MÆDICA - a Journal of Clinical Medicine

STATE-OF-THE-ART

# Advanced glycation endproducts (AGE) and their role in the pathogenesis of chronic complications of diabetes mellitus

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*ABSTRACT*

*Diabetes mellitus is a chronic disorder characterized by hyperglycemia and long-term complications affecting the blood vessels, eyes, nerves, and kidneys. The exact underlying mechanisms are still under investigation, but increased glycation, especially of proteins, and the subsequent accumulation of advanced glycation endproducts (AGE) seem to have an important role in this process. AGE can cause structural and functional changes of proteins, lipids and nucleic acids, and lead, through their interaction with specific receptors (RAGE), to an activation of inflammation and increased oxidative stress.*

*In recent years, increasing attention has been paid to exogenous AGE (from diet and cigarette smoke). Diet is the most important source of exogenous AGE, with the highest content found in heat-treated foods, especially lipid- and protein-rich foods, typical of Western diets. The AGE content of ingested foods depends on nutrient composition, temperature, method and duration of heat application. It has become more and more evident that dietary AGE represent an important source of circulating and tissue AGE and manifest similar pathogenic effects to their endogenous counterparts. Reducing the dietary ingestion of AGE could represent a novel attractive alternative for preventing the micro- and macrovascular complications of diabetes mellitus.*

**Key words:** diabetes mellitus, glycation, AGE, chronic complications, diet

Diabetes mellitus (DM) is a chronic disease characterized by hyperglycemia, resulting from absolute or relative insulin deficiency. Hyperglycemia has a key role in the pathogenesis of these complications, but the exact underlying mechanisms are still insufficiently explained. One of the mechanisms that have been intensively studied is increased glycation, especially of proteins, and the subsequent accumulation of advanced glycation endproducts (AGE). ❑

## 1. FORMATION OF AGES

The process which leads to the formation of AGE, also known as the Maillard reaction, was described in the early 1900's, when it was observed that amino-acids heated in the presence of reducing sugars developed a characteristic yellow-brown colour (1).

AGE, best known in the context of DM as the derivatives of abnormal glucose or mitochondrial

superoxide generation (2) are a heterogenous group of molecules which result from the non-enzymatic reaction between a carbonyl group from reducing sugars (as glucose) and a free amino-group of proteins, lipids and nucleic acids. The first step of this reaction is freely reversible and leads to the formation of a Schiff base. This reaction occurs over a period of hours; the Schiff base then rearranges (over a period of days) to form a more stable ketoamine or Amadori product and this reaction is practically irreversible (3).

These glycated compounds can undergo further reactions, generating dicarbonyl intermediates (eg. 3-deoxyglucosones: 3-DG, methylglyoxal: MG) and finally poorly characterized structures, advanced glycation end-products (Figure 1) (3). These glycation reactions occur over a period of weeks and were therefore believed to affect predominantly long-lived proteins, but shorter lived compounds such as lipid constituents, nucleic acids and even intracellular growth factors are also affected (4).

The spontaneous reaction of protein glycation depends on the degree and duration of hyperglycemia and is concentration-dependent in the early rather than in the later stages of the Maillard reaction, and is therefore enhanced in diabetes (5). Not all AGEs have been identified and the exact mechanisms underlying their formation remain unclear (3).

AGEs can be classified into three categories (3):

1. fluorescent cross-linking AGEs: e.g. pentosidine, crossline
2. non-fluorescent cross-linking AGEs: e.g. glyoxal lysine dimer (GOLD), methylglyoxal lysine dimer (MOLD)
3. non-cross linking AGEs: e.g. pyrraline, N-carboxymethyllysine (CML)

If oxidation accompanies glycation, the resulting products are also known as glycoxidation products, for example pentosidine and CML (6).

