

Advanced Glycation End Products and Diabetic Cardiovascular Disease

Anand Prasad, MD,* Peter Bekker, MD,† and Sotirios Tsimikas, MD†

Abstract: Advanced glycation end products (AGEs) are formed by a nonenzymatic reaction of sugar moieties (eg, glucose, fructose, glycolytic adducts) with the free amino groups on amino acid residues of proteins. A growing body of data demonstrate that AGEs are intimately involved in the pathophysiology of cardiovascular disease by stimulating inflammation, contributing to atheroma formation, and modulating vascular stiffness. The role of AGEs as potential biomarkers for disease presence and prognosis in patients with diabetes mellitus remains an active area of study. Epidemiologic and angiographic studies suggest that AGE levels may be related to the presence and extent of atherosclerosis, and may predict future outcomes in select populations. The present review summarizes the relevant evidence supporting the role of advanced glycation in promoting atherosclerosis and the epidemiologic studies demonstrating an association between AGEs and diabetic cardiovascular disease.

Key Words: diabetes mellitus, atherosclerosis, advanced glycation

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Emerging basic and clinical data over the past 2 decades have helped expand our understanding of diabetic vascular disease. It is now clear that the presence of diabetes mellitus (DM) is not only a potent risk factor for the development of atherosclerosis, but is viewed as a cardiovascular disease (CVD) equivalent.¹ Despite this realization, efforts to reduce the development and progression of systemic atherosclerosis in this population have been limited. CVD remains the primary cause of death in patients with DM, and continues to contribute to significant morbidity in these individuals.^{2,3}

The deleterious effect of hyperglycemia on microvascular complications has been well established, but the interaction of glucose control and macrovascular disease remains unclear. Although the underlying etiologies for these data are multifactorial, the disconnect between aggressive glycemic control and a clear reduction of cardiovascular (CV) events appears to be a consistent finding in several large clinical trials.⁴ The pathophysiologic focus responsible for the large vessel atherosclerosis of DM must be expanded beyond consideration of hyperglycemia to include the role of oxidative stress and oxidative lipid and protein modification. In this context advanced glycation end products (AGEs) may play an important role in the development of diabetic CV complications.

FORMATION AND PATHOPHYSIOLOGIC SIGNIFICANCE OF AGEs

AGEs are formed by the nonenzymatic reaction of sugar moieties (eg, glucose, fructose, glycolytic adducts) with the free amino

groups on amino acid residues of proteins.⁵ This reaction was first described by Louis Camille Maillard in the early 1900s and has been important in the food science field as the basis of “browning” of foodstuffs in response to heat.^{6,7} Clinically important Maillard reactions may also occur between sugars and lipoproteins (glycated low-density lipoprotein [LDL])⁸ and with nucleic acids (glycated DNA resulting in the potential for mutations).^{9–11} Furthermore, similar processes involving peroxidation of lipids may result in the generation of advanced lipoxidation end products (ALEs).¹²

The common driving force for the generation of all these products is oxidative stress, and the pathways for generation of several AGEs and ALEs are interrelated (Fig. 1). This complex relationship emphasizes that production of these entities is not simply dependent on hyperglycemia for generation and can occur via alternative nonglucose-centered pathways. The steps leading to the ultimate formation of AGEs begin with a reversible series of nonenzymatic interactions between sugars and proteins leading to the intermediate structures: the Schiff base and Amadori products. In the context of hyperglycemia, the Amadori products are related to the concentration of glucose and may rise or fall depending on the degree of glucose control. A clinically familiar Amadori product is glycated hemoglobin (HbA1c%), which reflects short-term (<12 weeks) glycemic control.¹³ It is important to note that glucose itself is not as potent a glycation substrate as glycolytic intermediates such as dihydroxyacetone-phosphate, glyceraldehyde-3-phosphate, glyoxal, methylglyoxal, and 3-deoxyglucosone.¹⁴ In addition, other potent moieties driving AGE formation include fructose, fructose-3-phosphate, glyceraldehyde-3-phosphate, and 3-deoxyglucosone.¹⁵ Of these compounds, methylglyoxal is of particular interest as it is a very potent glycating agent. The Amadori products themselves undergo slow rearrangements, ultimately leading to the formation of stable AGEs. This process occurs over months; therefore, the detection of AGEs in tissue has focused on long-lived structural proteins (such as collagen) in the skin, eyes, and kidneys.^{16,17}

