

## ARIC Manuscript Proposal #2207

PC Reviewed: 9/10/11  
SC Reviewed: \_\_\_\_\_

Status: A  
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Priority: 2  
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**1.a. Full Title:** Associations of C-reactive protein over six years with incident diabetes, cardiovascular events and mortality

**b. Abbreviated Title (Length 26 characters):** CRP, Diabetes, CVD, Death

### 2. Writing Group:

Writing group members: Christina M. Parrinello; Pamela L. Lutsey; Christie M. Ballantyne; Aaron R. Folsom; Jim S. Pankow; Elizabeth Selvin; others welcome

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

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**3. Timeline:** We plan on submitting the completed manuscript to the ARIC subcommittee within one year.

#### 4. Rationale:

C-reactive protein (CRP) is an acute-phase reactant produced in the liver, and is secreted into the bloodstream in response to the presence of pro-inflammatory cytokines. It is therefore considered a marker of inflammation, which has been implicated in the development of atherosclerosis<sup>1-3</sup>, insulin resistance and diabetes<sup>4,5</sup>. High CRP has been widely studied and associated with cardiovascular disease (CVD)<sup>6-10</sup>, incident diabetes<sup>11-18</sup> and all-cause mortality<sup>9,10</sup> in various populations.

It is unclear whether changes in CRP or trajectories of CRP over time are associated with increased risk of incident CVD, incident diabetes or mortality. Previous papers that have used a single measure of CRP as the exposure were unable to account for the time-varying nature of CRP as a marker of inflammation, which may better characterize a person's long-term inflammatory state. It is possible that an increase in CRP may reflect an acute change in health status (such as atherosclerotic plaque instability or rupture), which would lead to increased risk of an event. In this case, we may expect either change in CRP or most recent CRP level to be most predictive of outcomes. Alternatively, exposure to long-term sustained inflammation may drive atherogenesis and/or insulin resistance, in which case we may expect to observe strongest associations between sustained high levels of CRP and outcomes.

A few recent studies have assessed the association between change in CRP over several years and subsequent CVD/mortality. Either increase in CRP or the final (follow-up) CRP was shown to be associated with increased risk of mortality<sup>19-21</sup>. However, in the Cardiovascular Health All Stars Study change in CRP was not associated with increased risk of CVD<sup>20</sup>. In the Whitehall II Study, CRP was consistently higher in both persons who died of CVD and persons who developed diabetes compared to those who did not die or develop diabetes, and the trajectories of CRP over time were similar between the "case" and comparison groups<sup>22</sup>.

Many studies have assessed the addition of CRP to risk prediction models – both for prediction of CHD and diabetes. These studies have added a single measure of CRP to a model that includes traditional risk factors. CRP slightly improves the prediction of CHD risk<sup>7,23</sup>. Results of studies assessing the addition of CRP in prediction models for diabetes have been equivocal. While some have shown that the addition of one measure of CRP improves prediction of impaired fasting glucose, impaired glucose tolerance, or diabetes<sup>24,25</sup>, others have reported no improved prediction of incident Type 2 diabetes/hyperglycemia<sup>26-29</sup>. However, a clinically important question is whether having more than one CRP measurement is useful in risk prediction. For instance, could we improve risk stratification by knowing if a person has sustained high levels of CRP as opposed to one high CRP measurement then a 2<sup>nd</sup> low measurement?

Although CRP is a well-studied and well-known inflammatory biomarker, there is a lack of data regarding longitudinal changes in a community-based population. Currently, it is not known whether changes in CRP or long-term CRP status are clinically relevant. We propose to characterize CRP status in ARIC participants over six years ("six-year CRP

status”), by categorizing people into clinically relevant groups (persistently low, low to medium, low to high, medium to low, persistently medium, medium to high, high to low, high to medium and persistently high). We will compare the strength of associations of baseline CRP, follow-up CRP, and six-year CRP status with incident CVD, incident diabetes and mortality. Further, we will assess whether the addition of six-year CRP status to models containing the traditional risk factors improves prediction of each CVD and diabetes. This study will have the benefit of having more than a decade of follow-up from the Visit 4 examination (1996-1998) through 2010.

## **5. Main Hypothesis/Study Questions:**

**Aim 1:** To characterize the magnitude of six-year change in CRP and the six-year CRP status in all ARIC participants. To compare the magnitude of change across certain subgroups of participants (i.e. diabetes status, BMI category, gender, race/ethnicity), as well as in those who experience the outcome versus those who do not.

**Aim 2:** To assess the associations between baseline (visit 2) CRP, follow-up (visit 4) CRP, and both baseline/follow-up CRP with incident CVD, incident diabetes and mortality.

**Aim 3:** To assess the association between change/six-year CRP status in CRP and incident CVD, incident diabetes and all-cause mortality.

**Aim 4:** To assess the addition of six-year CRP status/change in CRP to risk prediction models for each incident CVD and diabetes.

## **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).**

### **Design and Methods**

Study population: The study population will consist of ARIC participants who have CRP measurements and key covariates available for both visits 2 (1990-1992) and 4 (1996-1998), who do not have a history of CVD or diabetes.

Study design: We will conduct a prospective cohort analysis to assess the association between CRP levels at two points in time six years apart, as well as six-year CRP status, and incident CVD, incident diabetes and all-cause mortality after visit 4. We will use a time-to-event analysis. Secondly, we will assess the addition of six-year CRP status to risk prediction models for each CVD and diabetes. For these analyses, we will calculate the area under receiver operating characteristic (ROC) curves (AUC) to compare

discrimination of models. We will also calculate the net reclassification index (NRI) to assess improvement in risk stratification.

