ARIC Manuscript Proposal # 3336

PC Reviewed: 1/8/18	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1. a. Full Title: Association between sRAGE, FIB-4 score and liver enzymes

b. Abbreviated Title (Length 26 characters): sRAGE, FIB-4 and liver enzymes

2. Writing Group Members: Marci Laudenslager, Dan Wang, Jeanne Clark, Natalie Daya, Elizabeth Selvin, Victor Chen, Mariana Lazo; Others welcome

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. ML

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3. Timeline: All data are presently available. We anticipate an accelerated timeline for this manuscript and aim for manuscript submission to the ARIC Publications Committee approximately six months following proposal approval.

4. Rationale: Nonalcoholic fatty liver disease (NAFLD) is the most common form of liver disease.[1] The prevalence of NAFLD has at least doubled over the past twenty years as a consequence of current national health trends in obesity and type 2 diabetes.[1,2] Although the majority of patients with NAFLD are without clinically significant hepatocellular injury, many progress to nonalcoholic steatohepatitis (NASH), fibrosis and end stage chronic liver disease through complex inflammatory mechanisms that are poorly understood.[3] NAFLD has additionally been linked to hepatocellular carcinoma (HCC) and elucidation of pathways integral to this progression is key in the identification of potential targets for early intervention and subsequent prevention of irreversible hepatocellular injury and malignant transformation.[4]

Advanced glycation end products (AGEs) are glycosylated proteins known to trigger inflammatory pathways through binding to and subsequent activation of the receptor for advanced glycation end products (RAGE).[5-7] RAGE is expressed on a variety of cell types including endothelial cells and monocytes; activation has generally been shown to influence proinflammatory and profibrotic pathways through activation of TNF-α and NFκB.[8-10] The soluble receptor for advanced glycation end products (sRAGE) is released from the cell surface following proteolytic cleavage of RAGE, often by metalloproteinases.[11-12] Once released into the serum, sRAGE may act as a decoy receptor for AGEs thereby reducing AGE binding to RAGE and attenuating activation of inflammatory and pro-fibrotic pathways.[5,7,12,13]

Lower levels in circulating levels of sRAGE have been observed in diabetes, cardiovascular disease, renal disease and polycystic ovarian syndrome.[12-17] Alterations in the RAGE signaling pathway have additionally been observed in murine models for NAFLD and NASH.[5,6,10] While several studies have sought to evaluate the role of sRAGE in human NAFLD, these studies were small and primarily cross-sectional. Palma-Duran et al.[18] reported a 1.7-fold lower levels of sRAGE in age, sex and BMI matched normoglycemic patients with NAFLD (defined by liver enzyme elevation and steatosis noted on ultrasound; n=58) as compared to healthy controls. Yilmaz et al.[19] demonstrated a significant negative correlation between sRAGE and ALT, though this study was a small cross-sectional analysis with a heterogeneous subject distribution (NASH (n=40), borderline NASH (n=8), simple fatty liver (n=9), healthy controls (n=14)). Presence and degree of steatosis and inflammation were established by both ultrasound and liver histology. This study was performed in the Turkish population and analysis did not account for racial or ethnic differences. These factors, together with small sample size, significantly limit the generalizability of study results. Finally, Zelber-Sagi and collegues[20] reported significantly lower levels of sRAGE in NAFLD compared to controls following adjustments for age, gender, BMI and fasting insulin (NAFLD, n=55; Control, n=93). Hepatic steatosis was defined by steatosis on ultrasound and fibrosis estimated by a NAFLD Fibrosis Score (NFS) greater than -1.455. This study additionally demonstrated increases in sRAGE following a 3-month lifestyle intervention consisting of aerobic exercise and a specified dietary regimen.[20] Though statistical and design limitations exist in all aforementioned studies, each provides compelling preliminary data regarding potential associations between sRAGE and NAFLD. These studies further serve to highlight the need for a broader and more comprehensive investigation of the association between the RAGE pathway and NAFLD in humans.[18-20] Further, no studies have prospectively investigated the association between sRAGE and incident NAFLD.

