Chapter 20
Intake of Advanced Glycation Endproducts: Role in the Development of Diabetic Complications

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Introduction

Diabetes is a very common cause of hospitalization and is listed as a diagnosis in 12% of hospital discharges. This diagnosis has a substantial impact on medical costs since hospital stay for diabetic patients is 1–3 days longer than non-diabetics. Cardiovascular complications are a common cause of hospitalizations for diabetic patients, and diabetes is a listed diagnosis in 29% of cardiac surgery patients.[1–4]

The underlying pathogenesis of cardiovascular disease in diabetes includes increased oxidant stress (OS) and inflammation. The manifestations of the diabetic state include metabolic abnormalities, increased OS, and a chronic inflammatory state, which serve to accentuate each other and result in a cycle of increasing organ damage. Since data obtained over the past few years support the new and novel concept that the inflammatory response seen in diabetes results from the cumulative and sustained pressure from oxidant stress, it is important to decrease OS from all potential sources.[4–9] The origin of oxidants in diabetes was initially considered to be entirely endogenous, but there is now an agreement that the environment, especially the diet, is a substantial source of oxidants.[10] This being the case it is important to understand the significant contribution of the diet to OS in diabetics and seek to reduce this input.

Advanced glycation endproducts (AGEs) are among the most commonly encountered oxidants in food.[10] They belong to a class of toxic oxidant molecules, also called glycoxidants. High levels of toxic oxidant AGEs are thought to underlie many of the complications of diabetes.[11,12] One of the ways by which AGEs induce these changes is by generating reactive oxidant species (ROS), which promote the formation of more AGEs, in a vicious action/reaction cycle, which progressively increases oxidative stress (OS) and the risk for both micro- and macrovascular disease (Fig. 20.1). Activation of receptors which recognize AGEs results in activation of downstream signaling pathways (including NFκB) which induce the production of pro-inflammatory cytokines and pro-angiogenic factors, leading to increased OS.[9]

AGE deposition in aging blood vessels and other tissues is well documented, and its role in the pathogenesis of cardiovascular disease and diabetic nephropathy is supported by the fact that structurally unrelated inhibitors of AGE formation provide protection against these diseases.[13–33] The number and breadth of these studies are an indication of the importance of blocking the formation and actions of AGEs. Understanding how AGEs interact with cells and tissues (Fig. 20.2), and how they can be prevented or treated, is an important challenge in the management of diabetes and its complications, across all medical disciplines. This chapter focuses on a non-pharmacological approach to the management of OS in diabetic patients.

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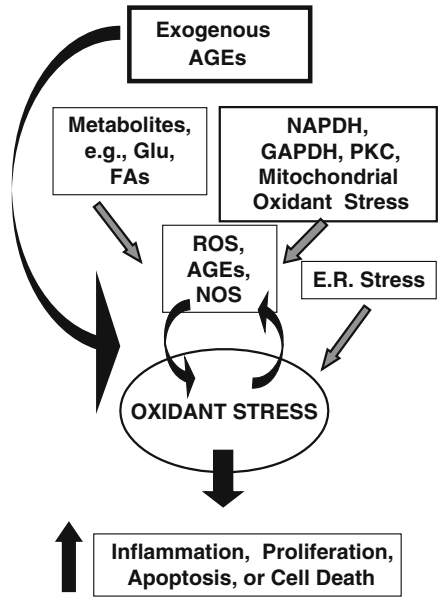

Fig. 20.1 Cellular/tissue injury in chronic disease is due to increased oxidant stress from exogenous and endogenous sources

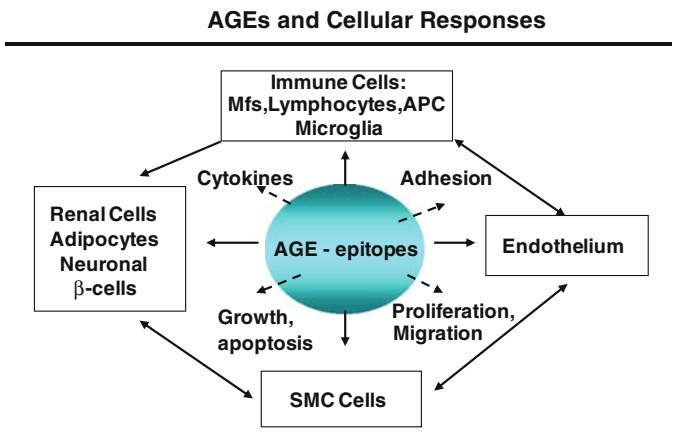

Fig. 20.2 AGEs interact with most cell types in the body, eliciting a variety of responses, depending on cell type and underlying state of OS

Toxic Oxidants (AGEs) and Cellular Responses

The glycoxidation pathway makes a fundamental contribution to OS, which underlies aging-mediated vascular disease and diabetes-related vascular and renal complications.[3,7,8,11,12,21,34–39] There are two sources of AGEs, *endogenous* and *exogenous* (*diet and smoking*[34]). It was previously thought that most AGEs are generated *endogenously* in diabetics by spontaneous reactions between the carbonyl groups of reducing sugars, ascorbate, and other carbohydrates and amino acids (lysine, arginine) or cystine-containing amino-peptides, nucleic acids, and lipids.[39,40–46] However, regular foods are now known to be a major source of AGEs in diabetics, as well as in non-diabetics.[47,48] The term AGEs, while often referring to non-reactive terminal products, such as $^{\epsilon}N$-carboxymethyllysine (CML) or pentosidine, also includes a broad range of reactive precursors, including 1- or

3-deoxyglucosone as well as methylglyoxal (MG) and their derivatives, a common one being hydroimidazolone, MG-H1. The latter are formed largely by non-oxidative mechanisms from triose phosphate intermediates during anaerobic glycolysis and are elevated in diabetes and aging.[49,50] Amine-containing lipids also form advanced lipoxidation endproducts (ALE), such as 4-hydroxy-nonenal or CML, and other lipid analogues.[11,30,40,41,49–53] Glycoxidation products of proteins and lipids (AGE/ALE) can accelerate the generation of reactive oxygen species (ROS), leading to oxidative and "carbonyl" stress.[38] Autoxidation of glucose is also accompanied by the generation of reactive oxygen species (ROS), such as superoxide radicals.[4,54] ROS enhance glycation and both mechanisms can promote atherogenesis and other complications related to diabetes or aging.[4] In fact, the direct cellular and tissue toxicity of certain AGEs, such as 1- or 3-deoxyglucosone or methylglyoxal derivatives, is now well established.[54]

