Relationship Between Levels of Advanced Glycation End Products and Their Soluble Receptor and Adverse Outcomes in Adults With Type 2 Diabetes

Merlin C. Thomas,[1] Mark Woodward,[2,3,4] Bruce Neal,[2] Qiang Li,[2] Raelene Pickering,[1] Michel Marre,[5] Bryan Williams,[6] Vlado Perkovic,[2] Mark E. Cooper,[1] Sophia Zoungas,[2,7] John Chalmers,[2] and Graham S. Hillis,[2,8] on behalf of the ADVANCE Collaborative Group

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OBJECTIVE

This study explored whether activation of the receptor for advanced glycation end products (RAGE) is implicated in the development of diabetes complications.

RESEARCH DESIGN AND METHODS

A case-cohort study was performed in 3,763 participants with prevalent diabetes in the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) trial. The hazard ratios (HRs) for death, major cardiovascular events, and new or worsening nephropathy were derived using Cox regression models, and the ability of sRAGE and AGE levels to reclassify the risk of nephropathy was assessed.

RESULTS

After adjustment for a range of possible confounders and other risk factors, sRAGE levels were associated with all-cause mortality (HR 1.11 for a 1-SD increase of log sRAGE [95% CI 1.00–1.22]; *P* = 0.045) and new or worsening nephropathy (HR 1.20 for a 1-SD increase of log sRAGE [95% CI 1.02–1.41]; *P* = 0.032). Circulating AGE levels were also independently associated with new or worsening nephropathy (HR 1.21 for a 1-SD increase [95% CI 1.08–1.36]; *P* = 0.001). Both markers also significantly improved the accuracy with which the 5-year risk of new or worsening nephropathy could be predicted (net reclassification index in continuous model, 0.25 for sRAGE and 0.24 for AGE levels).

CONCLUSIONS

In adults with type 2 diabetes, increased levels of sRAGE are independently associated with new or worsening kidney disease and mortality over the next 5 years. Higher levels of AGE are also associated with an increased risk of adverse renal outcomes. The AGE/RAGE axis may be of importance in the prevention and management of diabetes complications.

Diabetes is associated with an increased risk of adverse renal and cardiovascular outcomes, which are only partially reduced by intensive glycemic control. This may be because of the long-lasting (legacy) effects of poor glycemic control over many years (1). Potential mediators of this "metabolic karma" include the long-lasting posttranslational molecular modifications induced by hyperglycemia, known as advanced

[1]*Baker IDI Heart & Diabetes Institute, Melbourne, Victoria, Australia*
[2]*The George Institute for Global Health, The University of Sydney, Sydney, New South Wales, Australia*
[3]*The George Institute for Global Health, Nuffield Department of Population Health, University of Oxford, Oxford, U.K.*
[4]*Department of Epidemiology, Johns Hopkins University, Baltimore, MD*
[5]*Hôpital Bichat-Claude Bernard and Université Paris 7, Paris, France*
[6]*University College London and the National Institute for Health Research University College London Hospitals Biomedical Research Centre, London, U.K.*
[7]*School of Public Health and Preventive Medicine, Monash University, Melbourne, Victoria, Australia*
[8]*Department of Cardiology, Royal Perth Hospital, Perth, Western Australia, Australia*

Corresponding author: Mark Woodward, markw@georgeinstitute.org.au.

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glycation end products (AGEs) (2). Experimental data have linked AGEs to the development and progression of diabetes complications, including nephropathy, retinopathy, neuropathy, and cardiovascular disease (3–5). Posttranslational modification of functional groups on vulnerable proteins, lipids, and DNA targets has the potential to alter their structure, stability, and/or function (6,7). AGEs also stimulate pathogenic pathways after activation of the receptor for AGEs (RAGE) (8), which may represent the major mechanism by which AGEs lead to diabetes complications.

