> -phenacylthiazolium/ALT-711		AGEs, RAGE, and other changes in the heart of diabetic rat	[125]
		Clinical trial, in one study no effect on collagen crosslinking and vacuolar abnormalities in db rats	[78]
		<i>N</i> -phenacylthiazolium (PTB) and <i>N</i> -phenacyl-4,5-dimethylthiazolium (PMT) halides cleave model compounds but fail to cleave Maillard crosslinks in skin and tail collagen from diabetic rats	[126]
Thiamin/Benfotiamine		Postulated AGE breaking activity based on structure	
Pyridoxamine		Cleaves phenylpropane dione but not in vivo crosslinks	[126]
3. Anti-inflammatory drugs with anti-glycation properties			
Tenilsetam (thienyl-piperazinone)		Inhibits formation of the AGE-crosslinked amyloid peptide aggregates	[127]
		Inhibits in vitro crosslinking and fluorescence and pyrrolidine increase in renal and aortic tissue from db rats	[128]

Table 1 (continued)

Generic name	Brand name	Properties	References
Diclofenac (EXO-225)		Amadori product inhibitor [129], improved matrix deposition, glomerular and renal type IV coll expression in rats and urinary albumin excretion rate. Diclofenac is a widely studied and clinically approved anti-inflammatory agent under the name Voltaren.	[129] [61]
Aspirin (acetylsalicylic acid)		Delays cataract and decreases glycation better than salicylic acid Prevents glycation-induced changes in corneal and scleral collagen Decreases skin pentosidine in NIDDM subjects after 1-year therapy Partially protects enzyme inactivation by glycation Delays cataract and attenuates glycation of crystallins in vitro and in vivo	[130] [131–133] [134] [135] [136,137]
Spermine, Spermidine		Inhibits enzymes and protein structure changes due to glycation in vitro	[138]
4. Renoprotective drugs with AGE inhibition activity			
(a) ACE-inhibitor			
Captopril		Inhibits the fluorescence development associated with glycation of proteins	[139]
Ramipril		Inhibits fluorescent AGEs and oxidation markers	[140–142]
Temocaprilat		Inhibits in vitro the formation of pentosidine carboxymethyl lysine (CML)	[121,122]
(b) AIIRT-blocker			
Olmesartan		Inhibits in vitro the formation of pentosidine and carboxymethyl lysine (CML) Lowers renal pentosidine Acts by chelating transition metals and inhibiting various oxidative steps, but not trapping carbonyls.	[121,122] [143]
Losartan candesartan irbesartan termisartan valsartan		As for olmesartan in terms of AGE blocking properties	[121,122]
(c) Hypotensive drugs			
Hydralazine		Antihypertensive, lowers renal pentosidine Traps RCOs, chelates transition metals, and scavenges mildly carbon-centered and hydroxyl radicals	[143] [122]
5. Metabolically active drugs and vitamins with anti-AGE properties			
Aminoguanidine		Inhibitor of diamine oxidase, histaminase Nitric oxidase synthase Semicarbazide sensitive amine oxidase	[144] [82] [83]
Pyridoxamine (pyridorin)		Inhibitor of semicarbazide sensitive amine oxidase Improves lactate/pyruvate ratio in db	[84,145] [146]
Metformin		Reduces methylglyoxal levels Decreases lens, nerve, and kidney AGE products Traps methylglyoxal and glyoxal to form triazepinones Decreases collagen AGE products in db dogs	[147] [148] [149] [150]
Thiamine (vitamin B1), Benfotiamine		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 Lowers a number of renal and plasma AGEs and oxidation markers and prevents diabetic nephro- and retinopathy by diverting “excess” glycolytic intermediates toward the reductive pentose pathway Correct matrix defects from EC cultured in high glucose Benfotiamine corrects db neuropathy and lowers nerve AGE levels Both thiamine and benfotiamine have been found efficacious against diabetic neuropathy in humans	[151] [95,96] [152,153] [154] [155,156]
Aldose reductase inhibitors			
Sorbinil		Suppresses pentosidine formation in galactosemic rat lens But did not suppress renal fluorescence in db rats	[102] [157]
Eparlestat		Lowers erythrocyte and plasma CML and 3-DG-imidazolone in db patients	[158–160]
Ponalrestat		Ponalrestat retards fluorescence in aorta from db rat	[161]

Table 1 (continued)

