Published in final edited form as:

J Diabetes Complications. 2015 March ; 29(2): 180–185. doi:10.1016/j.jdiacomp.2014.11.008.

sRAGE, Inflammation, and Risk of Atrial Fibrillation: Results from the Atherosclerosis Risk in Communities (ARIC) Study

Mahmoud Al Rifai, MD MPH¹, Andrea L.C. Schneider, MD PHD^{1,2}, Alvaro Alonso, MD PhD³, Nisa Maruthur, MD MHS^{1,2}, Christina M. Parrinello, MPH¹, Brad C. Astor, PhD MPH⁴, Ron C. Hoogeveen, PhD⁵, Elsayed Soliman, MD⁶, Lin Y. Chen, MD⁷, Christie M. Ballantyne, MD⁵, Marc K. Halushka, MD PhD⁸, and Elizabeth Selvin, PhD MPH^{1,2}

¹Department of Epidemiology, Johns Hopkins University Bloomberg School of Public Health and the Welch Center for Prevention, Epidemiology and Clinical Research

²Department of Medicine, Johns Hopkins University School of Medicine

³Division of Epidemiology and Community Health, School of Public Health, University of Minnesota

⁴Departments of Medicine and Population Health Sciences, University of Wisconsin School of Medicine and Public Health

⁵Department of Medicine, Section of Cardiovascular Research, Baylor College of Medicine and Houston Methodist DeBakey Heart and Vascular Center

⁶Epidemiological Cardiology Research Center (EPICARE), Department of Epidemiology and Prevention, and Department of Internal Medicine-Cardiology, Wake Forest School of Medicine

⁷Cardiovascular Division, Department of Medicine, University of Minnesota Medical School

⁸Department of Pathology, Johns Hopkins University School of Medicine

Abstract

Objective—Advanced glycation end products (AGEs) may cause inflammation by binding to their cellular receptors (RAGE). Soluble RAGE (sRAGE) acts as a decoy receptor for AGEs and may prevent inflammation. Chronic low-grade inflammation is a risk factor for cardiovascular disease, including atrial fibrillation (AF).

Methods—We studied 1,068 participants in a subsample of the Atherosclerosis Risk in Communities (ARIC) Study who had baseline measurements of sRAGE (mean age 56, 60% female, 21% black). Inflammation was assessed using measurements of high sensitivity C-reactive

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Corresponding Author: Elizabeth Selvin PhD MPH, 2024 East Monument Street, Suite 2-600, Baltimore, Maryland 21205, [Phone: 410-614-3752, Fax: 410-955-0476, eselvin@jhu.edu](mailto:eselvin@jhu.edu).

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DISCLOSURE

No potential conflicts of interest relevant to this article were reported by any authors.

protein (hsCRP), fibrinogen, gamma-glutamyl transferase (GGT) and white blood cell (WBC) count. AF events were identified using ECG data, hospitalization discharge codes, and linkage to the National Death Index.

Results—Compared to the highest quartile (>1272.4 pg/mL), the lowest quartile of sRAGE (<714 pg/mL) was associated with higher baseline levels of inflammation (hsCRP ≥ 3 mg/L: OR=2.21 [95% CI 1.41–3.49], fibrinogen ≥ 400 mg/dL: OR=4.31 [95% CI 1.50–12.41], GGT ≥ 36 U/L in women and ≥ 61 U/L in men: OR= 5.22 [95% CI 2.66–10.22], WBC $>6.2 \times 10^9$ /L: OR=2.38 [95% CI 1.52–3.72]). sRAGE was not prospectively associated with 6-year change in inflammatory markers (hsCRP or GGT). There was no significant association of sRAGE and risk of AF (HR 1.49 [95% CI: 0.80–2.78] for the 1st vs 4th quartile of sRAGE).

Conclusions—sRAGE was strongly inversely associated with markers of inflammation at baseline, but not prospectively. sRAGE was not significantly associated with incident AF. This supports a role for sRAGE in attenuating current inflammation, but it remains unclear whether sRAGE plays a role in the development of AF.