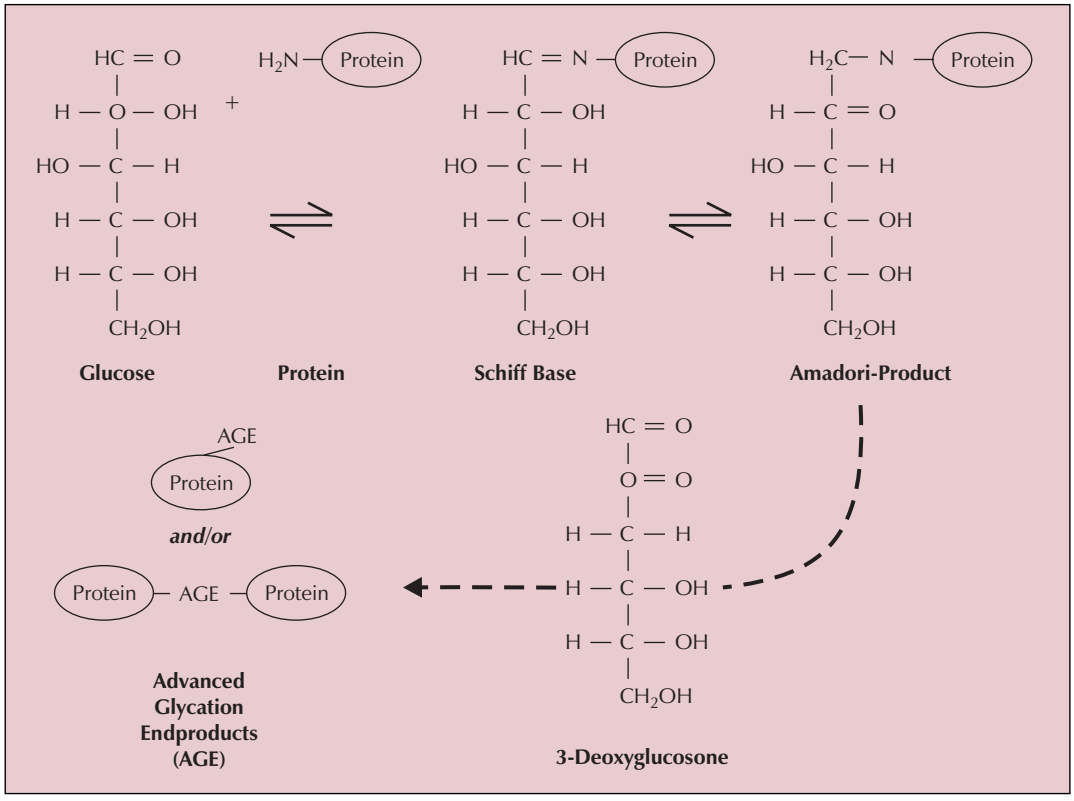


**FIGURE 1.** Formation of AGEs. The initial reaction between the amino-group of a protein and glucose leads to the formation of a Schiff base, which then rearranges to form a more stable ketoamine or Amadori product. Amadori products generate dicarbonyl intermediates (e.g. 3-deoxyglucosone) and finally the advanced glycation end-products (after reference 3).

AGEs can also form from lipid peroxidation, receiving the name of advanced lipoxidation end-products (ALEs) (7). The number of structurally identified AGEs is growing and CML is one of the better characterized end-products used as a marker of AGE/ALE in laboratory studies. ❑

## 2. EFFECTS OF AGES AT CELLULAR AND TISSUE LEVEL

### A. Receptor-independent effects

The free radicals that derive from glycation can cause protein fragmentation and oxidation of nucleic acids and lipids (8). By modifying the structure and function of different proteins, lipoproteins and nucleic acids, AGEs can alter enzymatic activity, decrease ligand binding, modify protein half-life and alter immunogenicity, thus being implicated in the pathogenesis of diabetes complications (4). The physiological consequences of AGE-proteins cross-link formation are, among others, sclerosis of renal glomeruli, thickening of the capillary basement membrane and atherosclerosis development (9). All these changes also occur physiologically, with advancing age, but are accelerated in diabetes (1).

