HHS Public Access

Author manuscript

Dig Liver Dis. Author manuscript; available in PMC 2022 July 01.

Published in final edited form as:

Dig Liver Dis. 2021 July ; 53(7): 873–878. doi:10.1016/j.dld.2021.02.005.

Association between the soluble receptor for advanced glycation end products (sRAGE) and NAFLD in participants in the Atherosclerosis Risk in Communities Study

Marci Laudenslager, MD, MHS[a], **Mariana Lazo, MD, PhD, ScM**[b], **Dan Wang, MS**[c], **Elizabeth Selvin, PhD, MPH**[a,c], **Po-Hung Chen, MD**[d], **James S. Pankow, PhD, MPH**[e], **Jeanne M. Clark, MD, MPH**[a,c]

[a]The Johns Hopkins School of Medicine, Department of Medicine, Division of General Internal Medicine, Baltimore, MD

[b]Drexel University Dornsife School of Public Health, Department of Community Health and Prevention and the Urban Health Collaborative, Philadelphia, PA

[c]The Johns Hopkins Bloomberg School of Public Health, Department of Epidemiology, Baltimore, MD

[d]The Johns Hopkins School of Medicine, Department of Medicine, Division of Gastroenterology and Hepatology, Baltimore, MD

[e]University of Minnesota School of Public Health, Division of Epidemiology and Community Health, Minneapolis, MN

Abstract

Background: Inflammation is key in the pathogenesis of Nonalcoholic Fatty Liver Disease (NAFLD) – a common progressive liver disease. The soluble receptor for advanced glycation end products (sRAGE) attenuates inflammatory signaling; low levels of sRAGE are correlated with increased inflammation.

Aim: We sought to describe associations between sRAGE and NAFLD.

Corresponding Author: Marci Laudenslager, MD, MHS, 2024 E. Monument Street, Suite 2-604C, Baltimore, MD 21287, Telephone: 410-502-3770, Fax: 410-955-0476, mlauden1@jhmi.edu.
Marci Laudenslager, MD, MHS – The Johns Hopkins School of Medicine, Department of Medicine, Division of General Internal Medicine;
Mariana Lazo, MD, PhD, ScM – Drexel University Dornsife School of Public Health, Department of Community Health and Prevention and the Urban Health Collaborative;
Dan Wang, MS – The Johns Hopkins Bloomberg School of Public Health, Department of Epidemiology;
Elizabeth Selvin, PhD, MPH – The Johns Hopkins Bloomberg School of Public Health, Department of Epidemiology and The Johns Hopkins School of Medicine, Department of Medicine, Division of General Internal Medicine;
Po-Hung Chen, MD – The Johns Hopkins School of Medicine, Department of Medicine, Division of Gastroenterology and Hepatology;
James S. Pankow, PhD, MPH - University of Minnesota School of Public Health, Division of Epidemiology and Community Health;
Jeanne M. Clark, MD, MPH – The Johns Hopkins School of Medicine, Department of Medicine, Division of General Internal Medicine and The Johns Hopkins Bloomberg School of Public Health, Department of Epidemiology;

Conflicts of Interest: None

Methods: We conducted a cross-sectional analysis of 1,088 Atherosclerosis Risk in Communities (ARIC) Study participants and used logistic regression to investigate the associations between sRAGE and NAFLD defined by elevated liver enzymes and fibrosis score.

Results: In this community-based sample (n=1,088, mean age 56 years, 61% female, 78% Caucasian), persons in the lowest vs. highest quartile of sRAGE had significantly higher odds of elevated ALT (OR 2.82, 95% CI 1.18–6.76) but not elevated AST (OR 1.16, 95% CI 0.45–2.99); persons in the lowest vs. highest quartile had significantly lower odds of elevated FIB-4 index (OR 0.56, 95% CI 0.37–0.84).

Conclusions: We found an inverse cross-sectional association between sRAGE and liver inflammation; this is consistent with prior studies linking low sRAGE to inflammatory states. However, we observed a direct association between sRAGE and fibrosis. Our findings suggest that sRAGE is dynamic in NAFLD and patterns may vary with different stages of disease.

Keywords

Receptor for Advanced Glycation End Products; Biomarkers; Inflammation; NAFLD

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common form of chronic liver disease in the United States and worldwide.(1) The prevalence of NAFLD has at least doubled over the past twenty years as a consequence of trends in obesity and type 2 diabetes; presently it is estimated that 24% of adults in the US are affected with NAFLD.(1–3) Approximately 25% of patients with NAFLD progress to nonalcoholic steatohepatitis (NASH), an important cause of cirrhosis and hepatocellular carcinoma (HCC).(4, 5) Further, NASH is currently the second leading cause of liver disease in adults in the United States awaiting liver transplantation.(6) Despite increasing prevalence and burden of disease, the mechanisms that drive development and progression of NAFLD remain poorly understood.

The current paradigm of NAFLD pathophysiology emphasizes insulin resistance, oxidative stress and inflammation and targeting of such pathways may be clinically beneficial in patients with NAFLD.(7) Signaling through the receptor for advanced glycation end products (RAGE) affects key elements of NAFLD pathophysiology – insulin resistance, cellular metabolism, cellular motility and apoptosis – through oxidative and inflammatory mediators.(8) Elucidation of the role of RAGE signaling in NAFLD may therefore identify pathways in NAFLD pathogenesis and potential targets for therapeutic intervention.