INTRODUCTION

The accelerated atherosclerosis and subsequent vascular damage that occur in diabetes may be due in part to nonenzymatic glycation of proteins and lipids and deposition of advanced glycation end products (AGEs)^{1–3}. AGEs may contribute to vascular disease in non-diabetic individuals as well⁴. The interaction of AGEs with their cell-bound receptors (RAGE) results in oxidative damage and the production of matrix metalloproteinases (MMPs), which cleave cell-bound RAGEs and produce soluble RAGE (sRAGE)⁵. sRAGE competes with RAGE by acting as a decoy receptor and may therefore prevent inflammation^{5,6}.

Low levels of sRAGE have been shown to be independently associated with incident coronary heart disease, diabetes, metabolic syndrome and death^{7,8} while high levels are associated with incident chronic kidney disease⁹. In cross-sectional studies low sRAGE levels are associated with atrial fibrillation (AF)^{10–12}, atherosclerosis, aortic stiffness, diabetes, obesity, hypertension and high sensitivity C-reactive protein levels^{5,7,13–15}.

We aimed to characterize the association between sRAGE and incident AF in a community-based setting. Because inflammation plays a role in the development of cardiovascular disease (CVD)^{16,17}, including AF¹⁸, we sought to determine the cross-sectional association of sRAGE with markers of inflammation (high sensitivity C-reactive protein (hsCRP), gamma-glutamyl transferase (GGT), fibrinogen, and white blood cell count (WBC))^{19–23}. Since prior studies have established that the AGE-RAGE-sRAGE axis is associated with development of CVD^{7,24,25}, we also examined the association of sRAGE with temporal changes in inflammatory markers to further address the role of sRAGE as a marker of current inflammation and also its potential role as a marker of future risk of AF.

We hypothesized that 1) lower levels of sRAGE would be associated with increased inflammation both at baseline and prospectively; 2) lower sRAGE levels would be associated with an increased risk of AF; and 3) that the association of sRAGE with AF would be partially mediated by inflammation.

PARTICIPANTS AND METHODS

Study Design

The Atherosclerosis Risk in Communities (ARIC) Study is a community-based prospective cohort of 15,792 middle-aged adults from four U.S. communities (suburbs of Minneapolis, Minnesota; Washington County, Maryland; Forsyth County, North Carolina; Jackson, Mississippi). The first examination of participants (visit 1) took place during 1987–1989 with 3 subsequent follow-up visits occurring approximately 3 years apart, and a fifth visit occurring in 2011–2013. Visit 2 (1990–1992) is the baseline for the present study. A random sample of 1,218 participants with normal kidney function (estimated glomerular filtration rate >60 mL/min/1.73 m²) was selected from the 14,348 participants who attended visit 2. Of the 1,218 participants included in the random sample, 64 were excluded for prevalent CHD, 2 were excluded for prevalent AF, and 84 were excluded for missing covariates, leaving 1,068 participants included in the present analysis.

sRAGE Measurement

sRAGE was measured in 2010 by Enzyme-Linked Immunosorbent Assay (ELISA) (R&D Systems, Minneapolis, Minnesota) using samples that had been stored at -70°C since collection in visit 2. The intra-assay coefficient of variation (CV) was 2.8% and the inter-assay CV was 9.6%⁷.

Measurement of Inflammatory Markers

hsCRP was measured in 2012–2013 in serum stored at -70°C since collection in visit 2 using the Roche Modular P800 autoanalyzer (Roche Diagnostics, Indianapolis, Indiana)⁷. The coefficient of variation (CV) was 4.5%. hsCRP from visit 4 (1996–1998) stored plasma was measured using Siemens Dade Behring BN II²⁶. The reliability coefficient for the hsCRP assay was 0.99 based on 421-blinded replicates.

Fibrinogen was measured in 1987–1989 (visit 1) in stored plasma by the thrombin-time titration method with reagents obtained from General Diagnostics Organon Technica Company (West Chester, Pennsylvania)²⁷. The intraclass correlation coefficient (ICC) obtained from repeated testing on a sample of subjects over several weeks was 0.72.

