

## REVIEW

## Clinical Relevance of Advanced Glycation Endproducts for Vascular Surgery

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*Atherosclerosis is the main contributor to cardiovascular disease and leads to intimal plaque formation, which may progress to plaque rupture with subsequent thromboembolic events and/or occlusion of the arterial lumen. There is increasing evidence that the development or progression of atherosclerosis is associated with advanced glycation endproducts (AGEs). AGEs are a heterogeneous group of compounds formed by the non-enzymatic reaction of reducing sugars with proteins, lipids, and nucleic acids. An increased understanding of the mechanisms of formation and interaction of AGEs has allowed the development of several potential anti-AGE strategies. This review summarizes AGE formation and biochemistry, the pathogenic role of AGEs in cardiovascular disease, anti-AGE therapies and clinical relevance to vascular surgery.*

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### Introduction

In 1912, Maillard first reported the non-enzymatic reaction between reducing sugars and proteins to form protein–protein cross-links and yellow–brown pigmentation, also known as advanced glycation endproducts (AGEs). Although this phenomenon initially was described in food industry, i.e. the browning that occurs by preparing roasted turkey, Maillard already hypothesized that this reaction also may be important in long-term diabetic complications. Over the last decades, evidence has accumulated that AGEs play an important role in diabetic complications and in normal ageing and atherosclerotic degenerative disease.<sup>1</sup> With aging several structural and functional changes occur in the cardiovascular system and kidneys. These age-related changes significantly

contribute to increased morbidity and mortality. Although numerous mechanisms are certainly involved, it has been proposed that these changes are related partially to increased AGE accumulation.<sup>2</sup>

This review summarizes AGE formation and biochemistry, the pathogenic role of AGEs in cardiovascular disease and their potential relevance to vascular surgery.

### AGEs: Formation, Tissue Damage, and Clinical Assessment

The increased accumulation of tissue advanced glycation end-products (AGEs) is the consequence of a series of complex and protein reactions. AGE accumulation results from a combination of hyperglycemia, hyperlipidemia, oxidative/carbonyl stress and/or decreased renal clearance of AGE-precursors (Table 1).<sup>3,4</sup> The original Maillard hypothesis proposed that chemical modification of proteins by reducing sugars (glycation of proteins) altered the

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**Table 1. Possible sources of the increase in AGE formation**

Increased pro-oxidant activity (e.g. age, diabetes, inflammatory state, bio-incompatibility of dialysis membrane/solution, infection)
Decreased anti-oxidant activity (e.g. vitamins, selenium, glutathione system)
Decreased detoxification of AGE precursors (3-deoxyglucosone reductase)
Less efficient renal excretion of AGE precursors (including dietary intake)
Hyperglycaemia, insulin resistance
Abnormalities in lipid metabolism (e.g. diminished clearance, reduced activity lipoprotein lipase)

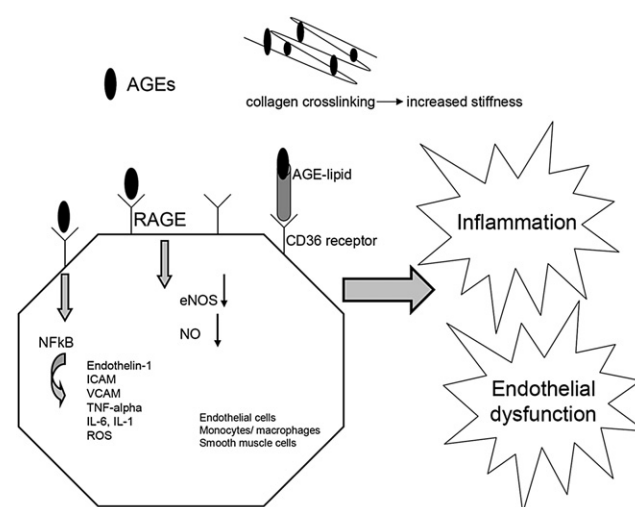
AGE = advanced glycation endproducts.

structure and function of tissue proteins, precipitating the development of diabetic complications.<sup>5</sup> Glycation involves the formation of chemically reversible early glycosylation products with proteins, Schiff bases and Amadori adducts (e.g. glycated hemoglobin; HbA1c). These early adducts underwent slow and complex rearrangements to form advanced glycation end-products (AGEs). The formation of glycoxidation products depends on oxidizing conditions and reactive oxygen species.<sup>6</sup> Furthermore, Maillard products also are formed via lipid-derived intermediates, resulting in advanced lipoxidation products (ALEs)<sup>6,7</sup> (all abbreviations used are summarised in Table 2). Decreased clearance of serum AGEs may increase tissue AGE accumulation and *de novo* formation, and absorption of AGEs from food or smoking may aggravate AGE accumulation.<sup>8–11</sup>

Assessment of tissue AGE accumulation may serve as a measure of cumulative metabolic stress and protein damage. Therefore, tissue AGE accumulation may reflect overall risk instead of single risk factors (e.g. diabetes, or hyperlipidemia) for developing organ damage.