Advanced glycation end products (AGEs) are glycosylated proteins known to trigger inflammatory pathways through binding to and subsequent activation of RAGE.(9–11) RAGE is expressed on a variety of cell types including endothelial and hepatic stellate cells (HCS)(12, 13); activation has been shown to influence proinflammatory and profibrotic pathways via increased signaling through TNF-α and NFκB.(14–16) The soluble receptor for AGEs (sRAGE) is released from the cell surface following proteolytic cleavage of RAGE.(17, 18) Cleavage to form sRAGE is enhanced by an inflammatory microenvironment rich in matrix metalloproteinases (MMP) and by AGEs themselves as

they accumulate in tissues and the surrounding vasculature.(9, 11, 18, 19) Once released into the serum, sRAGE may act as a decoy receptor for AGEs thereby reducing AGE-RAGE binding and attenuating activation of downstream inflammatory and pro-fibrotic pathways. (9, 11, 18, 19)

Inverse associations have been observed between sRAGE and chronic diseases including diabetes, obesity, cardiovascular disease, renal disease, and polycystic ovarian syndrome. (18–23) Further, hepatic steatosis and hepatocellular injury through RAGE-dependent inflammation, stellate cell activation and hepatic insulin resistance have been noted in murine models of NAFLD.(9, 10) Specifically, an AGE-rich diet has been shown to cause liver inflammation, injury and fibrosis via a RAGE-dependent pathway in mice.(9) Additionally, RAGE knockout models have demonstrated improved hepatic glucose metabolism despite a high fat diet challenge.(24) Though studies in humans are limited, several small cross-sectional analyses have, concordant with murine studies, suggested a role for RAGE in NAFLD and specifically found an inverse association between sRAGE and hepatic steatosis.(25)

Our objective was to investigate the cross-sectional associations between sRAGE and markers of hepatocellular injury (liver enzymes) and fibrosis (FIB-4 index) in participants from the Atherosclerosis Risk in Communities (ARIC) Study, a large community-based cohort. We hypothesized that low levels of sRAGE would be independently associated with prevalent NAFLD with inflammatory and fibrotic stages of disease defined by elevated liver enzymes and fibrosis score, respectively.

2. Materials and Methods

2.1 Study population

The design and methods of the ARIC study have been previously described.(23, 26) Briefly, subjects were recruited from four centers in the United States (Forsyth County, North Carolina; Jackson, Mississippi; Minneapolis, Minnesota; and Washington County, Maryland) and examined during the following study time points: 1987–1989 (visit 1), 1990–1992 (visit 2), 1993–1995 (visit 3), 1996–1998 (visit 4), 2011–2013 (visit 5), 2016–2017 (visit 6) and 2018–2019 (visit 7). Health surveys, physical measures, and blood specimens were obtained at each study visit. The population for the current study comprises a subsample of 14,348 participants who attended study visit 2. sRAGE measurements were obtained from a random sample of 1,289 participants with normal kidney function (estimated glomerular filtration rate >60 mL/min/1.73 m^2). We imputed platelet levels for 246 patients with missing platelet data at visit 2 from platelet levels obtained at visit 1. Visit 1 and visit 2 platelet levels were highly correlated with a Spearman's correlation of 0.74. The final sample size of the current study was 1,088 after excluding those with missing sRAGE, liver enzymes, platelet data to calculate FIB-4, other covariates of interest and self-reported excessive alcohol use (defined as ≥20 grams per day for females and 30 grams per day for males). A flow diagram detailing study subject selection and exclusions is included in supplementary methods (Supplementary Figure 1). Written informed consent was obtained from all study subjects. Institutional Review Boards at all participating study sites

approved this work in protocols that conform to ethical guidelines established in the 1975 Declaration of Helsinki.

2.2 Sample Processing and Measurement of sRAGE

sRAGE was measured from stored, frozen plasma samples using the Quantikine Human RAGE Immunoassay Kit (R&D Systems, Inc., Minneapolis, Minnesota). The intra and inter-assay coefficients of variation were 2.8% and 9.6%, respectively. Stability of sRAGE measurements was previously assessed and levels across a 3-year period were found to be highly reliable with an intra-class correlation of 0.76 and a Pearson's correlation of 0.78.(27)

2.3 Sample Processing and Measurement of Liver Enzymes

Levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured from samples stored at −80°C and collected from ARIC participants at study visit 2. Samples were assayed using Roche Diagnostics reagents and the Roche Modular P800 Chemistry Analyzer (Indianapolis, Indiana).(28)

2.4 Assessment of Liver Enzymes and FIB-4 Index Elevation

ALT and AST elevation were defined as levels greater than the 95th percentile based on gender-specific distributions of each enzyme in a subgroup of normal weight participants (BMI <25 kg/m^2) without diabetes (ALT >27 in females and 33 in males; AST > 31 in females and 33 in males).(29) With respect to FIB-4 index ((age*AST)/(platelet count*√ALT)), elevated likelihood of advanced fibrosis was defined as a FIB-4 index greater than or equal to 1.30.(30) This includes patients who are at intermediate (1.3 ≤ FIB-4 index <2.67) and high (FIB-4 index ≥2.67) risk for advanced fibrosis.

