Advanced Glycation End Products Predict Loss of Renal Function and High-Risk Chronic Kidney Disease in Type 2 Diabetes

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OBJECTIVE

To evaluate the association of a multicomponent advanced glycation end product (AGE) panel with decline in kidney function and its utility in predicting renal function loss (RFL) when added to routine clinical measures in type 2 diabetes.

RESEARCH DESIGN AND METHODS

Carboxymethyl and carboxyethyl lysine and methylglyoxal, 3-deoxyglucosone, and glyoxal hydroimidazolones were measured in baseline serum and plasma samples, respectively, from Action to Control Cardiovascular Risk in Diabetes (ACCORD) (n = 1,150) and Veterans Affairs Diabetes Trial (VADT) (n = 447) participants. A composite AGE score was calculated from individual AGE z scores. The primary outcome was a sustained 30% decline in estimated glomerular filtration rate (eGFR) (30% RFL in both cohorts). Secondary outcomes (in ACCORD) were 40% RFL, macroalbuminuria, and high-risk chronic kidney disease (hrCKD).

RESULTS

After adjustment for baseline and follow-up HbA_{1c} and other risk factors in ACCORD, the AGE score was associated with reduction in eGFR (β-estimate −0.66 mL/min · 1.73 m^2 per year; P = 0.001), 30% RFL (hazard ratio 1.42 [95% CI 1.13–1.78]; P = 0.003), 40% RFL (1.40 [1.13–1.74]; P = 0.003), macroalbuminuria (1.53 [1.13–2.06]; P = 0.006), and hrCKD (1.88 [1.37–2.57]; P < 0.0001). AGE score improved net reclassification (NRI) and relative integrated discrimination (IDI) for 30% RFL (NRI 23%; P = 0.02) (relative IDI 7%; P = 0.009). In VADT, the AGE score calculated by the ACCORD-derived coefficients was associated with 30% RFL (1.37 [1.03–1.82]; P = 0.03) and improved NRI (24%; P = 0.03) but not IDI (P = 0.18).

CONCLUSIONS

These data provide further support for a causal role of AGEs in diabetic nephropathy independently of glycemic control and suggest utility of the composite AGE panel in predicting long-term decline in renal function.

Diabetic kidney disease (DKD) is a major complication of diabetes and increases risk for end-stage renal disease and mortality. DKD risk is related to chronic

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hyperglycemia (1) and can be reduced by tight glycemic control in individuals with type 1 diabetes and early type 2 diabetes (2,3). In contrast, intensive glycemic control had only modest benefit in DKD progression in those with more chronic type 2 diabetes (4), raising the possibility that additional factors associated with long-lasting diabetes that are not readily improved by intensive glycemic control may play a prominent role in worsening of kidney function.

Advanced glycation end products (AGEs) are long-lived chemical intermediates formed by reactions of chemically reactive sugars with proteins, lipids, and nucleic acids (5). AGE-modified proteins undergo intracellular proteolysis, forming AGE-free adducts that are released into the circulation where they can contribute to systemic effects. Given the principal role of the kidney in AGE clearance and reabsorption, increased AGE accumulation may enhance renal dysfunction and DKD (6). Previous studies in well characterized but modest-sized type 1 and type 2 diabetes cohorts have shown a significant association between serum concentrations of several individual AGE-free adducts and DKD progression (7–10).

In the current study, we tested two different large cohorts of individuals with type 2 diabetes undergoing intensive glucose lowering to determine if AGEs predict worsening of kidney function and if addition of AGE burden to traditional clinical risk factors will improve prediction of DKD risk. We hypothesized that the association between baseline AGEs and DKD is independent of glycemic control.

RESEARCH DESIGN AND METHODS

The prospective association between AGE-free adduct levels and decline in renal function was assessed in 1,150 randomly selected participants of the Action to Control Cardiovascular Risk in Diabetes (ACCORD) (clinical trial reg. no. NCT00032487, clinicaltrials.gov) with available baseline serum samples. All samples and data were provided by National Heart, Lung and Blood Institute BioLINCC. A secondary cohort to validate the prediction of an AGE score generated from ACCORD was a subgroup of 447 participants of the Veterans Affairs Diabetes Trial (VADT) (NCT00000620) previously studied for associations between AGEs and cardiovascular outcomes (11).

As described previously, ACCORD was a factorial randomized clinical trial in 10,251 individuals with type 2 diabetes (12). Participants were randomized to an intensive or standard glucose-lowering group and simultaneously enrolled in a blood pressure or lipid-lowering trial. The lipid-lowering trial tested the effect of fenofibrate versus placebo. The active glucose-lowering period ended after a mean of 3.7 years, with an additional 17 months of monitoring until the active lipid and blood pressure periods ended. Serum creatinine levels were measured every 4 months for the first year, followed by annual and study closeout measurements (13). Urine albumin and creatinine levels were measured annually and at study closeout. In the observational follow-up study lasting 3.5 years following the closeout visit (14), the measurements were repeated at either 24 or 30 months and again at either 54 or 60 months. Serum creatinine was determined on a Roche Double Modular P Analytics Analyzer (Roche Diagnostics, Indianapolis, IN). Urine albumin was determined on a Siemens BN II Nephelometer (Siemens Healthcare Diagnostics, Tarrytown, NY). Urine creatinine was determined by a modified Jaffé reaction.

