AGE, RAGE, and Diabetic Nephropathy

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Abstract

Diabetes is a disease that is present worldwide and which is associated with a large number of potential complications including chronic kidney disease (CKD). Several factors have been implicated in the development of the latter, including advanced glycation end-products (AGEs), which are formed from the interaction between sugar and proteins. AGE toxicity may be triggered via different mechanisms, especially by receptor binding. Immunohistochemical studies have demonstrated the presence of AGEs in all renal structures (vessels, glomeruli, tubules, and the interstitium). They appear to be involved in the exacerbation of renal injury observed during diabetic nephropathy. At present, no specific treatment is yet available, although several therapeutic approaches are under development.

Keywords

Advanced glycation end-products, diabetic nephropathy, EMT, podocytes, RAGE, treatment

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Diabetic nephropathy (DN) has become the most frequent cause of terminal renal failure in western countries. Its natural course has been well described and includes the development of functional and structural degenerative changes in the kidney. More specifically, glomerular membrane thickening, mesangial matrix expansion, microvascular changes, arteriolar hyalinosis, and tubular degeneration are characteristic aspects of overt DN. In view of the marked increase in the incidence of type 2 diabetes, over the next few years it will be vital to determine the mechanisms underlying this pathology. Hyperglycemia is known to be implicated in the development of the above-mentioned degenerative changes and as a hyperglycemic state is associated with the formation of advanced glycation end-products (AGEs) it is likely that the latter play a major role in the pathogenesis of the changes that occur during DN. At the present time, new therapeutic approaches to limit the progression of DN are being investigated. Thus a fuller understanding of the mechanisms underlying AGE formation and the inhibitors thereof is of major importance in this respect.

Introduction to Advanced Glycation End-products

AGEs are toxic compounds that are formed from a link between a reducing sugar and an amino group on the lysine and arginine residue of certain proteins. Their toxicity, which is triggered by a chronic hyperglycemic state associated with diabetes, can act in three different ways: via deposition in the tissues, through *in situ* formation, or by receptor activation.

Advanced Glycation End-product Formation

Three pathways can lead to AGE formation: the Maillard reaction, the polyol pathway, and glycoxidation (see *Figure 1*).[1]

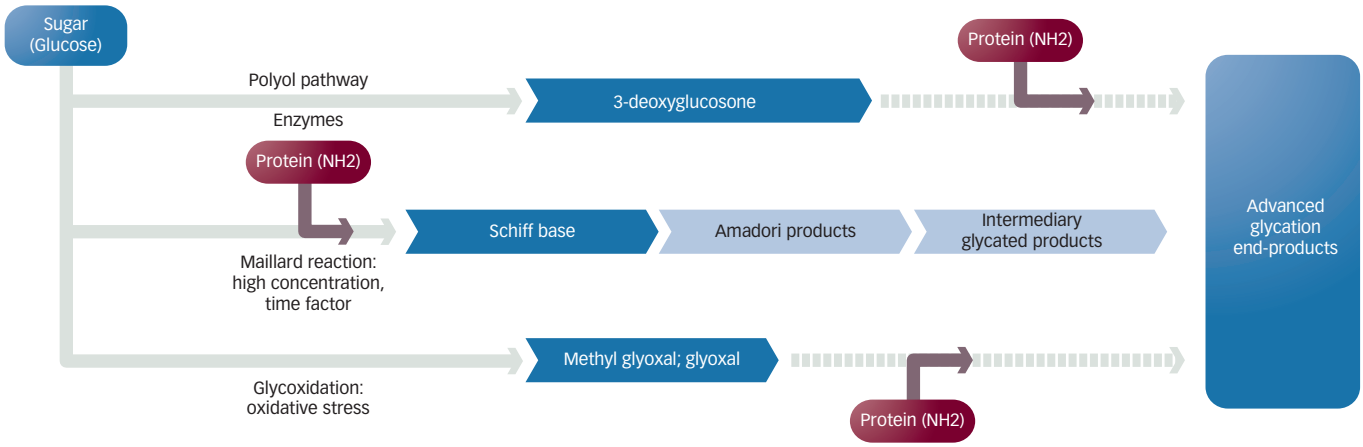
Glycation, which was first described in 1912 by LC Maillard constitutes the main pathway; the activation of this process depends on the glucose concentrations, time and temperature and several stages are required before AGEs can be formed.[2] The reaction between sugar and proteins leads to the formation of a Schiff base within a short time period (minutes or hours), which then undergoes rearrangements to yield Amadori products. The latter products are subject to further rearrangements, following which they become intermediary glycation products such as glycated hemoglobin, which is used in clinical practice. The final step of the reaction leading to the formation of AGEs requires longer periods of time (i.e. months).[3]