One major mechanism by which AGEs may contribute to the development of vascular complications is through effects on the structure and function of extracellular matrix components (Fig. 1).<sup>12</sup> These AGE-dependent changes may increase vascular stiffness and permeability and contribute to extracellular matrix accumulation (Table 3). Decreased degradation

of basement membrane components and increased binding of plasma proteins may further enhance accumulation. For instance, AGE modifications of plasma lipoproteins may increase vascular deposition of low density lipoproteins (LDL), as a consequence of impaired LDL receptor-mediated clearance, to enhance inflammation and the development of atherosclerosis.<sup>13</sup> Interactions between AGEs and AGE-specific receptors (RAGE) are a second major mechanism by which AGEs contribute to vascular pathology.<sup>14</sup> Although RAGE was first described as a receptor for AGEs, an emerging view is that RAGE is a multiligand receptor of the immunoglobulin superfamily. By binding to such receptors, AGEs induce or perpetuate intracellular transduction mechanisms to produce an array of surface receptors (e.g. endothelial adhesion molecules) and cytokines (e.g. interleukin-1, and tumour necrosis factor  $\alpha$ ) (Fig. 1). AGE accumulation also is directly related to endothelial nitric oxide synthase (eNOS) production and thereby endothelial dysfunction,<sup>15</sup> endothelial RAGE have been proposed as the major key in such interaction (Table 1).



**Fig. 1.** Extracellular advanced glycation endproducts (AGEs) may bind several proteins, including lipids and collagen. The crosslinking of collagen increases vascular stiffness and alters the extracellular matrix. The binding of AGEs to RAGE on e.g. endothelial cells induces a signaling cascade with nuclear factor kappa beta (NF-kB) as key signaling factor. NF-kB increases the transcription of different proteins, including endothelin-1, ICAM (intercellular adhesion molecule), VCAM (vascular cell adhesion molecule), TNF-alpha (tumour necrosis factor) and interleukines (IL). This cascade aggravates vascular inflammation and the production of ROS (reactive oxygen species). Furthermore, AGE-RAGE interaction induces endothelial dysfunction by its effect on endothelial nitric oxide (NO) synthase (eNOS). AGEs activate monocytes, causing increased expression of CD36 receptors leading to increased AGE-lipid (e.g. AGE-LDL) uptake and foam cell formation.

**Table 2. Abbreviations**

AGEs	Advanced glycation endproducts
ALEs	Advanced lipoxidation endproducts
HbA1c	Glycated hemoglobin
RAGE	Receptor for AGEs
esRAGE	Endogenous secretory RAGE
sRAGE	Soluble RAGE
eNOS	Endothelial nitric oxide synthase
AFR	Autofluorescence reader
IMT	Intima-media thickness
MMP	Matrix metalloproteinase

**Table 3. Mechanisms by which AGEs may contribute to cardiovascular complications**

Increased stiffness of connective tissue
Endothelial cells dysfunction: increased permeability, procoagulant state
Mononuclear cell activation: increase in vascular matrix
Modified LDL: atheroma formation
Quenching NO: endothelial dysfunction, defective artery relaxation
Reactive oxygen intermediates: increasing oxidative stress
Proinflammatory responses

LDL = low density lipoproteins, NO = nitric oxide.