The estimated glomerular filtration rate (eGFR) was calculated by the Chronic Kidney Disease Epidemiology Collaboration equation (15). To account for the rapid increase and reversal in creatinine levels associated with use or discontinuation of fenofibrate (16), all follow-up serum creatinine levels while on active fenofibrate treatment were normalized to the change in serum creatinine levels occurring from baseline to the first available scheduled visit. Urine albumin excretion was estimated by albumin-to-creatinine ratio (uACR).

A detailed description of VADT and the subgroup used in the present analysis has been previously published (11,17). The primary treatment goal of VADT was to achieve a 1.5% difference in HbA_{1c} between those randomized to intensive versus standard therapy, while achieving optimal levels of other conventional cardiovascular risk factors. The median follow-up of active trial was 5.6 years. Serum and urine creatinine and urine albumin were measured annually by the VADT central laboratory at Tufts University. The eGFR and uACR were calculated as described above.

Five dicarbonyl-derived AGE compounds, methylglyoxal-derived hydroimidazolone (MG-H1), glyoxal-derived hydroimidazolone, 3-deoxyglucosone hydroimidazolone, Nε-carboxymethyl lysine, and Nε-carboxyethyl lysine, were measured by liquid chromatography–tandem mass spectrometry using internal stable heavy isotope–substituted standards, as described previously (8). Analyses were performed in a blinded fashion on the serum (ACCORD) or plasma (VADT) filtrate following centrifugation through 10,000-cutoff Amicon filters. An Agilent model 6490 Triple Quadrupole MS System with a 1290 Rapid Resolution LC System was used for analyte detection. All AGEs were separated and analyzed in a single run using a single Waters X-select HSS T3 2.5 μm · 2.1 mm · 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 interassay coefficients of variation varied from 3.6 to 9.6%.

The primary outcome was renal function loss (RFL), defined as a 30% decline in eGFR at two consecutive visits at least 90 days apart. The time of an event was defined as the time of the first occurrence. Secondary outcomes included any first occurrences of 40% eGFR decline, macroalbuminuria (uACR $>$300 mg/g), and high-risk chronic kidney disease (hrCKD), defined by Kidney Disease: Improving Global Outcomes as eGFR $<$30 mL/min · 1.73 m^2 with uACR $<$30 mg/g or eGFR $<$45 with uACR 30–299 or eGFR $<$60 with uACR $>$300. RFL of both 30 and 40% are valid surrogate kidney end points in clinical trials enrolling individuals with relatively preserved kidney function (18,19).

Statistical analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC). P values <0.05 were considered statistically significant. AGE levels outside the 4 SDs were winsorized to the respective mean and 4 SDs. All values were then natural logarithm transformed and standardized to 1 SD. An overall AGE score defined as the mean of standardized individual AGEs was the primary exposure in all analyses. Differences between the groups at baseline were compared by Student t test or by nonparametric alternative (Wilcoxon test) for

continuous variables and by χ^2 or Fisher test for categories. Univariate and multivariable linear regressions were used to test for associations of AGE score with clinical and demographic characteristics. Mixed linear regression with random effects of subjects was used to test the association between baseline AGE score and change in eGFR. Cox proportional hazards regression was used for the time-to-event analysis. Proportional hazards assumptions were formally confirmed by cumulative sums of Martingale residuals, with *P* values from the Kolmogorov-type supremum test. All prospective models were adjusted for the randomization arm and relevant baseline covariates. All continuous variables in the models were natural log transformed and normalized to 1 SD. A constant of one was added to uACR to enable a log transformation of zero values. Time-dependent area under the receiver-operating characteristic curve, category-free net reclassification improvement (NRI), and integrated discrimination improvement (IDI) were calculated to quantify improvement in model performance after adding AGE score to clinical and demographic risk factors according to the 10-year outcomes risk estimates from the Cox proportional hazards risk models. The CIs for the NRI and IDI values were computed using 100 bootstrap samples. For validation in VADT, the AGE score calculated using regression parameters for individual AGEs (log transformed) from ACCORD was tested for association with change in eGFR and 30% RFL. Power calculation for the ACCORD random sample size selection was based on the initial estimate of a hazard ratio (HR) of 1.42 found in our VADT subcohort, which was previously measured and available. At least 255 events were required to detect this level of risk at 80% power and $\alpha = 0.05$. Our determination indicated that we would need 1,150 ACCORD participants for a more conservative estimate of 300 events. However, this estimate did not account for the initial eGFR declines in the fenofibrate arm of the study, as described above, leaving fewer true RFL events.

