Advanced Glycation End Products, Oxidation Products, and the Extent of Atherosclerosis During the VA Diabetes Trial and Follow-up Study

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OBJECTIVE

To determine whether plasma levels of advanced glycation end products and oxidation products play a role in the development of atherosclerosis in patients with type 2 diabetes (T2D) over nearly 10 years of the VA Diabetes Trial and Follow-up Study.

RESEARCH DESIGN AND METHODS

Baseline plasma levels of methylglyoxal hydroimidazolone, Nε-carboxymethyl lysine, Nε-carboxyethyl lysine (CEL), 3-deoxyglucosone hydroimidazolone and glyoxal hydroimidazolone (G-H1), 2-aminoadipic acid (2-AAA), and methionine sulfoxide were measured in a total of 411 participants, who underwent ultrasound assessment of carotid intima-media thickness (CIMT), and computed tomography scanning of coronary artery calcification (CAC) and abdominal aortic artery calcification (AAC) after an average of 10 years of follow-up.

RESULTS

In risk factor–adjusted multivariable regression models, G-H1 was associated with the extent of CIMT and CAC. In addition, 2-AAA was strongly associated with the extent of CAC, and CEL was strongly associated with the extent of AAC. The combination of specific advanced glycation end products and oxidation products (G-H1 and 2-AAA) was strongly associated with all measures of subclinical atherosclerosis.

CONCLUSIONS

Specific advanced glycation end products and metabolic oxidation products are associated with the severity of subclinical atherosclerosis over the long term and may play an important role in the "negative metabolic memory" of macrovascular complications in people with long-standing T2D.

Atherosclerosis is accelerated in patients with type 2 diabetes (T2D) and is a major cause of their cardiovascular disease (CVD) events. Although the critical role of chronic hyperglycemia in the development and progression of atherosclerosis and CVD events is well established (1,2), intensive glycemic control over 3–6 years did not reduce the progression of atherosclerosis and development of CVD in people with long-standing T2D (2–5). However, the longer follow-up periods (≥10 years) of

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several studies (6,7) have revealed that people receiving more intensive glucose-lowering therapy have a lower incidence of CVD events, even after the glycemic difference between treatment groups is lost. Although the underlying mechanisms for this delayed improvement in vascular outcomes is not well established, this long-lasting effect of poor glycemic control has been referred to as "negative metabolic memory" (8). The long-lasting reactive intermediates of hyperglycemia, such as advanced glycation end products (AGEs) and oxidative stress products (OxPs) are considered to be key players in the "glycemic memory" of vascular endothelial cells (9). AGEs are formed by the nonenzymatic reaction of glucose and other glycating compounds, such as methylglyoxal, glyoxal, and 3-deoxyglucosone, which are increased under hyperglycemic conditions (9). However, oxidative stress may also accelerate the formation of specific AGEs, such as glycoxidation products pentosidine, Nε-carboxymethyl lysine (CML), and possibly glyoxal hydroimidazolone (G-H1). On the other hand, AGE formation may lead to oxidative stress, and so the formation of AGEs and OxPs are intertwined (10). AGEs may not only be end products, but they may also be active intermediates in the cross-linking of proteins and the formation of reactive oxygen species. The oxidative process affects a variety of side amino acid groups, some of which are converted to carbonyl compounds (11), such as CML. Ongoing oxidation or mitochondrial oxidation of amino acids such as lysine can form stable OxPs such as 2-aminoadipic acid (2-AAA) that are increased in aging and diabetes (12).

AGEs and OxPs can damage vascular cells by different mechanisms. One frequently reported pathway is AGE binding to their purported (and relatively promiscuous) receptors on cells, such as macrophages, vascular endothelial cells, and vascular smooth muscle cells, although this has not been consistent for all AGEs (13). Other mechanisms include, among others, binding to and altering the function of intracellular proteins, the activation of vascular NADPH oxidase, and the uncoupling of endothelial nitric oxide synthase (8,14).

As AGEs are formed on long-lived proteins such as collagens, they have been measured in skin in several studies. Skin collagen–bound AGEs predicted future progression of microvascular disease, progression of carotid intima-media thickness (CIMT), left ventricular mass, and the severity of coronary artery calcium in subjects with type 1 diabetes (T1D) participating in the Epidemiology of Diabetes Interventions and Complications (EDIC) study (15–17). In addition, cross-sectional associations between skin autofluorescence and macrovascular complications in T2D patients were reported in several studies (18,19). However, data on long-term longitudinal associations between plasma levels of AGEs and OxPs with the extent of subclinical atherosclerosis in different vascular beds in T2D patients are lacking.