Once formed, AGEs may have multiple deleterious consequences related to alteration of CV structure and vascular inflammation (Fig. 2). Advanced glycation products such as pentosidine and N'-carboxymethyllysine (CML) have cross-linking properties and have been associated with the development of vascular stiffening.^{18–20} Pharmacologic disruption of these cross-links has been exploited as a potential therapy for hypertension and congestive heart failure.^{21–24} AGEs can also stimulate proinflammatory pathways including activation of nuclear factor κ -light-chain-enhancer of activated B cells by interaction with the receptor for AGE.²⁵ Glycation of atheroma collagen may also be important in lesion progression and vulnerability by predisposing to LDL deposition.^{26–28} Furthermore, glycation of LDL itself may increase its susceptibility to oxidation and subsequent development of atherosclerosis.⁸ AGEs also appear to react with and inactivate nitric oxide leading to endothelial dysfunction. Further linking of these biochemical findings with human atherosclerosis is a demonstration of the presence of AGEs within atherosclerotic plaques. Nakamura et al,²⁹ using immunohistochemical staining of autopsy-derived coronary artery segments from patients with DM demonstrated that AGE staining is diffusely distributed. However, there appears to be increased

From the *Department of Medicine, Division of Cardiology, University of Texas Health Science Center at San Antonio, TX; and the †Department of Medicine, Division of Cardiology, University of California, San Diego, CA.

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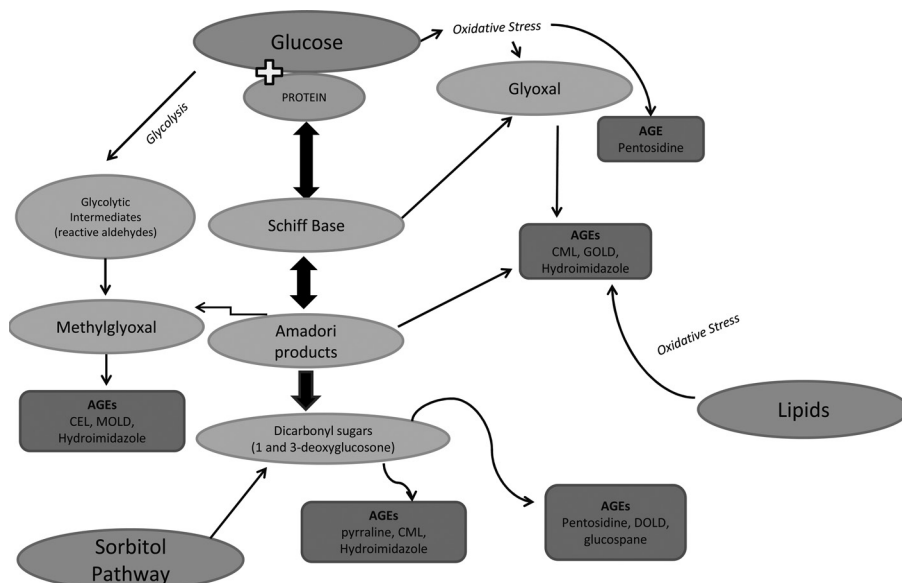
Correspondence: Anand Prasad, MD, FACC, FSCAI, Division of Cardiology, University of Texas Health Science Center at San Antonio, MC 7872, 7703 Floyd Curl Drive, San Antonio, Texas 78229-3900. E-mail: anandprasadmd@gmail.com.

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FIGURE 1. Multiple pathways involved in AGE formation. AGE formation is driven by oxidative stress. Multiple pathways can lead to generation of a variety of different AGEs including carboxyethyllysine (CEL), N'-carboxymethyllysine (CML), 3-deoxyglucosone-derived lysine dimer (DOLD), methylglyoxal lysine dimer (MOLD), glyoxal-derived lysine dimer (GOLD), and pentosidine.



