

Higher Plasma Soluble Receptor for Advanced Glycation End Products (sRAGE) Levels Are Associated With Incident Cardiovascular Disease and All-Cause Mortality in Type 1 Diabetes

A 12-Year Follow-Up Study

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OBJECTIVE—To investigate the associations of plasma levels of soluble receptor for advanced glycation end products (sRAGE) with incident cardiovascular disease (CVD) and all-cause mortality in type 1 diabetes and the extent to which any such associations could be explained by endothelial and renal dysfunction, low-grade inflammation, arterial stiffness, and advanced glycation end products (AGEs).

RESEARCH DESIGN AND METHODS—We prospectively followed 169 individuals with diabetic nephropathy and 170 individuals with persistent normoalbuminuria who were free of CVD at study entry and in whom levels of sRAGE and other biomarkers were measured at baseline. The median follow-up duration was 12.3 years (7.6–12.5).

RESULTS—The incidence of fatal and nonfatal CVD and all-cause mortality increased with higher baseline levels of log-transformed sRAGE (Ln-sRAGE) independently of other CVD risk factors: hazard ratio (HR) 1.90 (95% CI 1.13–3.21) and 2.12 (1.26–3.57) per 1-unit increase in Ln-sRAGE, respectively. Adjustments for estimated glomerular filtration rate (eGFR_{MDRD}), but not or to a smaller extent for markers of endothelial dysfunction, low-grade inflammation, arterial stiffness, and AGEs, attenuated these associations to HR 1.59 (95% CI 0.91–2.77) for fatal and nonfatal CVD events and to 1.90 (1.09–3.31) for all-cause mortality. In addition, in patients with nephropathy, the rate of decline of GFR was 1.38 ml/min/1.73 m² per year greater per 1-unit increase of Ln-sRAGE at baseline ($P = 0.036$).

CONCLUSIONS—Higher levels of sRAGE are associated with incident fatal and nonfatal CVD and all-cause mortality in individuals with type 1 diabetes. sRAGE-associated renal dysfunction may partially explain this association. *Diabetes* 59:2027–2032, 2010

Recent studies have suggested a potential role of the receptor for advanced glycation end products (RAGE) in the development of vascular disease in individuals with diabetes (1). At the molecular level, RAGE is upregulated in atherosclerotic lesions in diabetes (2). RAGE-induced production of adhesion molecules (3–5) and inflammatory cytokines (4) could contribute to endothelial (4,5) and renal dysfunction (6), low-grade inflammation (4,5), and arterial stiffening, all of which may partially explain the increased cardiovascular disease (CVD) in diabetes.

We have recently shown, in a large cross-sectional study of type 1 diabetes (EURODIAB), that plasma levels of sRAGE were positively associated with macro- and microvascular complications, and also with endothelial and renal dysfunction, and low-grade inflammation as pathophysiological mechanisms that explained in part the associations of sRAGE with vascular complications (7). Whether sRAGE is associated with incident fatal and nonfatal CVD as well as all-cause mortality in individuals with type 1 diabetes has never been investigated. In addition, the extent to which any such associations could be explained by markers of endothelial and renal dysfunction, low-grade inflammation, arterial stiffness, and AGEs is also not known. We hereby address these questions in a 12-year prospective follow-up study.

RESEARCH DESIGN AND METHODS

Study population and design. In 1993, 199 of all 242 albuminuric patients >18 years of age attending the outpatient clinic at the Steno Diabetes Center agreed to participate and were enrolled in a prospective observational study. Diabetic nephropathy was diagnosed according to the following criteria: persistent macroalbuminuria (>300 mg/24 h) in at least two of three previous consecutive 24-h urine collections, presence of retinopathy, and absence of other kidney or urinary tract disease. In addition, 192 patients with persistent normoalbuminuria (i.e., urinary albumin excretion [UAE] rate <30 mg/24 h) and matched for age, sex, and duration of diabetes were also enrolled as control subjects (8). The present study refers to 339 of the original 391 patients included in the cohort; details on inclusion/exclusion criteria and study main outcomes are depicted in a flow chart (see the online appendix,

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available at <http://diabetes.diabetesjournals.org/cgi/content/full/db09-1509/DC1>). The study was approved by the local ethics committee, in accordance with the Helsinki Declaration, and all patients gave their informed written consent.