GGT was measured in 2012–2013 in serum stored at -70°C since collection in visit 2 using the Roche Modular P800 autoanalyzer (Roche Diagnostics, Indianapolis, Indiana). The CV was 5.1% at 39 U/L and 2.9% at 171 U/L. GGT at visit 4 was measured in stored plasma using Olympus AU400²⁸. Intraassay coefficient of variation was 9.3% for GGT.

WBC count was measured in visit 2 using standard methods.

Definition of Atrial Fibrillation

AF events were ascertained using a standard definition in the ARIC Study, which includes ECGs performed at study visits, hospitalization discharge codes for AF or atrial flutter (ICD-9 codes 427.31 or 427.32) not accompanied by a code for cardiac surgery and AF or

atrial flutter listed as cause of death²⁹. Participants were administratively censored for events on December 31, 2010.

Statistical analysis

Baseline characteristics of the population were calculated overall and by quartile of baseline sRAGE levels. Logistic regression models were used to evaluate the association of elevated (versus normal/moderate) inflammatory markers by quartile of sRAGE using the highest quartile of sRAGE as the reference. P-value for trend across the quartiles was calculated using the median of each quartile. We defined elevated hsCRP as ≥ 3 mg/L⁷, elevated fibrinogen as ≥ 400 mg/dL³⁰ and elevated GGT as ≥ 36 U/L in women and ≥ 61 U/L in men³¹. As there were few people with elevated WBC ($\text{WBC} > 11 \times 10^9/\text{L}$), we divided WBC levels into tertiles and defined elevated WBC as the highest tertile ($> 6.2 \times 10^9/\text{L}$). Since hsCRP and GGT were also measured in samples obtained 6 years later at visit 4 (1996–1998), we evaluated the association of baseline sRAGE with 6-year change (modeled continuously per 1-SD log-transformed sRAGE) and incident elevations in hsCRP and GGT at visit 4 among persons with normal/moderate hsCRP or GGT at visit 2, respectively. Note: fibrinogen and WBC were not available at visit 4.

Cox proportional hazards models were used to investigate the association of baseline sRAGE with incident AF. In this analysis, we modeled sRAGE continuously (per 1 standard deviation of log-transformed sRAGE) and also categorized into quartiles with the highest quartile serving as the reference group. We also modeled the continuous association of sRAGE with incident AF using a restricted cubic spline with 4 knots placed at the 5th, 35th, 65th, and 95th percentiles.

Model 1 was adjusted for age (years), sex (male, female), and race-center (Minnesota whites; Maryland whites; North Carolina whites; North Carolina blacks; Mississippi blacks. People who were neither white nor African American and the few African Americans in the Minnesota and Washington County cohorts were excluded). Model 2 was adjusted for all variables in Model 1 plus smoking status (current, former, never), alcohol use (current, former, never), blood pressure medication use (yes, no), systolic blood pressure (mmHg), diastolic blood pressure (mmHg), total cholesterol (mg/dL), diabetes status (non-fasting glucose ≥ 200 mg/dL, fasting glucose ≥ 126 mg/dL, self-reported physician diagnosis of diabetes, or diabetes medication use), and body mass index (kg/m^2).

As sensitivity analyses, we studied the association between sRAGE and risk of AF stratified by race and formally tested for multiplicative interaction between sRAGE and race. We repeated the same analysis for sRAGE and AF risk stratified by baseline diabetes status. We also categorized visits 2 and 4 hsCRP into quartiles and studied the association with risk of AF using the first quartile as the reference. Furthermore we calculated change in hsCRP between visits 2 and 4 ($\text{hsCRP} = \text{visit 4 hsCRP} - \text{visit 2 hsCRP}$) and hazard ratios of incident AF per 1 unit of log-transformed hsCRP.

All reported p-values were two-sided and $p < 0.05$ was considered statistically significant. All analyses were performed using Stata/IC version 13.1 (StataCorp, College Station, Texas).