Not only structural proteins, but also plasma proteins and lipoproteins are affected by glycation (apo-lipoprotein B, HDL and LDL) and this contributes to the accelerated atherosclerosis in diabetes (10).

### B. Receptor-mediated effects

AGEs' interaction with their cellular receptors plays an important role in the pathogenesis of diabetes complications. Several receptors have been identified (11), but the best studied is the receptor for AGE (RAGE) (12). RAGE is a member of the immunoglobulin superfamily of cell surface molecules and is a multiligand receptor; it is expressed on a wide range of cells including smooth muscle cells, macrophages, endothelial cells, podocytes, astrocytes and microglia (12). Expression of RAGE is enhanced during diabetes and inflammation (3). RAGE acts as a signal transduction receptor for CML (the major AGE in vivo), although it most probably also interacts with other AGEs (13). On macrophages, interaction of AGEs with RAGE causes oxidative stress and activation of the transcription factor kB (nuclear factor kB, NF-kB); the process involves the activation of $p21^{ras}$/mitogen-activated protein (MAP) kinase signalling cascade, which then activates NF-kB (3). NF-kB modulates gene transcription for endothelin-1, tissue factor and thrombomodulin, generation of pro-inflammatory cytokines (IL-1α, IL-6, TNF-α), as well as enhanced expression of adhesion molecules (VCAM-1, ICAM-1) (6) (Figure 2). In endothelial cells, oxidative stress increases following binding of AGEs with RAGE; oxidative stress also increases after infusion of AGEs in animals (14).

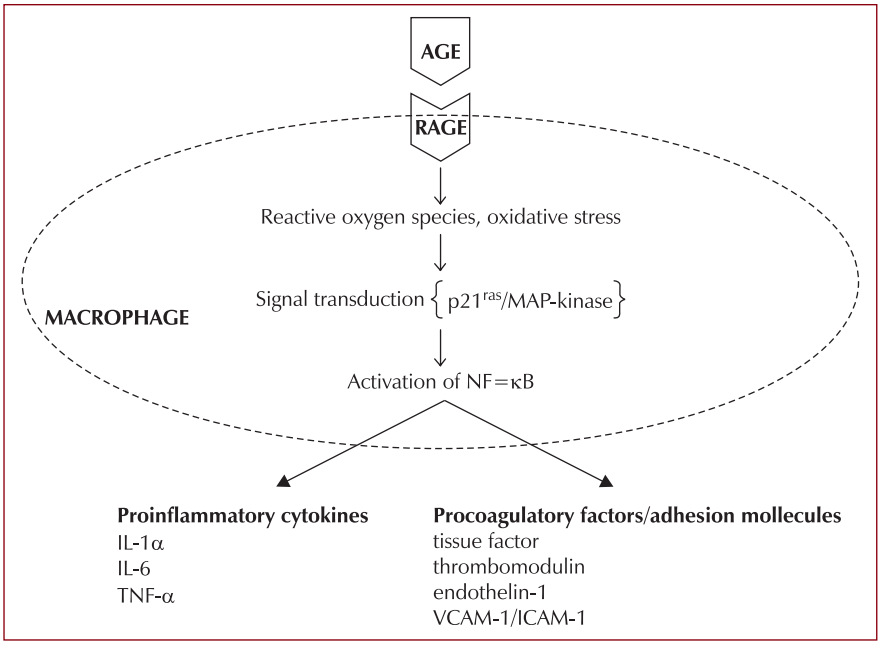


**FIGURE 2. Interaction of AGEs with RAGE.** The binding of AGE to RAGE induces oxidative stress and activation of cellular signalling, causing secretion of cytokines and mediators of inflammation, vasoconstriction and coagulation (modified after reference 3).