One potential marker for the expression of RAGE and activation of the AGE/RAGE axis is circulating soluble RAGE (sRAGE), a C-truncated isoform largely produced by proteolytic cleavage of the membrane-bound form via the action of metalloprotein sheddases (9). Tissue RAGE and circulating sRAGE are both increased in individuals with diabetes, paralleling increased levels of circulating AGEs and other RAGE ligands, including S100a8/9 and high-mobility group protein box-1, whose actions lead not only to RAGE activation but also to the autoinduction of RAGE expression (10–12). We and others have previously shown that sRAGE is independently associated with the development of cardiovascular complications and mortality in adults with type 1 diabetes (13,14). Similar findings have been reported in patients in type 2 diabetes without prior cardiovascular disease from the Collaborative Atorvastatin Diabetes Study (CARDS), although microvascular outcomes were not described in that report (15). In this current case-cohort study, we explore the association between levels of circulating AGEs, sRAGE, and adverse outcomes (death, cardiovascular complications, and new or worsening nephropathy) in adults with type 2 diabetes who participated in the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) trial.

RESEARCH DESIGN AND METHODS

Study Sample

The ADVANCE study has been described in detail previously (16–18). Participants with type 2 diabetes at increased risk of cardiovascular events were recruited from 20 countries in Asia, Australasia, Europe, and North America. Participants were also required to be aged ≥55 years and have a history of cardiovascular disease or one or more additional cardiovascular risk factor. The study made two randomized comparisons: a double-blind assessment of the efficacy of a fixed combination of perindopril and indapamide (2 mg/0.625 mg for 3 months, increasing to 4 mg/1.25 mg if tolerated) versus placebo, and an open-label evaluation of an intensive glucose-lowering regimen using modified release gliclazide, with a target glycated hemoglobin (HbA_{1c}) of ≤6.5%, versus standard guideline-based glycemic control. The study randomized 11,140 participants, and the median duration of follow-up was 5 years.

Nonfasting blood samples were taken at baseline, anticoagulated with EDTA, and stored centrally at −80°C for a median of 7.8 years before analyses. Stored plasma samples were available from all countries involved in ADVANCE, except China and India, giving a total base population of 7,376 patients. A case-cohort study population was constructed from a random sample of 3,500 individuals taken from these 7,376 participants plus 697 additional individuals who had a cardiovascular event, a microvascular complication, or died during follow-up.

Baseline data included demographic and clinical information, including age at diagnosis, presence and severity of diabetes complications, antidiabetic therapy, and other regular medications. Weight, height, urinary albumin-to-creatinine ratio (ACR), serum creatinine, fasting lipid levels, and HbA_{1c} were also measured. The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation (19). Levels of baseline high-sensitivity cardiac troponin T (hs-cTnT), N-terminal pro-B type natriuretic peptide (NT-proBNP), and hs-CRP were also measured in stored samples (20–22).

sRAGE was measured by ELISA (Quantikine; R&D Inc., Minneapolis, MN). Intra-assay coefficients of variability were <7%, and between-assay coefficients of variability were <9%, as previously described (10). Circulating AGEs were estimated by the level of AGE-associated autofluorescence (excitation, 370 nm; emission, 440 nm) in EDTA-treated plasma samples, adjusted for protein quantity.

Study End Points

The study end points were all-cause death, major cardiovascular events (cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke), and new or worsening nephropathy (defined as the development of macroalbuminuria, doubling of serum creatinine to a level of at least 200 μmol/L, need for renal replacement therapy, or death due to renal disease). All outcomes were validated by an independent adjudication committee.

Statistical Analyses

Baseline characteristics were summarized by ordinal categories defined by the tertiles of sRAGE and AGE levels. Tests for linear trend were performed across the thirds of each biomarker to identify possible covariates associated with sRAGE and AGE using linear regression. Hazard ratios (HRs) for log-linear effects of sRAGE and AGE levels on each of the three studied outcomes were obtained from weighted Cox regression models for case-cohort analyses in the SAS package. sRAGE levels were log-transformed to remove the effects of skewness. Results were expressed per unit SD, rounded up to whole numbers (for log sRAGE, 0.927 log[pg/mL], rounded to 1.0; and for AGEs, 93.23 relative fluorescent units [RFU]/mg protein, rounded to 100).

