

# Is AGE accumulation a therapeutic target for diabetic complications?

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The formation of advanced glycation endproducts (AGEs) has been recognised as an important pathophysiological mechanism in the development of vascular complications in diabetic patients.<sup>1</sup> Nonenzymatic glycation involves the condensation reaction of the carbonyl group of sugar aldehydes with free amino groups of proteins, resulting in the rapid formation of a Schiff base. Subsequently, this labile adduct undergoes rearrangements to a more stable Amadori product. Only a small number of these intermediate Amadori products are oxidised and can give rise to irreversibly formed AGEs, such as the cross-link pentosidine and N<sup>ε</sup>-(carboxymethyl)lysine (CML). Because of the slow formation, it was long believed that AGEs accumulate only on long-lived extracellular proteins. However, a rapid intracellular and extracellular AGE formation on short-lived proteins has attracted attention. Of importance for the intracellular Maillard reaction are glycolytic intermediates such as the dicarbonyl compounds glyoxal, methylglyoxal and 3-deoxyglucosone. In the context of intracellular glycation, it is important to emphasise that of all sugars, glucose has the slowest rate in the glycation reaction.

Several mechanisms have been proposed by which AGEs lead to diabetic complications: 1. the accumulation of AGEs in the extracellular matrix causing aberrant cross-linking, resulting in a decrease in the elasticity of vessels, 2. the binding of circulating AGEs to the receptor of AGEs (RAGE) on different cell types and activation of key cell signalling pathways such as NF- $\kappa$ B activation with subsequent modulation of gene expression and 3. intracellular AGE formation leading to quenching of nitric oxide and impaired function of growth factors.<sup>2</sup> Because of the many deleterious effects of AGEs on vascular structure and function, prevention or reversal of AGE accumulation is an attractive therapeutic target. The data presented by Mentink *et al.* in this issue suggest that six months of optimised metabolic control by insulin therapy is not accompanied by a decrease in circulating

AGEs.<sup>3</sup> The fact that optimised metabolic control decreased markers of endothelial function but did not affect circulating AGEs suggests either the involvement of other circulating AGEs than the ones detected in this study or that other AGE-induced mechanisms such as cross-linking and intracellular glycation may be responsible for endothelial dysfunction. On the other hand, other hyperglycaemia-induced biochemical pathways, such as the sorbitol pathway or protein kinase C activation, may be involved in endothelial dysfunction.

These results encourage us to make some comments on the detection of different AGEs. Mentink *et al.* have measured different AGEs with immunoassays and high performance liquid chromatography (HPLC). Immunoassays are often used for the quantification of AGEs, but for several reasons the use of antisera for quantitative immunoassays of protein-bound AGEs is questionable. One reason is that the specificity of the antibodies is often difficult to define with certainty and thus far no monospecific antibodies are commercially available. Another reason is that proteins used to block nonspecific binding in immunoassays may also contain AGE epitopes and thus interact with the antibody. In addition, because of steric constraints, not all AGE epitopes on the protein may be available for interaction with the antibody. Finally, there is evidence for the presence in plasma of factors competing for the reaction between the anti-AGE antibody and its antigen. These factors include anti-AGE autoantibodies and, possibly, complement. As a consequence, AGE immunoassays may only yield semiquantitative results and these should be interpreted with care. The possibility that the results of the study by Mentink *et al.* are due to imperfections of the immunoassays can not be excluded.<sup>3</sup> A better approach for the quantitative determination of specific AGE epitopes in proteins is to use a specific analytical technique for the analysis of these AGEs in protein hydrolysates.<sup>4</sup> A major restriction of this approach is that not all AGE epitopes are stable during the harsh

conditions used for protein hydrolysis. Fortunately, CML is stable under the conditions used for acid protein hydrolysis and can be measured accurately by analytical techniques as performed by Mentink *et al.*<sup>3</sup> Application of this kind of analytical technique in the laboratory could lead to a more comprehensive understanding of the role of nonenzymatic chemistry in disease.

The fact that Mentink *et al.* found that CML, which is a major AGE and a ligand for RAGE, did not decrease by improvement of metabolic control suggests that not hyperglycaemia, but accumulation of reactive dicarbonyl intermediates and/or increased oxidative stress and lipid peroxidation play a more important role in the generation of this compound. This is corroborated by recent findings showing that oxidants generated by NADPH oxidase play a crucial role in CML formation.<sup>5</sup> In addition to the formation, renal dysfunction is the most important determinant in CML levels in diabetic patients.<sup>4</sup>