2.5 Measurement of Additional Covariates

Self-reported age, race and gender were obtained from participant interview data. BMI was obtained from physical examination measurements. Hypertension (HTN) was defined as systolic blood pressure (SBP) ≥ 140 mm Hg or diastolic blood pressure (DBP) ≥ 90 mm Hg or self-reported use of blood pressure lowering medication within a 2 week period prior to visit 2. Type 2 diabetes was defined by A1C ≥ 6.5%, self-reported diagnosis, physician diagnosis or medication use. Hypercholesterolemia was defined as a total cholesterol level ≥200 mg/dL or medication use. Hypertriglyceridemia was defined as a triglyceride level ≥150 mg/dL. Low high-density lipoprotein (HDL) was defined as levels <40 mg/dL in males and <50 mg/dL in females. Tobacco and alcohol use were assessed by self-report and categorized as never, former or current use. For patients reporting current alcohol use, quantity was reported as total grams of alcohol consumed per week. Excessive alcohol use was defined as consumption of ≥20 grams per day for females and 30 grams per day for males.

2.6 Statistical Analysis

Descriptive statistics were used to characterize the study population by quartiles of sRAGE. In addition, sRAGE was dichotomized into low sRAGE (defined as the lowest quartile) versus a reference group composed of the remaining three quartiles. sRAGE was modeled

using both quartiles and a dichotomized approach in a manner consistent with current literature and in accordance with our physiologic interest in low sRAGE as a marker of inflammation in disease.(19, 23) Liver enzymes and FIB-4 indices were measured as continuous variables and analyzed as dichotomous outcome variables (elevated versus not elevated as previously described). Given the low prevalence of participants with a high FIB-4 index (FIB-4 index ≥2.67), we were unable to analyze subjects in the high FIB-4 category alone and instead combined subjects with intermediate and high FIB-4 indices for analysis.

Univariable and multivariable logistic regression models were used to estimate prevalence odds ratios while controlling for potential confounders. Multivariable models included age, race, gender, diabetes status (yes/no), BMI (kg/m^2), hypertension (yes/no), total cholesterol (mg/dL), HDL (mg/dL), triglycerides (mg/dL), tobacco use (current, former or never) and alcohol consumption (current, former or never). Age was excluded from FIB-4 analyses. We additionally modeled sRAGE as a continuous variable using restricted cubic spline models with the 75^{th} percentile as the reference group while adjusting for age, race and gender. We further evaluated the relationship between sRAGE and FIB-4 index using robust linear regression and bootstrap analyses.

3. Results

3.1 General characteristics of subjects

Baseline characteristics of the study population (n=1,088) by quartiles of sRAGE are shown in Table 1. Compared with higher quartiles, those in the lowest quartile of sRAGE were more likely to be male, African American, and have obesity, diabetes, hypertension, hypercholesterolemia, elevated CRP and former tobacco use (Table 1). Median ALT was higher in lower versus higher quartiles of sRAGE and those in the lowest quartile were more likely to have elevated ALT though this finding was not statistically significant (p = 0.057). Median and elevated AST measures were similar across quartiles. In contrast, elevated FIB-4 index was less likely in those in lower quartiles of sRAGE (Table 1). Median FIB-4 index likewise increased significantly with increasing quartiles of sRAGE (p = 0.001)(Table 1). Approximately 38% of patients had an elevated FIB-4 index (36.6% with an intermediate FIB-4 index – 1.3 ≤ FIB-4 index <2.6; 1.8% with a high FIB-4 index – FIB-4 index ≥2.67) while 62% of patients in the study population had a non-elevated FIB-4 index (low FIB-4 index; FIB-4 index <1.3). The distribution of FIB-4 categories within the patient population and corresponding distributions of sRAGE within each category are noted in Supplementary Table 1. sRAGE was significantly negatively correlated with ALT (Spearman correlation −0.06, p 0.036) and positively correlated with FIB-4 index (Spearman correlation 0.10, p 0.002). A scatter plot further describing the direct relationship between sRAGE and FIB-4 index is noted in Supplementary Figure 2. sRAGE was not significantly correlated with AST (Spearman correlation 0.42, p 0.172). Low sRAGE was significantly negatively correlated with CRP (Spearman correlation −0.26, p <0.001). Positive correlations were noted between ALT and AST and AST and FIB-4 index; a negative correlation was noted between ALT and FIB-4 index (Supplementary Figure 3).