Data and Resource Availability

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request as permitted by the Carl T. Hayden Veterans Affairs Medical Center Institutional Review Board and Veterans Affairs research policy.

RESULTS

Baseline characteristics of the 1,150 participants selected for the present analysis were similar to those within the full ACCORD cohort (Supplementary Table 1). At baseline, 557 (48%) participants had normal eGFR ($\geq$90 mL/min · 1.73 m^2), 493 (43%) had mildly reduced eGFR (60–89), and 100 (9%) had impaired kidney function ($<$60). Baseline uACR was available in 1,098 individuals; 280 (26%) had microalbuminuria (uACR 30–300 mg/g) and 59 (5%) had macroalbuminuria ($>$300), while hrCKD was present in 13 participants.

Higher AGE score was associated with older age, non-Hispanic White ethnicity, history of cardiovascular disease (CVD), lower diastolic blood pressure, longer diabetes duration, lower eGFR, and presence of micro- and macroalbuminuria, but not with HbA_{1c} (Supplementary Table 2). After adjustment for these variables, AGE score remained associated with non-Hispanic White ethnicity (*P* = 0.008), history of CVD (*P* = 0.005), diabetes duration (*P* = 0.008), and eGFR ($P < 0.0001$). Generally similar associations were seen for individual AGEs. Prospectively, higher AGE score was associated with greater eGFR decline (annual estimated change –0.63 mL/min · 1.73 m^2; *P* = 0.005), and this effect was independent of the risk factors listed above (including baseline HbA_{1c}) or cumulative mean of follow-up HbA_{1c}.

As shown in Table 1, 1,100 participants had at least two follow-up eGFR measurements. During a median follow-up of 4 years, 30% RFL was noted in 172 (16%) individuals. Compared with those who did not experience this outcome, affected individuals had a longer history of diabetes and higher baseline systolic blood pressure, uACR, and baseline HbA_{1c}. Similar differences were observed for the secondary outcomes of 40% RFL, macroalbuminuria, and hrCKD (Table 1).

Kaplan-Meier curves showed separation in risk for 30% RFL between those with low and high AGE scores (defined by the median AGE score) starting at year ~2 and increasing through the end of the study (Fig. 1*A*). The risk of 30% RFL increased continuously across AGE-score quartiles, with the top quartile of the AGE score showing the strongest association with 30% RFL compared with the bottom quartile (Fig. 1*B*). This was confirmed by a significant association between continuous AGE score and 30% RFL (*P* = 0.003) (Fig. 1*C*). In contrast, there were no significant associations between baseline HbA_{1c} (*P* = 0.06) or follow-up HbA_{1c} with 30% RFL in the time-varying model (*P* = 0.4). The association between AGE score and 30% RFL did not differ between groups defined by study arm, sex, ethnicity, age, baseline systolic blood pressure, HbA_{1c}, eGFR, or uACR; however, it differed by diabetes duration (*P* = 0.006 for interaction) and was significant only in those with a longer diabetes history ($P < 0.0001$ vs. *P* = 0.6) (Fig. 1*D*).

There was a nearly linear increase in risk for the secondary outcomes 40% RFL, macroalbuminuria, and hrCKD with each higher quartile of the AGE score (Fig. 2*A–C*). The continuous AGE score also showed a strong association with 40% RFL (*P* = 0.003), macroalbuminuria (*P* = 0.03), and hrCKD ($P < 0.0001$) (Fig. 2*D*). Individual AGEs, in general, showed significant associations with renal outcomes, although with smaller HRs compared with the AGE score (Supplementary Fig. 1). The associations between baseline or follow-up HbA_{1c} and any of the secondary renal outcomes were not statistically significant (Supplementary Fig. 1*B–C*). In the subgroup analyses, AGE score showed a significantly stronger (*P* = 0.02 for interaction) association with 40% RFL (and similar trends for other secondary renal outcomes) in those with lower baseline HbA_{1c} (*P* = 0.0003 vs. *P* = 0.3) (Supplementary Table 3). There was also evidence of a significant interaction between AGE score and sex, with significant associations occurring in women for incident macroalbuminuria and hrCKD (*P* = 0.03 and *P* = 0.02 for interaction, respectively).