Baseline investigations. Plasma levels of sRAGE were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Quantikine; R&D systems, Minneapolis, MN) according to the manufacturer's protocol. Briefly, a monoclonal antibody generated against the NH₂-terminal extracellular domain of human RAGE was used to capture sRAGE from plasma. Captured sRAGE was detected with a polyclonal anti-human sRAGE antibody. After washing, plates were incubated with streptavidin-horseradish peroxidase, developed with appropriate substrate, and OD450 was determined using an ELISA plate reader. Measurements were performed in duplicate and the intra- and interassay coefficient of variation (CV) values were 2 and 17.5%, respectively.

Measurements of other biomarkers and risk factors have been described in detail elsewhere (8,9) and in the online appendix.

Follow-up and study end points. All patients were followed up to the last visit at the Steno Diabetes Center, until 1 September 2006 or until death ($n = 82$) or emigration ($n = 3$). All patients were traced through the national register during autumn 2006. If a patient had died before 1 September 2006, the date of death was recorded and the primary cause of death was obtained from the death certificate, which was reviewed by two independent observers. Additional available information from necropsy reports was also included. All deaths were classified as cardiovascular unless an unequivocal noncardiovascular cause was established. In all patients alive at the end of follow-up, nonfatal CVD data were retrieved from their patient files at Steno Diabetes Center or other hospital records. The primary end point was a combination of fatal and nonfatal CVD (i.e., myocardial infarction, percutaneous coronary intervention, coronary bypass grafting, amputation due to ischemia, vascular surgery for peripheral atherosclerotic disease, and stroke), and the secondary end point was all-cause mortality.

Statistical analysis. All analyses were performed with SPSS version 15.0 for Windows (SPSS, Chicago, IL).

Variables with a skewed distribution were log_e-transformed before further analyses. Comparisons of baseline characteristics between groups were performed with Student's *t* or χ^2 tests, as appropriate. The associations between Ln-sRAGE and study end points were investigated with Cox proportional hazards regression models adjusted first for sex, age, duration of diabetes, case-control status, and A1C; second for other traditional cardiovascular risk factors; and third for the use of renin-angiotensin-aldosterone system inhibitors and/or other antihypertensive treatment or whether subjects did or did not withhold their medication before baseline examinations. Further adjustments for markers of renal dysfunction (i.e., estimated glomerular filtration rate [eGFR_{MDRD}] or Ln-UAE rate), low-grade inflammation (average of the *z* scores of Ln-interleukin-6, Ln-C-reactive protein, soluble intracellular adhesion molecule-1 [sICAM-1], and Ln-secreted phospholipase-A2), endothelial dysfunction (average of the *z* scores of soluble vascular cell adhesion molecule-1 and sICAM-1), and arterial stiffness (i.e., pulse pressure) were added into this model to ascertain the extent these could explain (i.e., attenuate the strength of) the association between Ln-sRAGE and study end points. The cross-sectional associations between Ln-sRAGE and markers of these pathophysiological mechanisms and AGEs [average of the *z* scores of Ln-pentosidine, N^ε-(carboxymethyl)lysine, and N^ε-(carboxyethyl)lysine] were examined with the use of linear regression analyses.

Finally, we investigated whether the associations listed above differed between patients with normoalbuminuria versus nephropathy by adding interaction terms between Ln-sRAGE and case-control status to our models; whenever the *P* value of such interactions were <0.1, results were presented for the two groups separately.

RESULTS

During the course of follow-up (median 12.3 years [interquartile range 7.6–12.5]), 82 individuals (24.2%) died; 85 (25.1%) suffered a fatal ($n = 48$) and/or nonfatal ($n = 53$) CVD event. Individuals with incident CVD events or who had died at follow-up had at baseline higher levels of Ln-sRAGE and a more adverse atherosclerotic risk (Table 1).

