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Racial Differences in Circulating Levels of the Soluble Receptor for Advanced Glycation Endproducts in Middle-Aged and Older Adults

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Abstract

Background—Low levels of the soluble receptor for advanced glycation endproducts (sRAGE) have been implicated in a number of chronic diseases. Previous studies indicate that sRAGE levels are ~30% lower in Blacks compared to Whites. However, the reasons for these differences are unclear.

Purpose—We aimed to identify predictors of circulating sRAGE biomarkers among Black and White adults at high cardiac risk.

Methods—Serum levels of total sRAGE, endogenous secretory RAGE (esRAGE), carboxymethyl-lysine (CML, a major RAGE ligand), and their ratios were measured in 99 Blacks and 454 Whites.

Results—Blacks had a more adverse cardiovascular risk profile, as well as lower median levels of total sRAGE (972 vs. 1564 pg/ml) and esRAGE (474 vs. 710 pg/ml) compared to Whites ($p<0.0001$). In addition, the proportion of esRAGE was higher in Blacks (47% vs. 44%, $p=0.02$), as were the CML/total sRAGE (0.89 vs. 0.56 ng/pg) and CML/esRAGE (1.72 vs. 1.20 ng/pg)

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

TEB and WGH designed the research study; TEB and WGH were responsible for data collection; TEB and XL were responsible for statistical analysis and data interpretation; TEB drafted the manuscript; and all authors revised it critically for important intellectual content and gave final approval of the version to be submitted.

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ratios ($p<0.0001$). Racial differences persisted after adjustment for key covariates including age, gender, tobacco use, comorbidities, BMI, blood pressure, glucose, insulin, triglycerides, C-reactive protein, and renal function ($p<0.05$). Race alone accounted for nearly half of the variability in total sRAGE levels (10.6%; model explained 23.9%). In stratified analyses, gender and heart rate were independently associated with total sRAGE and esRAGE in Whites, while CML and C-reactive protein were associated with total sRAGE in Blacks.

Conclusions—We identified several independent predictors of sRAGE biomarkers. Notably, Black race was associated with an adverse AGE/RAGE profile, including lower sRAGE and higher CML/sRAGE ratios.

Keywords

race; soluble RAGE; endogenous secretory RAGE; advanced glycation endproducts; carboxymethyl-lysine

1. Introduction

The receptor for advanced glycation endproducts (RAGE) is a multi-ligand cell-surface protein that belongs to the immunoglobulin superfamily and binds a number of pro-inflammatory ligands including advanced glycation endproducts (AGEs), members of the S100/calgranulin family, high mobility group box 1, and beta-amyloid peptide [1]. RAGE is expressed in a wide variety of cells and tissues where it modulates immune responses through multiple intracellular signaling pathways. Since its discovery nearly 25 years ago, RAGE has been implicated in the pathophysiology of cardiovascular, metabolic, neurological, and inflammatory diseases [2].

Soluble forms of RAGE (sRAGE) are generated by proteolytic cleavage of the membrane-bound receptor or by alternative splicing [3, 4]. sRAGE is thought to play a protective role by acting as a decoy receptor that attenuates RAGE-ligand interactions at the cell surface [5, 6]. Although there are numerous studies investigating sRAGE levels in both healthy and diseased populations, most papers report on total sRAGE or the endogenous secretory RAGE (esRAGE, a splicing variant that lacks the transmembrane domain), but not both. Emerging data indicate that circulating isoforms generated by different mechanisms may reflect different aspects and stages of disease [7–9]. However, more research is needed to identify potential factors that regulate the release of circulating sRAGE isoforms.

Race is likely an important factor as several studies have shown that total sRAGE levels are significantly lower in Blacks compared to Whites [10–13]. One study reported similar findings for esRAGE, albeit in a small number ($n=25$) of Afro-Caribbean patients with type 2 diabetes [10]. A recent genetics analysis showed that variants in the RAGE gene (*AGER*) do not account for racial differences in total sRAGE levels, suggesting that other biological or environmental factors are likely important [14]. Given that low sRAGE levels have been associated with type 2 diabetes, cardiovascular disease, and Alzheimer's disease [15, 16] (i.e., diseases that disproportionately affect Black individuals), it is possible that the observed racial differences in sRAGE may contribute to racial disparities in chronic disease risk. However, it is currently unclear whether traditional cardiovascular risk factors

contribute to these differences. Therefore, the main objectives of this study were to determine whether race is an independent predictor of total sRAGE and esRAGE levels in Black and White adults and whether the associations between cardiovascular risk factors and sRAGE levels differ by race. Importantly, sRAGE levels were assessed in conjunction with carboxymethyl-lysine (CML), a major AGE that binds to RAGE, and glomerular filtration rate (GFR), a measure of renal function, which collectively provide a more complete picture of the AGE-RAGE axis and are critical to the interpretation of circulating sRAGE levels [15, 16].