The second route, the polyol pathway, begins with the production of a reactive form of sugar: certain enzymes (e.g. aldose reductase or sorbitol deshydrogenase) form intermediate compounds such as 3-deoxyglucosone. This molecule can then react with the proteins and finally result in the formation of AGEs.

The third pathway, glycoxidation, yields reactive intermediary molecules called carbonyl compounds, such as glyoxal and methylglyoxal, which are produced by glucose autoxidation. These more recently identified products are highly reactive and can interact with proteins to form AGEs.

There are a large number of AGE compounds, which have not been well characterized. Although no official classification is yet available, AGEs can be differentiated according to their various characteristics including, fluorescence, antigenicity, or the formation of cross-linking. AGEs can be identified by high-performance liquid chromatography (HPLC) and mass

Figure 1: Advanced Glycation End-product Formation



spectrometry, or on the basis of their antigenic properties. The AGEs that have been the most fully described and studied to date are Nε-(carboxymethyl)lysine (CML) and pentosidine.

Mechanisms of Toxicity

AGE toxicity is mediated by three mechanisms. Firstly, AGEs can be deposited in the tissues, such as in the endothelial wall, the mesangium, or the skin. These deposits modify the structure and function of the tissues by increasing arterial stiffness and rigidity, and reducing extracellular matrix remodeling. Secondly, AGEs can be formed *in situ*, where they exert a profibrotic effect; and thirdly, AGEs can bind to cell receptors.

Five cell receptors have been identified: i) the macrophage scavenger receptor 1 (MSR1 or CD36); ii) the AGE receptor R1 (AGE-R1 or p60), corresponding to oligosaccharyl transferase 48; iii) the AGE receptor R2 (AGE-R2 or p90), corresponding to phosphoprotein 80K-H; iv) the AGE receptor R3 (AGE-R3 or galactin-3), a scavenger receptor that recognizes galactoside residues; and v) the receptor for AGEs (RAGE), which is the most widely studied type.[4–7] RAGE, which was first isolated from bovine lung, is the main cell-surface molecule implicated in the toxicity of AGEs.

The RAGE gene is present on human locus 6p21.3, next to the MHC class III protein family. It is a transmembrane receptor of the immunoglobulin superfamily and is expressed in different cells, e.g. in endothelial cells, monocytes/macrophages, smooth muscle cells, mesangial cells, mesothelial cells and nerve cells. RAGE is a multiligand receptor that can also bind s100 proteins or calgranulins (which belong to the group of proinflammatory cytokines); the high-mobility group B1 (HMGB1), also known as 'amphoterin'; lipopolysaccharides and β-amyloid peptide. RAGE can be expressed in three different forms: as full-length RAGE, or in two soluble forms (sRAGE).[8]

Consequences of Receptor for Advanced Glycation End-product Activation

AGE binding to RAGE activates multiple signaling pathways, generates oxidative stress and enhances membrane protein expression and cytokine production.[9]

In endothelial cells, AGE binding to RAGE activates signaling pathways in a series of steps involving MAP kinases (ERK1/2, p38, JNK), Rho GTPases (p21ras), phosphoinositol-3 kinase (PI3K), JAK/STAT, finally resulting in nuclear transcription factor activation, i.e. nuclear factor kappa-B (NF-κB) activation. NF-κB secondarily induces the expression of numerous pro-inflammatory molecules such as intercellular adhesion molecule (ICAM); vascular cell adhesion molecule (VCAM); tissue factor (TF) production; interleukins (interleukin 6 and 8); granulocyte/macrophage colony-stimulating factor (CSF); vascular endothelial growth factor (VEGF); macrophage chemotactic protein-1 release (MCP-1), and E-selectin. Moreover, this pathway leads to the generation by NADPH oxidase of reactive oxygen species, which have deleterious effects on the cells.[10] AGEs are inducers of apoptosis for mesothelial cells and pancreatic beta cells. RAGE activation is a critical step in triggering diabetic microvascular complications, that in fine can lead to the development of neuropathy, retinopathy, nephropathy, or macrovascular disease (e.g. atherosclerosis).