RESULTS

Participants in the lowest quartile of sRAGE at baseline (<714 pg/mL) were more likely to be male, African American, obese, and to have diabetes or hypertension. There were no differences in age across quartiles of sRAGE (Table 1). hsCRP, WBC and GGT were all significantly and inversely associated with sRAGE.

sRAGE and inflammation

Even after adjustment, high levels of each of the four inflammatory markers were strongly associated with low levels of sRAGE (Table 2). Compared to the highest quartile (>1272.4 pg/mL), the lowest quartile of sRAGE (<714 pg/mL) was consistently associated with higher baseline levels of inflammation (hsCRP 3 mg/L: OR=2.21 [95%CI 1.41–3.49], fibrinogen 400 mg/dL: OR=4.31 [95%CI 1.50–12.41], GGT 36 U/L in women and 61 U/L in men: OR= 5.22 [95%CI 2.66–10.22]) and WBC >6.2×10⁹/L: OR=2.38 [95%CI 1.52–3.72]). In contrast, baseline levels of sRAGE were not associated with 6-year change in hsCRP or GTT or incident elevated hsCRP and GGT levels at the follow-up visit approximately 6 years later (Table 3).

sRAGE and atrial fibrillation

Over a median follow-up time of 19 years, there were 106 incident AF cases. Low sRAGE was associated with higher risk of AF in unadjusted analyses (hazard ratio [HR]=1.82 [95% CI: 1.05–3.18]), in quartile 1 versus quartile 4. After adjustment for age, sex and race-center, the risk of AF was (HR=1.77 [95% CI: 0.97–3.22]). However, after comprehensive adjustment for risk factors, the risk for AF remained increased but was no longer significant (HR=1.49 [0.80–2.78] (Table 4). We observed similar results in the continuous analysis (per 1 SD of log-transformed sRAGE) (Table 4) and in the restricted cubic spline model, which had wide confidence intervals reflecting the small sample size and corresponding lack of precision (Figure 1). Absence of significant findings made analysis of mediation by inflammation unwarranted.

Sensitivity analysis

The risk of AF in each quartile of sRAGE was lower in blacks compared to whites, but there was no interaction between sRAGE and race (p=0.70) (Supplementary Table 1). The risk of AF in each quartile of sRAGE was higher in diabetics, however there was no interaction between sRAGE and diabetes (p=0.15). There was no association between visits 2 and 4 hsCRP or hsCRP and risk of AF (Supplementary Tables 2 and 3).

DISCUSSION

In this community-based population of middle-aged adults, we observed strong inverse cross-sectional associations between sRAGE and elevated levels of inflammatory markers (hsCRP, fibrinogen, GGT and WBC). In contrast, low sRAGE at baseline was not associated with change in hsCRP and GGT or future elevation at six years. Low sRAGE was associated with incident AF before but not after adjustment for other cardiovascular risk factors in this study population.

Our results confirm previous findings from a number of studies that have shown that sRAGE is inversely correlated with markers of inflammation. A study of persons with type 2 diabetes showed that sRAGE levels were lower in persons with diabetes compared to controls and that sRAGE was inversely associated with hsCRP levels and S100A12, a proinflammatory marker related to RAGE signaling³². It has been postulated that the AGE-RAGE axis may be relevant for cardiovascular risk even in the absence of diabetes^{7,33} and that inflammation is strongly linked to any role of sRAGE as a risk factor or bystander in the development of vascular disease^{16,17}. Given that oxidative damage caused by inflammation accumulates over time¹⁶ we decided to examine a possible prospective association of sRAGE and change in inflammatory status. The strong cross-sectional association of sRAGE and markers of inflammation but lack of prospective association suggests that sRAGE might only decrease current inflammation. If sRAGE is produced in response to inflammation caused by AGEs then it might not be reflective of chronic inflammatory status. These results help inform the time frame within which sRAGE exerts its supposed protective effects.

We did observe significant inverse associations of sRAGE with incident AF in crude and minimally adjusted analyses. However, after adjustment for cardiovascular risk factors this association was attenuated and no longer statistically significant. The absent association between hsCRP and AF in our study might also explain the non-significant findings of sRAGE and AF. Furthermore, coronary heart disease results from atherosclerotic damage caused by inflammation within the vessel layers^{17,35}. Our results suggest that sRAGE may be a less relevant marker of future risk of non-atherosclerotic cardiovascular outcomes, despite the role of inflammation as an independent risk factor for the initiation and perpetuation of atrial fibrillation¹⁸.