After adjustments for age, sex, case-control status, A1C, and duration of diabetes, the incidence of fatal and nonfatal CVD increased with a hazard ratio (HR) of 2.00 (95% CI 1.19–3.36), and the incidence of all-cause mortality increased with a HR of 2.44 (1.46–4.07), per unit increase

in baseline levels of Ln-sRAGE (Table 2, model 1, Fig. 1A and B, respectively). The associations between Ln-sRAGE and study end points were attenuated, but remained significant after adjustments for other CVD risk factors and the use of medication: HR 1.90 (1.13–3.21) for fatal and nonfatal CVD events and 2.12 (1.26–3.57) for all-cause mortality (Table 2, models 2 and 3).

Further adjustment for eGFR_{MDRD} attenuated the associations between Ln-sRAGE and incident fatal and nonfatal CVD as well as all-cause mortality by 28 and 14%, respectively (Table 2, model 4). Adjustments for Ln-UAE rate, pulse pressure, and AGEs attenuated these associations to a smaller extent, whereas adjustments for markers of endothelial dysfunction and low-grade inflammation did not (Table 2, models 5–9), despite the adverse associations between Ln-sRAGE and these variables (Table 3).

However, the adverse associations between Ln-sRAGE on the one hand and baseline levels of eGFR_{MDRD}, inflammation, endothelial dysfunction, and AGEs on the other were stronger in individuals with nephropathy than in those with normoalbuminuria (Table 3).

Additional analyses. We investigated the role of eGFR in the associations between Ln-sRAGE and end points further and found 1) that in subjects with nephropathy, higher baseline Ln-sRAGE levels were associated with steeper declines in eGFR_{MDRD} as well as GFR as estimated according to the 3.7-MBq ⁵¹Cr-EDTA method (GFR_{EDTA}) over the course of follow-up and 2) that the decline in (e)GFR attenuated the associations between Ln-sRAGE and study outcomes (see the online appendix).

DISCUSSION

The main findings of this study were twofold. First, in patients with type 1 diabetes, higher levels of plasma sRAGE are associated with incident fatal and nonfatal CVD as well as all-cause mortality, independently of other 'traditional' cardiovascular risk factors. Second, these associations could be partially explained by sRAGE-associated impairment in renal clearance, particularly in patients with nephropathy. Our findings are in agreement with studies that have examined the associations of sRAGE with CVD, but these were limited by cross-sectional designs (7,10–12). This is the first prospective study that has investigated the associations between plasma sRAGE and incident fatal and nonfatal CVD as well as all-cause mortality in a large sample of individuals with type 1 diabetes and has also addressed potential mechanisms that could explain the associations observed.

We hypothesized sRAGE to act as a putative marker of RAGE expression. The adverse associations found between sRAGE and CVD but also between sRAGE and markers of renal and endothelial dysfunction, low-grade inflammation, pulse pressure, and AGEs reported herein, which are in agreement with others (7,10–15), supported this hypothesis. However, sRAGE reflects the total pool of soluble RAGE in plasma and thus consists of different variants. These can result from alternative splicing (16) or from proteolytical cleavage of the membrane-bound RAGE (17). The exact functions of sRAGE in plasma are unknown, but these may differ across different variants. Indeed, several studies have reported inverse and thus "protective" associations between endogenous secretory RAGE (esRAGE) and (surrogate markers of) CVD (11,18–23). We have measured the total pool of plasma sRAGE only and therefore cannot discern whether the different

TABLE 1
Baseline characteristics according to the occurrence of cardiovascular events and death during follow-up