3.2 Association between sRAGE and NAFLD

In multivariable analyses, persons in the lowest quartile of sRAGE had a significantly higher odds of elevated ALT compared to those in the highest quartile (Table 2, adjusted OR 2.82, 95% CI 1.18–6.76). There was no significant association between low sRAGE and elevated AST (adjusted OR 1.16, 95% CI 0.45–2.99). There was a significantly lower odds of elevated FIB-4 index in patients in the lowest versus the highest quartile of sRAGE (adjusted OR 0.56; 95% CI 0.37–0.84) corresponding to a 44% reduction in the odds of elevated FIB-4 index in the lowest versus the highest quartile of sRAGE (Table 2). Further, there was a significantly higher FIB-4 index with each increasing quartile of sRAGE in both simple and fully adjusted logistic regression models (P for trend 0.02) (Table 2). Spline model analyses and multivariable analyses of associations between dichotomized sRAGE (lowest vs. other quartiles) and elevated ALT, AST and FIB-4 index yielded similar results (Supplementary Figure 4, Supplementary Table 2). We further evaluated the observed direct relationship between sRAGE and FIB-4 using robust linear regression and found that FIB-4 index was significantly lower in the lower quartiles as compared to the highest quartile (Q1: Adjusted β = −0.16, p <0.001; Q2: Adjusted β = −0.07, p 0.04; Q3: Adjusted β −0.10, p 0.004). Bootstrap analysis yielded similar results.

4. Discussion

In this community-based sample, we found that lower levels of sRAGE were independently associated with liver injury as inferred by elevated levels of ALT. While we observed an inverse relationship between sRAGE and liver injury, we found a direct association between sRAGE levels and FIB-4 index in a cross-sectional analysis. Finally, low sRAGE was correlated with high CRP consistent with previous reports identifying sRAGE as a key inflammatory pathway biomarker.

The foundation of our analysis of the RAGE pathway in NAFLD is based upon prior studies investigating RAGE activity in metabolic disorders and specifically those in which inflammation and insulin resistance play a central role. Analyses of RAGE activity in cardiovascular disease, diabetes and obesity have suggested that sRAGE may function as a decoy receptor for AGEs thereby preventing RAGE-mediated inflammatory signaling and downstream effects including thrombosis, atherosclerosis and insulin resistance.(8, 23, 31, 32) It has, therefore, been postulated that high levels of sRAGE may serve in a protective role in these disorders whereas low levels may indicate injury and a proinflammatory state.(23, 31) Further studies in diabetes, however, have challenged this paradigm for chronic disease and have suggested that constitutive pathway activation may lead to high, rather than low, levels of sRAGE.(13, 31, 33) This pattern has been attributed to increased proteolytic conversion of RAGE to its soluble receptor isoform in the setting of chronic pathway activation.(33, 34) Chronic disease and inflammation, therefore, may result in higher levels of sRAGE due to increased production of sRAGE via constitutive pathway activation in a manner that may be mediated by induction of MMPs.(9, 11, 18, 19) Given these findings along with shared clinical and pathophysiologic associations between these aforementioned disorders and NAFLD, others have sought to investigate whether RAGE signaling may likewise influence NAFLD pathogenesis. Studies, in fact, have found that an AGE-rich diet

causes liver inflammation, injury and fibrosis via a RAGE-dependent pathway in murine models.(9) Further, RAGE knockout models have demonstrated improved hepatic glucose metabolism despite a high fat diet challenge.(24) Though studies in humans are limited, several have likewise suggested a role for RAGE signaling in NAFLD and, specifically, an inverse association between sRAGE and hepatic steatosis. A recent study by Palma-Duran et al. described high AGE, low sRAGE and an elevated AGE/sRAGE ratio in 50 normoglycemic patients with NAFLD as compared to 58 age, sex and BMI-matched healthy controls.(25) Larger studies investigating patterns of sRAGE in human NAFLD are lacking in the current literature and, further, patterns in more advanced fibrotic disease have not been undertaken.(25) We sought to describe patterns of sRAGE in both inflammatory and fibrotic stages of sRAGE in a large, community-based cohort.

Our findings support current literature linking sRAGE to liver inflammation and further contribute novel insights with respect to sRAGE patterns in more advanced stages of NAFLD. While our findings are consistent with studies that found an inverse relationship between sRAGE and hepatic inflammation, we somewhat surprisingly noted a direct relationship between sRAGE levels and FIB-4 index.(25) These findings perhaps punctuate the importance of sRAGE in chronic hepatic inflammation and its progression to more advanced fibrotic disease. The results of our analyses, taken together, suggest that higher, rather than lower, levels of sRAGE may be associated with hepatic fibrosis. In considering mechanisms previously considered in diabetes, it is possible that persistent RAGE axis activation in hepatic fibrosis encourages overexpression of RAGE thereby increasing conversion of RAGE to sRAGE via MMPs in a hepatic microenvironment that is rich in both inflammatory cytokines and reactive oxygen species (ROS). In summary, low sRAGE may be noted in early disease and inflammation whereas higher levels of sRAGE may be evident with further injury and advanced fibrosis when inflammation tends to be less prominent.

There are several limitations to the current study. First, our limited sample size prevented subgroup analyses, for example, by racial category. Second, while the observational nature of this study suggests sRAGE is involved in NAFLD pathogenesis, we cannot infer causation. Third, we used liver enzymes elevation and FIB-4 index as surrogate markers for NAFLD as ultrasound imaging and/or biopsy data were not available in the ARIC study. While imaging and biopsy data are valuable diagnostic modalities, the widespread use of these tools for screening purposes is often limited by cost and the invasive nature of liver biopsy, in particular.(2, 30, 35, 36) Noninvasive scores have been developed as screening tools in this setting and FIB-4 index is a validated approach to predicting liver fibrosis in patients with NAFLD.(2, 30, 37–39) We did not exclude viral hepatitis as a potential etiology of liver enzymes elevation in this study. Previous studies have found the prevalence of hepatitis C to be exceedingly low (0.8%) among ARIC participants and we therefore do not expect viral hepatitis to be a significant contributor to liver injury and fibrosis in this sample.(40) Finally, the prevalance of high FIB-4 index was low in this study and this may be attributed to the fact that this is a community-based population. The low prevalence of high FIB-4 index prevented further analyses of those patients with the highest likelihood of having advanced fibrosis; further studies are needed in order to evaluate patterns of sRAGE in this patient population.