The data on sRAGE and AF has been cross-sectional in nature so far with varying results. A study by Raposeiras et al showed higher sRAGE levels in a small sample of cardiac outpatients who had AF¹⁰. Yan et al showed that endogenous sRAGE (esRAGE) levels were lower while hsCRP levels were higher in AF patients who underwent coronary angiography and that this association was stronger in diabetics¹¹. On the other hand, Zhao et al showed that while esRAGE levels were lower in AF patients, cleaved RAGE (cRAGE) was higher suggesting that these 2 markers may be involved in the pathogenesis of AF¹².

sRAGE levels and risk of atrial fibrillation are lower in blacks compared to whites^{7,29} however there was no effect measure modification by race in the association between sRAGE and AF in our study. Similarly sRAGE levels are lower in diabetics⁷ who have a higher risk of AF compared to non-diabetics³⁶, but there was no effect measure modification by diabetes status.

AGEs bind to their receptors (RAGEs), activate inflammatory pathways and lead to the production of matrix metalloproteinases (MMPs). C-truncated RAGE is a cellular form of RAGE, which is cleaved by MMPs to form sRAGE. sRAGE traps RAGE ligands (AGEs, HMGB1, S100b) or competes with them for RAGE binding and can therefore protect against RAGE binding and decrease inflammation^{5,6}. Low levels of sRAGE could mean that more AGEs are available to bind their cellular receptors, thereby increasing inflammation,

which is reflected by higher levels of inflammatory markers. There is no conclusive evidence so far on the feedback loops and signaling pathways involved in sRAGE^{37,38} which would help clarify the role of sRAGE with respect to chronic inflammation.

Our study has certain limitations that should be considered in the interpretation of these results. Small sample size was an important limitation of our study, which likely affected power to detect a moderate association between baseline sRAGE and incident AF. An additional limitation was that we only had a single measurement of sRAGE at baseline. sRAGE varies over time due to both physiologic and external factors like exercise and medications^{39–41}, however its long-term reliability is similar to other common biomarkers including total cholesterol and WBC count, which makes it a useful measure of long-term risk in epidemiologic studies⁴². Furthermore we only had total sRAGE measurements and thus could not study the individual components (cleaved RAGE; cRAGE and endogenous secretory RAGE; esRAGE) and their distribution in each sRAGE quartile. hsCRP and GGT were measured using different methods in visit 4 than visit 2 which could have affected analysis of longitudinal changes in these markers. We used fibrinogen at visit 1 which might not be reflective of inflammation 3 years later at visit 2. In any observational study, there remains the possibility of residual confounding. Ascertainment of incident AF events relied on ICD-9 codes and study visit ECGs, possibly resulting in under-ascertainment.

However our study also has a number of important strengths. These analyses were performed in a biracial community-based population with long-term follow-up and repeated measurements of hsCRP and GGT at 6 years after baseline. sRAGE levels were measured using an ELISA assay with inter- and intra-assay coefficients of variation <10% indicating good reliability⁴². Rigorous measurement of cardiovascular risk factors according to well-established protocols is another strength of our study.

In summary, we showed that sRAGE is strongly inversely associated with markers of inflammation at baseline, but not associated with 6-year change or future elevations in inflammatory marker. sRAGE was not associated with incident AF after adjustment for cardiovascular risk factors, possibly because of limited power in this study sample. Our results support an inverse relationship of sRAGE with current inflammation but it is not possible to infer a causal role in this observational setting. The role of sRAGE and the relevance of the AGE-RAGE axis for future cardiovascular risk deserve further examination

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

This research was supported by National Institutes of Health/National Institute of Diabetes and Digestive and Kidney Diseases Grant R01 DK076770 and a grant from the American Heart Association to Dr. Selvin. Dr. Schneider and C.M. Parrinello were supported by NIH/NHLBI T32 HL007024. Dr. Alonso was supported by grant 09SDG2280087 from the American Heart Association. The ARIC Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C).

The authors thank the staff and participants of the ARIC Study for their important contributions

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Figure 1.