	Fatal or nonfatal CVD event (n = 85)	No CVD event (n = 254)	P	Patients dead at follow-up (n = 82)	Patients alive at follow-up (n = 257)	P
Sex (% M/F)	61/39	60/40	0.901	68/32	58/42	0.156
Age (years)	44.7 ± 9.0	40.3 ± 9.6	<0.001	45.5 ± 9.9	40.1 ± 9.2	<0.001
Duration of diabetes (years)	30.7 ± 8.8	26.8 ± 7.5	<0.001	30.3 ± 10.0	27.0 ± 7.1	0.006
Nephropathy (%)	75	41	<0.001	78	41	<0.001
Retinopathy: no/simplex/proliferative (%)	8/37/55	21/45/34	<0.001	6/37/57	21/45/34	<0.001
A1C (%)	9.5 ± 1.5	8.9 ± 1.4	<0.001	9.6 ± 1.5	8.8 ± 1.4	<0.001
Total cholesterol (mmol/l)	5.70 ± 1.10	4.99 ± 1.12	<0.001	5.80 ± 1.13	4.97 ± 1.10	<0.001
HDL (mmol/l)	1.43 ± 0.42	1.55 ± 0.55	0.033	1.52 ± 0.48	1.52 ± 0.54	0.961
Triglycerides (mmol/l)	1.24 (0.93–1.70)	0.81 (0.63–1.19)	<0.001	1.28 (0.90–1.66)	0.81 (0.64–1.16)	<0.001
Creatinine (μmol/l)	104 (77–150)	80 (71–92)	<0.001	105 (78–147)	79 (72–92)	<0.001
Estimated GFR _{MDRD} (ml/min/1.73 m ²)	65.5 ± 29.1	86.8 ± 21.1	<0.001	65.5 ± 27.8	86.6 ± 21.9	<0.001
GFR _{EDTA} (ml/min/1.73 m ²)*	60.8 ± 31.3*	84.7 ± 30.1*	<0.001	60.6 ± 30.6*	84.6 ± 30.5*	<0.001
UAE rate (mg/24 h)	644 (33–1,940)	17 (7–525)	<0.001	720 (82–2,012)	16 (7–468)	<0.001
Systolic blood pressure (mmHg)	157 ± 24	136 ± 20	<0.001	159 ± 24	136 ± 19	<0.001
Diastolic blood pressure (mmHg)	85 ± 13	79 ± 12	0.001	86 ± 14	79 ± 11	<0.001
Mean arterial pressure (mmHg)	109 ± 15	98 ± 13	<0.001	111 ± 15	98 ± 13	<0.001
Pulse pressure (mmHg)	73 ± 21	57 ± 15	<0.001	73 ± 21	57 ± 15	<0.001
Renin-angiotensin-aldosterone system inhibitors (%)	51	20	<0.001	50	20	<0.001
Other antihypertensive agents (%)	64	28	<0.001	68	27	<0.001
Lipid-lowering agents (%)	0	0	—	0	0	—
Continuation of medication (%)	32	10	<0.001	30	11	<0.001
Smoking: never/former/current (%)	28/19/53	37/17/46	0.513	26/17/57	38/18/44	0.085
Soluble RAGE (ng/ml)	1.02 (0.80–1.41)	0.97 (0.72–1.37)	0.057	1.12 (0.83–1.53)	0.96 (0.72–1.33)	0.001
N ^ε -(carboxymethyl)lysine (μmol/l)	3.60 ± 1.12	3.54 ± 0.84	0.634	3.56 ± 1.32	3.55 ± 0.75	0.967
N ^ε -(carboxyethyl)lysine (μmol/l)	1.02 ± 0.28	0.92 ± 0.19	0.004	1.03 ± 0.30	0.92 ± 0.18	0.003
Pentosidine (pmol/mg)	49.3 (35.7–71.8)	40.8 (34.0–49.0)	0.001	51.8 (34.2–73.1)	41.0 (34.2–48.9)	0.001
C-reactive protein (mg/l)	1.59 (0.64–3.22)	0.96 (0.41–2.09)	0.008	1.42 (0.59–3.26)	1.02 (0.44–2.16)	0.021
Secreted phospholipase A2 (μg/ml)	4.40 (2.80–7.00)	4.00 (2.70–6.23)	0.329	4.05 (2.80–6.55)	4.00 (2.70–6.55)	0.948
Interleukin-6 (pg/ml)	2.18 (1.52–3.45)	1.49 (0.99–2.35)	<0.001	2.42 (1.75–3.89)	1.44 (1.00–2.21)	<0.001
Soluble vascular cell adhesion molecule-1 (sVCAM-1) (ng/ml)	1,045 ± 269	984 ± 346	0.141	1,100 ± 346	967 ± 318	0.001
Soluble intracellular adhesion molecule-1 (sICAM-1) (ng/ml)	771 ± 258	726 ± 272	0.182	805 ± 286	715 ± 260	0.008
Low-grade inflammation score	0.21 ± 0.57	−0.07 ± 0.68	0.001	0.23 ± 0.65	−0.07 ± 0.65	<0.001
Endothelial dysfunction score	0.13 ± 0.70	−0.04 ± 0.81	0.074	0.28 ± 0.85	−0.09 ± 0.75	<0.001
AGEs score	0.26 ± 1.17	−0.09 ± 0.79	0.012	0.27 ± 1.32	−0.08 ± 0.72	0.024