2. Methods

2.1. Study Population

This study included middle-age and older adults who were recruited from Forsyth County, NC and surrounding areas to participate in a prospective, observational study designed to identify cardiovascular abnormalities that predict incident congestive heart failure [17, 18]. Eligible participants were 55 to 85 years of age and at high risk for their first episode of congestive heart failure based on the presence of at least one of the following risk factors: hypertension, type 2 diabetes, or coronary artery disease. Hypertension was defined according to the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High

Blood Pressure as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 85 mmHg, or concurrent use of anti-hypertensive medications [19]. Type 2 diabetes was defined as a random plasma glucose ≥ 200 mg/dL, fasting glucose ≥ 126 mg/dL, or concurrent use of anti-glycemic medication based on criteria established by the American Diabetes Association [20]. Coronary artery disease was defined in accordance with American College of Cardiology and American Heart Association guidelines [21]. Major exclusion criteria included left main or 3-vessel coronary artery disease; moderate to severe aortic stenosis or other significant valvular disease; and myocardial infarction, acute coronary syndrome, or angina within the preceding year. All participants provided written informed consent prior to participation. This study was approved by the Institutional Review Board at Wake Forest School of Medicine.

2.2. Clinical and Laboratory Assessments

The present analysis includes data collected at the baseline clinic visit. Age, gender, race, and tobacco use were assessed by self-report using interviewer-administered questionnaires. Participants were asked if they ever smoked cigarettes (including start/stop date) and if they were currently smoking. Blood pressure (BP, using a conventional mercury sphygmomanometer) and heart rate were measured by a trained nurse between 8:00 am and 10:00 am, with the participant in a seated position for a period of 10 minutes. Systolic BP and diastolic BP were recorded as the mean of two measurements. Body mass index (BMI) was calculated from measured height and weight using a standard wall-mounted stadiometer and calibrated scale and standard cut-points were used to define normal weight ($\text{BMI} < 25 \text{ kg/m}^2$), overweight ($\text{BMI} = 25\text{--}29 \text{ kg/m}^2$), and obese ($\text{BMI} > 30 \text{ kg/m}^2$). Blood samples were collected by venipuncture in the morning following an overnight fast. Serum triglycerides,

total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, creatinine, and C-reactive protein were measured using standardized hospital laboratory methods. GFR was estimated using the CKD-EPI equation [22], and decreased kidney function was defined as a $\text{GFR} < 60 \text{ ml/min/1.73 m}^2$. Fasting glucose was measured with the glucose hexokinase method (Bayer Diagnostics, Tarrytown, NY) and fasting insulin was determined by a chemiluminescent immunoassay on an IMMULITE analyzer (Diagnostics Products Corporation, Los Angeles, CA). An estimate of insulin resistance was calculated from fasting glucose and insulin using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) equation [23].

sRAGE biomarkers were measured in thawed serum samples that were previously frozen and stored at -80°C . Total sRAGE was measured by an enzyme-linked immunosorbent assay (ELISA) that utilizes a monoclonal antibody raised against amino acids in the N-terminal extracellular domain of RAGE (R&D Systems, Minneapolis, MN). This assay quantifies all circulating sRAGE including secreted isoforms (i.e., esRAGE) and those generated by proteolytic cleavage. esRAGE was quantified separately using an ELISA that utilizes a polyclonal antibody raised against a unique 16-amino-acid peptide in the C-terminal region (B-Bridge International, Mountain View, CA). CML was measured using a competitive ELISA that uses the CML-specific monoclonal antibody 4G9 (Microcoat, Penzberg, Germany) [24]. In our laboratory, the intra-assay and inter-assay coefficients of variation are $<10\%$ for total sRAGE, esRAGE, and CML.