In the reclassification analyses, after addition of the AGE score to standard clinical and demographic factors (age, sex, race/ethnicity, systolic blood pressure, diabetes duration, HbA_{1c}, eGFR, and uACR), there were no significant changes in the integrated area under the curve for 30% RFL (*P* = 0.14), 40% RFL (*P* = 0.16), macroalbuminuria (*P* = 0.5), or hrCKD (*P* = 0.4). With the AGE score included, continuous NRI (i.e., a

Table 1—Baseline characteristics of the current ACCORD subcohort by incident renal events

	30% RFL*		40% RFL		Macroalbuminuria		hrCKD	
	Cases	Noncases	Cases	Noncases	Cases	Noncases	Cases	Noncases
N	172	928	200	919	99	784	107	762
Age, years	63 ± 6	62 ± 6	63 ± 6	62 ± 6	64 ± 7†	62 ± 6	64 ± 6†	62 ± 6
Male sex, %	54	55	51	54	63	54	53	55
White race, %	69	64	68	63	65	65	69	63
BMI, kg/m^2	33 ± 6	32 ± 5	33 ± 5	32 ± 5	32 ± 6	32 ± 5	34 ± 5	32 ± 5
History of CVD, %	33	33	35	33	39	31	41†	30
Statin use, %	69	62	63	63	70	63	69	64
Systolic BP, mmHg	140 ± 19†	136 ± 17	141 ± 20†	136 ± 17	139 ± 20†	135 ± 16	142 ± 22†	135 ± 16
Diastolic BP, mmHg	75 ± 11	75 ± 11	75 ± 11	75 ± 11	74 ± 10	75 ± 11	75 ± 11	75 ± 11
Diabetes duration, years	11 (7, 17)†	10 (5, 15)	13 (7, 18)†	10 (5, 15)	10 (7, 16)†	9 (5, 14)	12 (7, 18)†	9 (5, 15)
HbA_{1c}, %	8.6 ± 1.1†	8.3 ± 1.0	8.5 ± 1.0†	8.4 ± 1.0	8.5 ± 1.1	8.3 ± 1.0	8.5 ± 1.1	8.3 ± 1.0
HbA_{1c}, mmol/mol	70 ± 12†	68 ± 14†	70 ± 11†	67 ± 11	69 ± 12	67 ± 11	69 ± 12	67 ± 11
Total cholesterol, mg/dL	185 ± 43	183 ± 40	188 ± 45	184 ± 40	178 ± 35	183 ± 41	181 ± 38	183 ± 41
LDL cholesterol, mg/dL	105 ± 34	105 ± 34	106 ± 35	105 ± 34	99 ± 32	105 ± 34	101 ± 34	106 ± 34
HDL cholesterol, mg/dL	41 ± 11	42 ± 11	42 ± 11	42 ± 11	41 ± 12	42 ± 11	41 ± 10†	42 ± 11
Triglycerides, mg/dL	150 (114, 223)	153 (104, 225)	156 (111, 230)	153 (105, 225)	167 (118, 235)	151 (104, 220)	175 (129, 250)†	150 (104, 218)
uACR, mg/g	30 (10, 131)†	12 (6, 31)	29 (11, 121)†	13 (7, 37)	65 (29, 133)†	11 (6, 25)	72 (30, 238)†	11 (6, 26)
eGFR, mL/min · 1.73 m^2	83 ± 16	85 ± 17	84 ± 16	85 ± 17	77 ± 17†	86 ± 16	71 ± 17†	87 ± 15

Data are %, mean ± SD, or median (25th, 75th percentile). BP, blood pressure. *30% RFL reflects sustained decline at two consecutive visits. †$P < 0.05$ vs. noncases by χ^2, Student *t*, or Mann-Whitney nonparametric test.

proportion of cohorts correctly upgraded or downgraded in their eventual risk) was significant for 30% RFL (23%; P = 0.02) and macroalbuminuria (32%; P = 0.03) (Table 2). IDI, calculating the difference between slopes of prediction curves, demonstrated a significant improvement with AGE score for 30% RFL (6.9%; P = 0.009) and 40% RFL (5.2%; P = 0.03).

Of the 447 VADT participants with AGE measurements, 101 developed sustained 30% RFL (median time 3.3 years) (Supplementary Table 4). The AGE score, defined by ACCORD estimates, was associated with change in eGFR (0.69 mL/min · 1.73 m^2 per year; P = 0.001) and with risk for 30% RFL (HR 1.37 [95% CI 1.03–1.82]; P = 0.03) (Supplementary Fig. 2). AGE score calculated as an average of VADT individual AGE *z* scores or by ACCORD-derived estimates for individual AGEs showed higher HRs compared with the individual AGEs. Addition of the AGE score derived from ACCORD estimates to standard clinical factors resulted in a significant improvement in NRI (24%; P = 0.03) and no change in IDI (P = 0.18) for 30% RFL (Table 2).