Implications of Advanced Glycation End-product/Receptor for Advanced Glycation End-product Activation in Diabetic Nephropathy

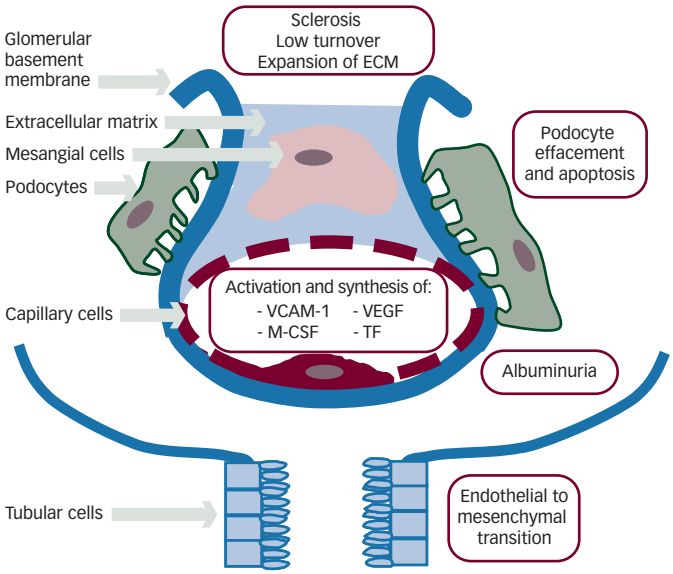
Advanced Glycation End-products in the Kidney

Immunohistochemical studies have detected AGEs throughout the kidney: in the mesangium, endothelium and the tubular and glomerular basement membranes (GBM). Their localization is not pathognomonic, but the distribution and nature of AGEs seem to differ between DN and other nephropathies: CML is the major AGE that is detected essentially in the mesangium and vessel walls, following by the tubular basement membranes and the GBM. On the contrary, pentosidine is preferentially located in the interstitial collagen and is present to a lesser degree in the other renal structures (vessel walls, mesangium, GBM, and tubular basement membrane).[9,11,12] All renal structures may be affected by AGE- and RAGE-induced lesions because RAGE is expressed by mesangial, epithelial, and vascular cells (see *Figure 2*).

Advanced Glycation End-products and Podocytes

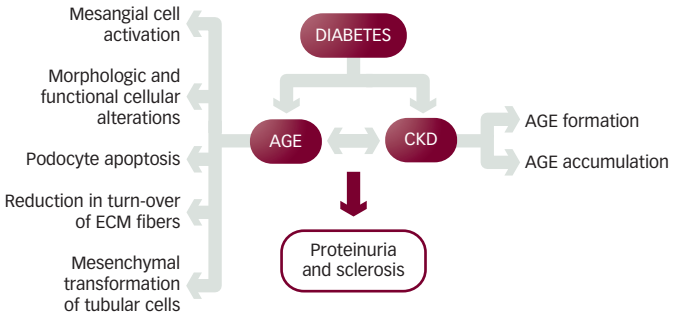
The podocytes, which play a key role in the development of DN, undergo numerous degenerative changes due to AGE activity, essentially through RAGE, which becomes upregulated.[13,14] AGE-rich culture medium and glycated collagen IV have been found to induce podocyte apoptosis via induction of the transcription factor FOXO4 and Bim expression.[15]

Figure 2: Effect of Advanced Glycation End-products on Renal Structure



ECM = extracellular matrix; M-CSF = macrophage–colony-stimulating factor; TF = tissue factor; VCAM-1 = vascular cell adhesion protein-1; VEGF = vascular endothelial growth factor.

Figure 3: Vicious Circle between Advanced Glycation End-products, Diabetes, and Chronic Kidney Disease



AGE = advanced glycation end-products; CKD = chronic kidney disease; ECM = extracellular matrix.