2.3. Statistical Analyses

All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC). Variables with a right skewed distribution (total sRAGE, esRAGE, CML, fasting glucose, fasting insulin, HOMA-IR, triglycerides, C-reactive protein, creatinine) were log transformed prior to further analysis. Clinical characteristics were compared between Blacks and Whites using t-tests for continuous variables or Chi-square tests for categorical variables. Total sRAGE and esRAGE levels were compared in subgroups of interest including age, gender, tobacco use, BMI category, decreased kidney function, diabetes, hypertension and coronary artery disease, overall and by race. Interaction terms were examined to determine if associations differed by race. Pearson correlation coefficients were used to determine associations between sRAGE levels and age, BMI, and other clinical and laboratory variables overall and by race. Analysis of covariance was used to examine racial differences in total sRAGE and esRAGE, as well as the ratios of esRAGE/total sRAGE, CML/total sRAGE, and CML/esRAGE. For each outcome, four models were fitted: model 1 was adjusted for age and gender; model 2 was adjusted for tobacco use, BMI, diabetes, hypertension, and coronary artery disease in addition to model 1 covariates; model 3 was adjusted for fasting glucose, fasting insulin, triglycerides, diastolic BP, heart rate, and C-reactive protein in addition to model 2 covariates; and model 4 was adjusted for CML and GFR in addition to model 3 covariates. CML was not included as a covariate in models with CML/total sRAGE and CML/esRAGE as the outcome. Other independent predictors were also identified from model 4. Furthermore, race-stratified analyses were performed using model 4 to identify race-specific independent predictors of sRAGE biomarkers. For all tests, p -values ≤ 0.05 were considered statistically significant.

3. Results

3.1. Clinical Characteristics of Study Population

A total of 454 White and 99 Black participants were included in this analysis. As shown in Table 1, Black participants were younger and more likely to be female compared to White participants. Blacks were also more likely to have diabetes and hypertension, but less likely to have decreased kidney function and coronary artery disease. In addition, Blacks had higher BMI, GFR, C-reactive protein, and diastolic blood pressure, but lower triglycerides compared to Whites ($p<0.05$ for all).

3.2. sRAGE Biomarker Levels in Blacks and Whites

Blacks had significantly lower median (interquartile range) levels of total sRAGE and esRAGE compared to Whites (Figure 1): 972 (742, 1343) pg/ml vs. 1564 (742, 1343) pg/ml ($p<0.001$) and 474 (324, 733) pg/ml vs. 710 (525, 974) pg/ml ($p<0.0001$), respectively. esRAGE accounted for 47% of total sRAGE levels in Blacks (Figure 2A), whereas the proportion was slightly lower in Whites (44%, $p=0.02$). In addition, the CML/total sRAGE and CML/esRAGE ratios were significantly higher in Black participants (Figure 2B): 0.89 (0.63, 1.12) ng/pg vs. 0.56 (0.40, 0.74) ng/pg ($p<0.001$) and 1.72 (1.09, 2.42) ng/pg vs. 1.20 (0.86, 1.69) ng/pg ($p<0.0001$), respectively. In addition to Black race, younger age (55–65 years) and diabetes were associated with lower total sRAGE levels, whereas decreased kidney function and coronary artery disease were associated with higher levels (Table 2). Similarly, esRAGE levels were lower in individuals with diabetes, but higher in those with decreased kidney function and coronary artery disease. Obese individuals had significantly lower levels of total sRAGE and esRAGE compared to both normal weight and overweight individuals. Overweight individuals also had significantly lower total sRAGE compared to those with normal weight, but esRAGE levels were similar.

Racial differences in total sRAGE and esRAGE persisted across Black and White subgroups of interest (Table 2), with no significant race x subgroup interactions. Among White participants, obesity, male gender, and absence of decreased kidney function were associated with lower total sRAGE levels and/or esRAGE levels ($p<0.05$ for all). Overweight and obesity were also associated with lower total sRAGE levels in Black participants, whereas no significant differences were observed in esRAGE levels across Black subgroups.