Six models with different sets of potential confounding variables were fitted for each of the biomarker/outcome combinations:

- model 1, with age, sex, and randomized treatment;
- model 2, with, additionally, duration of diabetes, current smoking, systolic blood pressure, BMI, HbA_{1c}, plasma glucose, total and HDL cholesterol, triglycerides, and a history of macrovascular complications;
- model 3, with, additionally ACR and eGFR;
- model 4, which added the other biomarker (i.e., sRAGE or AGE levels) to model 2;
- model 5, which added hs-cTnT, NT-proBNP, hs-CRP, and use of β-blockers to model 3; and
- model 6, which added the other biomarker (i.e., sRAGE or AGE levels) to model 5.

Analyses were also performed stratified by age (>67 or ≤67 years), sex, a history of macrovascular complications, a history of microvascular complications, eGFR (<60 or ≥60 mL/min/1.73 m^2), and HbA_{1c} (<7% or ≥7%) adjusted for the variables described in model 2, except a prior history of macrovascular complications. Log-likelihood ratio tests were conducted to test the nonlinearity effect of sRAGE and AGE level to outcomes by comparing a model with categorical biomarkers and a model with continuous biomarkers.

Because our data tended to have relatively high levels of sRAGE compared with earlier studies in diabetes (13–15), a sensitivity analysis was run repeating the main analyses of association in a restricted population including only those below the second tertile of sRAGE or AGE, as appropriate.

Discrimination was evaluated through C-statistics for 5-year risk, accounting for censoring, and compared between the full clinical model (with the covariates in model 3) and when adding each biomarker individually. In addition, the ability to reclassify the 5-year risk for new and worsening nephropathy was assessed by the integrated discrimination index (IDI) and net reclassification improvement (NRI) methods, using techniques suitable for survival data applied to the random subcohort (23,24).

RESULTS

Of the 4,197 participants in this study, blood samples from 434 (10%) were missing or unsuitable for analysis. The remaining 3,763 subjects in this cohort had a mean age of 66.9 years and a median duration of diabetes of 7.9 years (Tables 1 and 2); 92% were Caucasian. Of these participants, 942 (25%) had eGFR <60 mL/min/1.73 m^2 and 1,098 (29%) had micro- or macroalbuminuria; overall, 1,700 (45%) had eGFR <60 mL/min/1.73 m^2 or albuminuria. Circulating levels of sRAGE and AGEs were highest in patients with poorer renal function, higher urinary ACRs, dyslipidemia, or advanced age. Circulating concentrations of sRAGE and AGEs were associated with levels of hs-cTnT and NT-proBNP and with a history of heart failure. AGE levels, but not sRAGE levels, were higher in patients with poor glucose control or a prolonged duration of diabetes before enrolment.

During a median of 5 years of follow-up, 689 participants (18%) died, 683 (18%) suffered a major cardiovascular event, and 272 (7%) developed new or worsening nephropathy. Individuals with higher levels of sRAGE were more likely to experience each of these adverse outcomes, after adjusting for age, sex, and randomized treatment (Table 3). When the random subcohort was divided into thirds, participants with higher concentrations of sRAGE were at increased risk of all three outcomes (Supplementary Fig. 1). After adjusting for several other clinical risk

Table 1—Baseline demographic, clinical, and laboratory data classified by sRAGE levels (pg/mL)