Adjusted[†] hazard ratio (95% CI) for the continuous association of baseline sRAGE with incident atrial fibrillation during a median of 19 years of follow-up

* [†]Model adjusted for age, gender, race-center. sRAGE was modeled as a restricted cubic spline centered at 75th percentile. Knots were placed at the 5th, 35th, 65th, and 95th percentiles of sRAGE. The plot was truncated at 1st and 99th percentiles.

Table 1

Baseline characteristics overall and by quartile of sRAGE, ARIC Study, 1990–1992.

	Overall (N=1068)	Quartile 1 (sRAGE 119.4–713.8 pg/mL (n=265)	Quartile 2 (sRAGE 713.8–968.1 pg/mL (n=269)	Quartile 3 (sRAGE 968.1–1272.4 pg/mL (n=267)	Quartile 4 (sRAGE 1272.4–4650.4 pg/mL (n=267)
Age (years), mean (SD)	56.3 (5.6)	56.3 (5.7)	56.2 (5.4)	56.5 (5.9)	56.3 (5.5)
Gender, %					
Females, %	60.1	52.4	57.6	59.2	71.2
Males	39.9	47.6	42.4	40.8	28.8
Blacks, %	21.4	46.0	20.8	12.7	6.0
Whites, %	78.6	54.0	79.2	87.3	94.0
Current smoker, %	17.8	15.5	20.5	18.7	16.5
Current alcohol consumption, %	58.5	54.7	58.7	62.6	58.1
Hypertension, [†] %	31.3	43.4	29.0	28.1	24.7
Diabetes, [‡] %	10.7	16.6	11.2	9.4	5.6
Total cholesterol 200 mg/dL, %	56.7	57.7	65.1	54.7	49.1
BMI 30 kg/m ²	28.7	44.9	29.4	28.8	11.6
hsCRP (mg/L), median (IQR)	2.0 (1.0–4.2)	3.2 (1.6–6.1)	2.1 (1.0–4.6)	1.8 (0.9–3.5)	1.6 (0.8–3.2)
hsCRP 3mg/L, %	36.1	52.1	37.2	28.5	26.6
Fibrinogen (mg/dL), median (IQR)	290 (258–329)	295 (261–343)	293 (260–330)	282 (254–329)	285 (256–318)
Fibrinogen 400 mg/dL, %	5.1	9.8	5.2	3.4	1.9
GGT (U/L), median (IQR)	20 (14–31)	27 (19–40)	19 (14–32)	19 (14–28)	16 (11–23)
GGT 36 U/L in women and 61 U/L in men, %	12.7	23.0	12.6	9.7	5.6
WBC ($\times 10^9$ /L), median (IQR)	5.5 (4.7–6.6)	5.7 (4.8–6.9)	5.6 (4.6–6.6)	5.6 (4.7–6.6)	5.2 (4.5–6.1)
WBC 6.2×10^9 /L, %	33.9	38.9	36.1	36.7	24.0

[†] Hypertension defined as diastolic blood pressure 90 mmHg, systolic blood pressure 140 mmHg, or use of blood pressure-lowering medications

[‡] Diabetes defined as non-fasting glucose 200 mg/dL, fasting glucose 126 mg/dL, self-reported diabetes diagnosis, medication use

Table 2

Adjusted[†] odds ratios (95% confidence intervals) for the cross-sectional association of sRAGE with elevated inflammatory markers at baseline

	hsCRP 3mg/L	Fibrinogen 400 mg/dL	GGT 36 U/L in women and 61 U/L in men	WBC 6.2×10 ⁹ /L
Quartile 1 (sRAGE 119.4–713.7 pg/mL)	2.21 (1.41–3.49)	4.31 (1.50–12.41)	5.22 (2.66–10.22)	2.38 (1.52–3.72)
Quartile 2 (sRAGE 713.8–968.0 pg/mL)	1.35 (0.89–2.05)	2.28 (0.78–6.63)	2.30 (1.18–4.46)	1.63 (1.08–2.47)
Quartile 3 (sRAGE 968.1–1272.3 pg/mL)	0.87 (0.57–1.33)	1.59 (0.51–4.90)	1.74 (0.88–3.46)	1.70 (1.13–2.55)
Quartile 4 (sRAGE 1272.4–4650.4 pg/mL)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
P-value for trend [*]	0.001	0.003	<0.001	<0.001

[†] Adjusted for age (years), gender (male, female), race-center (Minnesota whites, North Carolina Whites, Maryland whites, North Carolina blacks, Mississippi blacks), smoking status (current, former, never), alcohol use (current, former, never), blood pressure medication use (yes, no), systolic blood pressure (mmHg), diastolic blood pressure (mmHg), total cholesterol (mg/dL), diabetes status (yes; no), BMI (kg/m²).