Therefore, in the current study we sought to determine whether baseline plasma levels of AGEs and OxPs are associated with the extent of CIMT, coronary artery calcification (CAC), and abdominal aortic artery calcification (AAC) over an average of 10 years of follow-up in the VA Diabetes Trial (VADT). We also examined whether this relationship was altered by intervening improved glucose control.

RESEARCH DESIGN AND METHODS

Participants

The data for the current study come from the Risk Factors, Atherosclerosis,and Clinical Events in Diabetes (RACED) follow-up substudy of the VADT. The design and active portion of the VADT and the main results were published previously (4). In brief, during the VADT, the effect of intensive glucose-lowering therapy on a composite primary CVD outcome was assessed during a median follow-up time of 5.6 years. After the conclusion of the active intervention phase of the VADT, participants were returned to usual care within their primary care clinics, but most participants agreed to additional data collection in the VADT follow-up (7). The current analysis uses nearly 5 years of additional follow-up after the end of the VADT. In addition, subclinical measures of atherosclerosis, CIMT by B-mode ultrasound, and CAC and AAC by computed tomography (CT) scanning, were determined after an average follow-up time of 10 $\pm$ 2 years in the RACED follow-up study (during the VADT and VADT Follow-up Study). Study sites that had access to appropriate imaging centers and were geographically representative of the national distribution of VADT sites were selected for this substudy of the VADT, as previously described (5).

Measurements of AGEs and OxPs

AGEs and OxPs were measured in plasma samples by liquid chromatography–mass spectrometry using internal stable heavy isotope–substituted standards (PreventAGE Health Care). Analysis was performed in a blinded fashion on the plasma filtrate after centrifugation through 10-kDa cutoff Amicon filters. This fraction contains free AGEs and OxPs as well as peptides of varied sizes, and the analytical method measured the free products. An Agilent model 6490 Triple Quadrupole LC/MS System with a 1290 Rapid Resolution LC System was used for analyte detection. All AGEs and OxPs were separated and analyzed in a single run using a single Waters X-select HSS T3 2.5 μm × 2.1 μm × 150 mm column with a mobile phase gradient of methanol/water with 0.20% heptafluorobutyric acid and a total analysis time of 19 min. The following seven biomarkers were measured: five dicarbonyl-derived AGE compounds—Nε-carboxymethyl lysine (CML), Nε-carboxyethyl lysine (CEL), glyoxal hydroimidazolone (G-H1), methylglyoxal hydroimidazolone (MG-H1), and 3-deoxyglucosone hydroimidazolone (3DG-H), and two oxidation products, methionine sulfoxide (MetSO) and 2-aminoaipic acid (2-AAA). Analyte interassay coefficients of variation were determined using a pooled plasma control measured in the 38 analytical runs performed during sample analysis. Interassay coefficients of variation varied from 3.6% (2-AAA) to 9.6% (G-H1).

CIMT Measurement

Prior to initiation of the study, all ultrasound technologists received training at the coordinating center at the University of Southern California to ensure the uniformity of measurement among centers. High-resolution B-mode carotid artery ultrasound was used to image the far wall of the right distal common carotid artery under a standardized protocol (20). The scans were sent to the University of Southern California Core Imaging and Reading Center. Automated boundary detection was used to locate the lumen-intima and media-adventitia echo boundaries at subpixel resolution to analyze ultrasound images (21). Quality control procedures included a comparison of scans, including repeat scans, to ensure that standardized procedures were followed.

Coronary and Abdominal Calcium Measurements

Coronary and abdominal aortic calcium were determined by using multidetector CT cardiac scanning as previously described (5,22). A standardized protocol was in place at all participating field sites that included detailed instructions for identification, storage, and transport of all image data as previously detailed (5,22). All scans were adjusted for phantom attenuation at each level. Since attenuation is slice and patient specific, this facilitates automatic adjustment for other patient-related factors that may influence image quality, such as BMI and muscle mass (23). The Agatston score (24) as well as calcium volume scores (25,26) were generated using Reading Center software.

