ARIC Manuscript Proposal # 1890

PC Reviewed: 1/10/12	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Determinants of sRAGE and its Association with Cardiovascular Disease, Diabetes, and Mortality in a Community-based Population

b. Abbreviated Title (Length 26 characters): sRAGE and Cardiovascular Risk

2. Writing Group:

Writing group members: Elizabeth Selvin; Marc K. Halushka; Andreea Rawlings; Ron C. Hoogeveen; Christie M. Ballantyne; Josef Coresh; Brad Astor; others welcome

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. __ES___ **[please confirm with your initials electronically or in writing]**

First author: Elizabeth Selvin, PhD, MPH
Associate Professor of Epidemiology and Medicine
Johns Hopkins Bloomberg School of Public Health
Address: 2024 E Monument St, Suite 2-600, Baltimore MD 21287

Phone: 410-614-3752 Fax: 410-955-0476
E-mail: lselvin@jhsph.edu

ARIC author to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).

Name: **Brad Astor**
Division of Nephrology
Address: University of Wisconsin School of Medicine and Public Health
5149 MFCB
1685 Highland Avenue
Madison, WI 53705
Phone: (608) 262-0361 Fax: (608) 262-6743
E-mail: bcastor@medicine.wisc.edu

3. Timeline: We anticipate a rapid timeline for this manuscripts and plan to have a manuscript submitted to the ARIC Publications Committee ~6 months from approval of the proposal.

4. Rationale:

Advanced glycation end products (AGEs) are of interest as novel biomarkers because they are the postulated etiologic link between hyperglycemia and diabetic complications. AGEs can also bind a variety of receptors. When stimulated by AGEs, the receptor for AGEs (RAGE) induces inflammation and is thought to fuel progression of chronic disease through nuclear factor (NF)-kB mediated signaling (1-3). Circulating AGE and RAGE levels are influenced by a number of endogenous (glucose, inflammation) and exogenous (smoking, diet) factors known to play a role in risk of cardiovascular disease and diabetes. Nonetheless, it is unclear if circulating AGE receptor levels provide useful information regarding risk of long-term complications.

RAGE is a transmembrane-spanning receptor for AGEs, S100/calgranulins, amyloid-β peptide and other molecules. RAGE is found on inflammatory cells and endothelial cells. The C-terminus of the protein is located on the extracellular surface. The N-terminus of RAGE is essential in activating pro-inflammatory NF-κB mediated signaling. The soluble receptor for advanced glycation end products (sRAGE) is the isoform of RAGE found in serum and is formed by proteolytic cleavage of RAGE. sRAGE has been described as a "sponge" for AGEs and may have protective functions as it lacks the N-terminus and cannot activate NF-κB signaling. Levels are dependent on cell surface RAGE levels (4).

Recent studies have demonstrated epidemiological associations of serum sRAGE with cardiovascular disease in persons with diabetes or kidney disease (5-8) but few studies have been conducted in a general population and using robust ELISA methods (9-14). A recent case-cohort study published in *Diabetes* showed that low levels of sRAGE were associated with an increased risk of coronary heart disease but not stroke in an observational re-analysis of data from the Collaborative Atorvastatin Diabetes Study, a randomized clinical trial with 3.9 years of follow-up (14). Using data from Ancillary Study 2006.16, we propose to characterize the epidemiology of serum sRAGE in a subsample of the ARIC Study population and investigate the long-term prospective association with cardiovascular outcomes, diabetes, and total mortality.

5. Main Hypothesis/Study Questions:

There is evidence from laboratory studies that soluble circulating RAGE (sRAGE) counteracts the detrimental effects of RAGE by binding serum AGEs (15, 16), suggesting that low levels of sRAGE may be a marker of long-term chronic disease risk. Thus, the objectives of this study were:

Aim 1: To identify determinants of sRAGE in a community-based population.

Hypothesis 1: sRAGE levels will be inversely associated with age, body mass index, C-reactive protein, and will be lower among men, persons with diabetes, blacks, current smokers, and persons with poor kidney function.

Aim 2: To characterize the association of sRAGE with risk of incident coronary heart disease, stroke, diabetes, and all-cause mortality.