3.3. Correlations with sRAGE Biomarkers

Total sRAGE and esRAGE levels were highly correlated overall ($r=0.74$, $p<0.0001$) and in both Blacks ($r=0.54$, $p<0.0001$) and Whites ($r=0.75$, $p<0.0001$). As shown in Table 3, total sRAGE levels were positively associated with age, creatinine, and CML and inversely associated with BMI, fasting glucose, fasting insulin, HOMA-IR, C-reactive protein, and GFR in the overall group ($p<0.05$ for all). Similar correlations were observed for esRAGE levels and for White participants (except for age and heart rate). In Blacks, total sRAGE levels were positively associated with CML and inversely associated with BMI, fasting glucose, and C-reactive protein ($p<0.05$ for all). None of the clinical variables were significantly associated with esRAGE levels in Blacks, although many of the correlation coefficients were greater or of similar magnitude as in Whites.

3.4. Independent Predictors of sRAGE Biomarkers

Using analysis of covariance, Black race remained significantly associated with lower levels of total sRAGE ($p<0.001$) and esRAGE ($p<0.0001$) after adjusting for demographics (age, gender), tobacco use, BMI, comorbidities (coronary artery disease, diabetes, hypertension), and cardiometabolic risk factors (fasting glucose, fasting insulin, triglycerides, diastolic BP, heart rate, C-reactive protein) (Table 4). Further adjustment for CML and GFR had little effect on the association between race and sRAGE levels. Total sRAGE was also lower in men ($p=0.002$), inversely associated with fasting glucose ($p=0.05$) and heart rate ($p=0.02$), and positively associated with CML ($p=0.01$). esRAGE was inversely associated with male gender ($p=0.05$), age ($p=0.01$), and heart rate ($p=0.005$). Replacing fasting glucose and insulin with HOMA-IR did not change the overall results. HOMA-IR was not independently associated with either total sRAGE or esRAGE. Race alone explained nearly 14% of the variability in total sRAGE levels and 6% of the variability in esRAGE levels. Even after inclusion of additional variables (i.e., in the fully adjusted model), race remained a primary predictor of both total sRAGE (10.6% of the variability, while the model explained 23.9%) and esRAGE (4.6% of the variability, while the model explained 13.8%).

Using similar analyses we found that Black race remained significantly associated with higher esRAGE/total sRAGE ($p=0.03$), CML/total sRAGE ($p<0.0001$) and CML/esRAGE ($p<0.0001$) ratios, after adjustment for cardiovascular risk factors. Other independent predictors included age, which was inversely associated with esRAGE/total sRAGE ratio ($p=0.002$) and positively associated with CML/esRAGE ratio ($p=0.02$). Additionally, male gender and heart rate were positively associated with CML/total sRAGE and CML/esRAGE ratios ($p<0.01$ for all). Racial differences in the CML/sRAGE ratios were partly due to CML, as Blacks had significantly higher CML levels compared to Whites after adjustment for other risk factors (954 vs. 884 ng/ml, $p=0.009$).

3.5. Race-Specific Associations with sRAGE Biomarkers

Race-stratified models were run to further explore which variables were most predictive of sRAGE levels in Blacks and Whites separately. For White participants, both total sRAGE and esRAGE levels were lower in men and inversely associated with heart rate ($p<0.01$ for all). Total sRAGE was also inversely associated with GFR ($p=0.01$). Furthermore, male gender and heart rate were positively associated with both CML/total sRAGE and CML/esRAGE ratios ($p<0.01$ for all), while age was inversely associated with esRAGE/total sRAGE ratio ($p=0.05$). Among Black participants total sRAGE was positively associated with CML ($p=0.007$) and inversely associated with C-reactive protein ($p=0.05$), while GFR ($p=0.04$) and age ($p=0.04$) were inversely associated with esRAGE/total sRAGE ratio. None of the variables tested were independently associated with esRAGE, CML/total sRAGE, or CML/esRAGE in Blacks.

4. Discussion

Total sRAGE and esRAGE have emerged as important biomarkers and/or mediators of cardiovascular, metabolic, neurological and inflammatory diseases [1, 2]. Although both high and low sRAGE levels have been associated with various disease states, low levels are

generally thought to reflect impaired endogenous protection [15, 16]. Growing evidence indicates that Blacks have significantly lower levels of total sRAGE and esRAGE compared to Whites, and our study not only confirms these racial differences, but also shows for the first time that the balance of key components in the AGE/RAGE pathway also differs by race. These novel findings shed light on the interplay between circulating biomarkers of sRAGE and its major ligand.

