**ARIC Manuscript Proposal # 1623**

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|---|---|---|
| **PC Reviewed: 3/9/10** | **Status: A** | **Priority: 2** |
| **SC Reviewed: _____** | **Status: _____** | **Priority: ____** |

**1.a. Full Title**: Apolipoprotein B, Apolipoprotein A1 and Standard Lipid Measures in the Prediction of Incident Coronary Heart Disease: The Atherosclerosis Risk in Communities (ARIC) Study

**b. Abbreviated Title (Length 26 characters)**: Apolipoproteins and CHD risk

**2. Writing Group**: Chiadi E. Ndumele; Josef Coresh; Salim Virani; Kunihiro Matsushita; Christie M. Ballantyne; Roger S. Blumenthal; Brad Astor; others welcome

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


**First author**: Chiadi E. Ndumele, MD
Address: Candidate for Masters in Health Science Degree
Welch Center for Prevention, Epidemiology and Clinical Research, and the Johns Hopkins Bloomberg School of Public Health
2024 E. Monument Street, Suite 2-600
Baltimore MD 21287

Phone: 410-955-0495 Fax: 410-955-0476
E-mail: cndumel2@jhmi.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: Josef Coresh
Address: Professor
Johns Hopkins University
2024 E. Monument Street, Suite 2-600
Baltimore MD 21287

Phone: 410-955-0495 Fax: 410-955-0476
E-mail: coresh@jhu.edu


**3. Timeline**: We expect all apolipoprotein assays from Visit 4 to be complete by March 2010. Analyses will be initiated once all data are cleaned. We aim to submit this manuscript to the ARIC publications committee in <6 months from this date.

## 4. Rationale:

Dyslipidemia is a major risk factor for coronary heart disease (CHD)[1]. Standard lipid measurements, including concentrations of total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides, predict future CHD and are utilized in current cardiovascular screening algorithms[2]. In recent years, assays have been standardized for the measurement of apolipoproteins, the structural proteins for the lipoproteins that transport lipids through the circulation[3]. There is longstanding interest in determining whether apolipoprotein concentrations are better predictors of cardiovascular disease than standard lipid measurements. Evaluations of apolipoprotein B and apolipoprotein A1 concentrations have drawn particular focus. One apolipoprotein B is present on the surface of each particle of very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL) and LDL; therefore apolipoprotein B measurements represent the aggregate of atherogenic particles in the circulation. In contrast, apolipoprotein A1 is found within HDL, and is therefore reflective of the concentration of anti-atherogenic particles.

Comparisons of the prognostic value of apolipoproteins and standard lipid measurements have yielded conflicting results: some studies have demonstrated superiority of apolipoprotein concentrations to standard lipids in predicting cardiovascular disease[4-6]. Other studies, including ARIC, have shown comparable predictive value, with no additional prognostic information gained by the measurement of apolipoproteins[7-10]. One factor that may have affected past comparisons of apolipoproteins and standard lipids is the limited reliability of the assays utilized. Earlier assays for apolipoproteins, including those used in prior ARIC analyses, were marked by poor precision, with coefficients of variation in the range of 12-17%[10]. In this study, apolipoproteins will be measured using newer, standardized immunoturbidometric assays (Siemens BN II) with coefficients of variation of < 5%.

It has also been hypothesized that the conflicting results of past studies may reflect the underlying risk of the study populations[11]. Apolipoprotein B measurements, which represent the number of atherogenic lipoproteins, may be more predictive than traditional assessments of cholesterol concentrations among higher risk individuals with elevated numbers of dense lipoprotein particles. Diabetes, obesity, the metabolic syndrome, and hypertriglyceridemia are most closely associated with the presence of small, dense atherogenic lipoproteins, as well as with increased cardiovascular risk[12]. We will compare the predictive value of apolipoproteins and standard lipid measurements in the overall ARIC cohort and within these high-risk subgroups, to help define optimal strategies for cardiovascular risk prediction.

## 5. Main Hypothesis/Study Questions:

**Aim**: To determine whether apolipoprotein B, apolipoprotein A1 and the apolipoprotein B/apolipoprotein A1 ratio are better predictors of incident CHD than standard lipid

measures. Comparisons of apolipoproteins and standard lipid measures will be performed in the overall ARIC cohort and within prespecified subgroups of individuals with diabetes (self reported physician diagnosis), obesity (body-mass index ≥ 30 kg/m2), hypertriglyceridemia (≥ 200 mg/dl), and the metabolic syndrome (as defined by the National Heart, Lung, and Blood Institute (NHLBI) - any 3 of the following conditions: abdominal obesity, hypertension, hypertriglyceridemia, low HDL-C, and insulin resistance).