Hypothesis 2: sRAGE will be inversely and independently associated with risk of coronary heart disease, stroke, diabetes, and all-cause mortality.

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).

Study population: sRAGE data are available for a random sample of 1289 ARIC participants ages 47-68 (visit 2, 1990-92) with estimated GFR >60 mL/min/1.73 m^2 who were included in Ancillary Study # 2006.16. The basis of this study population is the random sub-cohort of participants selected for the parent case-cohort study of incident chronic kidney disease. We will exclude those persons missing covariates of interest and those with prevalent cardiovascular disease by visit 2. The final sample size will be approximately 1200 participants.

Study Design: prospective cohort analysis of the association of sRAGE levels measured in a random subcohort of ~1200 participants at visit 2 with incident CHD and stroke events, and all-cause mortality. Using the most recent follow-up data available (through 2008), there have been 200 incident CHD events, 55 incident ischemic strokes, and 208 total deaths in this subsample (during a median follow-up of approximately 17 years). We will also examine the cross-sectional associations between sRAGE and other visit 2 variables measured at baseline, focusing on traditional cardiovascular disease risk factors.

Exposure: sRAGE measured by ELISA (R&D Systems, CV<3%) from stored plasma samples at ARIC visit 2.

Outcomes: incident CHD events (adjudicated events, procedures, and deaths), incident ischemic stroke (adjudicated), and all-cause mortality. Incident diabetes will be defined using two definitions: one that incorporates information from visits 2 and 4 (elevated glucose measurements, diabetes medication use, self-reported physician diagnosis); and a second that includes all post-visit 4 self-reported diabetes cases using diagnosis/diabetes medication use identified during the AFU telephone calls. Analysis of incident diabetes will be conducted after additionally excluding prevalent cases of diabetes at baseline.

Other variables of interest: We will examine the cross-sectional associations of sRAGE with age, sex, race-center, body mass index, total cholesterol, systolic and diastolic blood pressures, blood pressure-lowering medication use, smoking status, drinking status, dietary data (FFQ), diabetes status (self-reported history, medication use, glucose, HbA1c), C-reactive protein, physical activity level (Baecke index, visit 1), and education level (visit 1). These variables will also be considered as confounders in our prospective analyses of the association of sRAGE levels with incident cardiovascular events and total mortality.

Statistical Analysis: We will use Cox proportional hazards models to investigate the association between sRAGE and incident cardiovascular events, diabetes, and total mortality with adjustment for potential confounders (listed above). The proportional hazards assumption will be examined using log-(-log) plots and by testing risk factor-by-time interactions; if the assumption is violated the interactions term(s) will be kept in the model and the time-dependent nature of the risk will be interpreted accordingly. Quartiles will be used in initial analyses and we will generate linear splines with knots corresponding to the quartiles of the distribution of sRAGE. Restricted cubic splines will also implemented to obtain a smoother fit to the data.

We will use logistic regression models to investigate the cross-sectional associations between traditional cardiovascular risk factors measured at visit 2 and low sRAGE levels (first quartile vs the other four). We will conduct sensitivity analyses examining the associations of risk factors with very low sRAGE levels (first decile) and to examine potential effect modification by age, sex and diabetes status.

Possible project extension: In this subsample of the ARIC Study population, our preliminary analyses suggest significant independent associations between sRAGE and incident CHD events, but not stroke. Due to the design, we will have limited power to detect small effect estimates since the sample size is limited to ~1200 persons with valid sRAGE data who were included in the Ancillary Study # 2006.16 sub-cohort random sample. Nonetheless, we can potentially take advantage of the case-cohort design of the parent Ancillary Study and identify additional cardiovascular disease cases that may overlap with the other case groups being examined in the CKD and ESRD analyses. These additional cases can be "captured" and added to our existing cases occurring in the sub-cohort random sample. Prospective analyses can then be conducted with a weighted design with robust variance estimation (to account for the oversampling of cases) using a modified Cox proportional hazards model.