If alterations in the RAGE pathway follow similar trends in humans to what has previously been demonstrated in murine models, sRAGE may be an important early predictor for hepatocellular injury. Assessment of sRAGE may therefore allow for intervention at the preclinical stage and possible attenuation or prevention of downstream sequelae of constitutive activation of inflammatory signaling pathways. We propose both cross-sectional and prospective analyses to examine the associations between serum sRAGE and markers of hepatocellular injury (liver enzymes and fibrosis risk score [FIB-4]) through the utilization of measurements and clinical data obtained during ARIC study visits 2 and 4.

5. Main Hypothesis/Study Questions:

Specific Aim 1: Characterize the cross-sectional associations between sRAGE, liver enzymes levels and fibrosis risk score (FIB-4) from data obtained at ARIC visit 2

> Hypothesis 1: sRAGE will be inversely and independently associated with elevations in liver enzymes and FIB-4 score.

Specific Aim 2: Characterize the association between sRAGE and incident elevated liver enzymes and FIB-4 score elevation among persons with normal levels of liver enzymes at baseline.

> Hypothesis 2: sRAGE will be inversely and independently associated with incident elevations in liver enzymes and FIB-4 score.

6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).

This study involves cross-sectional and prospective components.

Cross-Sectional Analysis:

> Study Population: Sub-cohort of ARIC study participants who attended visit 2 (1990-1992) for whom sRAGE, liver enzymes and platelet count data are available.
>
> sRAGE measurements are available for a random sample of 2,553 ARIC participants who were included in Ancillary Study #2006.16. We will exclude those participants for whom covariates of interest are missing or were not obtained.
>
> Subjects with alcoholic and nonalcoholic fatty liver disease may share common pathophysiologic mechanisms with respect to the AGEs-RAGE axis. We therefore aim to explore these associations by including all subjects irrespective of alcohol use and subsequently performing stratified analyses by alcohol consumption.
>
> Study Design: Cross-sectional

Exposure: sRAGE measured by ELISA (R&D Systems, CV<3%) from stored plasma samples at ARIC visit 2. sRAGE will be modeled as a continuous variable and categorized into quartiles for statistical analyses.

Outcomes: Liver enzyme levels and FIB-4 score (calculated from age, AST, ALT and platelet count) at ARIC study visit 2. AST and ALT will be measured as continuous variables and categorized into binary variables with the following definitions: For both, ALT and AST, we will define liver enzyme elevation as a level greater than or equal to 40 U/l. With respect to FIB-4 score, subjects will be categorized into low, intermediate and high likelihood of fibrosis groups based upon cut-off values of <1.30, 1.30-2.67 and >2.67, respectively. [21-22]

Other variables of interest: Age, sex, race, education level, body mass index, diabetes status, hypertension, C-reactive protein and alcohol use.

Statistical Analysis: We will perform descriptive statistical analyses with stratification by the outcome variables of interest as well as subset analyses stratified by outcome and alcohol consumption. We will then use multivariable logistic regression models to investigate cross-sectional associations between sRAGE levels, liver enzymes and FIB-4 indices. sRAGE will be modeled as a continuous variable and also categorized into quartiles for statistical analysis. AST and ALT will modeled as binary variables (>=40 U/l, <40 U/l). With respect to FIB-4 score, subjects will be categorized into low, intermediate and high risk of fibrosis groups based upon cut-off values of <1.30, 1.30-2.67 and >2.67, respectively. [20-21] We will then analyze FIB-4 score according to the following group: FIB-4 greater than 1.30 (intermediate plus high likelihood of fibrosis) compared to a score less than or equal to 1.30 (low likelihood of advanced fibrosis) and FIB-4 greater than or equal to 2.67 (high likelihood of advanced fibrosis) compared to a score of 1.30 or less.