Exposures: CRP is the main exposure of interest. CRP will be examined both as a continuous variable (ln-transformed) and a categorical variable (using clinical cutoffs: <1 mg/L, 1-3 mg/L and >3 mg/L; using study population tertiles; and as a binary variable: increase vs. no change/decrease). We will also assess absolute and relative changes in CRP between the 2 study visits. In order to be most clinically relevant, six-year CRP status will be categorized as a 9-level variable to indicate either change in or persistence of CRP levels over time: persistently low, low to medium, low to high, medium to low, persistently medium, medium to high, high to low, high to medium and persistently high.

To note: A calibration study is currently underway to compare lab assays for ARIC visits 1-5, including the hsCRP assay at visits 2 and 4. If it is determined that there are systematic differences between these two assays and calibration is necessary, we will incorporate the necessary corrections into our study.

Outcome Definitions:

We will use the following endpoints: CVD (coronary heart disease (CHD), stroke, heart failure), diabetes and all-cause mortality. (CVD and mortality were adjudicated after 2004.)

*Cardiovascular disease:*

*Incident CHD:* CHD will be defined as definite/probable MI, cardiac procedures (including angioplasty/stenting and CABG), and deaths from CHD obtained from hospitalization data and deaths among ARIC participants.

*Incident stroke:* We will define stroke as fatal and non-fatal ischemic stroke.

*Incident heart failure:* We will define heart failure as hospitalizations and deaths attributed to heart failure.

*Incident Diabetes:*

Using the annual follow-up data, incident diabetes after V4 will be defined as self-report of diagnosis of diabetes by a physician or self-reported use of glucose-lowering medication. We may also conduct sensitivity analyses incorporating the fasting glucose measurements at visit 4.

*All-cause mortality:*

Death from any cause identified from V4 until the end of follow-up for this study, obtained from active surveillance.

Covariates:

Age, gender, race/ethnicity, field center, smoking, BMI, DBP, SBP, total cholesterol, HDL-c, LDL-c, triglycerides, statin use, antihypertensives, self-reported diabetes, glucose-lowering medication use, fasting glucose, A1c, OGTT, self-reported prevalent

CHD, alcohol consumption, education, eGFR/chronic kidney disease. We will adjust for covariate values at visit 2, and may additionally consider covariates as time-varying variables, and update values at visit 4 in prospective analyses.

Exclusions: Persons with prevalent CVD or prevalent diabetes at V4 will be excluded from the CVD and diabetes analyses, respectively. We will additionally exclude any participants who do not have CRP measures at both visits, or who are missing key variables. Lastly, we will exclude participants who reported race other than white/black, and black participants in the Minneapolis and Washington County cohorts.

Statistical Analysis:

*Aim 1:* We will characterize baseline, follow-up and change in CRP/six-year CRP status overall and by subgroups (i.e. gender, age group, race-center, diabetes status, etc.). To assess change in CRP, we will calculate the mean/median magnitude and percent change in CRP.

*Aim 2:* We will run several Cox proportional hazards regression models for each outcome, as follows: only baseline (visit 2) CRP; only follow-up (visit 4) CRP; both baseline and follow-up CRP simultaneously.

*Aim 3:* We will use Cox proportional hazards regression to assess the association between change in CRP/six-year CRP status and incident CVD, incident diabetes and mortality. We will conduct analyses considering CRP as both a continuous and categorical variable.

*Aim 4:* We will calculate the AUC to compare the ability of models with and without the addition of six-year CRP status to discriminate between people with and without the outcome of interest in the ARIC study. We will use Chambless & Diao's c-statistic, which enables the application of the c-statistic for survival data<sup>30</sup>. We will also calculate the net reclassification index (NRI) to determine if the addition of six-year CRP status improves risk stratification for each CVD and diabetes.

*Sensitivity analyses:*

1) We will conduct a sensitivity analysis excluding participants on lipid-lowering therapy (LLT) at visit 4; and

2) excluding people with high CRP values (e.g. >10 mg/L), which may be indicative of an acute infection.

Limitations:

- CRP was only measured at two time points, which may not capture the full scope of change in each individual over this time period.
- We may not be able to fully control for all possible biases, which could result in residual confounding in the association between CRP and CVD/mortality.
- There was loss to follow-up and deaths between visits 2 and 4, which could potentially result in selection bias.

7.a. Will the data be used for non-CVD analysis in this manuscript?  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?   
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  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.csc.unc.edu/ARIC/search.php>  
 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)?

*CRP and Venous Thromboembolism Incidence (MS1479) Folsom, A.*

*CRP, WBC and Heart Failure Incidence (MS1504) Folsom, A.*

*CRP and CVD (MS606) Folsom, A. R.*

*Association of high sensitive Troponin T (hs-cTnT), N-Terminal pro-brain natriuretic peptide (NT-proBNP) and high sensitivity C-reactive protein (hs-CRP) with cause-specific mortality: ARIC study (MS1811) Oluleye, O. W.*

*C-reactive protein and mortality in individuals with atrial fibrillation: the ARIC Study (MS1665) Hermida, J.*

*Reanalysis and simulation of Lp-PLA2/hs-CRP/CHD case-cohort study data (Ballantyne et al., Circulation 109:837-42, 2004) using all analysis data available for potential full cohort Breslow, N.*



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16. Laaksonen DE, Niskanen L, Nyssonen K, et al. C-reactive protein and the development of the metabolic syndrome and diabetes in middle-aged men. *Diabetologia*. Aug 2004;47(8):1403-1410.
17. Liu S, Tinker L, Song Y, et al. A prospective study of inflammatory cytokines and diabetes mellitus in a multiethnic cohort of postmenopausal women. *Archives of internal medicine*. Aug 13-27 2007;167(15):1676-1685.
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