Statistical Analyses

Statistical analyses were performed with the SAS statistical package (release 9.4; SAS Institute, Cary, NC). Means ± SD, medians (25th to 75th percentiles), and proportions are reported. Between-group differences in normally distributed continuous variables were assessed with *t* tests. Mann-Whitney *U* tests were used for variables with skewed distributions and the χ^2 test was used for proportions, respectively. *P* values were not adjusted for initial multiple comparisons; however, these analyses were used to identify the best predictors to examine in subsequent multivariable adjusted models. CAC and AAC were square root transformed to approach a normal distribution. The extent of CIMT, CAC, and AAC at follow-up were determined across quartiles of baseline AGEs and OxPs. ANOVA was used to test for significant differences across groups. In addition, post hoc comparisons between pairs of groups and linear trend tests were also conducted. The association of baseline AGEs and OxPs with the extent of CIMT, CAC, and AAC at follow-up were determined in a series of multivariable generalized linear regression models, adjusting for relevant variables. All AGEs and OxPs were used both as continuous variables (log transformed to minimize the effect of outliers) and categorical variables (comparing the highest quartile with quartiles I–III combined) in different regression models. In addition, results were confirmed using a stepwise variable selection model (forward in, backward out) to identify "best" predictors of CIMT, CAC, and AAC. Variables submitted to the selection procedure were as follows: age, duration of diabetes, prior CVD, history of hypertension, pack-years of smoking, on-trial variables (glomerular filtration rate [GFR], and levels of HbA_{1c}, HDL cholesterol, and triglycerides), and all AGEs and OxPs simultaneously (both as continuous and dichotomous variable in different models). Selection criteria required a *P* value <0.05 for a variable to enter and be retained in models.

To assess the possibility of effect modification by glucose-lowering treatment, pairwise interaction terms between treatment and AGEs and OxPs were evaluated. We also performed sensitivity analyses to examine the robustness of the results. Although square root transformation of calcium scores approached a normal distribution, for sensitivity testing we excluded people in the ≥99th percentile of CAC (Agatston score >6,000) and AAC values (Agatston score >35,000). In an additional analysis, we excluded 14 people in the upper quartiles with the highest values of specific AGEs and OxPs to make sure the associations were not just a result of a few people with extreme values. We also examined the effect of combined scores of two specific AGEs and OxPs on the severity of CIMT, CAC, and AAC. The combined scores (0 = both values low, 1 = one value high, 2 = both values high) were based on whether each of the two measurements was below (low) or within (high) quartile IV for each individual.

RESULTS

Baseline levels of AGEs and OxPs were available on a total of 411 participants (values are presented in Supplementary Table 1). After an average of 10 years of follow-up, of the 411 participants, 398 underwent ultrasound assessment of CIMT, whereas 353 and 345 participants, respectively, underwent CT scanning of CAC and AAC. Except for minor differences in age, pack-years of smoking, and history of CVD, no other differences existed between the 411 participants and the rest of the VADT participants (Table 1).

To determine whether baseline AGE and OxP values were associated with a subclinical measure of atherosclerosis at follow-up, the extent of CIMT, CAC, and AAC were examined by quartiles of AGEs and OxPs at baseline. As shown in Fig. 1*A–C*, participants with high levels of baseline G-H1 had a significantly greater extent of subclinical atherosclerosis (CIMT, CAC, and AAC) at follow-up ($P \leq 0.01$ for linear trend in all). A linear trend ($P \leq 0.05$) was also observed for the association between MG-H1, 3DG-H, and CEL, with both CAC and AAC, and between 2-AAA with CAC and CML with AAC. In addition, for several products, values in quartile IV

Table 1—Baseline characteristics

	RACED follow-up substudy participants (*n* = 411)	The rest of the VADT participants (*n* = 1,380)
Intensive treatment (%)	51	49
Age (years)	58 ± 8	61 ± 9*
Non-Hispanic white (%)	64	62
Female (%)	4	3
Smoking history (pack-years)	10 (0–30)	15 (0–40)*
History of hypertension (%)	69	73
Prior CVD (%)	34	42*
Diabetes duration (years)	11 ± 7	12 ± 7
BMI (kg/m^2)	31.2 ± 4.3	31.2 ± 4.5
HbA_{1c} (%)	9.4 ± 1.4	9.4 ± 1.5
Fasting glucose (mg/dL)	204 ± 65	204 ± 69
Total cholesterol (mg/dL)	181 ± 44	184 ± 48
LDL cholesterol (mg/dL)	115 ± 96	110 ± 48
HDL cholesterol (mg/dL)	36 ± 10	36 ± 10
Triglycerides (mg/dL)	158 (112–236)	163 (112–238)
GFR-MDRD (mL/min/1.73 m^2)	81 (69–93)	79 (67–95)

Data are means ± SD or median (25th to 75th percentiles), unless otherwise indicated. *P* values were determined by independent-samples *t* test, Wilcoxon test, or χ^2 test, as appropriate. *$P < 0.05$.