Other non-glucose dependent AGE pathways involve activated white blood cells, i.e., neutrophils, monocytes, and macrophages. Activated white blood cells produce enzymes, including myeloperoxidase and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, causing AGE formation by oxidation of amino acids.[4,26,44,55–59] Cell activation by AGEs, i.e. via binding to the AGE receptor RAGE can also promote ROS and AGE formation via the NADPH oxidase pathway, the myeloperoxidase pathway or possibly through the nuclear protein amphoterin (also termed High Mobility Group Box 1) which can activate RAGE and toll-like receptor 4, and thus amplify AGE formation.[11,25,60–63]

Another mechanism of AGE formation is the aldose reductase-mediated polyol pathway. Glucose entering the polyol pathway may directly form AGEs via reactive intermediates, i.e., glyoxal, methyl-glyoxal, or 3-deoxyglucosone, as well as via depletion of NADPH or glutathione, which result in raised intracellular ROS.[56] These changes indirectly result in the further formation of AGEs.[61] Since these two mutually enhancing processes are tightly linked, interventions targeting one will inevitably have an impact on the other.

Sources of Toxic Oxidants (AGEs) in Diabetic Patients

Endogenous Sources: Hyperglycemia promotes metabolic activity and is the best known endogenous pathway by which the levels of AGEs and OS are increased in cells.[9,24,27,44,48,52,60,64–68] However, increased OS leads to the oxidation of other sugars and/or lipids, which create dicarbonyl compounds that use highly reactive carbonyl groups to bind amino acids and form AGEs.[24,52] While hyperglycemia may be one cause of increased OS in diabetic patients, as noted above, other pathways may increase the levels and activity of enzymes, such as NADPH oxidase, which induce AGE formation by oxidizing amino acids in both inflammatory and parenchymal cells.[27,59,64] MG may be formed as an intracellular toxic product of glycolysis. It is metabolized to lactate by enzymes (glyoxylase I and II) that require the non-enzymatic conjugation of glutathione with MG.[49,69] Thus, MG is neutralized when the levels of glutathione are normal, a state that may be compromised in the presence of high OS, such as diabetes.

AGE-modified moieties, especially long-lived proteins and lipids, as well as nucleic acids may be removed by proteolytic digestion, degraded to inactive molecules, and then excreted by the kidneys. However, AGE-derived cross-links are particularly resistant to degradation.[70–72] AGE crosslinks may contribute to the increased levels and delayed clearance of oxidized lipoproteins, the inactivation of immune components, and increased sensitivity of diabetic patients to drug toxicity, infection, and ischemia.[1–4,8,22,58,73–77] In particular, the toxic effects of AGEs on tissue structure and function may include the formation of chemical cross-links within and between connective tissue components or between these elements and plasma constituents, which can impair vasodilation or LDL removal (due to the retention of molecules trapped in the sub-endothelium and/or by impairing recognition and uptake of AGE-modified LDL by the LDL receptor).[20,26,29,52,53,67,78,79] Since AGE-LDL is a particular form of glycoxidized lipoprotein which is cross linked and is thus retained in the aortic wall, it recruits macrophages and promotes their conversion to foam cells and/or the accumulation of smooth muscle cells. As such it is thought to be an efficient pro-atherogenic substance.[5,12,13,25,26,28,40,45,46,49,58,63,80–87]

By creating cross-links between components of the extracellular matrix, and thereby changing their physical properties, AGEs affect both their distensibility and elasticity of arteries. Both type 1 diabetes and type 2 diabetes subjects have increased arterial disease, which is associated with increased cardiovascular mortality based on diastolic dysfunction,[73,88] increased pulse-wave velocity, decreased arterial compliance, and formation of aneurysms. Decreased vascular elasticity due to cross-links, which are resistant to degradation and the normal turnover of collagen and elastin, may play a larger role than previously appreciated.[5,28,38,72,89] This interpretation is reinforced by the observation that AGE inhibition restores these properties in rodents.[13,26,28]

Exogenous Sources: Many studies suggest that the modern diet is also a significant source of toxic oxidants (AGEs) in both man and experimental animals and contribute to the development of diabetes and diabetic complications.[65,90] While it has long been appreciated that AGEs are present in food,[67,68] they were not considered to be an important contaminant, since bioavailability studies showed that "only a small amount" of AGEs was taken into the body.[32,66,86,87,91] This point of view now has to be revised for two reasons. First, the cumulative oxidant properties of AGEs are well known. Second, the amount of oxidants in the modern diet has dramatically increased, particularly the amount of AGEs, as documented throughout this review. In part, this can be traced to the widespread consumption of red meat, processed foods, and soft drinks, as well as the near universal use of pasteurization and other forms of food preservation using heat.[86,87,9] Thus, the amount of toxic oxidants, including AGEs, that is present in the diet is now cause for concern, even if "only" a fraction is absorbed. This fact led to several recent studies of the bioavailability, kinetics, and renal elimination of dietary-derived AGE in healthy adults[90] and diabetic patients, with or without impaired renal function[66], as well as in animals.[32] We found that ~10% of the AGEs in the diet are absorbed, of which 2/3 is incorporated into tissues and turn over very slowly; the other ~1/3 is excreted via the kidneys.[32,66] Since the amount of AGEs in food in the last 50 years has increased, and the lifespan of the population has increased, this amount of absorption assumes greater importance clinically.

While increasedOS and the propensity to develop cardiovascular diseases have been in part attributed to excess fat in the diet, it is now recognized that even diets with a modest amount of lipids may contain high levels of oxidants, generated when proteins or lipids are mixed with reducing sugars and processed under elevated temperatures, as in standard cooking (Table 20.1).[65,92,93] Prime examples of the resultant dietary oxidants include reactive carbonyl compounds, advanced glycation (AGE), and lipoxidation endproducts (ALE), containing CML and MG derivatives in large concentrations.[44,51,94,95] The compounds formed in food are identical to those formed endogenously. Together with the exogenous AGEs the endogenous AGEs contribute to the chronic inflammatory response associated with the complications of aging and diabetes.[8,41,49,52,54,86,91,96]

Table 20.1 Thermally modulated AGE content in common foods

Regular diet (U/mg)		Low-AGE diet	
Beef: *broiled*	5367	*BOILED*	2000
Chicken: *broiled*	5245	*BOILED*	1011
Salmon: *broiled*	1348	*RAW*	502
Potato: *fried*	*1522*	*BOILED*	17

The levels of AGEs in most foods, not only red meats, depend largely on the method of cooking (see potato). Data are shown as CML immunoreactivity based on ELISA.