Although the destruction of podocytes has been reported in patients with diabetes,[16] RAGE null mice with CKD, are protected from such podocyte destruction during diabetes, suggesting a preponderant role of AGEs in these lesions. At the onset of DN, the podocytes lose contact with the GBM. AGEs inhibit the expression of neuropilin-1, a transmembrane receptor involved in cell adhesion and also modify the expression of actin, a cytoskeletal protein which is very important in the maintenance of the glomerular slit diaphragm.[17] AGE activity reduces the expression of the zonula occludens, tight junctions which have been found in the slit diaphragm.[18] Other modifications have also been described, such as the reduced expression of α-actinin-4, an actin filament crosslinking protein required for normal podocyte adhesion.[19] All these changes result in increased albumin permeability through the podocyte monolayer. Thus, AGEs trigger the first steps leading to the development of proteinuria. AGE–RAGE binding in the podocytes also causes inflammation. For example, MCP-1 is upregulated by AGEs in general and more specifically by CML.

Advanced Glycation End-products and Mesangial Cells

Human mesangial cells express several membrane receptors for AGEs, including RAGE. AGEs directly induce the expression of various components of the extracellular matrix, such as the α-1 chain of collagen IV and fibronectin. They strongly modulate genes involved in matrix remodeling such as matrix metalloproteinases-2, tissue plasmin activator and their specific inhibitors. These events lead to reduced extracellular matrix protein turnover and promote the accumulation of AGEs in the mesangium.[18,20,21]

Advanced Glycation End-products and Tubular Cells

Glycated albumin present in the tubular cells is able to induce epithelial–mesenchymal transition (EMT). EMT is a biologic process in which a polarized epithelial cell undergoes numerous biochemical changes that enable it to assume a mesenchymal cell phenotype. A number of studies have reported that the cytokine implicated in EMT is transforming growth factor beta (TGF-β). *In vitro*, cell exposure to glycated albumin induces the *de novo* expression of α-smooth muscle actin, a mesenchymal marker, and reduces the expression of e-cadherin, an epithelial marker, as well as that of TGF-β. EMT is generally associated with modifications in cell engineering and with the enhancement of growth factors such as connective tissue growth factor, as well as with the production of cytokines, leading to degenerative changes, e.g. inflammation and fibrosis. Moreover, as previously observed in mesangial cells, AGEs exert different direct inflammatory and fibrotic effects on tubular epithelial cells via RAGE activation and NF-κB signal transduction. In tubular cells, AGEs reduce protection against oxidative stress via nitric oxide synthase inhibition.[22]

Diabetes and Advanced Glycation End-products—A Vicious Circle

Diabetes promotes AGE formation and participates in the development of CKD (see *Figure 3*). However, AGEs also exhibit toxic activity in chronic uremia, whether the renal disease is of diabetic or non-diabetic origin. Indeed, oxidative stress is major contributory factor in renal failure and regardless of the presence or not of diabetes, AGE levels are inversely correlated with the glomerular filtration rate (GFR). This constitutes a vicious circle. Diabetic patients present higher levels of circulating AGEs, and these levels are even higher in diabetic patients with microvascular complications such as retinopathy or nephropathy.[23] In type 1 diabetic patients, AGE skin deposits, as determined by autofluorescent analysis, are significantly higher in subjects with proteinuria. In end-stage renal disease, although AGE blood levels are significantly increased in hemodialysis patients, no difference in levels is observed between diabetic and non-diabetic patients.[11,24]

Treatment

Therapeutic Approaches

Since AGEs are closely associated with a diabetic state, research into therapeutic means of inhibiting their formation or preventing their degenerative effects is of major interest. This can be achieved in various ways: by decreasing the glucose levels directly associated with AGE formation, inhibiting the overproduction of intermediary glycation products, breaking down the neoformed AGEs and preventing their interaction with RAGE (see *Figure 4*).

Advanced Glycation End-products and Food Intake

AGEs can be formed endogenously as well as exogenously in processed foods which are orally ingested and absorbed into the system. The levels of urinary excretion of AGEs are dependent on their intake through the diet; however, in the case of DN patients, the renal excretion of orally absorbed AGEs is relatively attenuated. Daily dietary intake of AGEs constitutes an added chronic risk factor for vascular renal injury in patients with diabetes. As food processing can induce their formation, diet is therefore an important potential source of AGEs. However, only between 10 and 30 % of the ingested AGEs are absorbed by the organism. Their rate of formation depends on temperature, processing time and on the degree of moisture that is present. In western countries, the average diet is rich in AGEs due to the high sugar content in many foodstuffs and to the widespread availability of industrialized food products.[25,26]

It can therefore be concluded that the dietary restriction of AGE-containing food may significantly reduce AGE activity in diabetic patients, and possibly improve disease prognosis. In animal models, it has been reported that the reduction of AGE intake limits the development of proteinuria (which has a negative effect on kidney function) in non-obese diabetic and db/db mice, improves insulin sensitivity and also increases lifespan.