Data are means ± SD, medians (interquartile range), or percent. *Data of GFR_{EDTA} were only available in patients with diabetic nephropathy (n = 165).

variants of sRAGE have specific and potentially opposite associations with study outcomes. In addition, the extent to which levels of (e)sRAGE in plasma reflect the local concentrations and have the same effects as in tissues need to be further clarified.

The associations of sRAGE with CVD and all-cause mortality were attenuated when further adjusted for baseline eGFR, the former association being no longer statistically significant. However, this adjustment explained only 28% of the increased CVD risk associated with higher sRAGE. In addition, independent associations of both sRAGE and creatinine with CVD have been reported in individuals with diabetes (10). Furthermore, we found that sRAGE contributed to an accelerated decline in GFR, as estimated by eGFR_{MDRD} or by GFR_{EDTA} in subjects with diabetic nephropathy. This is in line with a recent study in which sRAGE was inversely associated with changes in eGFR in the course of 1 year of follow-up in a large population of elderly women (24). These findings support

the hypothesis of renal dysfunction being one of the intermediate factors linking sRAGE (as a reflection of RAGE expression) to vascular complications. However, we cannot discard the alternative (or concomitant) possibility, i.e., that baseline sRAGE levels may have been elevated as a consequence of impaired renal clearance. Indeed, our data also support this possibility; specifically, the association between higher baseline eGFR (per 10 ml/min per 1.73 m²) and incident CVD (HR 0.89 [95% CI 0.78–1.00]) was attenuated by ~24% when further adjusted for Ln-sRAGE (to HR 0.92 [95% CI 0.81–1.04]). These observations reflect the interrelationships between sRAGE and eGFR at baseline, the exact directions of which we cannot fully unravel in the present study. Nevertheless, and altogether, these observations support the view that higher sRAGE levels, due to, but also to a great extent independent of, impaired GFR, are positively associated with CVD and all-cause mortality. Further studies with repeated data

TABLE 2

Associations between plasma Ln-sRAGE and incident CVD events and all-cause mortality in the whole study population ($n = 339$)

	Fatal and nonfatal CVD			All-cause mortality		
	HR*	95% CI	<i>P</i>	HR*	95% CI	<i>P</i>
Model: adjustments						
1: age, sex, A1C, case-control status, and duration of diabetes	2.00	1.19–3.36	0.009	2.44	1.46–4.07	0.001
2: model 1 + mean arterial pressure, smoking status, and total cholesterol	1.87	1.13–3.08	0.014	2.29	1.40–3.76	0.001
3a: model 2 + renin-angiotensin-aldosterone system inhibitors agents	1.85	1.12–3.04	0.016	2.33	1.42–3.83	0.001
3b: model 2 + other antihypertensive agents	1.68	1.01–2.81	0.048	1.97	1.18–3.28	0.010
3c: model 2 + continuation of medication use at baseline examination	1.98	1.19–3.28	0.009	2.31	1.40–3.81	0.001
3: model 3a, 3b, and 3c	1.90	1.13–3.21	0.016	2.12	1.26–3.57	0.005
4: model 3 + eGFR _{MDRD}	1.59	0.91–2.77	0.106	1.90	1.09–3.31	0.023
5: model 3 + Ln-UAE	1.74	1.03–2.94	0.040	2.00	1.18–3.39	0.010
6: model 3 + inflammatory score	1.89	1.12–3.19	0.018	2.12	1.26–3.57	0.005
7: model 3 + endothelial dysfunction score	1.92	1.13–3.26	0.016	2.04	1.20–3.44	0.008
8: model 3 + pulse pressure	1.67	0.98–2.87	0.059	1.91	1.12–3.25	0.017
9: model 3 + AGEs score	1.74	1.02–2.98	0.042	1.98	1.16–3.38	0.012