The data on the effect of cooking temperature on the formation of AGEs, and perhaps other toxic oxidants, are based on direct measurements of CML, MG, and MDA (a lipid peroxide product) in food prepared by different methods.[65,96] Grilling or frying meat increases the amounts of toxic oxidants significantly more than meat prepared by boiling. Frying results in a vast increase in AGEs, compared to boiling. For instance, so-called French fries contain 100-fold more AGEs, compared to boiled potatoes. This results from the addition of lipids to carbohydrates and proteins under conditions of dry heat. The amount of AGEs in the diet largely depends on

the temperature during cooking and the degree of hydration (i.e., the amount of water present). For instance, cooking fish or chicken in water results in 4–6 times less AGEs, compared to cooking these foods in the absence of water. In addition, the amount of AGEs in cooked food is directly proportional to the fat content.

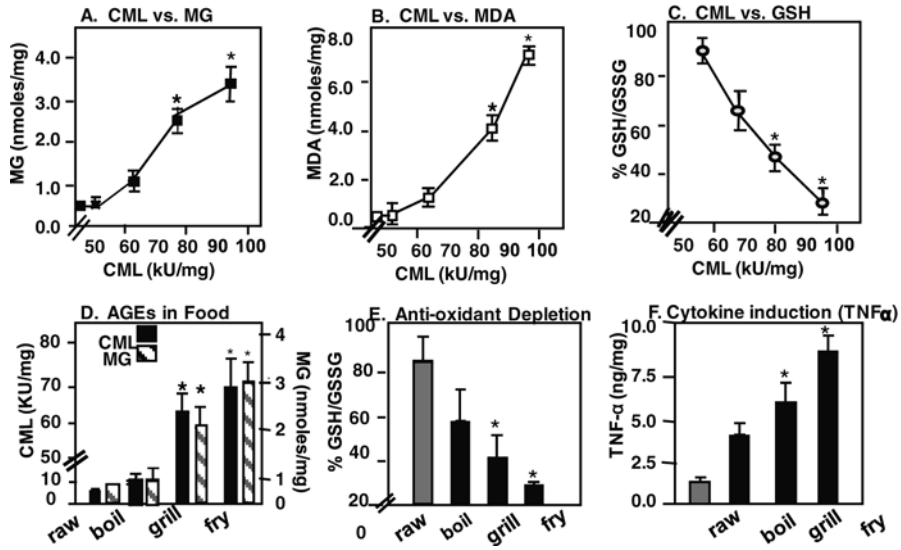

Fig. 20.3 Pro-oxidant and pro-inflammatory activity of food AGEs. **(a)** Correlation between food-derived protein AGEs (CML and MG-derivatives); **(b)** between CML and malondialdehyde (MDA, a lipid oxidation product); **(c)** addition of different amounts of food extracts to human endothelial cell (EC) cultures. CML levels inversely correlate with intracellular levels of glutathione, indicating that food AGEs induce cellular oxidant stress; **(d)** the amount of AGEs (CML and MG) in food (red meat) varies with method of cooking; **(e)** endothelial cell levels of anti-oxidant GSH vary, following the addition of food extracts prepared by different methods; **(f)** levels of the inflammatory cytokine, TNFα in EC, also vary with method of cooking following the addition of identical amounts of food extracts

The question is often raised as to the levels of specific AGEs in food. Therefore, we extracted red meat, prepared by different cooking methods and found that the amount of CML correlated with MG (Fig. 20.3a), that the amount of CML and MG correlated with the levels of MDA (an oxidized lipid) (Fig. 20.3b). In addition, both grilled and fried meat contained increased amount of both CML and MG, compared to either raw or boiled meat (Fig. 20.3c). While a relationship between the AGE content of foods, OS, and diabetic complications had previously been found, we directly tested whether food AGEs have biological activity, by preparing soluble extracts of these meat preparations, and then adding them to endothelial cells in vitro. The amount of intracellular anti-oxidants (i.e., glutathione) negatively correlated with the amount of AGEs in the preparations (Fig. 20.3d). The extracts also caused a decrease in anti-oxidant levels (GSH/GSSG ratio) (Fig. 20.3e), and promoted the release of TNFα (Fig. 20.3f). These data are of particular importance because they clearly show that AGEs are present in the food, and that their levels depend on the method of preparation. In addition, the data show that the AGEs present in the food are biologically active, since they directly cause increased OS and depletion of anti-oxidant defenses. Thus, both the type of food and the means by which it is prepared is critical in determining the total amount of oxidant AGEs present in food. We recently obtained direct proof that AGEs in the diet cause increased OS and raise the levels of circulating AGEs in mice. This was accomplished by adding a well-characterized AGE (MG) to a diet prepared with low heat, so that the initial content of AGEs in the food was reduced and that any changes observed would be directly related to the added AGEs. Examination of the mice from birth to 6 months of age revealed a linear rise in serum AGEs and increased OS levels in the mice fed the diet supplemented with MG (Fig. 20.4).[11] This critical finding provided direct proof of the ability of AGEs ingested via the diet to raise AGEs in the blood and lead to increased OS.

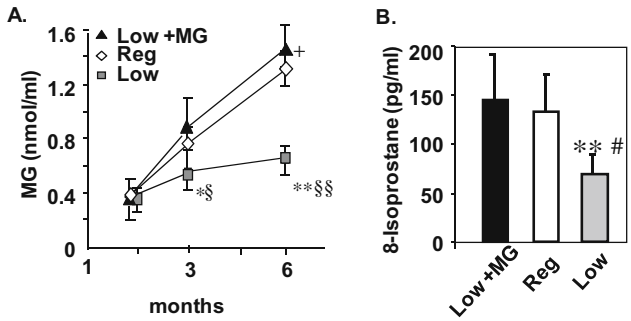

Fig. 20.4 Methyl-glyoxal (MG) derivatives in diet increase serum AGEs and OS. Pups from dams fed a low-AGE diet during gestation and nursing were pair-fed a low diet, a Low+MG, or a regular (Reg) diet at weaning. **(a)** Serum MG levels from weaning to 6 months of age. Low vs. Reg, $^{*}p<0.05$, $^{**}p<0.001$; Low vs. Low+MG, §$p<0.01$, §§$p<0.001$; Low+MG vs. Reg, + $p<0.05$. **(b)** 8-Isoprostane levels. Low vs. Low+MG, $^{**}p<0.01$; Low vs. Reg, #$p<0.01$

Reduction of Toxic Oxidants (AGEs) in Mice

Recent evidence indicates that the cycle of increased levels of AGEs and elevated OS can be interrupted by reducing glycation, either in the body (endogenous) or in the food (exogenous). While anti-oxidant or anti-AGE agents may prevent the formation of AGEs, a non-pharmaceutical intervention such as reducing the amount of AGEs in the food by simply reducing the heat applied may be the most cost-efficient, effective, and widely applicable way of decreasing exposure to oxidants. The reduction of dietary oxidants (AGEs) prevents many of the diabetic complications in several animal models and reinforces the need to continue the search for optimal interventions in the clinical setting.