Interaction of AGEs with RAGE plays an important role in the AGE-induced vascular dysfunction (15). In cultures of human endothelial cells, interaction of RAGE with heterogenous AGEs or CML-modified adducts resulted in increased levels of VCAM-1, ICAM-1 and E-selectin and increased adhesion of polymorphonuclear leucocytes to stimulated endothelial cells, effects reduced by RAGE blockade (16). Animal studies with soluble RAGE (which inhibit the interaction of AGEs with cellular RAGE) have shown a suppression of vascular lesion formation, as well as a reduction of vascular permeability (and hence dysfunction) (17). A "two hit" model has been proposed for explaining AGE-induced vascular dysfunction (17). According to this model, AGE-rich tissues (for example in diabetes) are populated by cells expressing high levels of RAGE and are subject to sustained AGE-RAGE interaction, resulting in chronic cellular activation. This activation constitutes a chronic, underlying first stimulus/hit in the model. A superimposition of a second stress, such as accumulated lipoproteins, results in exaggerated, chronic inflammation and accelerated atherosclerosis, typical of diabetes.

Another mechanism by which AGEs contribute to the vascular complications of diabetes is inhibition of prostacyclin production and induction of PAI-1, resulting in a predisposition to thrombogenesis (platelet aggregation and fibrin stabilization), effects demonstrated in human endothelial cells (18). These effects are mediated by RAGE, because they could be reversed by anti-sense DNA against RAGE mRNA. ❑

## 3. AGE METABOLISM

The principal mechanism for the degradation of AGE modified tissues and cells is supposed to be through extracellular proteolysis and specific and non-specific AGE receptors on scavenger cells, such as tissue macrophages (19). Macrophages degrade AGEs to low-molecular soluble peptides, known as "second generation AGEs", which are then cleared by the kidney (20). It is therefore clear that any deterioration in renal function results in AGE accumulation (6).

It has also been proposed that liver sinusoidal cells (such as Kupffer and endothelial cells) play an important role in the uptake – through endocytosis – of AGE proteins from the plasma (21). Defects in this system could therefore contribute to AGE accumulation independently of renal function (22). Insulin seems to have a positive role in this process, since it stimulates the AGE uptake in liver endothelial cells expressing macrophage scavenger receptors (22).

Intracellular protective systems (glyoxalase, lysozyme) also limit the accumulation of reactive AGE intermediates (19). ❑

## 4. AGES AND CHRONIC COMPLICATIONS OF DIABETES

AGEs have been implicated in the patho-genesis of aging, Alzheimer disease and chronic diabetes complications. The single unifying mechanism for hyperglycemia-induced diabetes complications seems to be increased generation of ROS and the AGE pathway is only one of the major pathways responsible for diabetic damage (in addition to the polyol pathway, the hexosamine pathway and the diacylglycerol pathway) (2).

### Diabetic retinopathy

Exposure of retinal cells to AGEs causes (through a PCK dependent pathway) up-regulation of vascular endothelial growth factor (VEGF), which stimulates angiogenesis and neovascularisation, both involved in the pathogenesis of proliferative diabetic retinopathy (23,24). AGEs also induce accelerated apoptosis of retinal capillary cells under in vitro conditions and could thus contribute to the development of diabetic retinopathy (25).

### Diabetic cataract

Subjects with diabetes are more likely to develop cataract and it also occurs at an earlier age. One of the possible causes is the glycation of lens crystallin, which has virtually no turnover, thus leading to opacification (26).

### Diabetic nephropathy

The diabetic renal disease is characterized by a thickening of the basement membrane, expansion of the mesangium, reduced filtration, albuminuria and ultimately renal failure. AGEs have been detected in renal tissues in amounts that correlate with the severity of diabetic nephropathy (27). In animal models, the injection of AGE-altered protein is followed by

a thickening of the basement membrane and expansion of the mesangial layer, thus further supporting the involvement of AGEs in diabetic nephropathy (28). The thickening of the basement membrane is, at least partly, due to the release of transforming growth factor β (TGF- β) (29). Several AGEs (CML, pyralline, pentosidine) have been identified in the renal tissue of diabetic patients with or without end-stage renal disease (ESRD), AGE accumulation increasing with severity of diabetic nephropathy (30).