*Hazard ratio for CVD morbidity and mortality or all-cause mortality per each unit increase in Ln-sRAGE levels at baseline.

on both sRAGE and GFR are needed to disentangle the temporal order of these associations.

There are limitations to our study. Samples for analyses of sRAGE and other biomarkers were taken at baseline only, which impedes evaluation of the impact of changes in these variables on study outcomes. In addition, the relatively high interassay CV in the measurement of plasma sRAGE, and the potential misclassification of nonspecific mortality as CVD-related mortality may have introduced nondifferential biases, in which case the estimates reported herein may have been underestimated. However, we cannot discard the possibility that possible underreporting of nonfatal CVD introduced some differen-

tial bias affecting our results. Finally, although our findings did not suggest strong mediating effects of endothelial dysfunction, low-grade inflammation, arterial stiffness, and AGEs in the associations between sRAGE and study outcomes, we cannot fully exclude their potential mediating role due to the use of a selection of markers representing these processes.

In conclusion, higher plasma sRAGE levels, as a reflection of RAGE expression, are associated with incident fatal and nonfatal CVD as well as all-cause mortality in type 1 diabetes, and this may partially be explained by sRAGE-associated renal dysfunction in patients with nephropathy.

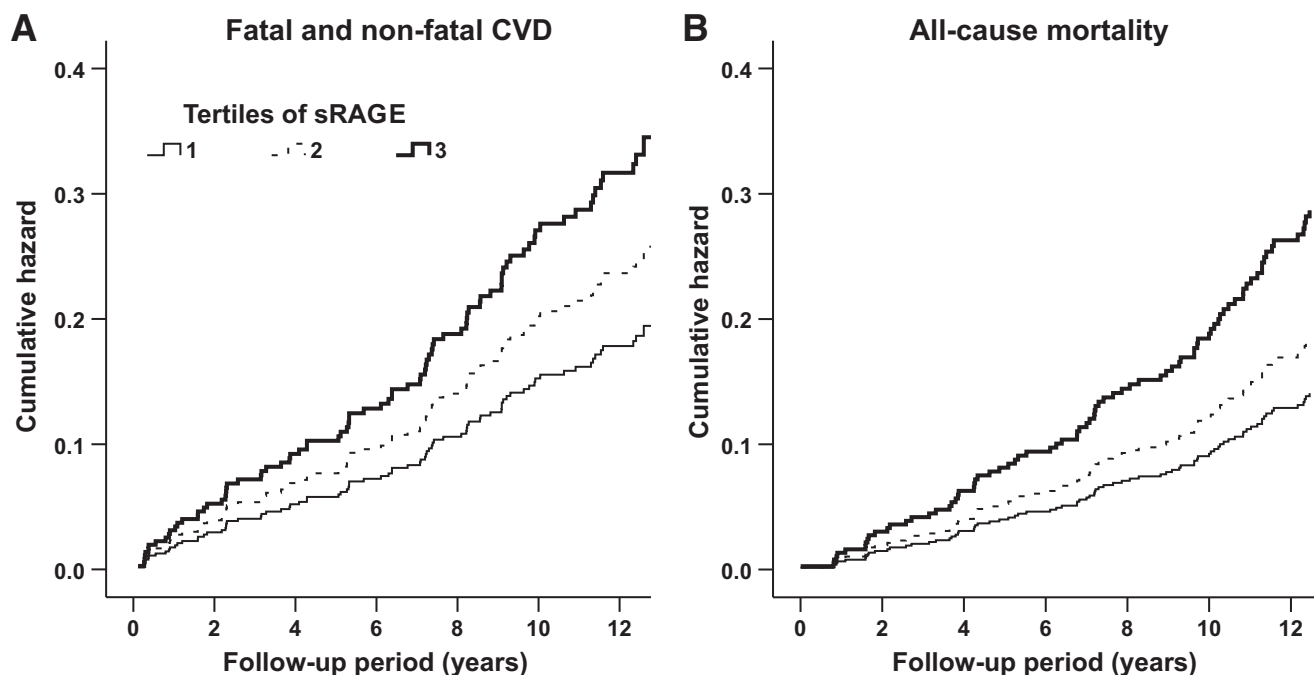


FIG. 1. Cumulative hazard for CVD morbidity and mortality (A) as well as all-cause mortality (B) across tertiles of plasma sRAGE. Data are adjusted for age, sex, case-control status, duration of diabetes, and A1C. Compared with patients in the lowest tertile of sRAGE, those in the middle and highest tertiles had increased risk for fatal and nonfatal CVD (HR 1.33 [95% CI 0.76–2.31] and 1.78 [1.03–3.06], respectively, *P* for trend 0.038) and all-cause mortality (1.31 [0.73–2.38] and 2.04 [1.17–3.55], respectively, *P* for trend = 0.010).