We have previously shown that sustained parenteral administration of AGEs, or feeding a high-AGE diet, reproduces many of the complications associated with diabetes.[48,66,68,78,97–100] The accumulation of advanced glycation endproducts (AGEs) begins in early life and progressively increases with time.[101] In this context, avoidance of early exposure to AGEs may determine susceptibility to certain diseases, including type 1 and type 2 diabetes. This concept was examined in non-obese diabetic (NOD) mice which have a high propensity to develop type 1 DM (~80% of young NOD females develop diabetes), which is attributed to an autoimmune process (Fig. 20.5, left upper panel).[98] We found that when these mice were exposed to a low-AGE diet the structure of their islets remained normal (Fig. 20.5, right upper panel), and the propensity to develop diabetes was sharply reduced. In fact, this "loss of susceptibility" could be traced to the maternal diet, since offspring of dams maintained on a low-AGE diet during gestation had a greatly reduced incidence of diabetes in the F1 generation, and diabetes was nearly absent in the F2 progeny.[98] The absence of diabetes resulted from preservation of intact pancreatic islets, the loss of several abnormalities in T lymphocytes, and the maintenance of low levels of OS (Fig. 20.6). This phenomenon was epigenetic in nature, since the progeny became diabetic, if they were returned to regular mouse chow, which has a high AGE content, similar to that of the modern human diet.[10,102]

We also tested other models of Type 2 DM, including db/db+/+ and high fat diet-induced type 2 DM in normal C57B6 mice, and found that both diabetes and complications were reduced.[22,28,48,97] Thus, the development of diabetes was blocked in both genetic and non-genetic models by reducing the intake of AGEs in the diet. A conclusion reached from these genetically and pathogenetically diverse models of diabetes was that the maintenance of lower levels of OS, by reducing the intake of AGEs (oxidants) in the diet may be a critical factor in the preservation of normal beta-cell function. Furthermore, the prevention of diabetes was effective in both those with a genetic background prone to the development of diabetes (type 1 and type 2), and the prevention of diabetes by diabetogenic interventions in otherwise normal strains. Furthermore, the data show that the levels of OS may be partly driven by the levels of toxic oxidants in the diet, and showed that the maternal diet was also an important source of oxidant AGEs in the fetus. Finally, the data show that these changes may be entirely preventable by

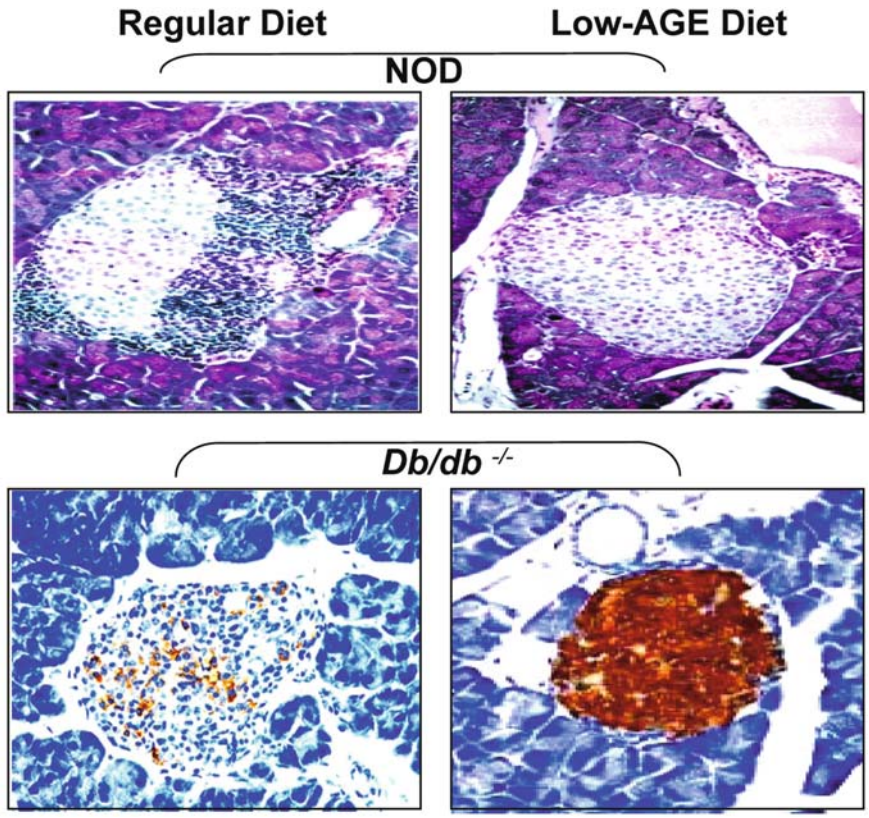

Fig. 20.5 Effect of dietary AGEs on islet morphology. **(a)** Non-diabetic F1 NOD mice, and **(b)** db/db +/+ mice were exposed to either regular (*left*) or low-AGE diet (*right panels*). NOD mouse pancreatic tissues stained by H&E (**a**) showed absence of the severe mononuclear cell infiltration after a low-AGE diet (for >12 mos). Islets of age-matched db/db+/+ mice (**b**), after L-AGE (×5 mos), stained for insulin showed intact insulin production compared to those on regular diet. Mag. ×400

modifying the preparation of the diet, without altering either the calorie content or particular nutrients. The latter point may be critical to normal fetal and post-natal development of diabetic mothers, and possibly even those with gestational diabetes. It also raises the question as to whether this may be important to the prevention of diabetes itself. Nonetheless, the conclusions from these initial studies are that consumption of diets that contain high amounts of AGEs can promote OS in apparently normal adults and that this can begin in the unborn fetus or in childhood. For instance, we and others found that infant formulae contain 28–389 fold (median=70) higher levels of toxic oxidant AGEs than maternal milk.[65,103] The CML in the infant formulae was absorbed and appeared in the urine.[103] Finally, the effect of oxidants in the diet may have other untoward effects as they may neutralize anti-oxidants in normal food, or anti-oxidants added for nutritional or pharmacologic purposes, and many vitamins are altered by oxidants or heat.[96]