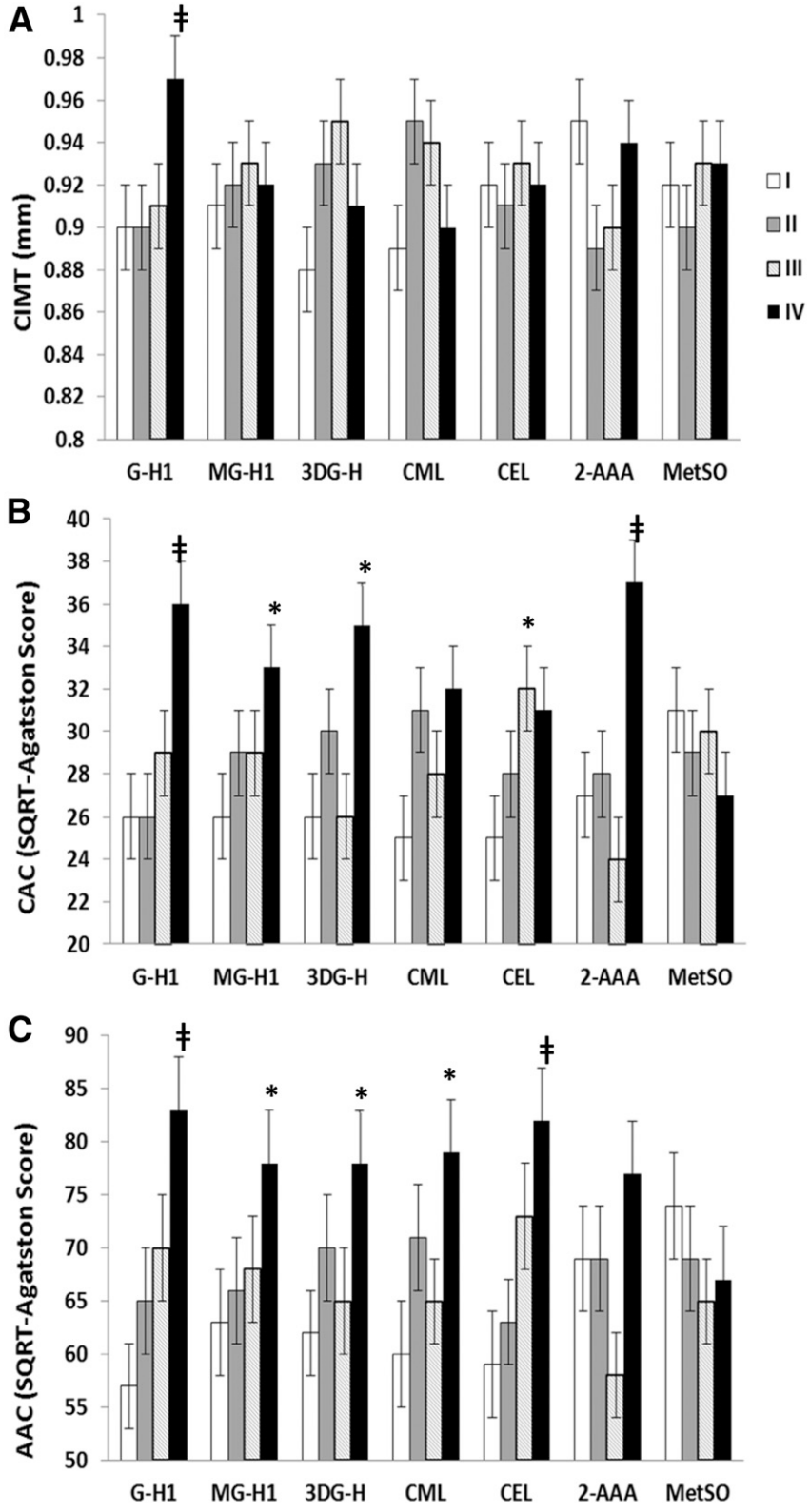

Figure 1—Subclinical atherosclerosis at follow-up by baseline quartiles of AGEs and OxPs. Means and SEs (error bars) of CIMT (n = 398, *A*) and the square root (SQRT) of Agatston scores for CAC (n = 353, *B*) and AAC (n = 346, *C*) by quartiles of AGEs and OxPs are shown. *$P \leq 0.05$ and ‡$P \leq 0.01$ for linear trend.

were associated with notably higher levels of one or more measures of atherosclerosis compared with other quartiles. For example, people in quartile IV of G-H1 had significantly ($P < 0.01$) higher CIMT, CAC, and AAC levels compared with quartiles I–III combined. Similarly, participants in quartile IV of 2-AAA had notably higher levels of CAC ($P < 0.01$) and AAC ($P = 0.05$) compared with quartiles I–III combined.