### Diabetic neuropathy

The mechanisms by which AGEs could contribute to the development of neuropathy in diabetes are multiple and not yet fully understood. AGE accumulation in vasa nervorum leads to wall thickening, ischaemia and occlusion, as well as to segmental demyelination (31). There is increased glycation of myelin in diabetes and glycated myelin is susceptible to phagocytosis by macrophages and can also stimulate macrophages to secrete proteases, which can contribute to nerve demyelination in diabetic neuropathy (32). In peripheral nerves from diabetic rats, AGEs reduce sensory motor conduction velocities, nerve action potentials, as well as peripheral nerve blood flow (33).

### Atherosclerosis and vascular dysfunction

AGEs have been identified in fatty streaks, atherosclerotic lesions, lipid-containing smooth muscle cells and macrophages from diabetic subjects (10). Increased glycation of LDL occurs in diabetes and glycated LDL is not recognized by the LDL receptor, but its uptake by scavenger receptors on macrophages and smooth muscle cells is enhanced and this could account for the hyperlipidemia and accelerated foam cell formation in DM (34). Moreover, glycation of HDL increases its turnover and reduces its efficiency during reverse cholesterol transport (35). AGE cross-linked arterial wall collagen can trap LDL and other plasma proteins, contributing towards accelerated atheroma formation in diabetes (36). Recently it has been shown that Lp(a), an independent CV risk factor, also undergoes glycation in diabetic subjects (37).

AGE adducts in the vessel wall interfere with endothelium-derived NO-synthase and the vasodilatatory activity of NO, thus further contributing to the vascular dysfunction in diabetes (38).

AGEs also promote secretion of several cytokines, including insulin-like growth factor-1 (IGF-1) and platelet derived growth factor (PDGF), which control migration of monocytes and macrophages and promote smooth muscle proliferation, an important step in atherosclerosis development (39). Recently, it has been shown that increased aortic MG, CML and CEL (carboxyethyllysine), as well as oxidative stress were associated with development of hypertension in rats (40).

In diabetic rats, enhancement of early and intermediate Amadori adducts of protein glycation showed a relationship with the development of endothelium impairment, supplying evidence for the role of these products in diabetic vasculopathy (41). ❑

## 5. DIETARY AGE

### A. Formation and metabolism

While the in-vivo formation of AGEs clearly mediates multiple pathological processes, it is becoming more and more obvious that exogenous sources of AGEs, such as diet and smoking may have significant impact on disease mechanisms. Diet is the most important source of exogenous AGE, with the highest content in heat-processed foods (42). AGE generation in foods during cooking depends on nutrient composition, temperature, method and duration of heat application (43).

Foods rich in lipids and proteins show the highest AGE levels; this may result from high levels of free radicals released during various lipoxidation reactions, which promote the formations of AGEs during the cooking of fats and meats. Glycoxidation and lipoxidation in these foods are promoted by heat, absence of moisture and presence of metals. Foods that are composed mostly of carbohydrates (starches, fruits, vegetables, milk) show the lowest AGE concentrations; however, in this group, commercially prepared breakfast foods and snacks (processed at high temperatures, over 230 °C) show significant AGE content (43).

Temperature and methods of cooking seem to be more critical to AGE formation than cooking time. For example, one serving of chicken breast boiled at 100°C for one hour yielded 5 times less AGEs than the same item broiled at 230°C for 15 minutes. The trend for AGE values achieved from the same ingredients with

different cooking methods is: oven frying (230°C) greater than deep frying (180°C), and broiling (225°C) greater than roasting (177°C) and boiling (100°C). Microwaving was shown to increase AGE content similar to boiling cooking methods (43). Strikingly, infant formula (Enfamil) was found to have a 100-fold higher AGE content than human or bovine milk (44).