Reduction of these toxic dietary AGE oxidants in diabetic mice attenuated diabetic vascular and kidney disease, acute vascular injury, and promoted wound healing.[48,67,68,75,76,104] Diabetic mice fed with standard laboratory diets (which have a high content of AGEs) developed vascular and renal lesions.[105] However, their age-matched cohorts fed with low-AGE isocaloric diets remain largely free of these changes, despite persistence of hyperlipidemia and diabetes.[47,54,67,68,75,76,97,106] A low-AGE diet also led to significant suppression of atherosclerotic plaque formation at the aortic root of diabetic/hyperlipidemic ($ApoE^{-/-}$) mice (Fig. 20.7).[67] In addition, a low-AGE diet provided protection of post-injury arterial inflammation and re-stenosis in $ApoE^{-/-}$ mice despite sustained hyperlipidemia.[78] As noted above, feeding diabetic mice a diet with lower levels of toxic

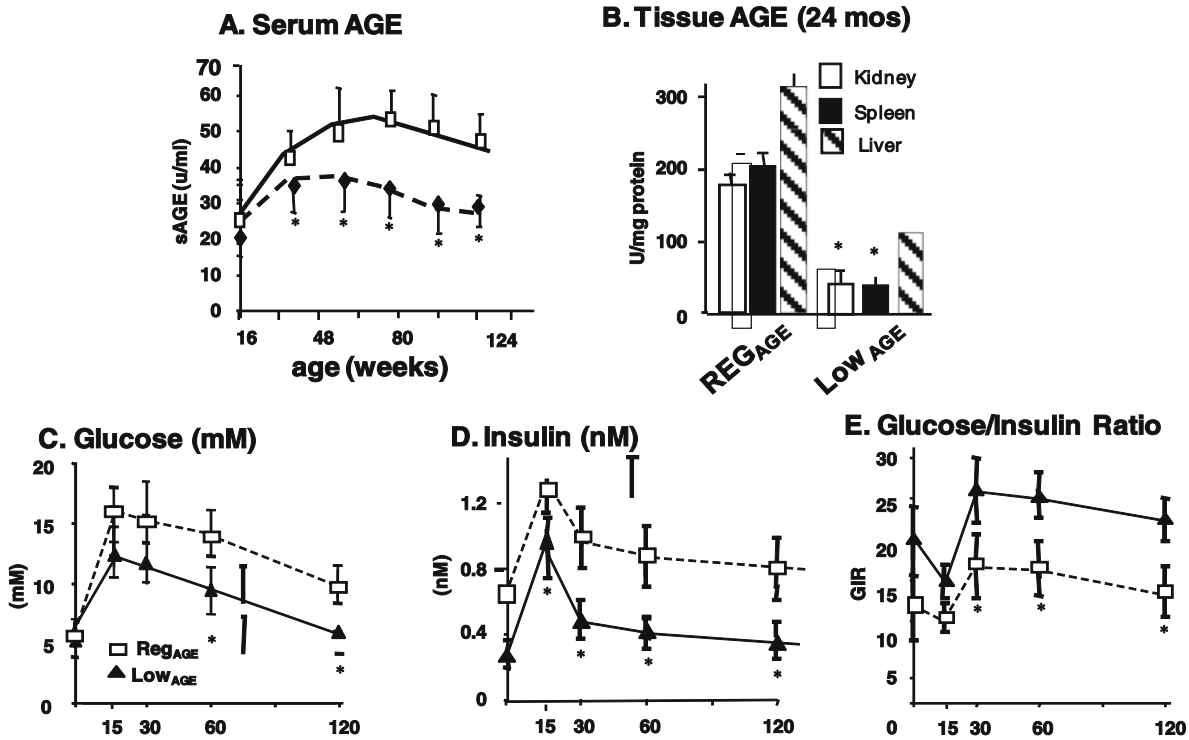

Fig. 20.6 A low-AGE diet suppresses age-associated diabetes. **a, b**: Serum and tissue AGE levels are reduced in normal mice pair-fed a low-AGE diet for life. Reg-AGE (*open symbols*), low-AGE diet (*closed symbols*). **(b)** Kidney, spleen, and liver AGE at 24 mos of age (n=8/group). **c–e**: Changes in glucose, insulin response to IGTT and GIR at 4 and 24 mos (n=6/group). Note: none of the low-AGE fed mice became diabetic by 3 years of age. All Reg-fed mice were diabetic at various age points before death (n=20/group)

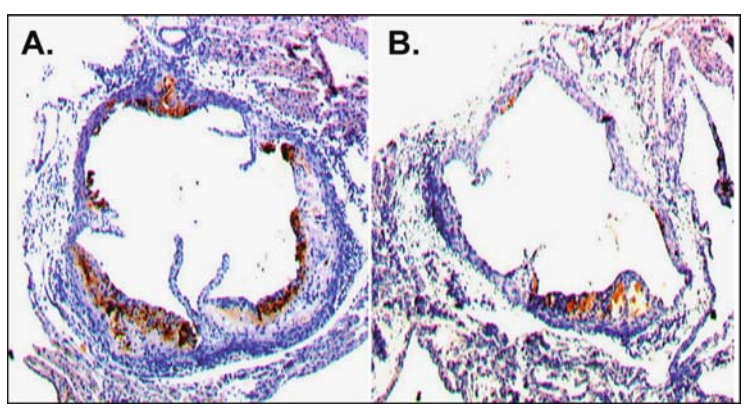

Fig. 20.7 Atheromatous lesion and inflammatory infiltrate are both inhibited in hypercholesterolemic mice (ApoE–/–) after feeding on a low-AGE diet for 5 mos. Aortic root sections from ApoE–/– mice fed a regular diet **(a)** or an AGE-restricted diet **(b)** are stained by a macrophage surface antigen-specific antibody (MOMA). Note the markedly decreased inflammatory cell infiltrate (Mag. ×200)

oxidants (AGEs) prevented the structural and functional changes of diabetic nephropathy in genetic models of type 1 and type 2 diabetes.[77,107] These studies support the proposed synergism between the levels of toxic oxidants in exogenous (diet) and the endogenously derived OS burden in diabetes. In addition, AGEs are antigenic, a property which may contribute to inflammation and atherosclerosis.[77,101]

In normally aging mice, long-term reduction in the intake of exogenous oxidants, without altering caloric intake, ameliorated OS, insulin resistance, and the incidence of diabetes, as well as significantly extending lifespan.[108] These diverse positive effects were attributed to the functional preservation of AGER1, anti-oxidant reserves, and the amelioration of aging-induced OS-response genes, including $p66^{Shc}$ and FOXO-1.[57,108,109]

Interventions which reduce the formation of ROS, such as calorie restriction, have long been known to promote lifespan extension.[43,110–112] Since calorie restriction is accomplished by reducing food intake, this effectively reduces the intake of AGEs as an obligate concomitant. We recently found that the well-known beneficial effects of a calorie-restricted diet could be due to the 40% reduction in food intake, primarily because it also restricts the intake of AGEs. In fact, when the AGE content of a calorie-restricted diet was increased, the benefits of calorie restriction on reducing OS, CVD, and CKD were lost.[12] We have shown that a long-term reduction of the intake of toxic AGEs decreases ROS, the expression of OS-response genes, cardiovascular and renal disease, and leads to an increased life span.[76,80,83,102,108,113] Furthermore, these effects were independent of calorie intake.