All AGEs correlated positively with each other (Spearman correlation coefficient ranged between 0.50 and 0.69, as shown in Supplementary Table 1). Thus, to avoid collinearity issues, we examined the effect of individual AGEs and OxPs in separate multivariable linear regression models. These analyses were used to identify best predictors to examine in subsequent multivariable adjusted models. As shown in Fig. 1, the association of AGEs and OxPs with the extent of atherosclerosis followed different patterns; thus, they were evaluated as both continuous and categorical (quartile IV vs. quartiles I, II, and III combined) variables. AGEs and OxPs that remained associated with atherosclerosis measures after adjustment for age, duration of diabetes, race/ethnicity, study treatment assignment, smoking, history of CVD and hypertension, and on-trial variables (HbA_{1c}, GFR, HDL cholesterol, and triglycerides) are shown in Table 2. The interactions between AGEs or OxPs with treatment assignment were not significant; thus, these were not included in the final models. G-H1 was significantly associated with CIMT both as a continuous and as a categorical variable (Models 1–2), and with CAC as a categorical variable (Model 3). In addition, 3DG-H (categorical, Model 4), CEL (continuous), and 2-AAA both as a continuous and as a categorical variable (Models 6 and 7) remained strongly associated with CAC. Only CEL, as a continuous variable, remained significantly associated with AAC (Model 8). Stepwise variable selection models also confirmed the results and identified G-H1 (for CIMT and CAC), 2-AAA (for CAC), and CEL (for AAC) as independent best predictors of subclinical atherosclerosis along with other known traditional cardiovascular risk factors (Supplementary Table 3).

In sensitivity analyses, after exclusion of individuals above the 99th percentile of CAC (n = 5) and AAC (n = 4), or after exclusion of participants above the 99th percentile of AGEs and OxPs (n = 14), the results did not change appreciably. Additional models examining differential relationships between AGEs or OxPs with atherosclerosis measures based on treatment assignment during the

Table 2—Multivariable linear regression models for significant AGEs and OxPs as predictors of subclinical atherosclerosis

		β ± SE	*P*
Dependent variable is CIMT			
Model 1	G-H1 (continuous)	0.09 ± 0.04	0.01
Model 2	G-H1 (quartile IV vs. I–III)	0.06 ± 0.02	0.01
Dependent variable is CAC			
Model 3	G-H1 (quartile IV vs. I–III)	5.53 ± 2.29	0.01
Model 4	3DG-H (quartile IV vs. I–III)	3.74 ± 2.24	0.09
Model 5	CEL (continuous)	4.27 ± 2.48	0.08
Model 6	2-AAA (continuous)	6.08 ± 2.74	0.03
Model 7	2-AAA (quartile IV vs. I–III)	6.84 ± 2.21	<0.01
Dependent variable is AAC			
Model 8	CEL (continuous)	13.77 ± 5.63	<0.01

Rows show results for the prediction of subclinical atherosclerosis by individual AGEs and OxPs (models 1–8). All models are adjusted for age, duration of diabetes, prior CVD, history of hypertension, pack-years of smoking, on-trial variables (GFR, HbA_{1c}, HDL cholesterol, and triglycerides). Both continuous variables (log-transformed) and dichotomous variables (quartile IV vs. quartiles I, II, and III combined) of AGEs and OxPs were used in different models. Replacing GFR with the albumin-to-creatinine ratio did not change the results.

VADT (intensive vs. standard) revealed no significant interactions.