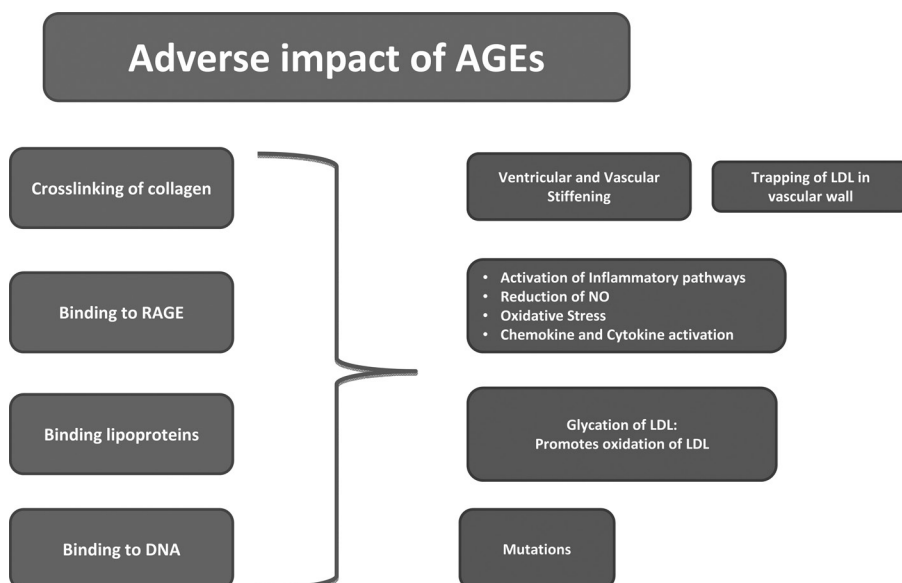
localization to the highly fibrous regions of the plaque surrounding the lipid core. A representative section from the study is shown in Figure 3.

Delineation of the specific AGEs (and ALEs) that are responsible for adverse changes in vascular function remains an active area of study. Complicating this evaluation is the fact that both endogenous and exogenous mechanisms result in the generation of a multitude of different interrelated AGE species. The majority of data with regard to the biological significance of specific AGEs come from indirect evidence and association studies. In general, several entities have been well studied with regard to both deleterious effect on ex vivo biochemical pathways and clinical relevance. For example, both methylglyoxal and CML are the principal molecules found in high AGE diets associated with clinically measurable vascular inflammation and are linked to the development of atherosclerosis,^{8,30} whereas pentosidine and CML have been linked to diabetic microangiopathy.^{17,31,32}

DIETARY SOURCES OF AGEs

The generation of endogenous AGEs primarily occurs through the mechanisms discussed earlier involving oxidative modification of glycolytic intermediates and lipid substrates. However, given the well-described role of the Maillard reaction in food science, considerable interest has focused on the contribution of diet to the whole body AGE pool. The total AGE content of a variety of foodstuffs has been quantified and generally the data reflect higher AGE levels in heat-treated foods.³³ An sharp increase in serum levels of AGEs can be detected after ingestion of a high AGE load meal.^{34,35} The consequences of AGE ingestion are not benign and may lead to vascular dysfunction. Negrean et al³⁴ noted marked impairment of macrovascular (brachial artery flow-mediated dilation) and microvascular function (skin microvascular hyperemic blood flow change), and elevation of markers of inflammation and oxidative stress in people with DM within 2 hours of ingesting a high AGE content meal. In a cross-over design, Vlassara et al³⁰ examined chronic high AGE ingestion

FIGURE 2. Adverse consequences of AGEs. RAGE, receptor for advanced glycation end products; LDL, low-density lipoprotein; and NO, nitric oxide.



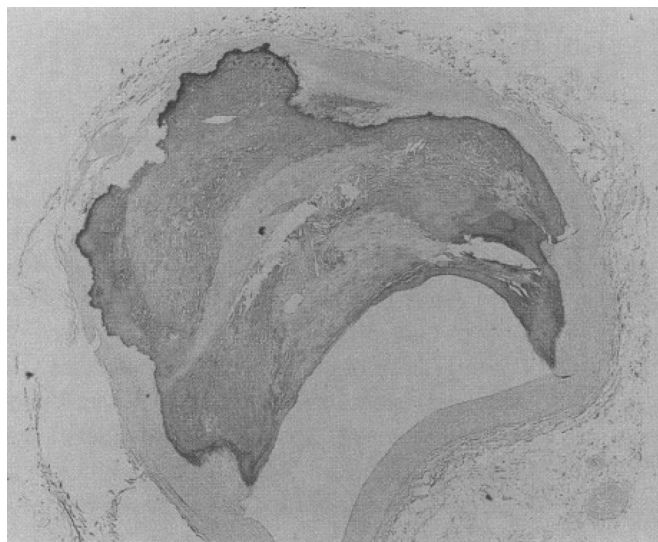


FIGURE 3. AGEs are localized within atherosclerotic plaque. Transverse section of a left anterior descending coronary artery from a patient with diabetes with immunohistochemical staining of AGEs. Reproduced with permission from Nakamura Y, Horii Y, Nishino T, et al. Immunohistochemical localization of advanced glycosylation end products in coronary atheroma and cardiac tissue in diabetes mellitus. *Am J Pathol.* 1993;143:1649–1656.