7.a. Will the data be used for non-CVD analysis in this manuscript? ____ Yes __X__ 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? ____ Yes ____ No
(This file ICTDER03 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/search.php

__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)?

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*_2006.16_____)
_X__ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* ___ 2006.15_____
_____ _____)

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

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.
_ES_

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.
_ES_

Literature Cited

1. Yan SF, Ramasamy R, Schmidt AM. Mechanisms of disease: advanced glycation end-products and their receptor in inflammation and diabetes complications. Nat Clin Pract Endocrinol Metab. 2008;4(5):285-93.
2. Yan SF, Ramasamy R, Schmidt AM. The receptor for advanced glycation endproducts (RAGE) and cardiovascular disease. Expert Rev Mol Med. 2009;11:e9. PMCID: PMC2670065.
3. Yan SF, Barile GR, D'Agati V, Du Yan S, Ramasamy R, Schmidt AM. The biology of RAGE and its ligands: uncovering mechanisms at the heart of diabetes and its complications. Curr Diab Rep. 2007;7(2):146-53.
4. Busch M, Franke S, Ruster C, Wolf G. Advanced glycation end-products and the kidney. Eur J Clin Invest. 2010;40(8):742-55.
5. Meerwaldt R, Links T, Zeebregts C, Tio R, Hillebrands JL, Smit A. The clinical relevance of assessing advanced glycation endproducts accumulation in diabetes. Cardiovasc Diabetol. 2008;7:29. PMCID: PMC2569910.
6. 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 Int.77(3):225-31.
7. Gross S, van Ree RM, Oterdoom LH, de Vries APJ, 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;84(5):659-63.
8. Monnier VM, Sell DR, Genuth SAUL. Glycation Products as Markers and Predictors of the Progression of Diabetic Complications. Annals of the New York Academy of Sciences. 2005;1043(1):567-81.
9. Lindsey JB, de Lemos JA, Cipollone F, Ayers CR, Rohatgi A, Morrow DA, et al. Association between circulating soluble receptor for advanced glycation end products and atherosclerosis: observations from the Dallas Heart Study. Diabetes Care. 2009;32(7):1218-20. PMCID: PMC2699719.
10. Falcone C, Emanuele E, D'Angelo A, Buzzi MP, Belvito C, Cuccia M, et al. Plasma levels of soluble receptor for advanced glycation end products and coronary artery disease in nondiabetic men. Arterioscler Thromb Vasc Biol. 2005;25(5):1032-7.
11. Semba RD, Ferrucci L, Fink JC, Sun K, Beck J, Dalal M, et al. Advanced glycation end products and their circulating receptors and level of kidney function in older community-dwelling women. American Journal Of Kidney Diseases: The Official Journal Of The National Kidney Foundation. 2009;53(1):51-8.
12. Semba RD, Ferrucci L, Sun K, Beck J, Dalal M, Varadhan R, et al. Advanced glycation end products and their circulating receptors predict cardiovascular disease mortality in older community-dwelling women. Aging Clinical And Experimental Research. 2009;21(2):182-90.
13. Yamagishi S-i, Adachi H, Nakamura K, Matsui T, Jinnouchi Y, Takenaka K, et al. Positive association between serum levels of advanced glycation end products and the soluble form of receptor for advanced glycation end products in nondiabetic subjects. Metabolism: Clinical And Experimental. 2006;55(9):1227-31.
14. Colhoun HM, Betteridge DJ, Durrington P, Hitman G, Neil A, Livingstone S, et al. Total soluble and endogenous secretory receptor for advanced glycation end products

as predictive biomarkers of coronary heart disease risk in patients with type 2 diabetes: an analysis from the CARDS trial. Diabetes. 2011;60(9):2379-85. PMCID: PMC3161327.
15. Wautier JL, Zoukourian C, Chappey O, Wautier MP, Guillausseau PJ, Cao R, et al. Receptor-mediated endothelial cell dysfunction in diabetic vasculopathy. Soluble receptor for advanced glycation end products blocks hyperpermeability in diabetic rats. J Clin Invest. 1996;97(1):238-43. PMCID: PMC507085.
16. Park L, Raman KG, Lee KJ, Lu Y, Ferran LJ, Jr., Chow WS, et al. Suppression of accelerated diabetic atherosclerosis by the soluble receptor for advanced glycation endproducts. Nat Med. 1998;4(9):1025-31.