Race alone accounted for nearly half of the variability in total sRAGE levels and one-third of the variability in esRAGE levels in fully adjusted models. Although age, gender, heart rate, fasting glucose, and CML were also independent predictors of total sRAGE and/or esRAGE, race was by far the strongest predictor in our study population. In contrast to prior studies citing impaired renal clearance as a primary determinant of sRAGE accumulation [25–27], GFR was not associated with total sRAGE or esRAGE after adjusting for additional risk factors overall or in Blacks. However, higher GFR was independently associated with lower total sRAGE levels in Whites. Previous reports indicate that metabolic, lifestyle, and genetic factors explain close to 50% of the variability in total sRAGE [14, 28]. Our fully adjusted model accounted for 24% of the variability in total sRAGE and 14% of the variability in esRAGE. Taken together, at least half of the variation in circulating sRAGE levels remains unexplained. Other unmeasured factors such as dietary AGE consumption, physical activity, and psychosocial stress may be relevant contributors to the observed racial differences in total sRAGE and esRAGE [29–31].

Few studies have investigated the relationship between circulating levels of different soluble forms of RAGE. sRAGE is generated by proteolytic cleavage of the extracellular domain at the cell surface (i.e., ectodomain shedding) or by alternative splicing [3, 4]. Ectodomain shedding not only serves to generate a decoy receptor, but is also a central mechanism by which RAGE mediates cell signaling and function [32]. As such, reduced or impaired RAGE shedding may have important implications for both normal physiology and disease, as is common with other membrane-bound immunoglobulin receptors. Similarly, abnormal alternative splicing may adversely affect gene expression by altering protein structure and function [33, 34]. Data from prior studies indicate that esRAGE constitutes ~20–60% of total sRAGE levels [10, 35–41], which are consistent with our findings of esRAGE/total sRAGE ratios of 44–47%. Notably, Black participants had a significantly higher esRAGE/total sRAGE ratio compared to Whites. Higher ratios were also associated with younger age (in both Blacks and Whites) and lower GFR (among Blacks only). Given the potential protection afforded by sRAGE in general, and RAGE shedding in particular, our findings of lower levels of both esRAGE and total sRAGE in Blacks compared to Whites, as well as the higher expression of membrane-bound RAGE observed in Black individuals [42, 43], warrant further investigation. Future research should clarify whether Black individuals with low sRAGE levels are more susceptible to chronic diseases associated with the AGE/RAGE pathway.

Binding of CML and other RAGE ligands to membrane-bound RAGE can stimulate ectodomain shedding and lead to an increase in sRAGE levels [3, 32]. Consistent with other studies [44, 45], our data show that both total sRAGE and esRAGE levels are positively associated with CML. Thus, sRAGE is likely an important mechanism to lower risk

associated with increased RAGE ligand accumulation. Although serum/plasma levels of total sRAGE are ~1,000 times lower than that of AGEs and other RAGE ligands [46], higher CML/sRAGE ratios may be associated with increased risk of cardiovascular and inflammatory diseases (even when the individual biomarkers are not) [47–49]. By representing the relative burden of ligand (bad) to soluble receptor (good), the CML/sRAGE ratio may be more reflective of the overall disease process. Given that the protective effect of sRAGE has consistently been shown in animal studies, the inconsistencies regarding the clinical significance of high vs. low sRAGE levels in humans likely reflect some dependency on the relative ability of sRAGE to protect against AGEs [15, 16]. In the present study, we observed a wide range of CML/sRAGE ratios, with a 30-fold difference between the highest and lowest ratio. More importantly, CML/total sRAGE and CML/esRAGE ratios were ~50% higher in Black versus White participants. These data further support the possibility that Blacks with a higher AGE burden and lower sRAGE levels have reduced endogenous protection against AGEs and may possess a higher risk of cardiovascular, metabolic, neurological, and inflammatory diseases.

Results from the stratified analyses suggest that some associations with sRAGE biomarkers differ by race. For example, male gender and higher heart rate were consistently associated with lower levels of total sRAGE and esRAGE and higher CML/sRAGE ratios in Whites, even after adjustment for other risk factors. Reasons for the gender differences are not clear; however, increased AGE/RAGE overexpression can impair calcium handling and gap junction function and thereby affect heart rate [50, 51]. In contrast, CML and C-reactive protein were independently associated with total sRAGE levels in Blacks. These findings suggest that Blacks may be particularly vulnerable to pathophysiological processes involving inflammation and oxidative stress, which are consistent with *in vitro* studies [52, 53]. We also observed a weaker correlation between total sRAGE and esRAGE in Blacks compared to Whites, which may suggest a dissociation of the mechanisms regulating sRAGE isoform generation. The implications of these racial differences are unknown and emphasize the need for more research to better understand why race (as a complex biological, social, and cultural construct) is important for the AGE/RAGE pathway and related diseases.