CONCLUSIONS

The current study demonstrates a strong independent association between a composite AGE score based on serum levels of five AGE-free adducts and future decline in kidney function in individuals with type 2 diabetes. The composite AGE score provided a more consistent and robust prediction of adverse renal outcomes than individual AGE compounds. A similar association between baseline AGE score and decline in future kidney function in a separate type 2 diabetes cohort demonstrates the consistency of this finding. Furthermore, a predictive model for the 30% RFL that included clinical risk factors plus AGE score derived using data from ACCORD also discriminated between participants who did and did not develop this outcome in VADT, indicating the potential predictive value of a standard AGE score across type 2 diabetes populations.

Because individual AGE-free adducts are formed by different pathways from specific precursors (20,21), blood levels of individual AGEs or their relationships to diabetes complications may be affected by a variety of clinical and demographic characteristics, such as race/ethnicity or history of CVD, which may vary among study cohorts. Therefore, the composite AGE score may provide a more accurate and consistent estimate of overall AGE burden and provide a better prognostic estimate of AGE-related risks across populations. Indeed, reclassification and model discrimination analysis supported the prognostic value of the AGE score for future decline in kidney function in advanced type 2 diabetes. When added to known risk factors for DKD, higher AGE score improved prediction of whether the individuals would or would not develop future sustained function loss. Notably, the reclassification and discrimination metrics for AGE score were also consistent with those reported previously for MG-H1 in the Native Americans (18).