(6 weeks) on markers of vascular inflammation (C-reactive protein [CRP], vascular cell adhesion molecule-1) in subjects with DM (majority without established CVD). The authors found that CRP increased by 35% after a high AGE diet and decreased by 20% on a low AGE diet. Vascular cell adhesion molecule-1 increased by 4% on the high AGE diet and decreased by 20% on the low AGE diet.

These data suggest that not only is high AGE content food associated with adverse markers of vascular function, but that lowering AGE content in the diet may be a therapeutic strategy in patients with DM above and beyond a low glycemic-low cholesterol diet. Ample data demonstrate that a diet low in saturated fats and rich in monounsaturated fats—the so-called Mediterranean diet—is associated with enhanced vascular function as assessed by flow-mediated dilation and with improved CV outcomes.³⁶ A randomized longitudinal assessment of a low AGE diet on CV function as compared with such traditional “heart healthy” diets has not been performed. The effect of a Mediterranean style diet itself, on AGE levels, has not been studied in detail. In general, fats, even mono- and polyunsaturated lipids, are rich in AGEs and the AGE content increases with heating, suggesting that avoidance of fat is important in a low AGE diet.³⁷ In addition, it appears that the method of heat treatment may be the most important modulator of the final AGE content of ingested foodstuffs. Recent data would suggest that for a given food substrate, cooking methods such as boiling, steaming, and stewing may result in less AGE content when compared with broiling or frying.³⁷ The particular AGEs in food that are responsible for vascular dysfunction and the long-term effect of a chronic high AGE diet on CV outcomes remain to be better defined.

SERUM LEVELS OF AGEs AS BIOMARKERS OF CVD

Population Studies

Serum AGE levels appear to be consistently elevated in patients with DM versus people without DM even when adjusted

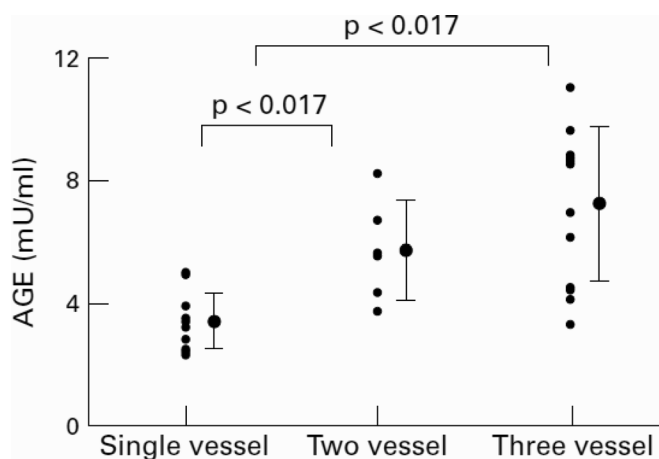


FIGURE 4. Serum AGE levels are related to the severity of coronary artery disease. There is a graded relationship between the extent of coronary artery stenoses and total AGE levels in patients with diabetes. Reproduced with permission from Kiuchi K, Nejima J, Takano T, et al. Increased serum concentrations of advanced glycation end products: a marker of coronary artery disease activity in type 2 diabetic patients. *Heart.* 2001;85:87–91.

for age and sex. However, elevated AGE levels are not simply a surrogate for hyperglycemia or diabetes but rather are independently correlated with the presence of macro- and microvascular disease. In a series of studies, Kilhovd et al^{38–40} evaluated the relationship between AGEs and CV events and mortality in a Finnish population with and without DM. In a random population sample of *nondiabetic* patients (535 male, and 606 female), clinical history, blood pressure data, glucose, lipid measurements, and total AGE (polyclonal anti-AGE enzyme-linked immunosorbent assay) were obtained.³⁸ The end points were all-cause death, non-CVD death, CVD death, and coronary heart disease death. Detailed data on myocardial infarction or stroke were not reported. Patients were followed for a median of 17.8 years. Baseline characteristics were markedly different between the men and women in the study, with women having significantly higher body mass index, LDL cholesterol, prevalence of hypertension and lower fasting glucose, total AGE levels, smoking prevalence, and history of myocardial infarction. Despite these differences, the authors found that increased serum AGE levels (in the top quartile) predicted total and coronary heart disease mortality in women but not in men, independent of traditional CV risk factors (including hs-CRP).