As the association of 2-AAA and G-H1 with CAC was robust and significant throughout all analyses, and seemed consistent with a threshold effect (highest in quartile IV), the effect of having high levels of one or both 2-AAA and G-H1 on the severity of CAC was investigated. Having elevated values of one or both G-H1 and 2-AAA (G-H1 >11 nmol/L and 2-AAA >1,816 nmol/L, based on IV quartiles) was associated with the severity of CAC, and also of AAC and CIMT, in a stepwise fashion (Fig. 2*A*–*C*). Adjustment for age and other significant risk factors for CAC selected in the stepwise model (Supplementary Table 1) did not change the results for CAC. After adjustment for age and other significant predictors of AAC and CIMT, participants having high levels of both G-H1 and 2-AAA (but not of just one) had significantly higher AAC and CIMT levels compared with participants having low levels of both (P = 0.03). Of note, the majority of participants with high 2-AAA and G-H1 levels had above median levels of other AGEs as well (91% had high levels of CEL and CML, 86% had high levels of 3DG-H, 71% had high levels of 3DG-H, 86% had high levels of MG-H1, and 80% had high levels of MetSO, respectively). Other combinations of AGEs or OxPs were less closely associated with atherosclerosis measures.

CONCLUSIONS

We report the novel finding that plasma levels of specific AGEs and OxPs are associated with future severity of subclinical measures of atherosclerosis in T2D patients. Specifically, plasma levels of G-H1, CEL, and 2-AAA were associated with the extent of CAC after an average follow-up of 10 years. G-H1 was also associated with the extent of CIMT after 10 years, while CEL was associated with long-term AAC.

These associations were robust and persisted after adjustments for other predictors of subclinical atherosclerosis including treatment assignment and mean levels of HbA_{1c} and lipids during the full follow-up period. Although all AGEs and 2-AAA levels were strongly and inversely correlated with GFR (r between −0.21 and −0.39, $P < 0.01$), adjustments for GFR during the study did not attenuate the findings. Importantly, intensive glucose control that achieved an HbA_{1c} difference of 1.5% between the treatment groups for a median of 5.6 years during the active phase of the VADT (5,6) also did not modify the strong association of baseline AGEs and OxPs with a subclinical measure of atherosclerosis.

As certain collagen AGEs fluoresce, skin intrinsic fluorescence can act as a novel marker of levels of collagen AGEs (27). Consistent with our study, a positive association between skin fluorescence and plasma levels of AGEs with CAC was reported in cross-sectional studies in patients with T1D (28,29) and those with T2D (18,19). Studies in patients with coronary artery disease also reported that serum levels of bound AGEs are related to the degree of coronary atherosclerosis both in patients with diabetes and in those without diabetes (30,31). Of particular relevance to our findings is the finding that within a subset of individuals (n = 127) from the Diabetes Control and Complications Trial (DCCT)/EDIC study of T1D, there were positive associations between multiple skin AGEs, including MG-H1 collected at the end of the DCCT glucose-lowering study, and the progression of CIMT between post-DCCT follow-up year 1 and year 6 (16). Of note, the association between skin AGEs and CIMT progression was no longer present by year 12 of the EDIC study (17), suggesting that the effect of baseline AGEs may influence atherosclerosis for at least 6 years but for <12 years. The EDIC study also reported that furosine, a derivative of the early glycation product Nε-fructosyl lysine, predicted the extent of CAC ~7–9 years later (16). In our study, the association between plasma levels of G-H1 with CIMT and G-H1 and of CEL and 2-AAA with CAC persisted over the 10 years of follow-up. Altogether, these studies provide strong evidence for an important relationship between early and late markers of glycation (within plasma and skin) with subclinical atherosclerosis both in patients with T1D and in patients with T2D. Of substantial interest, the associations were present in both the DCCT/EDIC study and our study many years after the measurement of the products and persisted even after accounting for years of glucose control after their measurement. As noted above, within our subset of patients from the VADT, associations of AGEs/OxPs persisted similarly in both standard and intensive treatment groups, despite the latter group having substantially lower HbA_{1c} values for 5.6 years. These findings are consistent with the concept that, once these products are formed during hyperglycemia, subsequent levels of glucose control over the next 5–10 years may have a modest influence on their link with vascular disease, perhaps contributing to the phenomena of negative metabolic memory (8).

Mechanistic studies conducted in vitro and in animal models have shown that AGE-induced vascular calcification is mediated in part by oxidative stress (32–34). This raises the possibility that some AGEs and OxPs may reflect the same process or perhaps may reinforce the action of each other. In line with this notion, in the Joslin Medalist study in T1D patients (35) the