Given the ubiquitous nature of AGE/RAGE signaling in metabolic and inflammatory pathways, it is tempting to speculate that the adverse AGE/RAGE profile in Blacks may confer an increased risk of numerous chronic diseases. While direct evidence of this is lacking, it does raise the possibility that targeting low sRAGE levels and high AGE levels may be useful treatment and/or prevention strategies in Blacks and other high-risk groups. Indeed, based on a limited number of animal and human studies, it is becoming increasingly clear that preventing AGE formation, inhibiting RAGE ligand binding or blocking RAGE signal transduction using both pharmacological and non-pharmacological approaches has the potential to modify pathological processes and alter the development and progression of disease [16, 54]. Furthermore, anti-AGE/RAGE therapies can likely be implemented in a variety of clinical and community settings, which has important public health implications.

The major strength of this study includes the simultaneous measurement of total sRAGE, esRAGE, CML, and GFR in Black and White participants at high cardiac risk. This novel

approach is a necessary first step in fully elucidating the diverse roles of the AGE/RAGE pathway in normal and diseased states [15, 16]. However, important limitations include the comparatively smaller sample size in Blacks, which may have reduced our ability to rigorously test associations with sRAGE biomarkers in this group. Participants were consecutively enrolled in the study with no *a priori* hypotheses regarding racial differences and no attempt to match on potential confounders; thus, we cannot exclude the possibility that some of the observed racial differences could be due in part to residual confounding. The lack of information available on physical activity and dietary habits also limits our interpretation of the results. In addition, given the commercial assays currently available, we cannot accurately determine the contribution of sRAGE isoforms generated by ectodomain shedding. We also cannot determine the cellular origin of the biomarkers measured in serum, which may be useful information as the relative distribution of sRAGE isoforms is likely tissue-specific [55, 56]. Nevertheless, the significance of circulating sRAGE as a biomarker in various diseases is supported by numerous studies. By measuring both esRAGE and total sRAGE in this study, we can begin to identify potential factors that may influence RAGE splicing and shedding in diverse populations.

In conclusion, we identified several independent predictors of biomarkers in the AGE/RAGE pathway among Black and White middle-aged and older adults, with some evidence of differences by race. Most notably, race was a strong predictor of these biomarkers, with Blacks having significantly lower levels of total sRAGE and esRAGE and higher ratios of esRAGE/total sRAGE, CML/total sRAGE, and CML/esRAGE. Whether these differences contribute to racial disparities in risk for AGE/RAGE-related diseases, including type 2 diabetes, cardiovascular disease, and Alzheimer's disease, remains to be elucidated.

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Abbreviations

AGE	advanced glycation endproducts
BMI	body mass index
BP	blood pressure
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CML	carboxymethyl-lysine
ELISA	enzyme-linked immunosorbent assay
esRAGE	endogenous secretory receptor for advanced glycation endproducts

GFR	glomerular filtration rate
HDL	high-density lipoprotein
HOMA-IR	homeostasis model assessment of insulin resistance
LDL	low-density lipoprotein
RAGE	receptor for advanced glycation endproducts
sRAGE	soluble receptor for advanced glycation endproducts

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Figure 1.

Racial differences in median levels of (A) total sRAGE and (B) esRAGE. The solid line within the boxes indicates the median; the bottom and top of the boxes indicate the 25th and 75th percentiles, respectively; the whiskers represent the minimum and maximum values;

Figure 2. Racial differences in median levels of (A) esRAGE/total sRAGE ratio and (B) CML/total sRAGE and CML/esRAGE ratios.