Human studies have confirmed that approximately 10% of ingested AGEs survive the digestive process and are transported, as small molecular weight particles into the blood stream, along with short peptides and aminoacids present in the digest. Only one third of absorbed AGEs is excreted renally within 48 hours in patients with diabetes and normal renal function (<5% in patients with diabetes and renal failure) (45). AGEs that are not cleared are incorporated in tissues and cells, where they remain biologically active and exert their pathological effects, similar to their endogenous counterparts (45).

### B. Predictive value of serum AGE

Serum levels of AGEs correlate with endothelial dysfunction and risk for cardiovascular events. A prospective study performed in Finland over 18 years showed that serum AGE levels predict total, cardiovascular disease and coronary heart disease mortality in a cohort of non-diabetic women (46). In healthy humans and T2DM patients, serum AGEs have been shown to be independently associated with endothelial dysfunction (47).

### C. Animal studies

Dietary AGE restriction resulted in significant reduction of circulating AGE levels and atherosclerosis progression (neointimal area after arterial injury) in hypercholesterolemic mice (48), while a high-AGE (HAGE) diet lead to a rise in serum AGE and accelerated atherosclerosis (49). Diabetic mice fed on a HAGE diet showed delayed wound healing and had higher serum AGE levels over a 3-month period compared to those placed on a low-AGE diet (LAGE) (50). A LAGE diet has also been shown to prevent the development of diabetic nephropathy in animal models, even in the face of persistent hyperglycemia (51) and to improve insulin sensitivity in the db/db mouse (52). Development of insulin resistance and T2DM during prolonged high-fat feeding in mice are linked to the excess of AGE/ALEs inherent in fatty diets (53). Very recently, evidence from animal studies points out that dietary restriction of AGEs extends the median life span, to a similar extent to marked caloric restriction (-40%), considered until now the only method for prolonging survival (54).

### D. Studies in humans

Dietary ingestion of glycotoxins influences the serum levels of AGE in human subjects. In patients with renal failure, dietary AGE content, independently of other diet constituents, correlates with circulating AGE levels (20). In healthy subjects, diet-derived AGEs have been shown to be major contributors to body's AGE pool and also to induce inflammation (55).

Dietary AGE restriction in non-diabetic patients with renal failure resulted in decreased serum levels of glycotoxins (56), an effect also observed in diabetic patients with normal renal function (57). A study performed in diabetic subjects, randomized to receive either a LAGE or a HAGE diet over 6 weeks, showed that LDL pooled from patients on HAGE diet was more glycated, more oxidized and stimulated NF-kB activity, thus enhancing its vascular toxicity (58). Another recent study in human subjects with diabetes compared two diets which differed by 6-fold in the AGE content, but were otherwise nutritionally equivalent. The HAGE diet led after 2 weeks to an increase in inflammatory mediators and markers of vascular dysfunction (serum TNF-α, VCAM-1, CRP, serum AGE and LDL-AGE), while all these parameters decreased at the end of the LAGE diet (57).

These effects underscore the potential role of a low-AGE diet in the primary prevention of atherosclerosis, and also in the prevention of restenosis after coronary angioplasty. ❑

## CONCLUSION

Increased glycation, especially of proteins, leading to the formation of AGE and their subsequent accumulation in blood and tissues play an important role in the pathogenesis of chronic complications of diabetes mellitus. The chemical structure of AGEs, their synthesis in vivo, the exact role in the pathogenesis of diabetes complications, as well as the interaction with their receptors are intensively being investigated. Various methods for reducing glycation or blocking RAGE represent interesting possibilities for preventing or delaying diabetes complications.

Dietary AGE have been shown to exert similar pathogenetic effects to their endogenous counterparts. Therefore, a simple dietetic intervention, which does not necessarily mean deprivation of certain foods, but only the preferred use of low-AGE-producing culinary techniques, could represent an attractive alternative for preventing the micro- and macrovascular complications of diabetes mellitus. ❑

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