**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 design**: We will compare the prospective associations of apolipoproteins and standard lipid measures with incident coronary heart disease, using ARIC Visit 4 as the baseline for all analyses. Visit 4 took place from 1996-1999 and is the only visit for which apolipoprotein B has been measured for all participants using newly standardized immunoturbidometric assays. Standard lipid measures are available for all participants at each of the ARIC examinations.

**Exposures**: Total cholesterol concentration, cholesterol concentrations in lipoprotein subfractions (LDL-C, HDL-C, Non-HDL-C), apolipoprotein B, and apolipoprotein A1. Cholesterol concentrations will be compared to their respective apolipoprotein concentrations, and cholesterol ratios will be compared to the apolipoprotein B/apolipoprotein A1 ratio as detailed in the table below:

| | **Lipoprotein subfractions** | **Apolipoproteins** |
|---|---|---|
| Comparison of Individual Measures | Total cholesterol<br>LDL-C<br>Non-HDL-C | Apolipoprotein B |
| | HDL-C | Apolipoprotein A1 |
| Comparison of Ratios | Total cholesterol/HDL-C<br>Non-HDL-C/HDL-C<br>LDL-C/HDL-C | Apolipoprotein B/Apolipoprotein A1 |

**Outcomes**: The primary outcome will be incident CHD, as defined by ARIC, occurring after Visit 4 through 2005 (or most current follow-up available). Secondary outcomes will include the specific endpoints of nonfatal MI, fatal MI/CHD, cardiac procedures, and CHD events excluding cardiac procedures.

**Exclusions**: Because this analysis is focused upon the predictive value of lipids in the primary prevention setting, we will exclude participants with known CHD prior to Visit 4 (self reported CHD at Visit 1, or adjudicated CHD events from Visit 1 to Visit 4). We

will also exclude participants missing covariates of interest and women taking hormone replacement therapy at baseline.

**Covariates**: Age, sex, race, smoking status, systolic and diastolic blood pressures, blood pressure medication use, statin use, family history of premature CHD, BMI, diabetes, and triglycerides.

**Main Analyses**: Cox proportional hazards models will be used to determine the associations of standard lipid measures (Total cholesterol, LDL-C, HDL-C and non-HDL-C), apolipoproteins, and cholesterol and apolipoprotein ratios with incident coronary heart disease after adjusting for covariates of interest.

1) We will assess the inter-relationships between the various lipid markers using scatterplots and the age-adjusted Spearman's partial correlation coefficient.
2) Adjusted hazards ratios and 95% CIs will be calculated for the risk associated with a 1 standard deviation higher level for each lipid measure, and for the risk associated with increasing quintiles of each lipid measure.
3) We will model each standard lipid measure and respective apolipoprotein levels simultaneously to examine the relative hazard of each adjusted for the other (e.g., non-HDL-C and apolipoprotein B). We will also model standard cholesterol ratios and the apolipoprotein B/apolipoprotein A1 ratio simultaneously to examine the relative hazard of each adjusted for the other (e.g., Total cholesterol/HDL-C and apolipoprotein B/apolipoprotein A1). We will consider the reduced power due to the correlation of the lipoprotein and apolipoprotein variables in the model and examine the increase in the standard error of the estimates in interpreting these models.
4) If the analyses above demonstrate meaningful differences in the relative hazard of CHD associated with apolipoproteins and standard lipid measures, we will: a) compare the receiver operator curves in relation to incident CHD for each measure; and b) calculate the net reclassification improvement for incident CHD risk associated with the measurement of apolipoproteins.
5) Statin Use – Sensitivity analyses will quantify statin use and conduct analyses censored at initiation of statin use (if sample size allows).
6) Fasting status – Some participants were not fasting. Similar to other published studies, we will conduct analyses stratified by fasting status[6, 8].

**Subgroup Analyses**: The comparisons of standard lipid measures and apolipoproteins described in the Main Analyses section above will also be performed within pre-specified subgroups: individuals with a) diabetes mellitus; b) obesity; c) hypertriglyceridemia; and d) the metabolic syndrome, as defined by the NHLBI. Analyses stratified by gender will also be performed.

**Secondary Analyses**: We will also compare the associations of standard lipid measures and apolipoproteins with subsets of incident CHD events – CHD events excluding procedures, non-fatal MI, fatal MI/CHD, and cardiac procedures.