Although the effects of dietary AGEs on human health remain controversial and open to debate,[27–30] Negrean and colleagues demonstrated that a high-AGE diet induces acute impairment of vascular function and promotes the development of inflammatory cytokines.[31] Moreover, it has been reported that a high-AGE diet may reduce lifespan. However, in these studies the specificity of dietary AGEs could not be clearly defined: the meals were overcooked, thus reducing the vitamin content and generating, apart from AGEs, other toxic molecules such as trans fatty acids and methylglyoxal.

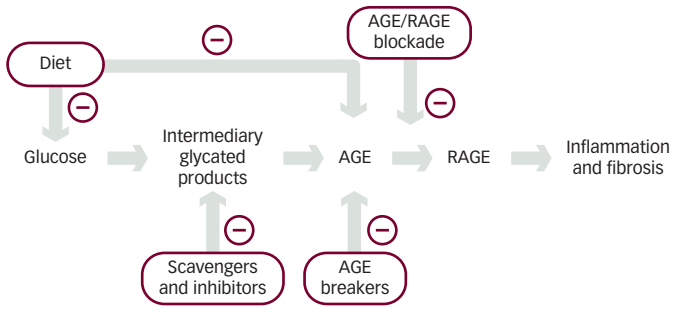
Inhibitors of Advanced Glycation End-product Formation

Another approach to reducing AGE accumulation is through the inhibition of its formation. One of the most widely studied molecules belongs to the aminoguanidine (AG) group, namely pimagedine.[32] AG is a competitive inhibitor of AGE formation, acting as a scavenger of dicarbonyl compounds as well as of other biological compounds. In rats exposed to IV injection of AGEs, AG prevented the development of glomerular hypertrophy, proteinuria, and glomerulosclerosis.[11] Nevertheless, clinical trials investigating the use of AG in patients with overt DN have so far been disappointing.

ACTION 1 was a randomized, double-blind, placebo-controlled trial comparing two dose levels of AG (150 and 300 mg daily) with placebo on the progression of nephropathy in 690 patients with type 1 diabetes. ACTION 2 was a similarly structured trial performed in 559 patients with type 2 diabetes. The results showed that the effects of AG administration were not as positive in human subjects as in animal models, and were associated with serious side effects including transient flu-like syndrome, anemia, and the induction of antinuclear and crescentic glomerulonephritis.[33]

Two vitamin derivatives have been tested as inhibitors of AGE formation. Benfotiamine, a lipophilic thiamine derivative, was previously considered as a likely candidate, but in a double-blind, randomized, placebo-controlled study performed in patients with type 2 diabetes and nephropathy, high-dose benfotiamine treatment for 12 weeks combined with angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) did not succeed in reducing proteinuria, despite an improvement in thiamine status.[34]

Figure 4: Therapeutic Pathways



AGE = advanced glycation end-product; RAGE = receptor for advanced glycation end-product.

Pyridoxamine, which is a vitamin B6 derivative, blocks Amadori-to-AGE conversion with carbonyl trapping and scavenging of metal ions *in vivo*. Phase 2 safety and efficacy trials have been completed and demonstrated a good safety profile.[35,36] These preliminary efficacy results are encouraging, but to date no Phase 3 trials are underway.

Aromatic compounds could constitute another treatment approach. Three compounds have been developed, namely LR-9, LR-74 and LR-90. Their mechanism of action is due to the chelation of metal ions, which suppresses hydroxyl radical production during sugar autoxidation and associated glycation reactions and inhibits post-Amadori AGE formation. In animal models, LR compounds have been found to prevent glomerulosclerosis, decrease AGE accumulation in the kidney, inhibit albuminuria and further increase plasma creatinine levels.[37,38] To date, these compounds have not been tested in humans.