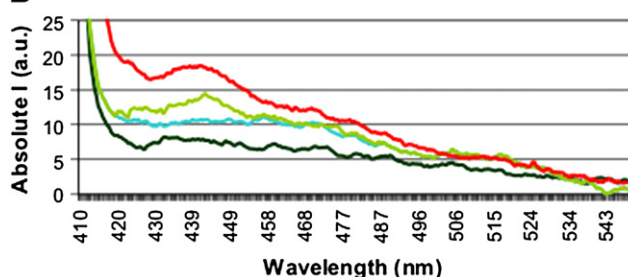
The characteristic fluorescence spectrum of AGEs at 440 nm, upon excitation at 370 nm, has been used to determine tissue AGE accumulation, for example in extracts from homogenates of skin biopsies.<sup>16</sup> Biochemical and immunochemical assays measure both fluorescent AGEs, like pentosidine, and non-fluorescent AGEs, such as carboxymethyl-lysine.<sup>17</sup> HPLC with tandem-mass spectrometry has evolved as the gold standard for specific AGE assays, but complexity, cost and lack of reproducibility of this technique limit its broad use. Moreover, blood and urine sampling of AGE do not necessarily reflect tissue AGE levels.<sup>18</sup>

Recently, we have developed an instrument, the AutoFluorescence Reader (AFR), designed to non-invasively and rapidly measure skin auto-fluorescence and therefore AGE accumulation (Fig. 2). Several studies have shown that skin auto-fluorescence measured by AFR is strongly related to AGE accumulation in healthy subjects, diabetic and hemodialysis patients over a broad age range.<sup>19,20</sup>

## AGEs and Atherosclerotic Disease

### *Carotid stenosis*

Carotid intima-media thickness (IMT) is positively associated with serum concentrations of AGEs.<sup>21</sup> Even after correction for other cardiovascular risk factors, increased carotid IMT remains correlated with raised AGE levels. Plasma levels of AGEs in patients on dialysis treatment are related to carotid IMT and predict further progression of IMT.<sup>22</sup> In an animal study, infusion of AGEs resulted in increased intima thickening of the carotid artery compared to control animals, independently of the presence of diabetes or hypercholesterolemia.<sup>23</sup> RAGE overexpression in plaques from patients undergoing carotid endarterectomy is associated with plaque inflammation and vulnerability.<sup>24</sup> Endogenous secretory receptor for AGEs (esRAGE) binds to AGEs and is capable of neutralizing AGE action. In type 1 diabetic patients,

**A****B**

**Fig. 2.** (A) The AutoFluorescence Reader illuminates a skin surface with an excitation light source between 300–420 nm. Only light from the skin is measured with a spectrometer. (B) Various fluorescence spectrum results from different subjects: healthy subject (black line), diabetic patient without cardiovascular complications (blue line), diabetic patient with peripheral artery occlusive disease (green line), hemodialysis patient with recent myocardial infarction (red line). I = intensity (a.u.).

circulating esRAGE is correlated inversely with carotid IMT.<sup>25</sup> Koyama *et al.* also observed an inverse relationship between esRAGE and carotid IMT in type 2 diabetic patients and non-diabetic subjects.<sup>26</sup> Thiazolidinediones are a class of drugs currently used clinically for their insulin-sensitizing activity. These drugs also inhibit binding of ligands to RAGE. A recent animal study found that thiazolidinediones inhibit RAGE expression at sites of carotid injury to inhibit neointima formation.<sup>27</sup>

### *Aortic and peripheral artery occlusive disease*

AGE accumulation also occurs in atherosclerotic plaques of the aorta<sup>28</sup> and is related to arterial wall stiffness, especially in hypertensive subjects, independent of age, diabetes or renal failure.<sup>29</sup> In cholesterol-fed animals, AGE deposition in the aortic wall increases with IMT, and treatment with statins reduced AGE deposition.<sup>30</sup>

Additionally, AGEs are thought to play a role in features of peripheral artery occlusive disease

(PAD). AGE levels are higher in type 2 diabetic patients with PAD compared to those without.<sup>31</sup> Furthermore, AGE contents are correlated inversely to ankle-brachial index, even after correction for other cardiovascular risk factors. Even in patients without clinically manifest PAD raised AGE levels are related to arterial wall thickness. For instance, women with a history of pre-eclampsia appear to have an increased risk for cardiovascular disease. We recently showed that in such women AGE accumulation was related to femoral artery IMT.<sup>32</sup>

### *Coronary heart disease*

Type 2 diabetic patients with coronary heart disease (CHD) have higher serum AGEs than patients without CHD. Serum AGEs correlate with CHD severity.<sup>33</sup> AGEs remain associated with CHD after correction for other cardiovascular risk factors. Plasma levels of esRAGE are significantly lower in non-diabetic men with CHD compared to those without.<sup>34</sup> The various AGEs bind RAGE. On the other hand RAGE also binds non-AGEs (non-advanced glycation endproducts) termed extracellular newly identified RAGE-binding protein (EN-RAGE), which may further promote inflammation within the plaque.<sup>35</sup> In coronary lesions from sudden cardiac death victims, expression of RAGE and EN-RAGE was significantly greater in diabetic subjects (versus non-diabetics) and was associated with apoptosis of smooth muscle cells and macrophages.<sup>36</sup>