Characteristic	Lowest third (≤1,119) *n* = 1,255	Middle third (>1,119 to <2,053) *n* = 1,253	Highest third (≥2,053) *n* = 1,255	Total *N* = 3,763	*P* for trend
Male sex, *n* (%)	823 (65.6)	758 (60.5)	715 (57.0)	2,296 (61.0)	<0.0001
Current smoker, *n* (%)	195 (15.5)	189 (15.1)	186 (14.8)	570 (15.1)	0.6163
History of a macrovascular event, *n* (%)	418 (33.3)	419 (33.4)	474 (37.8)	1,311 (34.8)	0.0190
Age, mean (SD), years	66.50 (6.37)	67.00 (6.64)	67.27 (6.80)	66.92 (6.61)	0.0037
BMI, mean (SD), kg/m^2	30.07 (5.08)	30.02 (5.39)	29.96 (5.32)	30.02 (5.26)	0.5873
Diabetes duration, mean (SD), years	7.50 (6.26)	8.25 (6.62)	7.89 (6.39)	7.88 (6.43)	0.1331
History of heart failure, *n* (%)	42 (3.3)	50 (4.0)	78 (6.2)	170 (4.5)	0.0006
Moderate or vigorous activity, *n* (%)	643 (51.2)	600 (47.9)	576 (45.9)	1,819 (48.3)	0.0075
Aspirin or other antiplatelet agent, *n* (%)	603 (48.0)	599 (47.8)	655 (52.2)	1,857 (49.3)	0.0381
Statin or other lipid-lowering agent, *n* (%)	543 (43.3)	575 (45.9)	546 (43.5)	1,664 (44.2)	0.9040
β-Blocker, *n* (%)	320 (25.5)	364 (29.1)	456 (36.3)	1,140 (30.3)	<0.0001
ACE inhibitor or ARB, *n* (%)	738 (58.8)	733 (58.5)	761 (60.6)	2,232 (59.3)	0.3501
Systolic blood pressure, mean (SD), mmHg	148.21 (21.85)	147.10 (21.74)	147.24 (21.22)	147.52 (21.61)	0.2612
Diastolic blood pressure, mean (SD), mmHg	82.17 (11.00)	81.74 (10.94)	80.87 (10.72)	81.59 (10.90)	0.0028
Total cholesterol, mean (SD), mmol/L	5.13 (1.15)	5.15 (1.24)	5.12 (1.13)	5.13 (1.17)	0.8774
HDL cholesterol, mean (SD), mmol/L	1.24 (0.34)	1.23 (0.33)	1.20 (0.32)	1.22 (0.33)	0.0005
Triglycerides, mean (SD), mmol/L	1.90 (1.18)	1.94 (1.20)	2.04 (1.25)	1.96 (1.21)	0.0055
HbA_{1c}, mean (SD), % HbA_{1c}, mean (SD), mmol/mol	7.38 (1.41) 57 (15.4)	7.42 (1.40) 58 (15.3)	7.43 (1.48) 58 (16.2)	7.41 (1.43) 58 (15.6)	0.3667
Urinary ACR, median (IQI), mg/mmol	12.90 (6.36, 34.48)	15.03 (6.43, 43.70)	16.80 (7.07, 46.94)	15.03 (6.81, 41.37)	0.0026
Glucose, mean (SD), mmol/L	8.53 (2.73)	8.51 (2.68)	8.36 (2.74)	8.46 (2.71)	0.1151
eGFR, mean (SD), mL/min/1.73 m^2	74.44 (15.60)	71.01 (17.04)	69.00 (17.62)	71.48 (16.92)	<0.0001
hs-CRP, median (IQI), mg/L	1.87 (0.89, 4.12)	1.80 (0.87, 4.02)	1.79 (0.86, 4.00)	1.81 (0.87, 4.05)	0.3563
hs-cTnT, median (IQI), pg/mL	5.00 (1.50, 10.00)	5.00 (1.50, 11.00)	6.00 (1.50, 13.00)	5.00 (1.50, 11.00)	<0.0001
NT-proBNP, median (IQI), pg/mL	66 (26, 151)	87 (35, 211)	124 (47, 335)	90 (35, 223)	<0.0001
AGEs, mean (SD), RFU/mg protein	118.71 (86.54)	137.81 (92.73)	155.51 (96.57)	137.34 (93.23)	<0.0001

ARB, angiotensin receptor blocker; IQI, interquartile interval.