Strengths of this study include rigorous and comprehensive participant evaluation and data collection through the ARIC study framework - this study population is a random subsample of ARIC study participants for whom sRAGE data were available. Though our sample size is relatively small, this is to our knowledge the largest study investigating the association between sRAGE in human NAFLD.

Our results support a growing body of literature that suggest a role for the RAGE pathway in the development and progression of NAFLD in humans. Targeted RAGE therapies have been proposed for use in metabolic and inflammatory diseases.(14, 41, 42) RAGE inhibition by anti-RAGE antibodies, RAGE peptide aptamers and siRNAs have been investigated in diabetic complications and murine models of myocardial ischemia.(14, 41–45) Further, the small-molecule RAGE inhibitor azeliragon is currently under investigation in patients with Alzheimer's Disease and impaired glucose tolerance.(46, 47) Small molecule inhibition of ligand-stimulated RAGE-Diaphenous 1 (DIAPH1) signal transduction has additionally been explored and proposed as a potential therapeutic modality as the cytoplasmic portion of RAGE has been demonstrated to specifically and effectively interrupt RAGE signaling.(45) Our work and work by others suggest that these agents may additionally benefit patients with NAFLD, a disorder for which there are presently no FDA-approved therapies.

In conclusion, low levels of sRAGE were independently associated with liver injury in a cross-sectional study of a large community based cohort. Conversely, a direct association was observed between sRAGE and FIB-4 index in a cross-sectional analysis suggesting sRAGE patterns may vary by extent and stage of disease. These findings support evidence in the current literature that suggest a role for RAGE signaling in NAFLD through its influence inflammatory mediators. Our results further serve to support the hypothesis that RAGE-targeted therapies may be an important consideration in NAFLD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

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

Funding Sources:

This work was supported in part by federal funds from the National Institutes of Health (NIH 5T32HL007180-44). The Atherosclerosis Risk in Communities study has been funded in whole or in part with Federal funds from the National Heart, Lung, and Blood Institute, National Institutes of Health, Department of Health and Human Services, under Contract nos. (HHSN268201700001I, HHSN268201700002I, HHSN268201700003I, HHSN268201700005I, HHSN268201700004I).

References

1. Younossi Z, Tacke F, Arrese M, Chander Sharma B, Mostafa I, Bugianesi E, et al. Global Perspectives on Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis. Hepatology. 2019 6;69(6):2672–82. [PubMed: 30179269]
2. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nature reviews Gastroenterology & hepatology. 2018 1;15(1):11–20. [PubMed: 28930295]