Skin autofluorescence also is increased in (non-diabetic) persons with stable coronary artery disease and is related to markers of inflammation.<sup>37</sup> Furthermore, skin autofluorescence is an independent marker of acute myocardial infarction and it predicts major adverse cardiac events after acute myocardial infarction.<sup>37</sup> AGE deposits have been demonstrated within cardiomyocytes, and serum levels of AGEs in type 1 diabetic patients are related to isovolumetric relaxation time of the left ventricle, a marker of left ventricular diastolic dysfunction.<sup>28,38,39</sup> Also in patients with end stage renal disease AGE content is related to left ventricular geometry and with several markers of diastolic function.<sup>39</sup>

### *Prediction of cardiovascular events and mortality*

AGEs are not only related to manifestations of cardiovascular disease, they also provide prognostic information. AGE content predicts adverse cardiac events in patients after cardiac surgery.<sup>40</sup> In patients with heart failure pentosidine, one of the most studied AGEs, is a predictor of cardiac events second only to

known risk factors such as left ventricular mass and B type natriuretic peptide.<sup>41</sup>

Increased serum levels of AGEs predict total, cardiovascular and coronary mortality in women with type 2 diabetes.<sup>42</sup> AGE level remained a strong predictor of survival, even after adjustment for confounding factors, including CRP. During long-term follow-up we also observed that AGEs (skin autofluorescence) were strong predictors of survival in both diabetic and hemodialysis patients.<sup>19,43</sup> In hemodialysis patients, skin autofluorescence is a strong predictor of cardiovascular mortality, together with history of CHD, low plasma albumin and high CRP. Multivariate analysis showed that skin autofluorescence was a stronger predictor of mortality than traditional risk factors, such as smoking and lipid profile. Although tissue AGEs are related to cardiovascular events/mortality, others have suggested that serum levels of AGEs do not predict future events. However, serum AGEs may be influenced by dialysis modalities, absorption from food and smoking.<sup>9,10,44</sup> High serum AGEs might even reflect a better nutritional status, which is associated with improved survival.<sup>45</sup> Recently, in dialysis patients we observed that skin autofluorescence, but not the serum levels of specific AGEs, predicted composite cardiovascular outcomes (combination of cardiovascular death and events (unpublished data)). Also others have shown that tissue fluorescence is a strong predictor of survival in "healthy" subjects.<sup>46</sup> Plasma fluorescent products significantly and independently predict coronary heart disease in subjects without history of cardiovascular disease.

### **Plaque Vulnerability**

There is increasing evidence that inflammation plays a central role in the progression of plaque erosion and rupture. For instance, plaque macrophages synthesize matrix metalloproteinases (MMPs), which are capable of degrading plaque constituents, and thereby are biomarkers of plaque vulnerability. In humans, RAGE overexpression has been associated with enhanced inflammatory reactions at the vulnerable region of the plaque in carotid endarterectomy specimens.<sup>24</sup> Plaque studies in-vitro indicated that AGEs increase MMP levels in a dose-dependent manner. In an intervention study it has been shown that statin treatment prior to carotid endarterectomy reduces inflammation, MMPs and RAGE expression.<sup>47</sup>

Apoptosis is a late hallmark of atherosclerosis associated with plaque instability and/or rupture.<sup>48</sup> Apoptosis might affect up to 50% of macrophages located in a ruptured plaque, a phenomenon which



is not seen in a stable atherosclerotic plaque. Burke *et al.* performed a postmortem analysis of coronary atherosclerotic plaques from patients with sudden coronary death.<sup>36</sup> Expression of RAGE in coronary plaques was associated with apoptotic smooth muscle cells and macrophages.

The AGE–RAGE axis seems relevant in the progression to vulnerable plaques. Administration of soluble RAGE (sRAGE) blocks AGE–RAGE interaction and stabilizes atherosclerotic lesions and complexity in non-diabetic animals.<sup>49</sup> Interventions specifically aimed at AGE-reduction may further help in understanding the role of AGEs–RAGE in the progression to vulnerable plaques.