Table 2—Baseline demographic, clinical, and laboratory data classified by AGE levels (RFU/mg protein)

Characteristic	1st AGE tertile (≤89) *n* = 1,235	2nd AGE tertile (>89 to <161) *n* = 1,230	3rd AGE tertile (≥161) *n* = 1,240	Total *N* = 3,705	*P* for trend
Male sex, *n* (%)	761 (61.6)	776 (63.1)	721 (58.1)	2,258 (60.9)	0.0762
Current smokers, *n* (%)	176 (14.3)	192 (15.6)	196 (15.8)	564 (15.2)	0.2818
History of a macrovascular event, *n* (%)	431 (34.9)	410 (33.3)	454 (36.6)	1,295 (35.0)	0.3704
Age, mean (SD), years	66.57 (6.33)	67.04 (6.60)	67.11 (6.90)	66.92 (6.61)	0.0429
BMI, mean (SD), kg/m^2	7.26 (6.02)	8.04 (6.57)	8.40 (6.68)	7.88 (6.43)	<0.0001
Diabetes duration, mean (SD), years	30.15 (5.30)	29.71 (5.09)	30.18 (5.42)	30.02 (5.26)	0.8789
History of heart failure, *n* (%)	51 (4.1)	47 (3.8)	71 (5.7)	169 (4.6)	0.0575
Moderate or vigorous activity, *n* (%)	576 (46.6)	599 (48.7)	620 (50.0)	1,795 (48.4)	0.0947
Aspirin or other antiplatelet agent, *n* (%)	613 (49.6)	623 (50.7)	583 (47.0)	1,819 (49.1)	0.1921
Statin or other lipid-lowering agent, *n* (%)	578 (46.8)	533 (43.3)	530 (42.7)	1,641 (44.3)	0.0422
β-Blocker, *n* (%)	416 (33.7)	359 (29.2)	347 (28.0)	1,122 (30.3)	0.0021
ACE inhibitor or ARB, *n* (%)	703 (56.9)	731 (59.4)	763 (61.5)	2,197 (59.3)	0.0197
Systolic blood pressure, mean (SD), mmHg	148.29 (21.71)	146.63 (21.76)	147.56 (21.43)	147.52 (21.61)	0.4010
Diastolic blood pressure, mean (SD), mmHg	82.30 (10.72)	80.92 (10.68)	81.46 (11.24)	81.59 (10.90)	0.0560
Total cholesterol, mean (SD), mmol/L	5.03 (1.08)	5.08 (1.11)	5.29 (1.31)	5.13 (1.17)	<0.0001
HDL cholesterol, mean (SD), mmol/L	1.22 (0.32)	1.23 (0.34)	1.22 (0.32)	1.22 (0.33)	0.7024
Triglycerides, mean (SD), mmol/L	1.89 (1.12)	1.93 (1.18)	2.05 (1.33)	1.96 (1.21)	0.0010
HbA_{1c}, mean (SD), % HbA_{1c}, mean (SD), mmol/mol	7.30 (1.31) 56 (14.3)	7.34 (1.36) 57 (14.9)	7.59 (1.60) 59 (17.5)	7.41 (1.43) 57 (15.6)	<0.0001
Urinary ACR, median (IQI), mg/mmol	13.50 (6.19, 39.78)	14.07 (6.27, 37.26)	16.16 (7.07, 46.82)	15.03 (6.81, 41.37)	0.4090
Glucose, mean (SD), mmol/L	8.35 (2.50)	8.37 (2.52)	8.65 (3.09)	8.46 (2.71)	0.0052
eGFR, mean (SD), mL/min/1.73 m^2	73.26 (16.02)	71.28 (16.42)	69.97 (18.14)	71.48 (16.92)	<0.0001
hs-CRP, median (IQI), mg/L	1.81 (0.87, 4.08)	1.68 (0.82, 3.91)	1.95 (0.93, 4.22)	1.81 (0.87, 4.05)	0.4594
hs-cTnT, median (IQI), pg/mL	5.00 (1.50, 10.00)	5.00 (1.50, 12.00)	6.00 (1.50, 12.00)	5.00 (1.50, 11.00)	0.0233
NT-proBNP, median (IQI), pg/mL	85 (34, 208)	86 (33, 210)	103 (40, 264)	90 (35, 223)	0.0105
sRAGE, median (IQI), pg/mL	1,336 (803, 2,050)	1,473 (890, 2,525)	1,749 (1,118, 2,895)	1,512.0 (915, 2,429)	<0.0001

For conventions, see Table 1.