3. Younossi ZM. Non-alcoholic fatty liver disease - A global public health perspective. Journal of hepatology. 2019 3;70(3):531–44. [PubMed: 30414863]
4. Diehl AM, Day C. Cause, Pathogenesis, and Treatment of Nonalcoholic Steatohepatitis. The New England journal of medicine. 2017 11 23;377(21):2063–72. [PubMed: 29166236]
5. Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. Gastroenterology. 2011 1;140(1):124–31. [PubMed: 20858492]
6. Wong RJ, Aguilar M, Cheung R, Perumpail RB, Harrison SA, Younossi ZM, et al. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. Gastroenterology. 2015 3;148(3):547–55. [PubMed: 25461851]
7. Day CP. Pathogenesis of steatohepatitis. Best practice & research Clinical gastroenterology. 2002 10;16(5):663–78. [PubMed: 12406438]
8. Asadipooya K, Lankarani KB, Raj R, Kalantarhormozi M. RAGE is a Potential Cause of Onset and Progression of Nonalcoholic Fatty Liver Disease. Int J Endocrinol. 2019;2019:2151302. [PubMed: 31641351]
9. Leung C, Herath CB, Jia Z, Andrikopoulos S, Brown BE, Davies MJ, et al. Dietary advanced glycation end-products aggravate non-alcoholic fatty liver disease. World journal of gastroenterology. 2016 9 21;22(35):8026–40. [PubMed: 27672297]
10. Leung C, Herath CB, Jia Z, Goodwin M, Mak KY, Watt MJ, et al. Dietary glycotoxins exacerbate progression of experimental fatty liver disease. Journal of hepatology. 2014 4;60(4):832–8. [PubMed: 24316518]
11. Yan SF, Ramasamy R, Schmidt AM. Mechanisms of disease: advanced glycation end-products and their receptor in inflammation and diabetes complications. Nature clinical practice Endocrinology & metabolism. 2008 5;4(5):285–93.
12. Fehrenbach H, Weiskirchen R, Kasper M, Gressner AM. Up-regulated expression of the receptor for advanced glycation end products in cultured rat hepatic stellate cells during transdifferentiation to myofibroblasts. Hepatology. 2001 11;34(5):943–52. [PubMed: 11679965]
13. Yamagishi S, Matsui T. Role of receptor for advanced glycation end products (RAGE) in liver disease. European journal of medical research. 2015 2 11;20:15. [PubMed: 25888859]
14. Hudson BI, Lippman ME. Targeting RAGE Signaling in Inflammatory Disease. Annual review of medicine. 2018 1 29;69:349–64.
15. Meghnani V, Wagh A, Indurthi VS, Koladia M, Vetter SW, Law B, et al. The receptor for advanced glycation end products influences the expression of its S100 protein ligands in melanoma tumors. The international journal of biochemistry & cell biology. 2014 12;57:54–62. [PubMed: 25310905]
16. Jiang JX, Chen X, Fukada H, Serizawa N, Devaraj S, Torok NJ. Advanced glycation endproducts induce fibrogenic activity in nonalcoholic steatohepatitis by modulating TNF-alpha-converting enzyme activity in mice. Hepatology. 2013 10;58(4):1339–48. [PubMed: 23703665]
17. Zhang L, Bukulin M, Kojro E, Roth A, Metz VV, Fahrenholz F, et al. Receptor for advanced glycation end products is subjected to protein ectodomain shedding by metalloproteinases. The Journal of biological chemistry. 2008 12 19;283(51):35507–16. [PubMed: 18952609]
18. Merhi Z Crosstalk between advanced glycation end products and vitamin D: A compelling paradigm for the treatment of ovarian dysfunction in PCOS. Molecular and cellular endocrinology. 2019 1 5;479:20–6. [PubMed: 30170183]
19. Loomis SJ, Chen Y, Sacks DB, Christenson ES, Christenson RH, Rebholz CM, et al. Cross-sectional Analysis of AGE-CML, sRAGE, and esRAGE with Diabetes and Cardiometabolic Risk Factors in a Community-Based Cohort. Clinical chemistry. 2017 5;63(5):980–9. [PubMed: 28280052]
20. Meerwaldt R, Links T, Zeebregts C, Tio R, Hillebrands JL, Smit A. The clinical relevance of assessing advanced glycation endproducts accumulation in diabetes. Cardiovascular diabetology. 2008 10 7;7:29. [PubMed: 18840258]
21. Basta G, Leonardis D, Mallamaci F, Cutrupi S, Pizzini P, Gaetano L, et al. Circulating soluble receptor of advanced glycation end product inversely correlates with atherosclerosis in patients with chronic kidney disease. Kidney international. 2010 2;77(3):225–31. [PubMed: 19924100]

22. Gross S, van Ree RM, Oterdoom LH, de Vries AP, van Son WJ, de Jong PE, et al. Low levels of sRAGE are associated with increased risk for mortality in renal transplant recipients. Transplantation. 2007 9 15;84(5):659–63. [PubMed: 17876282]
23. Selvin E, Halushka MK, Rawlings AM, Hoogeveen RC, Ballantyne CM, Coresh J, et al. sRAGE and risk of diabetes, cardiovascular disease, and death. Diabetes. 2013 6;62(6):2116–21. [PubMed: 23396398]
24. Song F, Hurtado del Pozo C, Rosario R, Zou YS, Ananthakrishnan R, Xu X, et al. RAGE regulates the metabolic and inflammatory response to high-fat feeding in mice. Diabetes. 2014 6;63(6):1948–65. [PubMed: 24520121]
25. Palma-Duran SA, Kontogianni MD, Vlassopoulos A, Zhao S, Margariti A, Georgoulis M, et al. Serum levels of advanced glycation end-products (AGEs) and the decoy soluble receptor for AGEs (sRAGE) can identify non-alcoholic fatty liver disease in age-, sex- and BMI-matched normo-glycemic adults. Metabolism: clinical and experimental. 2018 6;83:120–7. [PubMed: 29409822]
26. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. The ARIC investigators. American journal of epidemiology. 1989 4;129(4):687–702. [PubMed: 2646917]
27. Bower JK, Pankow JS, Lazo M, Christenson E, Hoogeveen RC, Ballantyne CM, et al. Three-year variability in plasma concentrations of the soluble receptor for advanced glycation end products (sRAGE). Clinical biochemistry. 2014 1;47(1–2):132–4. [PubMed: 24246851]
28. Hu EA, Lazo M, Selvin E, Hamilton JP, Grams ME, Steffen LM, et al. Coffee consumption and liver-related hospitalizations and deaths in the ARIC study. European journal of clinical nutrition. 2019 8;73(8):1133–40. [PubMed: 30341433]
29. Lazo M, Rubin J, Clark JM, Coresh J, Schneider AL, Ndumele C, et al. The association of liver enzymes with biomarkers of subclinical myocardial damage and structural heart disease. Journal of hepatology. 2015 4;62(4):841–7. [PubMed: 25433159]
30. Salomone F, Micek A, Godos J. Simple Scores of Fibrosis and Mortality in Patients with NAFLD: A Systematic Review with Meta-Analysis. Journal of clinical medicine. 2018 8 15;7(8).
31. Lazo M, Halushka MK, Shen L, Maruthur N, Rebholz CM, Rawlings AM, et al. Soluble receptor for advanced glycation end products and the risk for incident heart failure: The Atherosclerosis Risk in Communities Study. American heart journal. 2015 11;170(5):961–7. [PubMed: 26542505]
32. Bucciarelli LG, Wendt T, Qu W, Lu Y, Lalla E, Rong LL, et al. RAGE blockade stabilizes established atherosclerosis in diabetic apolipoprotein E-null mice. Circulation. 2002 11 26;106(22):2827–35. [PubMed: 12451010]
33. Yamagishi S Comment on: Selvin et al. sRAGE and risk of diabetes, cardiovascular disease, and death. Diabetes 2013;62:2116–2121. [PubMed: 23396398]
34. Yamagishi S, Matsui T. Soluble form of a receptor for advanced glycation end products (sRAGE) as a biomarker. Frontiers in bioscience. 2010 6 1;2:1184–95.
35. Ballestri S, Nascimbeni F, Baldelli E, Marrazzo A, Romagnoli D, Targher G, et al. Ultrasonographic fatty liver indicator detects mild steatosis and correlates with metabolic/histological parameters in various liver diseases. Metabolism: clinical and experimental. 2017;72:57–65 [PubMed: 28641784]
36. Nascimbeni F, Pais R, Bellentani S, Day CP, Ratziu V, Loria P, et al. From NAFLD in clinical practice to answers from guidelines. Journal of hepatology. 2013;59(4):859–71 [PubMed: 23751754]
37. Kim BK, Kim DY, Park JY, Ahn SH, Chon CY, Kim JK, et al. Validation of FIB-4 and comparison with other simple noninvasive indices for predicting liver fibrosis and cirrhosis in hepatitis B virus-infected patients. Liver international : official journal of the International Association for the Study of the Liver. 2010 4;30(4):546–53. [PubMed: 20074094]
38. Shah AG, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ, et al. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2009 10;7(10):1104–12. [PubMed: 19523535]
39. Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology. 2006 6;43(6):1317–25. [PubMed: 16729309]