Using a similar approach, the same group subsequently studied the relationship between serum AGE levels and mortality in 874 individuals with type 2 diabetes.⁴⁰ Patients were followed up to 18 years after enrollment. Again baseline characteristics were unbalanced between men and women, with women having significantly higher age, body mass index, LDL cholesterol, fasting glucose, glycated HbA1c%, a lower rate of current smoking, prior CVD, creatinine clearance, and total AGE levels. Similar to the data reported for patients without diabetes, adjusted mortality (total, non-CVD, and CVD) was significantly related to serum AGE levels in women but not in men. Female subjects with the highest quartiles of AGE levels had the highest risk of mortality independent of traditional CV risk factors or hs-CRP. The sex-based differences underlying the findings of both studies were unclear and to date the interaction of sex and advanced glycation remains uncertain.

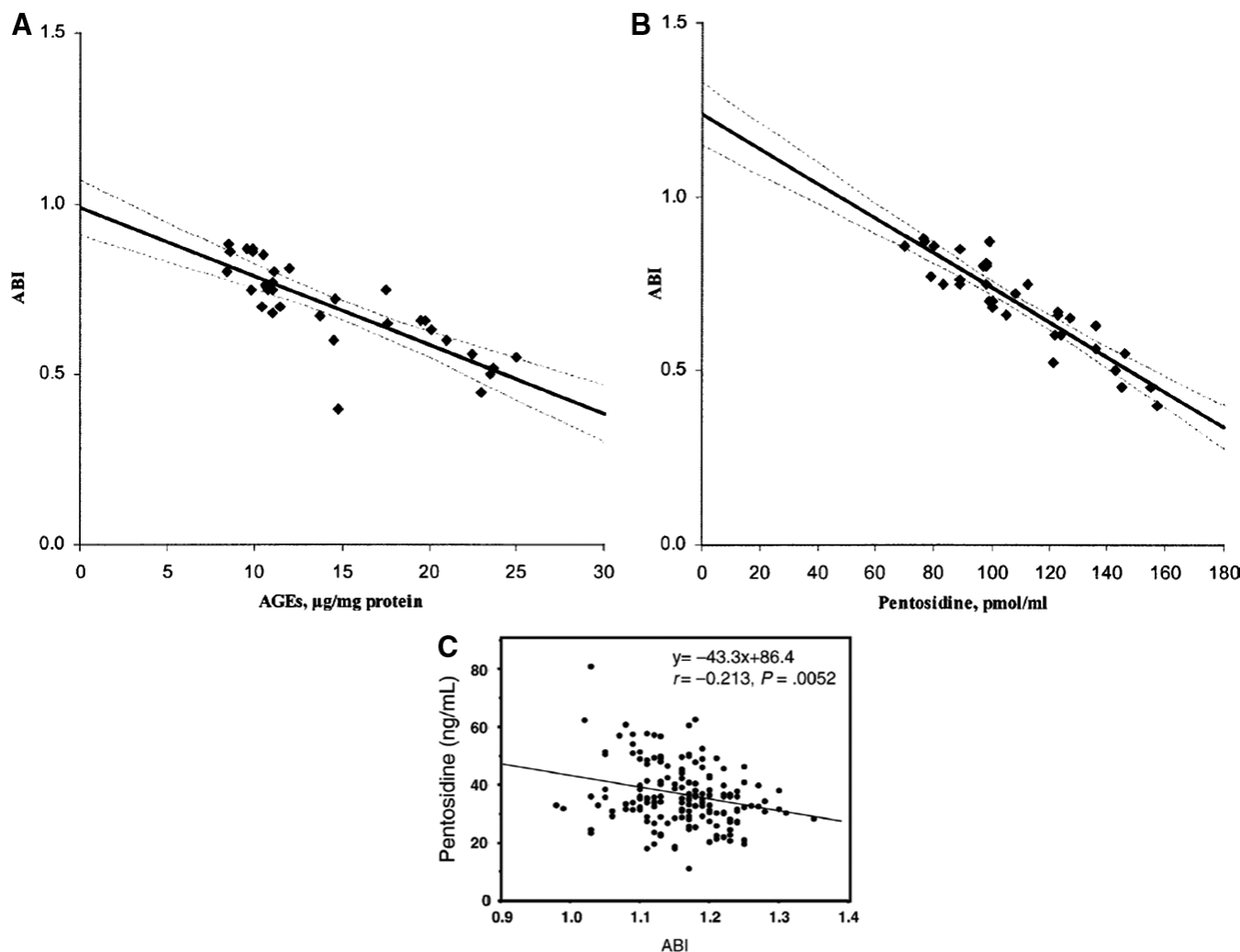


FIGURE 5. A and B, Inverse relationships between total AGE and pentosidine levels the ankle brachial index in patients with diabetes. Reproduced with permission from Lapolla A, Piarulli F, Sartore G, et al. Advanced glycation end products and antioxidant status in type 2 diabetic patients with and without peripheral artery disease. *Diabetes Care*. 2007;30:670–676. C: Inverse relationships between pentosidine levels and the ankle brachial index in healthy male nondiabetic subjects. Reproduced with permission from Takahashi R, Imamura A, Yoshikane M, et al. High serum concentrations of pentosidine, an advanced glycation end product, are associated with low normal value of ankle-brachial index in apparently healthy men. *Metab Clin Exp*. 2011;60:649–654.