Advanced Glycation End-product Breakers

Potential treatment strategies for these AGE-driven complications include not only the prevention of AGE formation but also the breakdown of existing AGE crosslinks. Alagebrium (formerly called ALT-711) is a derivative of phenacylthiazolium bromide, the crosslink-breaker prototype, with a chemical composition that is related to vitamin B1. AGE breakers cleave AGE–protein crosslinks *in vitro*. They thus provide an opportunity to reverse the development of previously formed histological lesions. In diabetic mice, alagebrium administration either early or late during the course of the disease resulted in decreased renal AGE accumulation and RAGE expression, proteinuria, and inflammatory factors.[39]

A new AGE breaker, TRC-4186, was found to significantly reduce urinary albumin excretion and also the severity of histopathological lesions in mouse models of type 2 diabetes. TRC4186 has been tested in Phase 1 clinical trials, with successful results being reported.[40,41] As regards Phase 2 trials, one such clinical trial has been reported, but could not be pursued further for financial reasons. It is clear that the action of AGE breakers has not yet been fully investigated and further studies are required.

Receptor for Advanced Glycation End-product Activation Blockers

As previously mentioned, AGEs exert part of their toxic effect through their interaction with RAGE. Moreover, in streptozotocin-treated diabetic mice compared to wild-type mice, RAGE null mice were found to be protected from developing DN, essentially from albuminuria, hyperfiltration, and glomerulosclerosis.[42] It has already been established that the intraperitoneal injection of anti-RAGE antibodies in diabetic mice reduces the development of proteinuria, hyperfiltration, and nephromegaly.[43] It is obvious that the blockade of this interaction has a therapeutic potential.

Another therapeutic possibility is that of soluble RAGE (sRAGE) injection. sRAGE is a circulating form of RAGE and is produced by cleavage of the membrane receptor, i.e. cleaved RAGE (cRAGE, by ADAM 10) or by alternative splicing (endogeneous secreted [es] RAGE). sRAGE acts as a decoy receptor and forms complexes with RAGE ligands. However, after they have been formed, it is not known whether these complexes are removed, destroyed or if they are deposited in the tissues. sRAGE is also able to prevent the negative effects of ligand–RAGE interactions. At the present time, these specific treatment approaches are not available for human therapeutic use.[44]

Other Advanced Glycation End-product Blockers

Certain currently prescribed medicinal drugs for the treatment of diabetics have a real anti-AGE action, such as the renin–angiotensin–aldosterone system blockers and statins.

Angiotensin-converting Enzyme Inhibitors/Angiotensin Receptor Blockers

These drugs have several anti-AGE effects. First, they limit AGE formation by trapping reactive carbonyl compounds and reducing oxidative metabolism. Then ACEIs interfere with the intracellular signaling pathway between RAGE and NF-κB. Moreover, they enhance sRAGE formation. Clinical studies in type 1 diabetic patients have demonstrated a significant increase in circulating plasma sRAGE levels in association with decreased blood levels of AGEs compared to those observed in control patients.[8,45]

Statins

Statins have demonstrated anti-AGE effects both *in vivo* and *in vitro*. *In vitro*, tubular cells are protected from AGE-related lesions in the presence of pravastatin or rosuvastatin.[46] In mesangial cells, the addition of statins to the culture medium reduces the expression of TGF-β and limits collagen IV synthesis.[47] In the diabetic mouse, atorvastatin has been found to exert a beneficial effect on DN, with reduced AGE accumulation, down-regulation of RAGE expression and upregulation of sRAGE in the kidney.[48] In non-diabetic CKD patients, treatment with atorvastatin over a one-year period reduced proteinuria in association with decreased AGE serum levels. In diabetic patients, atorvastatin induced a significant increase in serum sRAGE levels after a six-month treatment period, independently of low density lipoprotein (LDL) serum levels.[49]

Anti-diabetic Treatment

Metformin, which is used in the treatment of type 2 diabetes, has an anti-AGE effect in addition to lowering blood glucose levels. This effect is due to metformin's 'aminoguanidine-like structure', as a result of which it reduces AGE formation and in diabetic patients decreases methylglyoxal plasma levels. However, its use is limited in the case of severe CKD.

Conclusion

In conclusion, diabetes, a uremic state and also the type of diet can induce AGE formation and promote its accumulation in the tissues. Whereas the mechanisms involved in glycation were already first described 100 years ago, AGEs are becoming increasingly recognized as highly implicated in the development of DN, and it has now been established that all renal structures are involved in AGE-related lesions. Several different targets and therapeutic approaches appear promising in this regard, but further Phase 2 and 3 clinical studies are required before any definitive conclusions can be made. ■

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