40. Mehta SH, Brancati FL, Strathdee SA, Pankow JS, Netski D, Coresh J, et al. Hepatitis C virus infection and incident type 2 diabetes. Hepatology. 2003;38(1):50–6 [PubMed: 12829986]

41. Asadipooya K RAGE is a potential cause of onset and progression of nonalcoholic fatty liver disease. International Journal of Endocrinology. 2019 9 18, 2019;2019.

42. Litwinoff E, Hurtado Del Pozo C, Ramasamy R, Schmidt AM. Emerging Targets for Therapeutic Development in Diabetes and Its Complications: The RAGE Signaling Pathway. Clinical pharmacology and therapeutics. 2015 8;98(2):135–44. [PubMed: 25974754]

43. Ku SH, Hong J, Moon HH, Jeong JH, Mok H, Park S, et al. Deoxycholic acid-modified polyethylenimine based nanocarriers for RAGE siRNA therapy in acute myocardial infarction. Archives of pharmacal research. 2015 7;38(7):1317–24. [PubMed: 25559468]

44. Matsui T, Higashimoto Y, Nishino Y, Nakamura N, Fukami K, Yamagishi SI. RAGE-Aptamer Blocks the Development and Progression of Experimental Diabetic Nephropathy. Diabetes. 2017 6;66(6):1683–95. [PubMed: 28385802]

45. Manigrasso MB, Pan J, Rai V, Zhang J, Reverdatto S, Quadri N, et al. Small Molecule Inhibition of Ligand-Stimulated RAGE-DIAPH1 Signal Transduction. Scientific reports. 2016 3 3;6:22450. [PubMed: 26936329]

46. Galasko D, Bell J, Mancuso JY, Kupiec JW, Sabbagh MN, van Dyck C, et al. Clinical trial of an inhibitor of RAGE-Abeta interactions in Alzheimer disease. Neurology. 2014 4 29;82(17):1536–42. [PubMed: 24696507]

47. Study of Azeliragon in Patients With Mild Alzheimer's Disease and Impaired Glucose Tolerance. https://ClinicalTrials.gov/show/NCT03980730.

Table 1:

Baseline characteristics of the study population by quartiles of sRAGE

sRAGE Quartiles†	**Q1**	**Q2**	**Q3**	**Q4**	
Mean (Range), pg/mL	**555.4 (119.4–714.1) (n=272)**	**845.4 (714.7–970.0) (n=272)**	**1118.8 (970.3–1276.0) (n=272)**	**1712.0 (1277.1–4650.4) (n=272)**	**p-for-trend**
Age - mean (SD)†	56 (6)	57 (6)	56 (6)	56 (6)	0.925
Gender					<0.001
Male	122 (45)	113 (42)	115 (42)	79 (29)	
Female	150 (55)	159 (58)	157 (58)	193 (71)	
Race					<0.001
Caucasian	146 (54)	212 (78)	238 (88)	253 (93)	
African American	126 (46)	60 (22)	34 (13)	19 (7)	
BMI† (kg/m^2)					<0.001
<25	46 (17)	80 (29)	92 (34)	124 (46)	
25–<30	96 (35)	103 (38)	107 (39)	115 (42)	
≥30	130 (48)	89 (33)	73 (27)	33 (12)	
Diabetes‡	42 (15)	28 (10)	19 (7)	11 (4)	<0.001
Hypertension‡	118 (43)	89 (33)	73 (27)	69 (25)	<0.001
Hypercholesterolemia‡	164 (60)	178 (65)	156 (57)	140 (51)	0.010
Triglycerides ≥ 150 mg/dL	76 (28)	80 (29)	85 (31)	69 (25)	0.631
Low HDL cholesterol†‡	107 (39)	115 (42)	112 (41)	100 (37)	0.507
CRP†					<0.001
<1 mg/L	27 (10)	60 (22)	72 (26)	91 (33)	
1–<3 mg/L	100 (37)	106 (39)	120 (44)	111 (41)	
≥3 mg/L	145 (53)	106 (39)	80 (29)	70 (26)	
Alcohol Use					0.228
Current	138 (51)	149 (55)	168 (62)	155 (57)	
Former	71 (26)	58 (21)	46 (17)	46 (17)	
Never	63 (23)	65 (24)	58 (21)	71 (26)	
Tobacco Use					0.033
Current	37 (14)	51 (19)	48 (18)	43 (16)	
Former	132 (49)	106 (39)	98 (36)	93 (34)	
Never	103 (38)	115 (42)	126 (46)	136 (50)	
ALT (U/L) median (IQR)†	15 (9)	15 (8)	14 (7)	14 (7)	0.012
AST (U/L) median (IQR)†	19 (6)	20 (7)	19 (7)	20 (6)	0.291
Elevated ALT‡	21 (8)	14 (5)	15 (6)	10 (4)	0.057
Elevated AST‡	11 (4)	18 (7)	8 (3)	11 (4)	0.509
FIB-4 Index median (IQR)†	1.13 (0.52)	1.17 (0.55)	1.16 (0.54)	1.23 (0.60)	0.001

sRAGE Quartiles[†]	**Q1**	**Q2**	**Q3**	**Q4**	
Mean (Range), pg/mL	**555.4 (119.4–714.1) (n=272)**	**845.4 (714.7–970.0) (n=272)**	**1118.8 (970.3–1276.0) (n=272)**	**1712.0 (1277.1–4650.4) (n=272)**	**p-for-trend**
Elevated FIB-4 Index[‡]	89 (33)	108 (40)	100 (37)	120 (44)	0.018
Platelet count/mm^3 median (IQR)[†]	261 (72)	249 (75)	248 (80)	247 (59)	0.012

Data are presented as total number followed by percentage in parentheses (n(%)) unless otherwise indicated

[†]Q - Quartile, SD - Standard Deviation, BMI - Body mass index, HDL - High-density lipoprotein, CRP - C-reactive protein, ALT - Alanine aminotransferase, AST - Aspartate aminotransferase, IQR - Interquartile Range

[‡]Diabetes: HbA1C ≥6.5%, self-report, physician diagnosis or medication use; Hypertension: systolic blood pressure ≥ 140, diastolic blood pressure ≥ 90 or medication use; Hypercholesterolemia: total cholesterol ≥ 200 mg/dL or medication use; Low HDL cholesterol: <50 mg/dL in Females, <40 mg/dL in Males; Elevated ALT: >27 in Females, >33 in Males; Elevated AST: >31 in Females, >33 in Males; Elevated FIB-4 Index ≥ 1.30

Table 2:

Cross-sectional associations between sRAGE, elevated liver enzymes and FIB-4 index by quartiles of sRAGE

Outcome[†]	sRAGE Quartile	Unadjusted OR (95% CI)	p-value	Adjusted[‡] OR (95% CI)	p-value	p-for-trend
Elevated ALT	Q1	2.19 (1.01–4.75)	0.05	2.82 (1.18–6.76)	0.02	0.03
	Q2	1.42 (0.62–3.26)	0.41	1.44 (0.60–3.44)	0.41	
	Q3	1.53 (0.67–3.47)	0.31	1.59 (0.68–3.72)	0.28	
	Q4	1 (Reference)		1 (Reference)		
Elevated AST	Q1	1.00 (0.43–2.35)	1.00	1.16 (0.45–2.99)	0.76	0.33
	Q2	1.68 (0.78–3.63)	0.19	1.88 (0.84–4.19)	0.13	
	Q3	0.72 (0.28–1.82)	0.49	0.78 (0.30–2.00)	0.61	
	Q4	1 (Reference)		1 (Reference)		
Elevated FIB-4	Q1	0.62 (0.43–0.87)	<0.01	0.56 (0.37–0.84)	<0.01	0.02
	Q2	0.83 (0.59–1.17)	0.30	0.86 (0.59–1.24)	0.42	
	Q3	0.74 (0.52–1.04)	0.08	0.75 (0.52–1.08)	0.12	
	Q4	1 (Reference)		1 (Reference)		

[†]Elevated ALT: >27 in Females, >33 in Males; Elevated AST: >31 in Females, >33 in Males; Elevated FIB-4 Index: ≥1.3

[‡]Age (ALT and AST analyses only), Race, Gender, Diabetes (yes/no), BMI (kg/m^2), Hypertension (yes/no), Total Cholesterol (mg/dL), HDL (mg/dL), Triglycerides (mg/dL), Tobacco Use (current, former, never), Alcohol Use (current, former, never).