**Limitations**:

- New vs. old apolipoprotein assay – Generally, the new apolipoprotein assays have been performed at Visit 4, and the old apolipoprotein assays were performed at Visit 1 and 2. However, there is a subset of Visit 2 participants (in a chronic kidney disease case-control study) for whom the new apolipoprotein assay will be performed on stored specimen. This allows a direct prospective comparison of the new and old apolipoprotein assays in relation to incident CHD prediction within a subgroup of Visit 2 participants. In addition, we will examine and compare the association of mean (as well as median and percentiles) apolipoprotein levels versus age, stratified by sex-race group, across the ARIC visits.
- Statin use – The high prevalence of statin use in our study population inherently complicates evaluations of the associations of apolipoproteins and standard lipid measures with incident CHD.

**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)?**

**Sharrett AR, Ballantyne CM, Coady SA, Heiss G, Sorlie PD, Catellier D, Patsch W;**

**Atherosclerosis Risk in Communities Study Group. Coronary heart disease prediction from lipoprotein cholesterol levels, triglycerides, lipoprotein(a), apolipoproteins A-I and B, and HDL density subfractions: The Atherosclerosis Risk in Communities (ARIC) Study. Circulation. 2001 Sep 4;104(10):1108-13.**

**ARIC Proposal #1610: Associations between lipoprotein(a) levels and cardiovascular outcomes in African Americans: The Atherosclerosis Risk In Communities (ARIC) Study. Writing group members: Christie M. Ballantyne MD, Vijay Nambi MD, Salim S. Virani MD, Ariel Brautbar MD, Ron C. Hoogeveen PhD, Eric Boerwinkle PhD, Gerardo Heiss MD Ph D, A. Richey Sharrett MD DrPH, Josef Coresh MD PhD, Thomas H. Mosley MD, Joel D Morrisett PhD, Aaron R Folsom MD, Diane J. Catellier DrPH.**

**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**

**___ A. primarily the result of an ancillary study (list number* _____)**
**_x__ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* __2006.16_____ _____ _____)**

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

**12. 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.**

Reference List


(1) Pearson TA, Blair SN, Daniels SR et al. AHA Guidelines for Primary Prevention of Cardiovascular Disease and Stroke: 2002 Update: Consensus Panel Guide to Comprehensive Risk Reduction for Adult Patients Without Coronary or Other Atherosclerotic Vascular Diseases. American Heart Association Science Advisory and Coordinating Committee. *Circulation* 2002 July 16;106(3):388-91.

(2) Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 2001 May 16;285(19):2486-97.

(3) Marcovina S, Packard CJ. Measurement and meaning of apolipoprotein AI and apolipoprotein B plasma levels. *J Intern Med* 2006 May;259(5):437-46.

(4) McQueen MJ, Hawken S, Wang X et al. Lipids, lipoproteins, and apolipoproteins as risk markers of myocardial infarction in 52 countries (the INTERHEART study): a case-control study. *Lancet* 2008 July 19;372(9634):224-33.

(5) Pischon T, Girman CJ, Sacks FM, Rifai N, Stampfer MJ, Rimm EB. Non-high-density lipoprotein cholesterol and apolipoprotein B in the prediction of coronary heart disease in men. *Circulation* 2005 November 29;112(22):3375-83.

(6) Walldius G, Jungner I, Holme I, Aastveit AH, Kolar W, Steiner E. High apolipoprotein B, low apolipoprotein A-I, and improvement in the prediction of fatal myocardial infarction (AMORIS study): a prospective study. *Lancet* 2001 December 15;358(9298):2026-33.

(7) Di AE, Sarwar N, Perry P et al. Major lipids, apolipoproteins, and risk of vascular disease. *JAMA* 2009 November 11;302(18):1993-2000.

(8) Mora S, Otvos JD, Rifai N, Rosenson RS, Buring JE, Ridker PM. Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women. *Circulation* 2009 February 24;119(7):931-9.

(9) Ridker PM, Rifai N, Cook NR, Bradwin G, Buring JE. Non-HDL cholesterol, apolipoproteins A-I and B100, standard lipid measures, lipid ratios, and CRP as risk factors for cardiovascular disease in women. *JAMA* 2005 July 20;294(3):326-33.

(10) Sharrett AR, Ballantyne CM, Coady SA et al. Coronary heart disease prediction from lipoprotein cholesterol levels, triglycerides, lipoprotein(a), apolipoproteins A-I and B, and HDL density subfractions: The Atherosclerosis Risk in Communities (ARIC) Study. *Circulation* 2001 September 4;104(10):1108-13.

(11) Sniderman AD. Apolipoprotein B versus non-high-density lipoprotein cholesterol: and the winner is.. *Circulation* 2005 November 29;112(22):3366-7.

(12) Mudd JO, Borlaug BA, Johnston PV et al. Beyond low-density lipoprotein cholesterol: defining the role of low-density lipoprotein heterogeneity in coronary artery disease. *J Am Coll Cardiol* 2007 October 30;50(18):1735-41.