These experimental studies suggest that while factors such as heredity or nutrient intake play a large role in diabetes, the level of oxidants in the diet may be a key link to both the induction of diabetes and diabetic complications. AGEs may be a major contributor to these lesions, since they cause a wide spectrum of vascular abnormalities, including basement membrane thickening and endothelial injury, resulting in increased vascular permeability, a pro-thrombotic state, and decreased blood flow; all of which are traits of microvascular disease affecting the retina, kidneys, and peripheral nerves.[9,114–119] The specific role that AGEs play in causing microvascular disease is well documented in the kidney glomeruli.[100] However, the role of exogenous AGEs in retinopathy or neuropathy is not yet defined.

Elevated AGEs are also associated with macrovascular abnormalities in diabetic subjects, including coronary atherosclerosis.[14,27,73,120] AGEs decrease both endothelial cell nitric oxide levels and activity, by inhibiting endothelial nitric oxide synthase and prostacyclin or by quenching NO^{-}.[38,80,120] These changes, in conjunction with the effects attributed to protein kinase C activation, can further increase vasoconstriction.[117,118,121,122] AGEs increase expression of angiotensin II and endothelin in vascular smooth muscle cells, which also contribute to vasoconstriction, enhancing pro-inflammatory processes, and mitogenesis.[121,122] Activation of NFκB and activator protein-1 (AP-1) and other transcription factors by AGEs may lead to increased expression of adhesion molecules (e.g., ICAM-1, VCAM-1) and plasminogen activator inhibitor 1 (PAI-1), and contribute to chronic vascular dysfunction.[117]

In summary, these studies on diabetes and its complications introduce the view that the accumulation of advanced glycation endproducts (AGEs) may begin in utero (during the fetal period), continue in childhood, and progressively increases with normal aging. If the intake of AGEs is high, i.e., similar to that in the average modern diet, the baseline level of OS may be higher than "normal" and the rate of rise would be accelerated. This would reduce anti-oxidant defenses and result in an inflammatory state that may significantly alter innate immune responses to inflammatory processes. The end result could be the earlier onset of obesity and type 1 or type 2 diabetes, accompanied by macrovascular (atherosclerosis) and microvascular (kidney, retinal) diseases.[4,117,118]

Cellular Receptors that Recognize Toxic AGE Oxidants

Among the receptors that bind AGEs, AGER1 is the most extensively studied receptor involved in AGE endocytosis and processing (Fig. 20.8).[52,55,123,124] AGER1 is the principal receptor mediating the removal of excess extracellular AGEs, from both endogenous and exogenous origin. AGER1 is a ã50 kDa integral surface membrane protein that inhibits AGE-induced OS (as depicted in Fig. 20.9), via inhibition of RAGE, MAPK and Ras activation and phosphorylation of EGFR and ERK;[26] inhibition of phosphorylation of ser-36 of $p66^{Shc}$, a key pro-apoptotic adaptor protein involved in the phosphorylation and inactivation of members of the FOXO pathway,[109] which normally enhances anti-oxidants such as MnSOD and increases resistance to OS; and also via reduction of AGE-induced mitochondrial OS and mitochondrial injury in endothelial cells. Increased expression of the AGER1 transgene blocks ROS in vitro[55,57] and prevents vascular and kidney injury in mice.[137] These

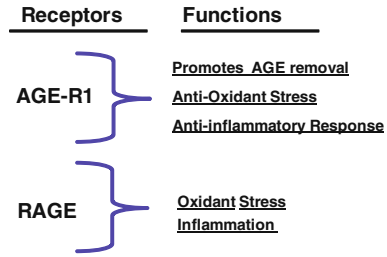

Fig. 20.8 Major AGE receptors and functions: AGER1 is localized on cell surface, mitochondria, and ER. RAGE is principally a cell surface receptor

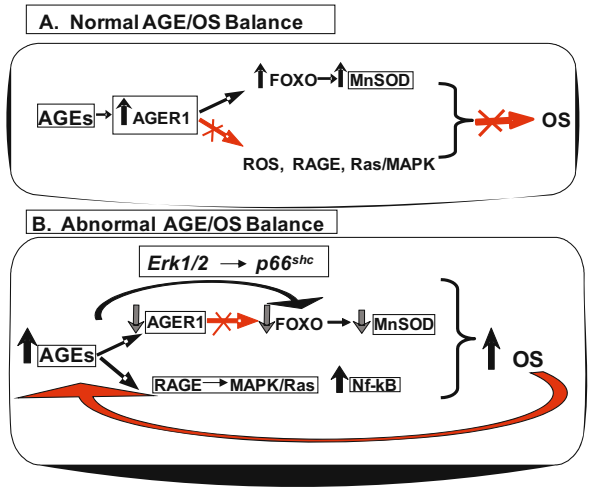

Fig. 20.9 Schematic representation of putative AGE-receptor interactions and their contribution to oxidant balance. **(a)** In the normal state AGER1 can suppress AGE- and ROS-dependent increased RAGE/Ras/MAPK and NFκB activity, as well as p66shc-dependent inactivation of FOXO. These oppose the formation of excess ROS from AGEs. **(b)** Chronic, poorly controlled diabetes, and/or sustained influxes of exogenous oxidants can reduce levels of AGER1 and its anti-OS actions, which will further increase ROS/AGEs

observations point to AGER1 as a potentially important multifunctional component of the anti-OS defense system. The levels of AGER1 are directly correlated with the levels of circulating AGEs in healthy adults, but they are reduced in those with chronically high levels of AGEs, such as found in patients with diabetes.[123,125] Our studies in both human subjects and experimental models show that AGEs and AGE receptors are a part of normal homeostasis.[10,62,102,125] When the levels of AGEs exceed the capacity of the body to detoxify them, they accumulate and raise the levels of OS. If the levels of AGER1 are sufficient, AGER1 actively counteracts this increase and maintains the normal oxidant balance. Elevated levels of AGEs, combined with low AGER1 expression, are a signal that the capacity for removal of AGEs has been exceeded, i.e., in diabetes and chronic kidney disease.[123] The sustained nature of the responses induced by AGEs, coupled with the continued intake of excess dietary oxidants in the Western diet, may constitute the basis for the progressive depletion of innate defenses in diabetes. These changes may include, or be due to, reduced levels of AGER1 (Fig. 20.9). Decreased AGER1 function, allowing AGEs to accumulate, can result in depletion of anti-oxidants and promote high OS. Thus, the maintenance of normal AGER1 levels may be critical to redox homeostasis.