factors, these associations were attenuated, largely through the adjustment for renal impairment, although the association between sRAGE and all-cause mortality and sRAGE with adverse renal events remained significant. By contrast, the association with cardiovascular events was no longer significant. In analyses restricted to the lowest two-thirds of sRAGE, associations with new or worsening nephropathy were largely preserved (Supplementary Table 1). Circulating levels of AGEs were associated only with an increased risk of new or worsening nephropathy. This relationship persisted even after adjustment for a wide range of potential confounding factors, including sRAGE levels (Table 3 and Supplementary Fig. 2). AGEs and sRAGE both improved the accuracy with which the 5-year risk of new or worsening nephropathy could be discriminated or correctly classified compared with only accounting for the other clinical and demographic measures considered (Table 4).

There was no evidence of nonlinearity ($P > 0.05$) between sRAGE or AGE levels and the three study outcomes. No significant interactions were found in subgroup analyses for either biomarker when considering the outcomes of death or cardiovascular events (Supplementary Fig. 3*A* and *B*). A similar stratified analysis showed the relationship between sRAGE levels and new or worsening nephropathy was most evident in patients with preserved renal function (Supplementary Fig. 3*C*).

CONCLUSIONS

Activation of RAGE by AGEs and other ligands has been suggested to be an important mediator of vascular complications in diabetes (4,25,26). The expression of RAGE is increased at sites of vascular injury, including in the diabetic kidney (4). Circulating levels of sRAGE are increased in individuals with diabetes, especially those with microvascular and/or macrovascular complications of their disease (13–15). In this report we also demonstrate that sRAGE levels are also independently associated with new-onset or worsening renal disease and all-cause mortality in individuals with type 2 diabetes from the ADVANCE trial. The current results are consistent with our previous data in patients with type 1 diabetes (13) and in patients with type 2 diabetes enrolled in CARDS (15), where sRAGE levels were positively associated with all-cause mortality. However, this is the first report confirming an independent additional association between sRAGE and progressive renal disease specifically in patients with type 2 diabetes. Taken together, these results highlight the potential importance of the AGE/RAGE axis in the pathogenesis of diabetes complications as well as their outcomes.

Table 3—HRs for adverse events according to increasing levels of AGEs and sRAGE

		sRAGE (log scale) (per 1 unit*)		Laboratory AGE value (per 100 RFU/mg protein*)	
Model	Outcome	HR (95% CI)	*P* value	HR (95% CI)	*P* value
1	All-cause death	1.20 (1.09–1.32)	0.0002	1.05 (0.97–1.14)	0.2193
	Major cardiovascular events	1.16 (1.05–1.29)	0.0038	1.06 (0.98–1.15)	0.1262
	New or worsening nephropathy	1.43 (1.23–1.67)	<0.0001	1.25 (1.12–1.38)	<0.0001
2	All-cause death	1.17 (1.06–1.29)	0.0015	1.00 (0.91–1.09)	0.9474
	Major cardiovascular events	1.14 (1.02–1.26)	0.0158	1.02 (0.93–1.11)	0.7392
	New or worsening nephropathy	1.39 (1.19–1.63)	<0.0001	1.21 (1.08–1.35)	0.0009
3	All-cause death	1.11 (1.00–1.22)	0.0451	0.97 (0.89–1.06)	0.5382
	Major cardiovascular events	1.08 (0.97–1.21)	0.1471	1.02 (0.93–1.12)	0.7121
	New or worsening nephropathy	1.20 (1.02–1.41)	0.0316	1.21 (1.08–1.36)	0.0013
4	All-cause death	1.17 (1.06–1.29)	0.0013	0.97 (0.89–1.06)	0.5522
	Major cardiovascular events	1.15 (1.03–1.27)	0.0102	0.99 (0.91–1.09)	0.8881
	New or worsening nephropathy	1.34 (1.15–1.57)	0.0002	1.17 (1.04–1.32)	0.0102
5	All-cause death	1.11 (1.01–1.21)	0.0323	0.98 (0.90–1.07)	0.6516
	Major cardiovascular events	1.09 (0.98–1.20)	0.1077	1.00 (0.91–1.09)	0.9688
	New or worsening nephropathy	1.32 (1.14–1.54)	0.0003	1.20 (1.07–1.35)	0.0015
6	All-cause death	1.11 (1.01–1.22)	0.0257	0.96 (0.88–1.06)	0.4385
	Major cardiovascular events	1.10 (0.99–1.22)	0.0687	0.98 (0.89–1.08)	0.7276
	New or worsening nephropathy	1.28 (1.10–1.49)	0.0017	1.17 (1.04–1.32)	0.0101