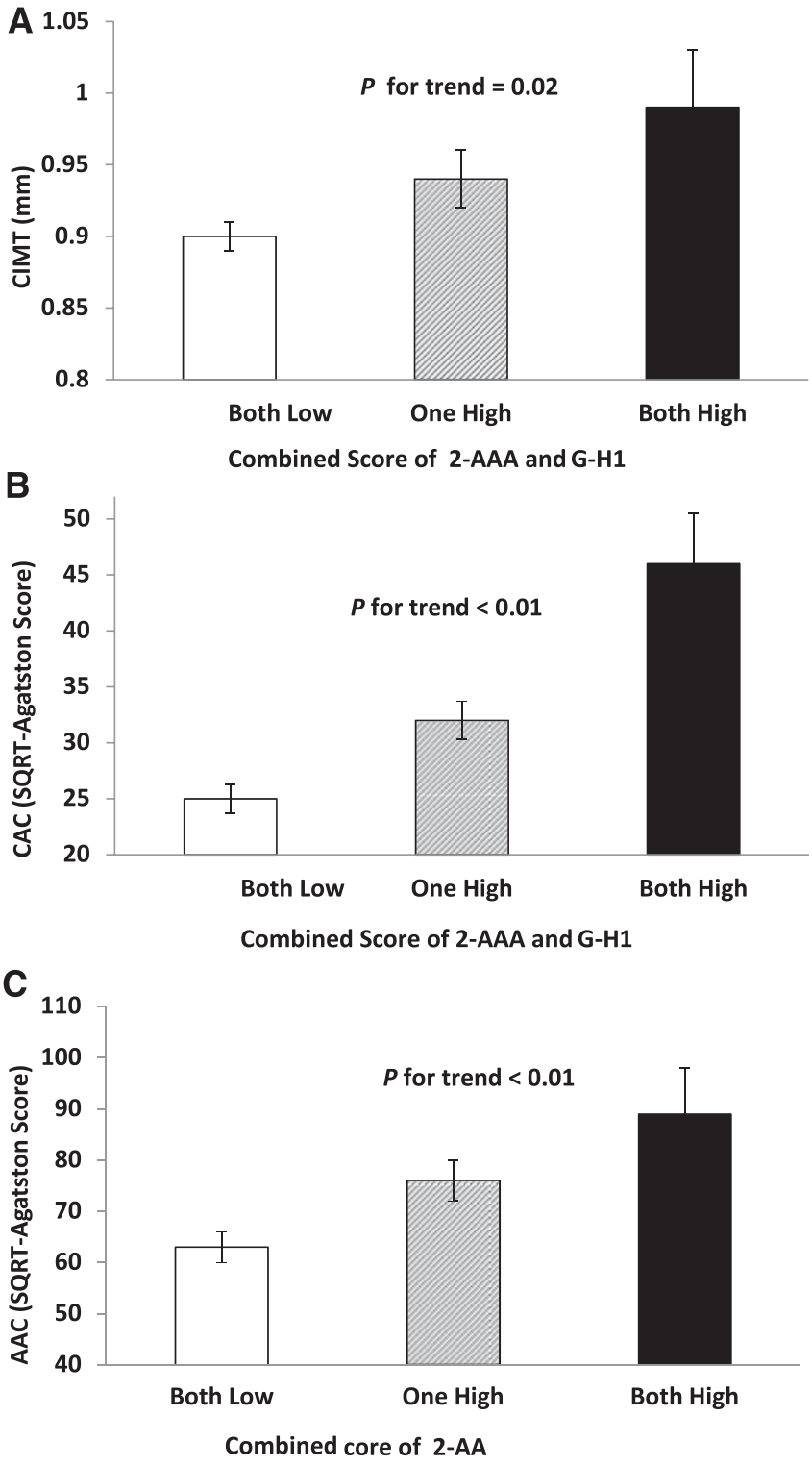

Figure 2—Subclinical atherosclerosis at follow-up by combined scores of High 2-AAA and G-H1. Means and SEs of CIMT and the square root (SQRT) of Agatston calcium scores by combined scores (high/low) of G-H1 and 2-AAA (based on IV quartiles; i.e., >11 nmol/L for G-H1, and >1,816 nmol/L for 2-AAA) are shown. The number of participants in each group: both low = 233, one high = 134, both high = 31, $P = 0.02$ for both high vs. both low (*A*); both low = 208, one high = 117, both high = 28, $P \leq 0.01$ for all pairwise comparisons (*B*); and both low = 204, one high = 115, both high = 27, $P \leq 0.01$ for all pairwise comparisons except quartile IV vs. quartile III (*C*).