### AGEs and the Diabetic Foot

In diabetic feet, both macrovascular complications, as described above, and microvascular complications (neuropathy) play important roles. Monnier *et al.* first described the relation between AGE accumulation in skin collagen and the severity of long-term microvascular diabetic complications.<sup>16</sup> Sugimoto *et al.* showed excess deposition of AGEs in human diabetic peripheral nerves.<sup>50</sup> In-vitro AGEs induced neuronal apoptosis in a synergistic action with nitric oxide.<sup>51</sup> We have recently shown in diabetic patients that skin autofluorescence is related to the severity of both diabetic neuropathy and foot ulceration.<sup>52</sup> Although for this study, the presence of peripheral macrovascular disease was an exclusion criterion, a complex interrelationship between neuropathy and microvascular disease exists in diabetic patients. Microvascular disease may worsen ulceration, and endothelial dysfunction has been demonstrated in relation to both diabetic neuropathy and foot ulceration.<sup>53</sup> AGE accumulation has been reported to worsen endothelial function and endothelial RAGE has been proposed as the pivotal molecule. RAGE blockade accelerates wound closure in diabetic mice and suppresses levels of cytokines such as tumor necrosis factor.<sup>54</sup> Therefore, AGE accumulation may play a role in diabetic foot ulceration by its effects on neuropathy, on vascular disease and on delayed wound repair.

### AGEs and Graft/Stent Patency

The origin of intimal hyperplasia development is multifactorial. AGEs contribute to the formation of neointimal hyperplasia following acute vessel wall injury.<sup>55,56</sup> Two independent animal studies showed

up-regulation of RAGEs and increased expression of AGEs in the vessel wall after injury. Blockade of the receptor by sRAGE directly after injury showed a beneficial effect on the occurrence of neointimal hyperplasia. In-stent re-stenosis of cardiac stents in (diabetic) patients might be attributed to increased AGEs and therefore inflammation at the site of the stent. In diabetic patients receiving cardiac stents an elevated level of serum AGEs appeared to be an independent risk factor for the development of angiographic re-stenosis.<sup>57</sup>

Cai *et al.* postulated a relation between graft failure and increased deposition of AGEs in the vessel wall, located at the site of intimal hyperplasia in arteriovenous grafts at the position of the venous anastomoses.<sup>58</sup> Serum AGEs are higher in hemodialysis patients with at least one thrombotic complication in their history compared to those without.<sup>59</sup> Further research is necessary to support positive effects of anti-AGEs medical treatment in diabetic or hemodialysis patients receiving vascular interventions.

### Anti-AGE Intervention

In diabetes, normalizing high glucose levels may help in decreasing AGE formation and accumulation.<sup>60</sup> The discovery of chemical agents that can inhibit glycation reactions may have potential therapeutic importance. For instance, pyridoxamine, has been shown to inhibit AGE formation and the formation of lipid-derived Maillard products; advanced lipoxidation endproducts (ALEs).<sup>61</sup> Pyridoxamine inhibits the development of renal and vascular complications in obese rats.<sup>62</sup> Other less-studied drugs have been shown to be potent inhibitors of AGE formation. Angiotensin converting enzyme inhibitors, angiotensin II type 1 receptor blocker, calcium antagonist, statins, all may decrease AGE formation, probably by reducing oxidative stress.<sup>63</sup>

A second approach has been focused on the cleavage of already formed AGEs protein-protein crosslinks. Compounds like 4,5-dimethyl-3 phenacylthiazolium, also known as ALT-711, have been tested widely. AGE-breakers have been shown to break preformed AGE crosslinks, and to improve arterial compliance in a phase 2 clinical trial in elderly.<sup>64</sup>

RAGE blockade and restricted dietary intake of AGEs are new fields of interest in anti-AGE therapies.

### Conclusion

AGEs measurements report the cumulative burden of hyperglycaemia, hyperlipidaemia, oxidative and

carbonyl stress, and renal dysfunction. As a measure of protein damage AGE may serve as an indicator of over-all risk for organ damage. Importantly, AGEs are related to the progression of vascular stenosis and plaque vulnerability. AGEs predict future cardiovascular morbidity and mortality. Of great interest is the relationship of AGEs with wound healing disturbances (e.g. diabetic foot) and its effect on stent / graft patency. Interventional studies offer the prospect of reducing AGE accumulation and cardiovascular risk. Finally, it is important to test whether AGE results have an effect on final treatment in cardiovascular disease, such as influencing indications for surgery (plaque vulnerability), and increasing operative success (patency). In conclusion, vascular AGE accumulation has numerous implications for vascular surgery.

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