Model 1: adjusted for age, sex, and randomized treatment groups; model 2: as model 1, but adjusted additionally for duration of diabetes, current smoking, systolic blood pressure, BMI, plasma glucose, HbA_{1c}, total cholesterol, HDL cholesterol, triglycerides, and history of macrovascular events; model 3: as model 2, but additionally adjusted for ACR and eGFR (by the Chronic Kidney Disease Epidemiology Collaboration equation); model 4: as model 2, but adjusted additionally for sRAGE or AGE, as appropriate; model 5: as model 3, but adjusted additionally for hs-cTnT, NT-proBNP, hs-CRP, and use of β-blockers; model 6: as model 5, but additionally adjusted for sRAGE or AGE, as appropriate. *Approximately 1 SD (on the raw scale sRAGE was measured in pg/mL).

It is important to note that studies of general population cohorts have reported that lower sRAGE levels may be associated with poor health outcomes (27,28). For example, in the Atherosclerosis Risk in Communities (ARIC) study in adults from the general population with normal renal function, lower levels of sRAGE were paradoxically associated with an increased risk of diabetes, coronary heart disease, and mortality during 18 years of follow-up (26). It is possible to speculate that in populations (like ADVANCE) with a high prevalence of renal impairment, reduced renal clearance of sRAGE serves to dramatically uncover the induction of RAGE expression that might not otherwise be observed in healthy adults.

In a case-cohort study nested within the ARIC study, sRAGE levels were positively associated with development of chronic kidney disease and end-stage renal disease (29). However, these associations were not significant after adjustment for eGFR, which, as in the ADVANCE study, was the major determinant of circulating sRAGE levels. By contrast, the association between sRAGE and all-cause mortality and sRAGE with adverse renal events in the ADVANCE study remained significant after adjustment for renal impairment, possibly because of a higher event rate and,consequently, greater power to observe an effect. Unlike ARIC, ADVANCE had few (<2%) black participants, in whom sRAGE levels are known to be lower (15). There may be an effect of treatment, such that the aggressive management of risks in patients with type 2 diabetes included in the ADVANCE study effectively eliminated the dominant role of conventional risk factors in the pathogenesis of adverse outcomes, while glucose and lipids remain strong determinants of outcome in untreated cohorts. In addition, it may be that increased levels of RAGE ligands and elevated levels of RAGE (as occurs in diabetes) are both required for the pathogenic aspects of this pathway to become manifest. This would be consistent with our finding that AGEs and sRAGE were both independently associated with renal outcomes in our cohort.

Although a causal relationship cannot be established from the presented observational data, the finding that activation of the AGE/RAGE axis may be associated with progressive renal disease is consistent with prior experimental data. Genetic depletion of RAGE is able to attenuate complications associated with diabetes (30), and treatment with truncated RAGE also significantly reduces the renal damage in diabetic mice (25). Similarly, the potential importance of AGEs as downstream mediators of renal damage has been demonstrated in studies using chemically disparate inhibitors of AGE formation to retard the development of diabetic kidney disease (31). Previous studies have reported a strong association between the presence and severity of kidney damage and circulating levels of sRAGE in patients with type 2 diabetes (11,32–34). This is partly determined by the reduced clearance of sRAGE in patients with renal impairment. However, we show here for the first time that, independent of baseline renal function and other risk factors for progressive renal disease, sRAGE was significantly associated with new or worsening nephropathy in adults with type 2 diabetes. Moreover, the improvements in the NRI achieved when adding sRAGE and/or AGE were substantial compared with other recognized biomarkers, suggesting these indices may be of value when stratifying the