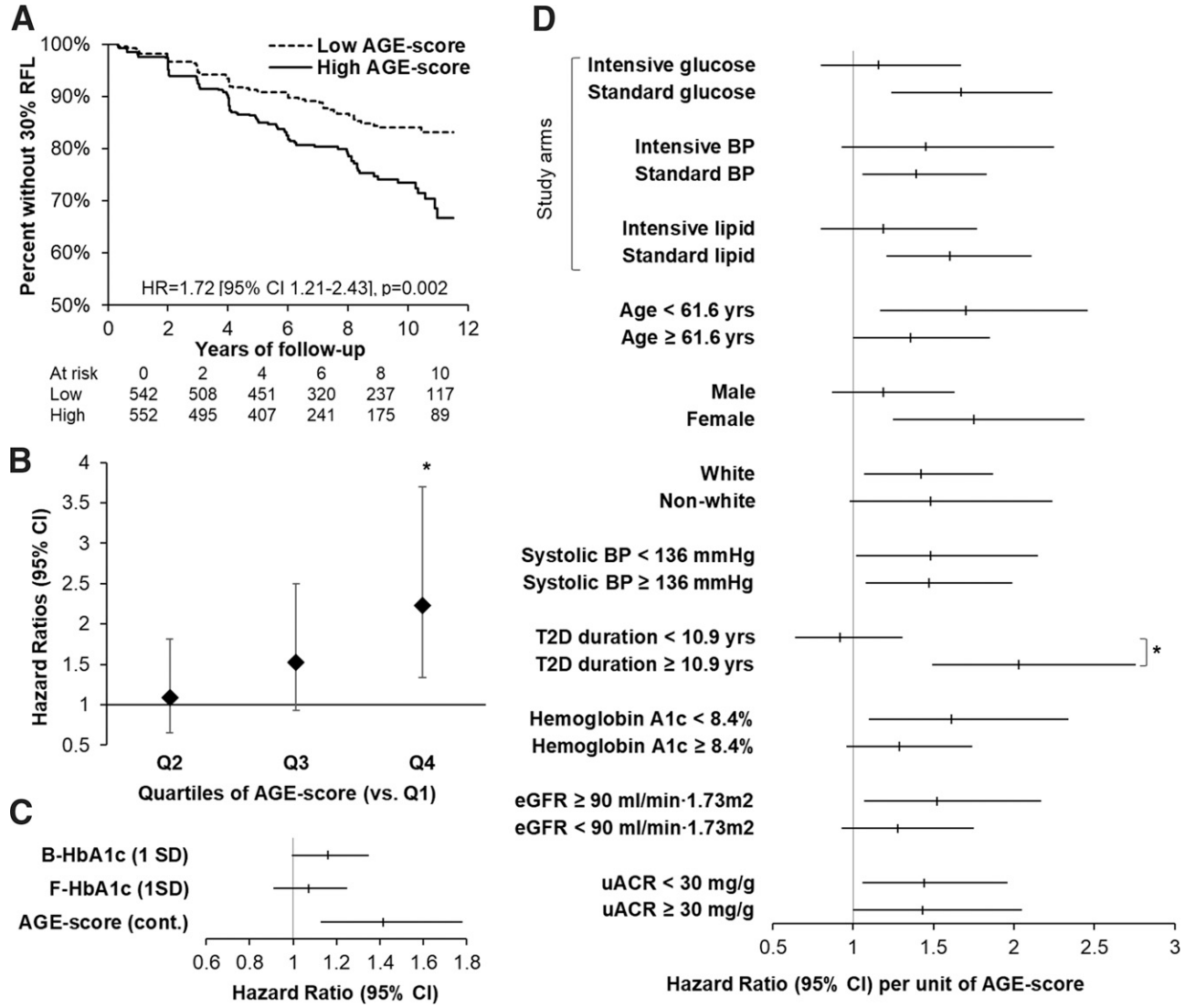

Figure 1—Association of baseline AGE score with 30% RFL in ACCORD. *A*: Kaplan-Meier curves for 30% RFL in those with high (at or above the median) and low (below the median) AGE scores. Also shown are the numbers of individuals at risk by year of follow-up. *B*: HRs and 95% CIs for 30% RFL by quartile (Q) (Q2–Q4 vs. bottom Q [Q1]) of AGE score. *C*: HRs (95% CIs) for baseline HbA_{1c} (B-HbA_{1c}), follow-up HbA_{1c} (F-HbA_{1c}), and continuous AGE score. F-HbA_{1c} was tested as a time-varying variable from all visits until the time of the event. *D*: HRs (95% CIs) for continuous AGE score by subgroup defined by categories or medians (age, systolic blood pressure [BP], type 2 diabetes [T2D] duration, and HbA_{1c}). Brackets with asterisk indicate a significant interaction term. All models were adjusted for study arm and baseline age, sex, race/ethnicity, T2D duration, systolic BP, B-HbA_{1c}, eGFR, and uACR. *$P < 0.05$ vs. bottom Q (*B*) and for interaction (*D*).

Although the present analysis focused on sustained 30% RFL as the primary outcome, a similar relationship also existed with the first occurrence of 40% RFL. Because this even larger decline in renal function was frequently occurring toward the end of the study follow-up, we were not able to determine its sustained nature. Nevertheless, large meta-analyses showed that both 30% and 40% RFL are strong predictors of future end-stage renal disease and mortality and are recommended as alternative end points in studies enrolling individuals with relatively preserved kidney function and a limited number of serious events over the follow-up period (18,19). Our previous study in a smaller cohort of Native Americans with type 2 diabetes showed a significant association of 40% RFL with two AGEs from the panel, MG-H1 and Nε-carboxyethyl lysine, while similar trends were seen for glyoxal-derived hydroimidazolone, 3-deoxyglucosone hydroimidazolone, and Nε-carboxymethyl lysine (10). The current study in a substantially larger and ethnically diverse cohort showed significant associations between most of the individual AGEs and both 30% and 40% RFL.

Baseline AGE score was also associated with development of macroalbuminuria and with transition to hrCKD. Because these outcomes (especially macroalbuminuria) are less dependent on GFR, our data provide further evidence that the association of AGEs with renal disease is not simply a reflection of reverse causation, with early declines in GFR enhancing AGE-free adducts levels. In fact, the association between higher AGE score and greater risk of adverse renal outcomes was present even in those with normal kidney function (eGFR $\geq$90 mL/min · 1.73 m^2). These results