^{*} P-value for trend represents linear trend across the medians of sRAGE quartiles.

Bolded data represents p<0.05.

Table 3

Adjusted[†] odds ratios (95% confidence intervals) of baseline sRAGE with 6 year change (continuous) and incident elevations[‡] in hsCRP (≥ 3 mg/L) or GGT (≥ 36 U/L in women and ≥ 61 U/L in men) at the 6 year follow-up visit

	hsCRP β (95%CI)	GGT β (95%CI)
Log-transformed sRAGE sRAGE (per 1 SD)	0.41 (−0.72–0.88)	0.70 (−1.77–3.17)
	hsCRP OR (95%CI)	GGT OR (95%CI)
Quartile 1 (sRAGE 119.4–713.7 pg/mL)	1.02 (0.57–1.85)	1.19 (0.46–3.08)
Quartile 2 (sRAGE 713.8–968.0 pg/mL)	1.28 (0.78–2.11)	0.93 (0.38–2.26)
Quartile 3 (sRAGE 968.1–1272.3 pg/mL)	0.96 (0.59–1.56)	1.31 (0.58–2.96)
Quartile 4 (sRAGE 1272.4–4650.4 pg/mL)	1 (reference)	1 (reference)
P-value for trend [*]	0.61	0.89

[†] Adjusted for age (years), gender (male, female), race-center (Minnesota whites, North Carolina Whites, Maryland whites, North Carolina blacks, Mississippi blacks), smoking status (current, former, never), alcohol use (current, former, never), blood pressure medication use (yes, no), systolic blood pressure (mmHg), diastolic blood pressure (mmHg), total cholesterol (mg/dL), diabetes status (yes; no), BMI (kg/m²)

[‡] Incident elevations were defined as hsCRP ≥ 3 mg/L or GGT ≥ 36 U/L in women and ≥ 61 U/L in men at visit 4 among those with hsCRP < 3 mg/L or GGT < 36 U/L in women and < 61 U/L in men at visit 2"

^{*} P-value for trend represents linear trend across the medians of sRAGE quartiles.

Table 4

Adjusted[†] hazard ratios (95% confidence intervals) for incident atrial fibrillation by quartile of baseline sRAGE.

	Atrial fibrillation events (n events / n total)	Unadjusted	Model 1	Model 2
Quartile 1 (sRAGE 119.4–713.7 pg/mL)	33 /265	1.82 (1.05–3.18)	1.77 (0.97–3.22)	1.49 (0.80–2.78)
Quartile 2 (sRAGE 713.8–968.0 pg/mL)	20 / 269	1.01 (0.55–1.88)	1.00 (0.53–1.89)	0.84 (0.44–1.60)
Quartile 3 (sRAGE 968.1–1272.3 pg/mL)	33 / 267	1.69 (0.97–2.95)	1.66 (0.95–2.90)	1.45 (0.81–2.57)
Quartile 4 (sRAGE 1272.4–4650.4 pg/mL)	20 / 267	1 (reference)	1 (reference)	1 (reference)
Log-transformed sRAGE sRAGE, pg/mL (per 1 SD)	106/ 1068	0.81 (0.67–0.98)	0.81 (0.65–1.00)	0.87 (0.69–1.10)

[†]Model 1: Adjusted for age (years), gender (male, female), race-center (Minnesota whites, North Carolina Whites, Maryland whites, North Carolina blacks, Mississippi blacks); Model 2: adjusted for all variables in Model 1 plus smoking status (current, former, never), alcohol use (current, former, never), blood pressure medication use (yes, no), systolic blood pressure (mmHg), diastolic blood pressure (mmHg), total cholesterol (mg/dL), diabetes status (yes; no), BMI (kg/m²)

Bolded data represents p<0.05.