Table 4—C-statistic, IDI, relative IDI, and NRI (each with their 95% CI) for new or worsening nephropathy compared with the base model[a]: sRAGE and AGE

Base model[a] C-statistic	0.8279 (0.7935–0.8623)
Base + sRAGE (log scale)	
C-statistic	0.8286 (0.7942–0.8630)
IDI	0.0147 (0.0101–0.0195)
Relative IDI (%)	10.1 (7.2–12.9)
NRI–continuous	0.2537 (0.0798–0.4290)
NRI–categorical[b]	0.2616 (0.1782–0.3634)
Base + AGE	
C-statistic	0.8281 (0.7936–0.8626)
IDI	0.0144 (0.0095–0.0206)
Relative IDI (%)	9.9 (6.9–13.3)
NRI–continuous	0.2415 (0.0607–0.4152)
NRI–categorical[b]	−0.0111 (−0.0381 to 0.0086)

Results are derived from the random subcohort (n = 3,500). [a]Base model includes age, sex, randomized treatment allocations, prior history of vascular disease, duration of diabetes, current smoking, systolic blood pressure, BMI, ACR, eGFR, HbA_{1c}, glucose, triglycerides, and total and HDL cholesterol. [b]Using cutoff points of 1.5% and 3% 5-year risk.

risk of incident or worsening nephropathy in patients with diabetes as well as being a potential target for novel treatments.

Some small cross-sectional studies have previously described an association between circulating levels of sRAGE and atherosclerosis burden in type 2 diabetes (28,35,36). However, we found no independent association between levels of sRAGE and cardiovascular outcomes in our cohort after adjusting for other risk factors, including baseline renal function. This is perhaps surprising given the clear vasculoprotective effects of RAGE deletion observed in animal studies of atherosclerosis (30) and in our previous report of an association between sRAGE and cardiovascular outcomes in adults with type 1 diabetes (13). However, the greatest benefit of glucose lowering in the ADVANCE study was observed on renal outcomes, while the same cardiovascular outcomes not linked to sRAGE cardiovascular outcomes were not significantly modified by intensive glucose control (16).

In the ADVANCE study, AGE levels were higher in patients with poor glycemic control or prolonged duration of diabetes before intervention, consistent with the nonenzymatic chemistry of the Maillard reaction, which is dependent on both glucose concentration and time. However, sRAGE levels were not associated with HbA_{1c} or diabetes duration. This may be reconciled because RAGE is a multiligand receptor and may be induced by a range of other proinflammatory ligands (e.g., S100) and by inflammatory processes via the nuclear factor-κB binding element in its promoter. This may partly explain the association with renal injury and mortality observed in this study.

The strengths of the current study include its large size, international recruitment in a well-characterized trial population that was closely monitored and treated uniformly, rigorous a priori definition of outcomes, completeness of follow-up, and adjustment for major risk factors. Some limitations include the potential for recruitment bias in a randomized controlled trial and using only single measures of the baseline variables of interest. Our indirect measure of AGEs was also crude compared with the more quantitative assays based on mass spectroscopy and ELISA. However, there is no evidence that more specific measures offer any advantages, and even skin autofluorescence has been shown to be a good predictor of AGE burden and of the risk of adverse outcomes (37). That associations demonstrated in this study may be due to confounding by unmeasured factors or ones that are difficult to quantify is also possible. For example, sRAGE concentrations may be associated with a range of occult differences in inflammatory or metabolic pathways that may themselves affect clinical outcomes in individuals with diabetes.

In summary, this large multicenter study in adults with type 2 diabetes demonstrates that sRAGE is positively associated with new-onset or worsening renal disease and all-cause mortality, independent of and in addition to chronic kidney disease, the major predictor of adverse outcomes in this cohort. These data support the accumulating body of experimental data demonstrating that activation of the AGE/RAGE axis is a key mediator of microvascular damage in diabetes but also suggest that it may not be a direct mediator of macrovascular complications. Genetic studies would be useful to explore whether any effects are truly causal.

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