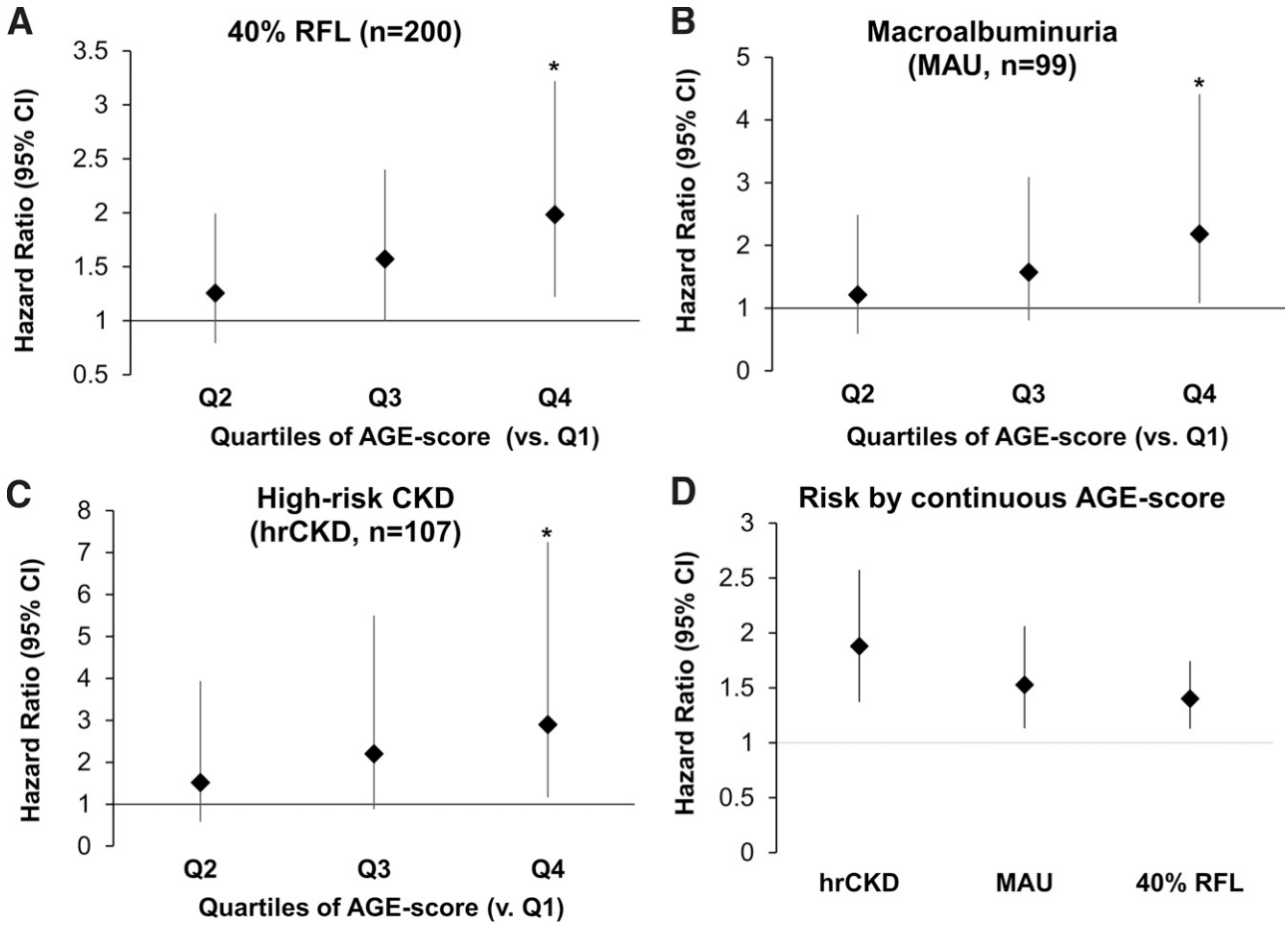

Figure 2—Association between AGE score and secondary renal outcomes in ACCORD. *A*: Forty percent RFL by quartile (Q) of AGE score (Q2–Q4 vs. bottom Q [Q1]). *B*: Incident macroalbuminuria [MAU] by Q of AGE score. *C*: hrCKD by Q of AGE score. *D*: HRs for the secondary outcomes for continuous AGE score. All models were adjusted for study arm and baseline age, sex, race/ethnicity, type 2 diabetes duration, systolic blood pressure, HbA_{1c}, eGFR, and uACR. *$P < 0.05$ vs. Q1.

also suggest that plasma AGEs have a broad-based pathophysiologic link with incident renal complications, because they are associated with two distinct renal events, worsening of both proteinuria and creatinine clearance.

The relationships between baseline AGE score and subsequent renal events persisted after accounting for, and were substantially stronger than, baseline and follow-up HbA_{1c} levels in both study populations. Consistent with previous reports from several cohorts, including our data from VADT (8,10,11), individual AGEs showed only a weak association with HbA_{1c}. Of relevance, the relationships of AGE-free adduct score with renal outcomes appeared stronger in

Table 2—Reclassification models after adding AGE score for 10-year risk of renal events in ACCORD and 5-year risk of sustained 30% RFL in VADT

	NRI						IDI	
	Cases, %		Noncases, %					
	Up	Down	Up	Down	NRI (CI), %*	*P*	Relative IDI (CI), %*	*P*
ACCORD								
Sustained 30% RFL	56	45	44	56	23 (8–41)	0.02	6.9 (1.1–13)	0.009
Any 40% RFL	53	47	45	55	16 (−4 to 34)	0.12	5.2 (0.5–10)	0.03
Macroalbuminuria	55	45	43	57	32 (−2 to 53)	0.03	3.9 (0.1–9.9)	0.11
hrCKD	56	45	42	58	26 (0.8–54)	0.06	3.2 (−0.1 to 9.4)	0.18
VADT								
Sustained 30% RFL	57	43	45	55	24 (1–48)	0.03	6.3 (−2.6 to 14)	0.18

The basic model included study arm and baseline age, sex, race, diabetes duration, systolic blood pressure, HbA_{1c}, eGFR, and uACR. *CIs (2.5–97.5%) were calculated using 100 bootstrap samples.

those with lower baseline HbA_{1c} levels. Bolstering our previous findings involving individual AGEs in VADT (11), we now report a strong positive association between duration of diabetes and AGE score in the much larger ACCORD subcohort. The importance of diabetes duration for AGE accumulation and development of DKD is further corroborated by a significant interaction between diabetes duration and AGE score in risk of 30% RFL, showing a significant association only in those with a longer history of diabetes. This may indicate a more prominent role of chronic hyperglycemia (and related pathways such as oxidative stress) in AGE formation compared with more recent ambient glycemic control. Thus, our data support the concept that the AGE-modified proteins, once formed, are long lasting, not readily modified by glucose control, and dominant risk factors for microvascular complications of diabetes, largely independent of glycemic control (22). This is also consistent with a potential contribution of the AGEs in the negative legacy effect of long-term poor glycemic control on microvascular diabetes complications in individuals with established diabetes (23,24). Congruent with the prominent role of AGEs in DKD, strategies that increased degradation and excretion or blocked action of AGEs prevented or slowed renal disease in experimental models of diabetes (25–28).