Generic name	Brand name	Properties	References
6. Antioxidants and free radical trapping agents			
Carnosine (β -alanyl-L-histidine)		Antioxidant and anticataract agent Inhibits AGE-crosslinked amyloid peptide aggregate Inhibits protein modification induced by a lysine-methylglyoxal-AGE Promotes the heat denaturation of glycated protein Traps ALEs and aldehydes	[162,163] [127] [164] [165] [166,167]
Flavonoid-rich herbal extracts		In vitro, inhibition of enzyme inactivation by methylglyoxal Suppress fluorescent AGEs	[168] [169]
Thiamine/Benfortiamine		Have also antioxidant properties	[97]
Curcumin		Chain-breaking antioxidant With anti-AGE properties And metal chelator properties	[170,171] [172] [173]
7. AGE inhibitors with chelating properties			
Aminoguanine		See above	[38]
Carnosine		See above	[38]
OPB-9195		See above	[38]
Olmesartan		See above	[121]
Pyridoxamine		See above	[38]
Pyrrolidinedithiocarbamate (PDTC)		Is a metal-chelating compound that exerts both pro-oxidant and antioxidant effects and is widely used as an antitumor and anti-inflammatory agent Prevents the AGE-induced increase in NF- κ B and AP-1 activity, VEGF mRNA up-regulation, and the resultant increase in DNA synthesis in microvascular EC	[174] [175]
Temocaprilat		See above	[121]
Tenilsetam		See above	[38]
Curcumin		See above	[173]
2,3-Diaminophenazine (NNC39-0028)		See above	[78]
AGE signaling pathway inhibitors			
Cerivastatin		A HMG-CoA reductase inhibitor, acts as AGE-RAGE signaling inhibitor, prevents the glyceraldehyde- and glyoxal-AGE-induced increase in NF- κ B and AP-1 activity, VEGF mRNA up-regulation, and the resultant increase in DNA synthesis in microvascular EC	[175]
Incadronate disodium		A nitrogen-containing bisphosphonate, inhibits in vitro AGE-induced increase in DNA synthesis, angiogenesis from human microvascular endothelial cells, NF- κ B and AP-1, and the subsequent up-regulation of VEGF mRNA levels in AGE-exposed EC	[176]
Pyrrolidine dithiocarbamate		Prevents the in vitro AGE-induced increase in NF- κ B and AP-1 activity, VEGF mRNA up-regulation, and the increase in DNA synthesis in microvascular EC	[175]
Curcumin		Prevents the AGE-induced increase in NF- κ B and AP-1 activity, VEGF mRNA up-regulation, and the resultant increase in DNA synthesis in microvascular EC Prevented immunoreactive AGE and collagen crosslinking but not fluorescence increase in tail and skin from db rat	[175] [172]
Other drugs			
Pioglitazone (thiazolidinedione)		A PPAR- γ agonist is also an inhibitor of glycation	[177]
Pentoxifylline		A cAMP-phosphodiesterase inhibitor is also an inhibitor of glycation	[177]
L-Lysine		Reduces HbA1c, collagen glycation, and albuminuria	[178]
Acarbose		α -Glucosidase inhibitors: reduces HbA1c, glomerular basement membrane glycation, and nephropathic process in diabetic rat Prevented skin and tendon collagen fluorescence increase in db rats	[179] [180]

chosen as marker and culprit of disease and which pathway should be targeted for intervention strategies?

Second, a growing number of AGE inhibitors have in common the ability to chelate metals. These include pyridoxamine, OPB-9195, aminoguanidine, curcumin, and the newly discovered LR-90 which is a urea-deriv-

ative. The importance of metals is further supported by Miyata's data showing ACE and angiotensin II receptor inhibitors have chelating anti-AGE properties. If so, how exactly are the metals involved in mediating the complications? Are these chelators inhibiting Fenton chemistry? Do they act on metallo-enzymes like the

semicarbazide sensitive amine oxidase (SSAO) that has been implicated in diabetic complications? Do they help remove metals deposited in organs critically involved in diabetic complications?

Third, a striking finding is that several drugs, which act on pathways primarily unrelated to glycation (as, e.g., the reno-protective agents), are found to inhibit AGE formation in vivo and ameliorate tissue dysfunction. This may mean that AGEs are just markers of the disease, or conversely, they are critically involved in the disease process. In support of the latter argument, several histological studies implicate co-localization of AGE formation and RAGE expression within the tissue lesion. Thus, an important future goal will be to precisely identify the cellular targets of glycation.

Fourth, how does one reconcile the fact that compounds as disparate in their structure as pyridoxamine, aminoguanidine, ACE inhibitors, and thiamine/benfotiamine have the same beneficial properties on multiple systems affected by diabetes, such as nerve, retina, and kidney in the rat? While the data are not homogeneous, how does one reconcile the Brownlee “Unifying Hypothesis of Diabetic Complications” with the mechanism of AGE inhibitors, which themselves are unlikely to act on the transketolase pathway. One hypothesis would propose that any drug that prevents diabetic renal disease, and renal losses of thiamine, vitamin C or other physiological antioxidants, is likely to also prevent complications elsewhere. This, however, might be applicable only to the diabetic rat, since humans can develop retinal lesions in absence of nephropathy.

Finally, the Brownlee hypothesis has placed central emphasis on superoxide production and GAPDH inactivation as the source of all evils. Indeed, a growing number of antioxidant therapies offer support for this hypothesis in animals and humans, and explanations have been advanced to explain the failure of Vitamin E to efficiently prevent diabetic complications [104]. Does this mean AGE formation is doomed to be an epiphenomenon and treating superoxide production will do the trick? An answer to that question is premature. Long-term clinical studies, with compounds like thiamine/benfotiamine, and investigations into their anti-AGE/chelation role will be needed to address that question.

The ongoing efforts to decrease carbonyl stress imply the Maillard reaction in vivo is deleterious. While ample in vitro evidence supports the notion that the structure and biology of molecules and cells can be adversely affected by reactive carbonyl compounds and endproducts of the Maillard reaction, in vivo demonstration of cause and effect is much more difficult. The same statement applies to other forms of stochastic damage, such as oxidant damage, whereby oxidative modifications to biological molecules often do not exceed a few percent of total molecules. This may change with better analytical tools, such as those offered for proteome analysis. Taken

together, however, both forms of damage, oxidant- and carbonyl-stress, are likely to contribute to total molecular damage and cellular dysfunction that is associated with the progression of chronic diseases such as diabetes, end stage renal disease, and Alzheimer’s disease.

Acknowledgments

The author wishes to acknowledge continued support by the National Eye Institute, the National Institute on Aging, the Juvenile Diabetes Research Foundation International, and the American Diabetes Association.

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