Recently, Nin et al⁴¹ examined the association of plasma AGE levels and CVD incidence and all-cause mortality in 339 middle-aged patients with type 1 DM (with and without diabetic nephropathy) who were free of apparent CVD. The authors specifically measured N'-carboxyethyllysine (CEL), CML, and pentosidine levels. The patients were prospectively studied with detailed baseline clinical data and biomarkers and the end points included a primary combined end point of fatal and nonfatal CVD (ischemic and revascularization events) and the secondary end point of all-cause mortality. After adjustments for age, sex, duration of diabetes, and HbA1c%, the incidence of fatal and nonfatal CVD was independently related to baseline AGE levels. This relationship was similar for all 3 measured AGEs. Unlike the studies by Kilhovd et al, there were no reported differences by sex in AGE levels (Dr. Johanna Nin, personal communication, 2011), though it should be emphasized that the populations

studied (type 1 DM versus type 2 DM) and AGEs measured (CEL, CML, pentosidine versus total AGE) were different in the 2 sets of studies.

Atherosclerotic Burden Studies

Although an examination of population cohorts demonstrates a potential link between AGE levels and CVD risk, angiographic studies suggest a relationship between the burden of atherosclerosis and AGE levels. Kiuchi et al⁴² measured total AGE levels in patients with DM having angina who were undergoing coronary angiography. The authors noted that AGE levels rose progressively with the severity of coronary artery disease (CAD) (Fig. 4) in a graded fashion. This relationship was independent of CV risk factors such as hypertension, hyperlipidemia, and smoking. In the noncoronary circulation, Lapolla et al⁴³ demonstrated an inverse

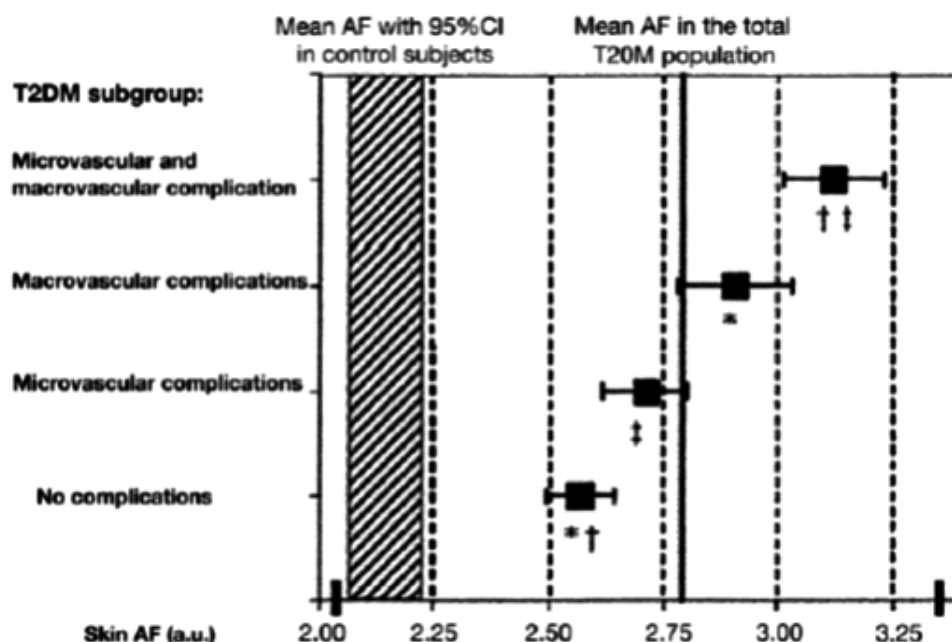


FIGURE 6. Skin autofluorescence is related to the severity of diabetic micro- and macrovascular complications. Mean skin autofluorescence with 95% confidence intervals (CIs) in different categories of complications in a population of patients with type 2 diabetes. Reproduced with permission from Lutgers HL, Graaff R, Links TP, et al. Skin autofluorescence as a noninvasive marker of vascular damage in patients with type 2 diabetes. *Diabetes Care*. 2006;29:2654–2659.