Subgroup analyses in the ACCORD cohort demonstrated significantly higher AGE-related risk for macroalbuminuria and hrCKD in women compared with men, indicating the prominence of AGEs in the development of proteinuria-associated DKD in women. Given the relatively small number of events in women in our study cohort, additional studies are needed to confirm this novel observation in a more representative setting. However, our findings are in line with the prior finding of increased susceptibility to albuminuria in women with worse glycemic control (29).

Our findings add to the growing evidence implicating AGEs in the pathophysiology of DKD (6). In circulation, AGEs are present as protein-bound fractions or free adducts, stemming predominantly from AGE-modified tissue proteins. In our previous studies, AGE-free adducts but not the protein-bound fraction showed an association with diabetes complications (30). Consistent with the primary role of kidneys in clearance of circulating AGE-free adducts, serum AGE levels in the current study were inversely associated with eGFR. The inverse association between various AGEs and kidney function has been reported previously by a number of cross-sectional or longitudinal studies (7–10). A portion of filtered AGE-free adducts is actively reabsorbed and resecreted in the proximal tubule (31). During renal transit and after tissue absorption, AGEs interact with RAGE and other matrix cell receptors and increase collagen expression in the renal interstitium and mesangium (32). This provides an opportunity for AGEs to induce irreversible cross-linked proteins in the glomeruli (33) and activate premature senescence in proximal tubule cells (34). Consistent with in vitro AGE toxicity, increased exposure to AGEs promoted microvascular dysfunction and DKD in animal models (35,36). Importantly, in humans with type 1 or type 2 diabetes, higher levels of multiple AGE-free adducts predicted worse morphologic features of diabetes nephropathy (8,10).

This study has several strengths. A highly precise quantitative technique was used for AGE measurement. The assay was designed to capture multiple AGEs reflecting distinct pathways of advanced glycation in a single run. The analyses were conducted in the largest type 2 diabetes cohort to date, and AGE score was validated in another distinct type 2 diabetes cohort, providing strong evidence for a role of AGE-free adducts in predicting DKD risk. Both cohorts had extensive baseline clinical and demographic characteristics, permitting comprehensive consideration of covariates and potential confounding effects. Key study metrics were measured regularly during the entire duration of both trials, with standardized protocols for measurement of serum creatinine, uACR, and other relevant risk factors. The large sample sizes allowed robust statistical analyses, with adjustment for many of these relevant variables. Standardized protocols were used for treatment of diabetes and other risk factors, reducing the likelihood for bias in treatment among those with different baseline AGE measures.

One limitation of the current study was the use of eGFR based on serum creatinine levels. Serum creatinine levels can be affected by nonrenal factors, including muscle mass, diet, fasting, muscle injury, and some medications, including fenofibrate (37). Previous analyses in ACCORD demonstrated that the increase in serum creatinine levels in the fenofibrate arm was transient, occurring within the first 4 months of onset and diminishing 3 months after discontinuation of fenofibrate (16). Notably, after correction for this initial increase in creatinine levels, the association between AGE score and renal outcomes was similar in the placebo and fenofibrate arms. Together with the similar associations between AGE score and RFL in VADT, this finding supports the validity of our correction approach in the ACCORD cohort. Moreover, our results are also consistent with smaller studies reporting associations between various individual AGE-free adducts and directly measured GFR (8,10). Because kidney biopsies were not collected in either the ACCORD or VADT study, we were unable to provide morphologic evidence for the presence and type of kidney damage. Nevertheless, previous small studies have shown an inverse association between individual AGE adducts and kidney function that was also accompanied by histologic changes consistent with diabetes nephropathy (8,10). Both ACCORD and VADT were conducted prior to the introduction of newer diabetes medications that have been shown to protect against diabetes nephropathy (38,39). Whether this protective effect includes modification of AGE formation, metabolism, or action remains to be elucidated.

In conclusion, we showed that circulating levels of multiple individual AGE-free adducts were associated with later kidney function decline in individuals with chronic type 2 diabetes. A composite score based on a panel of five AGE adducts showed a stronger association with renal outcomes than individual AGEs in two different cohorts of type 2 diabetes. Addition of the AGE score to standard clinical measures improved the prediction of substantial RFL. These data provide further support for a causal role of AGEs in DKD and suggest utility of this AGE panel in predicting the long-term risk of developing DKD.

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