Among the AGE receptors that trigger an inflammatory response, the best known is a multi-ligand protein, RAGE and its variants.[66,81] HMGB1 (amphoterin) has recently been identified as a major RAGE ligand.[60] Activated RAGE leads to the induction of ROS and an inflammatory response.[126–128] RAGE does not participate in AGE removal, but its extracellular domain (sRAGE) is present in circulation. Since, blood levels of sRAGE inversely correlate with the OS state, it has been suggested that sRAGE may bind and assist in clearing AGEs

and other oxidants.[129–132] While sRAGE levels have generally been found to be low in diabetic individuals, some have found that they are increased in diabetic patients with coronary vascular disease.[23,82]

Other entities that bind AGEs include AGER2, which is an 80–90 kDa protein possibly involved in early AGE signaling, and AGER3, a 30–35 kDa protein, which may make a contribution to both AGE removal and cell activation.[52,107] AGEs are also bound by ScR-II, CD36, lysozyme and other defensins.[133] While several AGE-receptor gene polymorphisms have been identified, none have been strongly linked to diabetes.[134] Toll-like receptor 4 also binds RAGE ligands (HMGB-1), although it is not known if it also binds AGEs.[60]

Reduction of Exposure to Toxic Oxidants (AGEs) in Humans

Several epidemiologic studies show that a rise in OS among clinically normal subjects may be important in the pathogenesis of insulin resistance and other metabolic diseases.[4,6,7,39,135] We found that the AGE content of regular meals or meals with a low AGE content given to normal subjects is readily reflected in the serum levels of AGEs and in the amount of AGEs excreted in the urine.[66,86,87] In addition, we recently found that serum AGEs correlated with oxidant stress in a cross-sectional study in normal non-diabetic adults of different ages (Fig. 20.10a).[88,125] Those who ate a diet with a low AGE content, had lower levels of markers of inflammation and oxidant stress, i.e., hsCRP, TNFα, and fibrinogen, whereas these levels were much higher in those consuming a diet with a higher AGE content. Of interest, a significant association was noted between serum AGE and HOMA, an indicator of insulin resistance.[88,125,136] The associations between serum AGEs, 8-isoprostane, and HOMA suggest that sAGE may be an important contributor to OS, prior to the onset of diabetes. Hyperglycemia has been assumed to be the source of the increased AGE levels found in diabetes, a condition associated with systemic OS.[3,64] None of the normal subjects in this study were hyperglycemic in the fasting state. Therefore, their higher fasting levels of serum AGE could not be explained on the basis of glycemia. This data provided strong additional evidence that the intake of AGEs in the diet very likely contributes to the increased levels of serum AGEs, and the subsequent increased systemic OS.

These data are consistent with previous observations in patients with diabetes and chronic kidney disease.[47,106] The use of CML-like AGEs as surrogates for other oxidants or carbonyl-rich products in the diet seems justified by the correspondence between the levels of CML-like AGEs in the diet and CML or MG in the circulation, as well as the highly significant correlation (r=0.7, p=0.0001) between sAGEs and the endogenous lipid peroxidation derivatives, 8-isoprostanes.[125]

Thus, the intake of AGEs contained in the usual adult diet may deliver excessive amounts of AGE oxidants to the body, which may promote cumulative changes in oxidant homeostasis with time. These changes may presage the emergence of metabolic and cardiovascular disturbances, heretofore believed to be part of the normal aging

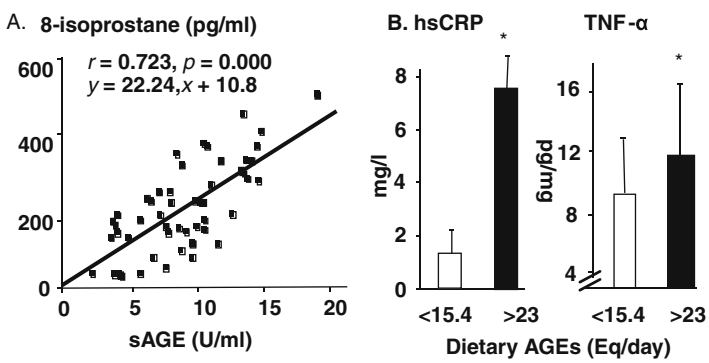

Fig. 20.10 **a.** Serum AGEs (CML) correlate with plasma lipid peroxidation products (8-isoprostanes) in non-diabetic subjects. **b**. Levels of inflammatory factors, hsCRP and TNFα, are elevated in healthy subjects consuming AGE-rich diets (>23 Eq/d), but not in those consuming a low-AGE diets (<15.4 Eq/d), $^{*}p$=0.025. Note: high-AGE consumers had a BMI >33

process.[4,6–8,35,37,88,137,138] The above data suggest that reduction of the intake of AGEs via the diet may inhibit the accumulation of toxic AGEs, increased oxidant stress, and slow or reduce the emergence of the diabetes and diabetes-related complications. Indeed, a reduced intake of AGEs via the diet in diabetic persons was associated with lowered serum AGEs, OS, and inflammatory markers, e.g., TNFα, hsCRP, and VCAM-1.[see references in 90,102] These findings led to the hypothesis that dietary AGEs, together with those made endogenously, could promote an excessive systemic glycoxidant burden, oxidant stress, and cell activation, all of which would enhance the "vulnerability" of tissues to injury. This is especially true of diabetic patients, since they have elevated levels of AGEs and OS, accompanied by decreased anti-oxidant reserves, at baseline. It is imperative that studies be undertaken in diabetic patients to determine if this approach would also improve diabetic complications.