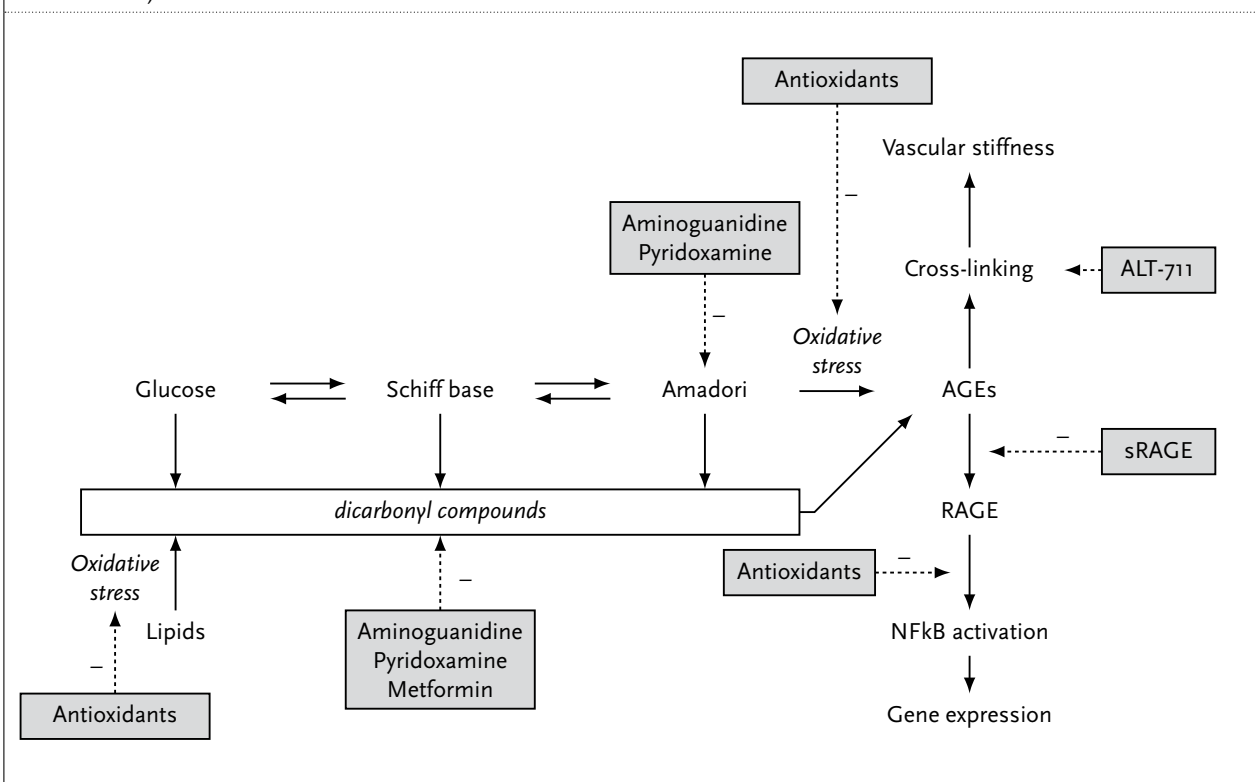
Despite these considerations, we can conclude that additional intervention therapies may be used to reduce AGE accumulation or to reduce AGE-induced effects, also in view of the difficulties in achieving optimal metabolic control in subsets of patients with diabetes. A short overview of the current status of these therapies is

presented in *figure 1*. For further detailed information, we refer you to an excellent review by Monnier.<sup>6</sup>

## INHIBITION OF AGE FORMATION

The first approach is to reduce the formation of AGEs by intervention at one of the many steps involved in the formation of AGEs, such as by aminoguanidine.<sup>7</sup> Aminoguanidine was the first compound designed to inhibit AGE formation and has undergone clinical trials. Despite promising early results, aminoguanidine is unlikely to be used for therapeutic purposes due to safety concerns and lack of efficacy. Metformin, which is routinely used in the treatment of type 2 diabetic patients, has some structural similarities to aminoguanidine. It reduces methylglyoxal, an important precursor of AGE formation in type 2 diabetes.<sup>8</sup> One may speculate whether the beneficial effects of metformin in type 2 diabetic patients, as reported in the UKPDS study, are related to these specific effects on AGE accumulation. Pyridoxamine is a natural intermediate of vitamin B<sub>6</sub> metabolism and is a potent inhibitor of the formation of AGEs.<sup>9</sup> Marked effects of pyridoxamine, such as delayed development of nephropathy and retinopathy, have been

**Figure 1.** Potential sites of intervention in the formation of AGEs (by aminoguanidine pyridoxamine, metformin and antioxidants), AGE cross-link breaking (by ALT-711) and AGE-mediated damage (by sRAGE and antioxidants)



demonstrated in diabetic rats. Pyridoxamine is currently being investigated in phase 3 of clinical trials for the treatment of diabetic nephropathy. All doses are well tolerated, without serious adverse effects. The initial results suggest that albuminuria is markedly reduced.

## REDUCTION OF AGE CROSS-LINKS

The second approach to reduce AGE-induced effects is to diminish AGE cross-links in cardiovascular tissue by 'AGE breakers'.<sup>10</sup> ALT-711 is the first drug from a new class of therapeutic agents that break established AGE cross-links. In a randomised, placebo-controlled trial eight weeks of ALT-711 treatment reduced pulse pressure and arterial compliance in elderly patients. In an open-label, observational study in stable patients with diastolic heart failure, 16 weeks of ALT-711 diminished left ventricular hypertrophy and improved indices of diastolic function. Other clinical trials demonstrated the antihypertensive effect ALT-711, while the prevalence of adverse events is low.

## INTERVENTION IN THE AGE-RAGE PATHWAY

The third approach to reduce the deleterious effects of AGEs is by intervention in the AGE-RAGE interaction or their induced signalling pathway.<sup>11</sup> The soluble form of RAGE (sRAGE) counteracts deleterious effects of AGEs, which suggests RAGE may be a new target for therapeutic intervention in diabetic disorders.

In addition to these approaches, numerous existing drugs against diabetic complications, both natural and pharmacological, are being investigated for their possible therapeutic potential and most of them have anti-AGEing effects. Thiamine and benfotiamine, drugs with antioxidant or metal-chelation properties, such as aspirin, ibuprofen, indomethacin, and flavonoids as well as angiotensin II receptor blockers and angiotensin-converting enzyme inhibitors, were reported to be

inhibitors in the formation of AGEs. The question is whether the biological activities of these drugs are (partly) due to AGE lowering. More specific studies are needed to address that question.

Although it is now well recognised that the accumulation of AGEs in tissues has an important role in the pathogenesis of diabetic complications, the major question remains which AGE(s) is or are the real bad guy(s) and what are the AGE pathways leading to their deleterious effects. Interfering in the glycation pathway may offer new treatments for glucose-derived vascular complications of diabetes.

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