relationship between the ankle brachial index (ABI) and AGE levels (total AGE and pentosidine) in an outpatient population of type 2 DM patients with peripheral arterial disease (PAD) (Fig. 5, A and B). More recently, Takahashi et al⁴⁴ studied 170 healthy men (free of CVD and without diabetes) and measured ABIs and serum pentosidine levels. Although the ABIs were nearly all above 1.0 in this population, there seems to be a significant inverse relationship between pentosidine levels and ABIs (Fig. 5C). In multivariable analyses, the ABI remained significantly associated with pentosidine levels. Although the data from both of the abovementioned ABI studies are compelling, the role of AGEs in PAD remains unclear. In the study by Lapolla et al,⁴³ the symptomatic stage of the patients was not reported and the population had no evidence of aortoiliac or femoropopliteal disease—rather only noncalcified infrapopliteal disease. The relationship of AGEs to PAD in patients with more extensive distribution of disease or calcified infrapopliteal vessels remains to be elucidated. Furthermore, the relationship between PAD and AGE levels independent of concomitant CAD requires further study. Nonetheless, these data reinforce the concept that AGE levels appear to be determined in part by the degree of atherosclerotic burden.

SKIN AUTOFLUORESCENCE TO DETECT AGEs

In addition to serum detection of AGE, skin accumulation of several AGEs, including pentosidine, CML, and CEL, may be measured by using noninvasive skin autofluorescence (SAF) readers. Using this method, SAF has been correlated with the presence and severity of microvascular disease in patients with DM and in individuals with renal failure.^{31,32} AF of components of the eye such as the cornea and lens has been related to the presence of AGE deposition and retinopathy.⁴⁵ AF also appears to provide prognostic information about macrovascular disease development and progression. SAF has been examined in the context of diabetic complication risk prediction models. In the UKPDS study,

for example, SAF provided added risk stratification power (on top of the traditional UKPDS risk score) in identifying patients at high risk for developing CVD.⁴⁶ Lutgers et al demonstrated that not only the presence but the degree of AF was related to the development of both micro- and macrovascular disease. The authors examined clinical data and SAF measurements obtained from 973 patients with type 2 DM and in 231 nondiabetic control patients. As expected, SAF was higher in the group with DM versus the control patients. More interestingly, within the cohort with DM there was a graded relationship between SAF and vascular complications (Fig. 6).⁴⁷ The degree of severity of established macrovascular disease may also be related to SAF levels. Mulder et al performed a small observational study on 88 individuals presenting with an acute ST segment elevation myocardial infarction, 81 patients with stable angina, and 32 controls without CVD. SAF was measured with an automatic reader in all groups. Mean SAF readings were higher in those patients with CAD versus controls and in the ST segment elevation myocardial infarction group as compared with the stable angina group. Furthermore, although the sample size was small and event rates low, SAF appeared to be related to subsequent mortality and clinical events (Fig. 7, A and B).⁴⁸ Finally, with regard to noncoronary atherosclerosis, Noordzij et al⁴⁹ recently examined SAF in 56 patients with carotid artery stenosis (with or without concomitant lower extremity PAD), and 56 healthy controls (matched for age and sex). There was a graded relationship between disease severity and SAF such that AF was not only higher in carotid stenosis patients versus healthy controls, but higher in carotid patients with PAD versus those without PAD. On multivariable analysis, age, smoking, DM, renal function, and PAD were determinants of AF levels; however, carotid stenosis in and of itself was not. Though limited by a small sample size and lack of direct detection of PAD or CAD, these data again suggest that an association may exist between peripheral atherosclerosis and AGEs.