TABLE 3

Associations between plasma Ln-sRAGE and potential mechanisms linking sRAGE to incident CVD and all-cause mortality

Dependent variable	Model	All (n = 339)			Normoalbuminuria (n = 170)			Nephropathy (n = 169)		
		β	95% CI	P	β	95% CI	P	β	95% CI	P
Baseline eGFR _{MDRD} *	1	-0.28	-0.36 to -0.20	<0.001	-0.04	-0.15 to 0.06	0.422	-0.41	-0.53 to -0.30	<0.001
	2	-0.25	-0.33 to -0.17	<0.001	-0.02	-0.09 to 0.06	0.702	-0.41	-0.53 to -0.29	<0.001
Ln-UAE	1	0.07	0.03 to 0.11	0.001						
	2	0.06	0.02 to 0.10	0.002						
Low-grade inflammation score†	1	0.03	-0.04 to 0.10	0.384	-0.05	-0.16 to 0.05	0.316	0.10	0.01 to 0.19	0.039
	2	0.03	-0.04 to 0.10	0.400	-0.08	-0.17 to 0.02	0.130	0.09	0.00 to 0.18	0.056
Endothelial dysfunction score‡	1	0.16	0.08 to 0.24	<0.001	0.07	-0.03 to 0.16	0.168	0.23	0.10 to 0.36	<0.001
	2	0.16	0.08 to 0.24	<0.001	0.07	-0.03 to 0.17	0.159	0.23	0.10 to 0.36	0.001
Pulse pressure	1	0.12	0.03 to 0.21	0.012						
	2	0.08	0.00 to 0.16	0.055						
AGEs score§	1	0.18	0.09 to 0.27	<0.001	0.05	-0.05 to 0.14	0.329	0.26	0.11 to 0.41	0.001
	2	0.17	0.08 to 0.26	<0.001	0.03	-0.06 to 0.12	0.525	0.27	0.12 to 0.41	0.001

β , standardized regression coefficient: indicates change in dependent variable (in SD) per 1-SD increase in Ln-sRAGE. Model 1, adjusted for age, sex, duration of diabetes, A1C, and case-control status when appropriate. Model 2, model 1 plus additional adjustments for smoking status, mean arterial pressure, total cholesterol, use of renin-angiotensin-aldosterone system inhibitors and other antihypertensive treatment, and continuation of medication at baseline examination. Symbols indicate significant interaction between sRAGE and case-control status, and therefore associations are also presented for each group separately (specifically, * $P < 0.001$, † $P = 0.044$, ‡ $P = 0.068$, and § $P = 0.021$).

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