Table 1

Clinical characteristics by race

Characteristic	Blacks (n=99)	Whites (n=454)
Age (yrs)	67 ± 9 [†]	70 ± 8
Females	68 (69%) [#]	216 (48%)
Diabetes	49 (49%) [†]	165 (36%)
Hypertension	97 (98%) [†]	408 (90%)
Coronary artery disease	12 (12%) [#]	146 (32%)
Decreased kidney function	15 (15%) [*]	116 (26%)
Current/former tobacco use	40 (45%)	195 (45%)
BMI (kg/m ²)	32.6 ± 6.7 [#]	29.6 ± 5.6
Systolic BP (mmHg)	145 ± 18	143 ± 17
Diastolic BP (mmHg)	79 ± 11 [†]	75 ± 10
Heart rate (bpm)	66 ± 11	67 ± 11
Total cholesterol (mg/dl)	173.0 ± 39.6	172.7 ± 42.8
HDL cholesterol (mg/dl)	47.1 ± 14.9	46.3 ± 14.2
LDL cholesterol (mg/dl)	106.1 ± 37.3	99.9 ± 36.9
Triglycerides (mg/dl) [#]	86.5 (60.0, 122.0) [#]	113.0 (81.0, 172.0)
Fasting glucose (mg/dl) [#]	107.5 (97.5, 122.5)	108.0 (99.0, 125.0)
Fasting insulin (uIU/ml) [#]	15.1 (8.8, 23.9)	15.0 (9.2, 22.9)
HOMA-IR [#]	4.1 (2.5, 6.7)	3.9 (2.4, 6.5)
C-reactive protein (mg/l) [#]	3.5 (1.5, 6.4) [†]	1.7 (0.8, 4.2)
GFR (ml/min/1.73 m ²)	82.4 ± 20.8 [#]	71.9 ± 17.1
Serum creatinine (mg/dl) [#]	0.9 (0.8, 1.1)	0.9 (0.8, 1.10)

Table values are mean ± SD, median (interquartile range), or N (% Yes).

[#] Analyses were performed using log-transformed variables;^{*} p 0.05,[†] p 0.01,[#] p 0.0001 compared to Whites.

Table 2
Median total sRAGE and esRAGE levels in subgroups of interest overall and stratified by race

Characteristic	N	Overall		Blacks		Whites	
		sRAGE (pg/ml)	esRAGE (pg/ml)	N	sRAGE (pg/ml)	esRAGE (pg/ml)	N
Age group							
<65 years	178	1378 (972, 1869)	697 (463, 936)	48	865 (685, 1354)	567 (327, 797)	130
65 years	375	1487 (1111, 1987)*	659 (474, 947)	51	991 (790, 1297)	442 (324, 686)	324
Gender							
Male	269	1416 (1061, 1925)	686 (479, 940)	31	894 (739, 1253)	571 (336, 747)	238
Female	284	1494 (1103, 2038)	656 (466, 944)	68	986 (781, 1384)	465 (322, 722)	216
Tobacco Use [#]							
Never	283	1523 (1056, 2052)	677 (475, 940)	49	879 (739, 1293)	480 (309, 772)	234
Ever	235	1416 (1120, 1931)	671 (464, 959)	40	1023 (764, 1413)	484 (352, 741)	195
BMI							
Normal	110	1639 (1201, 2135)	714 (555, 1135)	12	1360 (899, 1991)	593 (445, 781)	98
Overweight	204	1468 (1099, 2023)*	696 (487, 985)	60	882 (724, 1364) [‡]	466 (355, 712)	179
Obese	239	1355 (998, 1814) [‡]	610 (444, 869) [‡]	27	975 (750, 1240) [‡]	469 (315, 734)	177
Decreased kidney function							
No	422	1413 (1044, 1890)	674 (456, 921)	84	960 (750, 1318)	470 (323, 705)	338
Yes	131	1571 (1219, 2117) [‡]	677 (525, 1081) [‡]		979 (722, 1639)	536 (352, 829)	1603 (1285, 2188) [‡]
Diabetes							
No	339	1523 (1136, 2075)	721 (480, 967)	50	976 (724, 1523)	480 (352, 732)	289
Yes	214	1341 (991, 1760) [‡]	601 (445, 890)*	49	960 (785, 1264)	463 (309, 747)	165
Hypertension							
No	48	1536 (1112, 1920)	697 (521, 961)	2	930 (597, 1264)	566 (436, 695)	46
Yes	505	1449 (1063, 1954)	671 (465, 940)	97	972 (757, 1343)	474 (324, 733)	408
Coronary artery disease							
No	395	1420 (1029, 1935)	642 (454, 932)	87	960 (730, 1404)	466 (324, 736)	308
Yes	158	1528 (1144, 2085)*	714 (553, 1015) [‡]	12	1002 (912, 1204)	594 (336, 704)	146
							1565 (1204, 2115)
							739 (555, 1059)

Table values are median (interquartile range). P-values correspond to differences in sRAGE levels between subgroups of interest. sRAGE biomarkers were log-transformed for analysis.