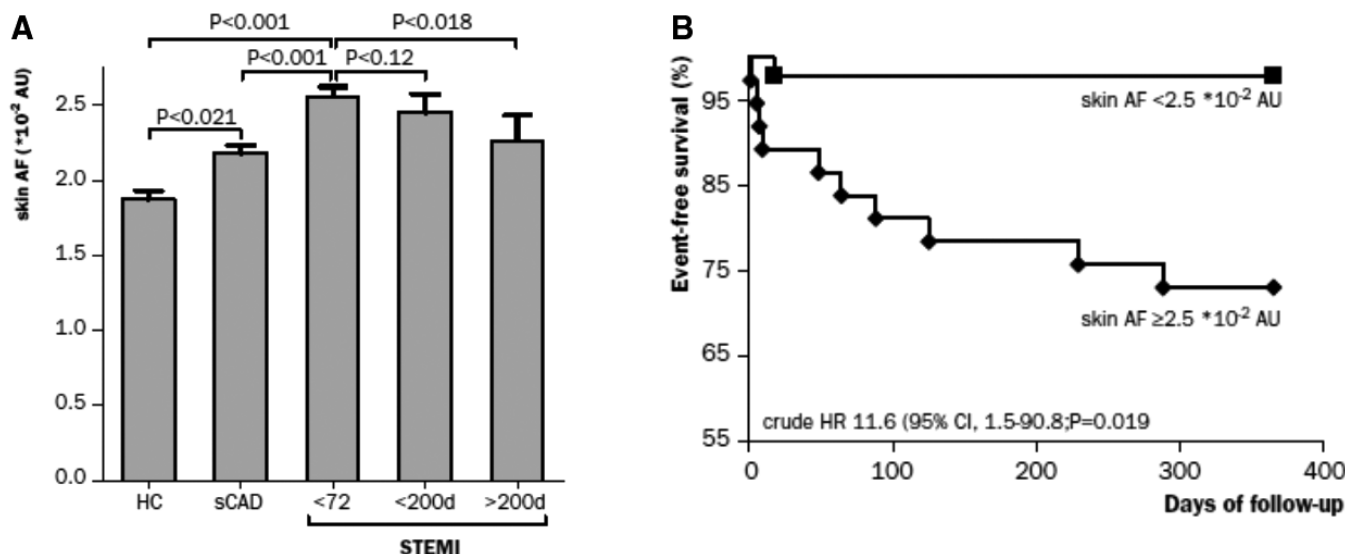


FIGURE 7. A, Skin autofluorescence in patients with stable coronary artery disease and in those presenting with an ST-segment elevation myocardial infarction. Data are compared with healthy controls. Bar charts represent mean skin autofluorescence (AF) + standard error in healthy age- and sex-matched controls (HC) and patients with stable coronary artery disease (sCAD) and <72 hours following ST-elevation myocardial infarction (STEMI). Two last bars represent follow-up measurements of STEMI patients, <200 days ($n = 15$; nonsignificant decrease) and >200 days ($n = 14$; significant decrease [$P = 0.018$]) after discharge. AU, arbitrary units. Reproduced with permission from Mulder DJ, van Haelst PL, Graaff R, et al. Skin autofluorescence is elevated in acute myocardial infarction and is associated with the one-year incidence of major adverse cardiac events. *Neth Heart J*. 2009;17:162–168. B, Outcomes in patients with ST segment myocardial infarction stratified by skin autofluorescence (AF). Kaplan-Meier estimates of survival during 1-year follow-up with regard to the composite end point of all-cause mortality and hospitalization for myocardial infarction or heart failure in patients with ST-elevation myocardial infarction in relation to skin autofluorescence below and above the median. Skin AF was $<2.5 \times 10^{-2}$ AU in 46 and $\geq 2.5 \times 10^{-2}$ AU in 42 patients. CI, confidence interval; AU, arbitrary units; and HR, hazard ratio. Reproduced with permission from Mulder DJ, van Haelst PL, Graaff R, et al. Skin autofluorescence is elevated in acute myocardial infarction and is associated with the one-year incidence of major adverse cardiac events. *Neth Heart J*. 2009;17:162–168.

CONCLUSIONS

The accumulated data regarding the role of AGEs in vascular disease would suggest that glycation may play an important role in mediating CV risk in patients with DM. Conceptually, these data are congruent with physiologic studies demonstrating the interplay of advanced glycation with inflammation, oxidative stress, and atheroma formation. From a clinical standpoint, additional population-based analyses (in both patients with DM and without DM) will be required to determine the independent effect of increased AGEs on CV outcomes. Furthermore, it remains uncertain at this time whether total AGE levels, SAF, or specific glycation moieties (such as CML or pentosidine) will best serve as clinically useful biomarkers in this context. Thus far, the associations between AGE levels and atherosclerosis presence and progression in individual subjects are based on relatively small studies, but suggest a graded response in coronary and peripheral circulation.

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