We propose the following models for statistical analysis:

In Model 1 we will control for demographic factors including age, sex, race and education level. In Model 2, we will further adjust by the following potential confounders: diabetes status (self-reported physician diagnosis, medication use, A1C≥6.5), hypertension (self-reported history or systolic ≥ 140 or diastolic blood pressure ≥90 or medication use), C-reactive protein and alcohol use (self-reported history) as assessed at visit 1.

Model 1: Age, sex, race, education level
Model 2: Age, sex, race, body mass index, diabetes status, hypertension, C-reactive protein and alcohol use

We will examine the presence of interaction by sex, race and alcohol consumption (elevated vs. otherwise with elevated alcohol consumption defined as >14 drinks/week in men and >7 drinks/week in women).

Prospective Analysis:

Study Population: Sub-cohort of ARIC study participants who attended visit 2 (1990-1992) and visit 4 (1996-1998) for whom sRAGE (visit 2 only), liver enzyme and platelet count data are available. sRAGE measurements are available for a random sample of ARIC visit 2 participants who were included in Ancillary Study #2006.16. We will exclude those participants for whom covariates of interest are missing or were not obtained. Subjects with prevalent liver enzyme elevation and/or FIB-4 score >1.30 at visit 2 will additionally be excluded from analysis.

Similar to the cross-sectional analyses, we aim to explore the associations by including all subjects irrespective of alcohol use and subsequently performing stratified analyses by alcohol consumption.

Study Design: We will conduct prospective analyses with visit 2 as baseline and will examine the associations between baseline levels of sRAGE and incident liver enzyme and FIB-4 score elevation at visit 4.

Exposure: sRAGE measured by ELISA (R&D Systems, CV<3%) from stored plasma samples at ARIC visit 2. sRAGE will be measured as a continuous exposure and subsequently categorized into quartiles for statistical analysis.

Outcomes: Incident elevation of liver enzymes and FIB-4 indices at visit 4, as categorized defined in the cross-sectional analyses.

Other variables of interest: Age, sex race, education level, body mass index, diabetes status, hypertension, C-reactive protein and alcohol use.

Statistical Analysis: We will examine the prospective association between baseline sRAGE and incident liver enzyme and FIB-4 score elevations, as defined above. We will use logistic regression models with adjustment for potential confounders noted above.

Limitations: The possibility of residual confounding remains despite adjustment for known potential confounders. The use of sRAGE as a surrogate marker of RAGE activation and liver enzymes and FIB-4 score as markers of hepatocellular injury and surrogate markers for NAFLD are limitations of this analysis. The use of only one measurement of sRAGE at visit 2 is an additional methodologic limitation.

7.a. Will the data be used for non-CVD analysis in this manuscript? __X__ Yes ____ No

b. If Yes, is the author aware that the file ICTDER03 must be used to exclude persons with a value RES_OTH = "CVD Research" for non-DNA analysis, and for DNA analysis RES_DNA = "CVD Research" would be used? __X__ Yes ____ No
(This file ICTDER has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript? ____ Yes __X__ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER03 must be used to exclude those with value RES_DNA = "No use/storage DNA"? ____ Yes ____ No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://www.cscc.unc.edu/aric/mantrack/maintain/search/dtSearch.html

___X___ Yes _____ No

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

MSP# 1890 Determinants of sRAGE and its Association with Cardiovascular Disease, Diabetes, and Mortality in a Community based Population

MSP# 1905 The Association of Lifestyle Factors with circulating levels of the Soluble Receptor for Advanced Glycation End Products

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? __X__ Yes ____ No

11.b. If yes, is the proposal

__X_ A. primarily the result of an ancillary study (list number*1995.09, 2008.10, 2009.16)

___ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* _____ _____ _____)

*ancillary studies are listed by number at https://www2.cscc.unc.edu/aric/approved-ancillary-studies

12a. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is **your responsibility to upload manuscripts to PubMed Central** whenever the journal does not and be in compliance with this policy. Four files about the public access policy from http://publicaccess.nih.gov/ are posted

in http://www.cscc.unc.edu/aric/index.php, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to PubMed central.

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