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* p 0.05,
/ p 0.01,
‡ p 0.0001 compared to referent group.
Data on tobacco use were missing for 10 Blacks and 25 Whites.

Table 3Correlates of total sRAGE[#] and esRAGE[#] levels overall and stratified by race

Characteristic	Overall		Blacks		Whites	
	sRAGE	esRAGE	sRAGE	esRAGE	sRAGE	esRAGE
Age	0.12 [†]	0.008	0.14	-0.07	0.07	-0.01
BMI	-0.19 [‡]	-0.13 [†]	-0.21 [*]	-0.004	-0.10 [*]	-0.10 [*]
Systolic BP	0.07	0.01	0.15	-0.03	0.07	0.03
Diastolic BP	-0.03	-0.04	0.01	-0.03	0.01	-0.005
Heart rate	-0.08	-0.12 [†]	-0.06	-0.02	-0.12 [†]	-0.17 [†]
Total cholesterol	0.01	-0.01	-0.03	0.03	0.02	-0.02
HDL cholesterol	0.02	0.04	0.04	0.07	0.03	0.04
LDL cholesterol	-0.03	-0.04	-0.06	-0.003	0.01	-0.03
Triglycerides [#]	0.07	0.03	0.06	0.05	-0.02	-0.05
Fasting glucose [#]	-0.13 [†]	-0.11 [†]	-0.26 [†]	-0.10	-0.12 [†]	-0.12 [†]
Fasting insulin [#]	-0.09 [*]	-0.11 [*]	-0.07	-0.14	-0.11 [*]	-0.10 [*]
HOMA-IR [#]	-0.12 [†]	-0.12 [†]	-0.15	-0.14	-0.13 [†]	-0.12 [*]
C-reactive protein [#]	-0.22 [‡]	0.17 [‡]	-0.24 [*]	-0.11	-0.16 [†]	-0.14 [†]
GFR	-0.21 [‡]	-0.14 [†]	-0.06	-0.06	-0.17 [†]	-0.10 [*]
Creatinine [#]	0.09 [*]	0.09 [*]	0.05	0.12	0.09	0.07
CML [#]	0.16 [†]	0.12 [†]	0.23 [*]	0.16	0.14 [†]	0.10 [*]

Table values are Pearson correlation coefficients. Sample sizes were as follows: n=516–553 for the overall group; n=89–99 for Blacks; n=427–454 for Whites;

[#] Analyses were performed using log-transformed variables;^{*} p 0.05,[†] p 0.01,[‡] p 0.0001

Table 4

Association between race and sRAGE biomarkers based on analysis of covariance

Biomarker	Model 1	Model 2	Model 3	Model 4
Total sRAGE (pg/ml)	−0.47 (0.05) [‡]	−0.42 (0.05) [‡]	−0.44 (0.06) [‡]	−0.43 (0.06) [‡]
esRAGE (pg/ml)	−0.37 (0.06) [‡]	−0.32 (0.06) [‡]	−0.34 (0.07) [‡]	−0.32 (0.07) [‡]
esRAGE/total sRAGE	0.04 (0.02) [*]	0.05 (0.02) [*]	0.04 (0.02) [*]	0.05 (0.02) [*]
CML/total sRAGE (ng/pg)	0.20 (0.02) [‡]	0.21 (0.02) [‡]	0.21 (0.03) [‡]	0.21 (0.03) [‡]
CML/esRAGE (ng/pg)	0.16 (0.03) [‡]	0.16 (0.03) [‡]	0.16 (0.03) [‡]	0.17 (0.03) [‡]

Table values are regression coefficients (standard errors) for Black race;

^{*}
p 0.05,[‡]
p 0.01,[‡]
p 0.0001;

All biomarkers were log transformed for analysis. Model 1 is adjusted for age and gender. Model 2 is adjusted for model 1 variables plus tobacco use, BMI, diabetes, hypertension, and coronary artery disease. Model 3 is adjusted for model 2 variables plus fasting glucose, fasting insulin, triglycerides, diastolic BP, heart rate, and C-reactive protein. Model 4 is adjusted for model 3 variables plus CML and GFR.