The Link Between Toxic AGEs and Oxidant Stress (OS)

For the last few decades, the focus has been on the oxidation and glycoxidation products generated by hyperglycemia. Extended studies, including those in type 1 and type 2 diabetes, have lent support to this view, at least in part.[89] However, very recently two other long-term trials raised serious doubts about the utility of strict normalization of glycemic levels in reducing diabetic complications.[1,2] These studies may redirect our thinking to embrace a more diverse realm of controls of risk factors in diabetic patients, perhaps with a focus on OS. It may include prospective on oxidants generated by other mechanisms, including the contribution of dietary AGEs as toxic oxidants. The evidence is increasing that the generation of OS is the basis of many diabetes complications and that it can be lowered by reducing the levels of toxic oxidants from either exogenous or endogenous sources.[64,66,106] The fact that reduction of AGE intake prevents type 1 and type 2 diabetes, as well as diabetic complications in different animal models, noted above corroborates the importance of external sources of AGEs in their pathogenesis and provides a strong impetus for novel clinical interventions.

Diabetes increases the incidence of large vessel diseases such as strokes, myocardial infarction, aortic atherosclerosis, aneurysms, and limb ischemia requiring amputation.[1–4,73] These complications often have catastrophic effects in the short term. Based on the studies in animals discussed above, it is reasonable to postulate that a high dietary intake of toxic AGEs in the usual Western diet may be a major contributor to atherosclerotic lesion severity in the presence of high circulating glucose levels in diabetic patients, an effect that may be preventable by reducing the amount of toxic oxidant AGEs in the diet. Clinical studies to examine this point are now in progress.

Acute Effects of Dietary AGEs in Normal and Diabetic Subjects

After normal subjects are given an AGE-rich meal, the levels of serum AGEs peak within 4–6 h, and return to baseline within 12 h (Fig. 20.11a, solid lines, right panel) and the urinary levels follow.[66] In these individuals, ∼70% of the absorbed oxidants in the diet remain in cells and tissues, and 30% is excreted in the urine.[66] These data point to the unrecognized potential for toxic oxidants from the diet to progressively accumulate over time, particularly in persons consuming a high-AGE diet. As discussed above, even persons who are "normal" in all other aspects can develop high levels of serum AGEs. Importantly, this suggests that the tissue levels of toxic oxidants may progress with time, if the intake remains high.

Since diabetic patients often have long-term elevation of OS and a concomitant decrease in anti-oxidant reserves, it is logical that they would have a decreased ability to deal with a high dietary load of oxidant AGEs. In fact, this is the case as shown by a study of diabetics with either microalbuminuria or overt proteinuria. Long before any clinical or laboratory signs of renal disease are apparent, diabetic patients with microalbuminuria have an impaired ability to excrete the increased load of toxic AGEs presented within the normal diet. Namely, after a high-AGE meal, diabetic patients with microalbuminuria and an estimated GFR in the normal range (Fig. 20.11b, broken line) had sharply increased levels of serum AGEs, and that these levels remained elevated for more than 30 hours. The far smaller peak of AGEs in the urine fell only when the serum AGE levels returned

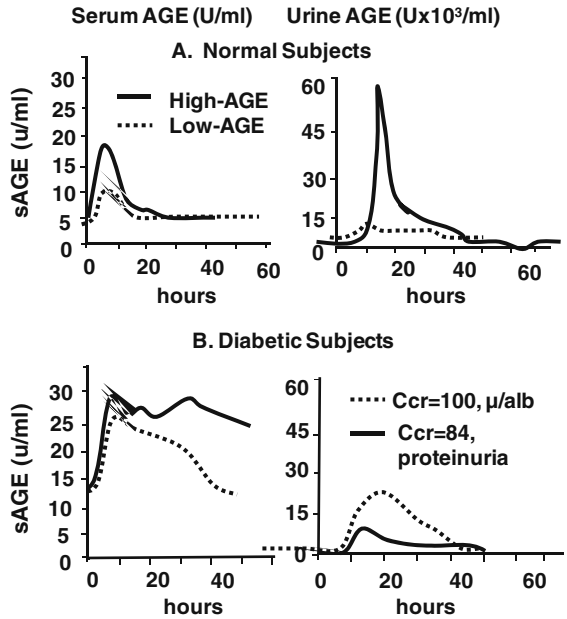

Fig. 20.11 **(a)** Serum and urine AGE kinetics in normal adults after a regular meal (high AGE) or a meal with 50% lower AGEs (low-AGE). **(b)** Diabetic patients with different degrees of diabetic nephropathy were fed the same amount of diet AGEs (regular meal): higher baseline and prolonged elevation of serum AGE levels corresponded to significantly reduced urine AGE levels in those with a lower GFR (*solid lines*). Note, neither group had severe disease but AGE excretion was markedly reduced in both groups

to baseline levels. On the other hand, patients with overt proteinuria, but with only a modestly decreased GFR (20%) (Fig. 20.11b, solid line), had a marked prolongation of the high levels of serum AGEs, while the amount excreted in the urine was significantly decreased. Note that since serum creatinine levels were in the normal range at this level of GFR, these patients would have been considered to have normal renal function by most physicians. Thus, diabetic patients with kidney lesions, as evidenced by albuminuria of any level, appear to have a marked impairment in their ability to handle oxidants presented in the diet. Importantly, this occurs long before physicians generally consider them to have "kidney damage." In addition, these data suggest that the kidney is a major site for the handling of toxic oxidants, and current clinical measures of kidney damage do not accurately measure critical abnormalities in this aspect of kidney function. Since toxic AGEs within the diet directly contribute to elevated OS, and to diabetic complications, the data provided by the studies presented above suggest that it is critical to lower the exposure of patients with diabetes to AGEs within the diet prior to the time they develop clinical evidence of renal disease. This intervention should be in addition to inhibiting the further increase of endogenous AGEs in the body due to elevated OS.

Conclusions

Oxidant stress (OS) is now recognized to be one of the major factors predisposing to diabetic complications. The sources of OS include both the intake of toxic oxidants (AGEs) and their formation intracellularly as part of the hypermetabolic state and other sources of OS. This chapter emphasizes the fact that the intake of toxic oxidants contained in food is a major factor in the pathogenesis of diabetic complications. Furthermore, we show that the amount of toxic oxidants present within the food can be very simply modified by changing the methods by which meals are prepared. The results of decreasing the intake of toxic oxidants by diabetic patients include a decrease in markers of inflammation, vascular injury, and OS. In animals, this is associated with a sharp reduction in the severity of established diabetic complications, the presence of an inflammatory state, vascular

injury, insulin resistance, and diabetes. Since reducing the level of toxic oxidants within the diet does not add to health care costs, does not change nutrient intake, and promises to substantially reduce diabetic complications, this intervention should be strongly considered by both physicians and patients in the day-to-day management of diabetic complications.

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