combination of high pentosidine (a glycoxidation product and minor cross-linking protein) level and the AGE, CEL was associated with a greater risk for any diabetes complication, including CVD, than high levels of either product alone. Protein modification by the formation of 2-AAA and G-H1 may also be pivotal for the formation of extracellular matrix and vascular calcification (29,36); thus, several of these products could work through complementary pathways to enhance vascular calcification or other measures of atherosclerosis. Interestingly, in our study the effect on atherosclerosis in several vascular beds of combined high levels of G-H1 (an AGE) and 2-AAA (an OxP) was greater than isolated high levels of G-H1 or 2-AAA, suggesting that the combination of these may be acting in concert to worsen vascular disease. It is important to note that the majority of participants with high levels of combined G-H1 and 2-AAA had high levels of other AGEs as well. It is therefore possible that other AGEs may contribute to this strong association between combined G-H1 and 2-AAA and atherosclerosis.

Oxidation species are in general very reactive and have a short half-life. In contrast, 2-AAA is an end product of metal-catalyzed oxidation of lysine and its immediate precursor allysine, and, as such, it is a more reliable marker of oxidative stress with a longer half-life like those of AGEs (37). Levels of 2-AAA increase as a consequence of oxidative deamination of the lysine residue in a high-glucose milieu (14) and therefore may be a uniquely good measure of oxidative stress. It has also been shown that 2-AAA can be produced by enzymatically catalyzed mitochondrial oxidation of lysine during its degradation (37,38). However, no matter the predominant source of 2-AAA, levels appear dramatically increased in patients with diabetes, renal disease, and sepsis (12).

An important caveat of this study is that participants in the VADT were older, predominantly male veterans with long-standing T2D. Thus, although the results are in line with the results in younger male and female T1D patients (18,19,28–31), extrapolation of the study findings to other populations must be done with caution. This study also does not allow us to make a definite claim of causation between AGEs and

OxPs with the extent of atherosclerosis. The absence of baseline measures of atherosclerosis in this cohort further complicates this assessment of causality. Another limitation of this study is that we did not have AGE and OxP measurements at the end of the active phase of the VADT. We therefore are not able to determine whether intensive glucose-lowering treatment could reduce these products and whether this might have reduced their association with vascular disease. However, the failure of treatment assignment to influence the results suggests that glucose-lowering treatment for this time frame may be insufficient to alter the relationships between these long-lived products and atherosclerosis.

Nevertheless, there are several strengths to this study. First, this is the largest longitudinal study of AGEs and OxPs in T2D patients with atherosclerosis to date. Second, we examined this relationship across multiple vascular beds, providing a thorough assessment of these products and whole-body atherosclerosis. Third, as shown in Table 1, this subset was representative of the entire VADT cohort and consisted of an ethnically diverse group from a large, carefully conducted U.S.-wide multicenter study and should be relatively representative of men with T2D who are at high risk for CVD. Moreover, as a result of a participant's age and CVD risk, there was a broad range of atherosclerosis, with few participants having zero vascular calcium, unlike the predominance of minimal vascular calcification in younger patients and in patients with T1D. Fourth, all participants were enrolled in one national health care system that provided consistent and similar health care for all participants throughout both the active treatment phase of the VADT and the subsequent post-treatment follow-up. This is supported by equally excellent blood pressure and lipid levels and uses of preventive medication, such aspirin, statins, and ACE inhibitors, within both intensive and standard treatment groups for all 10 years of follow-up (4,5,7).

There are also several potentially relevant clinical implications from these results. Recent trials have demonstrated that relatively short-term improvements in glucose levels are insufficient to reduce the development of vascular complications (3–6). This could be explained in part by the long-lasting relationship that appears to exist between AGEs and OxPs and atherosclerosis. This also emphasizes the need to start good glycemic control early in the course of diabetes treatment, thus limiting the long-term negative consequences of formation of these products. As plasma AGEs and OxPs may accumulate from several pathways, including via the diet (39), greater understanding of their sources and potential ways to limit their accumulation is needed. These data in T2D patients, along with previous results in T1D patients (15–17), provide further support for the development of therapies that may limit AGE/OxP formation or their ability to modify proteins.

In summary, these findings suggest that the effect of hyperglycemia and subsequent increased levels of AGEs and OxPs in patients with long-standing T2D may have long-lasting adverse effects on the development of macrovascular complications. This result, along with the finding that nearly 6 years of intensive glucose-lowering therapy was unable to modify these relationships, supports the possibility that AGEs and OxPs may help explain the concept of negative metabolic memory. These results could also help to explain why improvements in CVD outcomes with glucose-lowering therapy appear to require a substantially longer follow-up time (6,7,40).

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