

ARIC Manuscript Proposal # 1422

PC Reviewed: 09/09/08
SC Reviewed: _____

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Status: _____

Priority: 2
Priority: _____

1.a. Full Title: Relationship between Lipoprotein Cholesterol levels and Carotid artery plaque characteristics: the ARIC Carotid MRI study.

b. Abbreviated Title (Length 26 characters): Lipoproteins and carotid artery plaque characteristics.

2. Writing Group:

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

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3. Timeline: We plan to analyze the data as soon as approval is obtained. Manuscript will be prepared as soon as analysis is done. We plan to do the analysis, as well as prepare the manuscript for submission within 1 year.

4. Rationale:

The current National Cholesterol Education Program (NCEP) Adult treatment Panel (ATP III) guidelines have established low density lipoprotein cholesterol (LDL-C) as the primary target of therapy [1]. Though LDL-C does predict risk for coronary heart disease

(CHD) in the population, several studies have now shown that non-high density lipoprotein cholesterol (non-HDL cholesterol) [2, 3], and total apolipoprotein B (apo B) [3,4] levels may provide similar or better risk stratification. The rationale includes the fact that measurement of apo B, or non HDL cholesterol accounts for all the atherogenic lipoproteins [very low density lipoprotein (VLDL) cholesterol, Intermediate density lipoprotein (IDL) cholesterol, LDL-C, as well as lipoprotein (a)].

Apo A-1 is the major protein constituent of high density lipoprotein cholesterol (HDL-C), and has been shown to correlate inversely with risk for CHD [5]. The relationship between apo A-1 and HDL-C is not straightforward as each HDL particle may have 2-4 apo A-1 molecules. It has been suggested that apo B/ apo A-1 ratio may be used as a surrogate for the ratio of all atherogenic lipoprotein entering the intima of the blood vessels and the reverse cholesterol transport mechanism that removes this excess cholesterol. Based on this, it is possible that apo B/ apo A-1 ratio may also be an important predictor of events.

Though there is data available on the utility of these lipid parameters and cardiovascular events, the association between these lipid subfractions and the extent of atherosclerosis/ plaque vulnerability remain poorly defined. In one of the manuscripts from the ARIC carotid MRI study [6], Wagenknecht et al showed that though baseline (visit 1) total cholesterol and LDL-C levels were associated with an increase in total wall volume, they were not independent predictors for the presence of lipid rich core after adjustment for total wall volume. In this study, LDL-C levels at year 18 were associated with the size of the lipid rich core independent of the wall thickness. However, it is important to note that Wagenknecht et al.[6] did not investigate the association of levels of total apo B, non HDL cholesterol, as well as apo B/ apo A-1 ratio with carotid artery plaque characteristics in the carotid MRI substudy.

Therefore, we plan to investigate the following specific aims 1) to understand the association between traditional lipid measurements (total cholesterol, LDL-C, HDL-C, triglycerides, total chol/HDL-C ratio, non-HDL C, and non HDL-C/HDL-C ratio), as well as these apolipoprotein parameters (total apo B, apo B/ apo A-1 ratio) and measures of carotid atherosclerosis and plaque vulnerability in the ARIC carotid MRI study and 2) to understand if baseline measurements (visit 1) of these lipid fractions/ratios better correlate with the extent of carotid atherosclerosis and plaque vulnerability than measurements done on year 18 of the study.

5. Main Hypothesis/Study Questions:

Hypothesis 1:

Lipoprotein subfractions and their ratios (total apo B lipoprotein level, apo B/apo A-1 ratio, and non-HDL cholesterol) are better predictors of carotid wall volume than total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, total chol/ HDL cholesterol ratio, non HDL-C/ HDL-C ratio and apoA-1 in the ARIC cohort. Similarly, we hypothesize that baseline (visit 1) levels of these lipid fractions/ ratios will be better predictors of total wall volume than measurements done in year 18.

We hypothesize this based on the assumption that long standing presence of elevated levels of lipid subfractions would lead to a greater cumulative exposure and would lead to a greater increase in the extent of atherosclerosis compared to an elevated level of these lipoproteins only in year 18 of the study.

Please note that separate analyses will be carried out for baseline (visit 1) lipid measurements, as well as year 18 lipid measurements.

*Since the use of cholesterol lowering medications in ARIC cohort increased from 3% at baseline to 36% in carotid MRI substudy [6], analyses will be done first including all participants and then restricting it to participants who were not on any cholesterol lowering medications on either baseline visit or year 18 of the study.

Hypothesis 2:

Baseline (visit 1) total apo B lipoprotein level, and apo B/ apo A-1 ratios, non-HDL cholesterol are better predictors for the presence of high risk plaques compared to total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, total chol/ HDL chol ratio, nonHDL-C/HDL-C ratio, and apo A-1 in the AIRC cohort.

We expect that since apo B and non-HDL cholesterol measure all atherogenic lipoproteins [i.e. LDL, VLDL, IDL, Lipoprotein (a)], they might have a stronger association with measures of plaque vulnerability compared to other traditional lipid parameters. Similarly, apo B/ apo A-1 ratio representing the net cholesterol influx into the intima of the vessel might be better associated with measures of plaque vulnerability compared to traditional cholesterol measurements.

*For the reasons given above, analysis will be done first on all participants first and then restricted to participants who were not on any cholesterol lowering medications on either baseline visit or visit 5.

** Please note that since the presence of lipid rich core is highly dependant on vessel wall thickness, analyses involving lipid rich core will be restricted to participants with MRI maximum wall thickness ≥ 1.5 mm. In this population with maximum wall thickness ≥ 1.5 mm, 2 separate analyses will be done (one having maximum wall thickness as a covariate and one without having maximum wall thickness as a covariate for adjustment).

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).

Carotid MRIs were performed on nearly 2000 participants in the ARIC-MRI study. The ARIC MRI cohort included 1200 participants whose carotid artery wall thickness as measured by B mode ultrasound on visit 3 or 4 was approximately $\geq 68^{\text{th}}$ percentile (though the cut-offs varied depending on the field center). The IMT cut-offs were 1.35, 1.00, 1.28, and 1.22 mm at Forsyth County, Jackson, Minneapolis suburbs, and Washington County, respectively, representing the 73rd, 69th, 73rd, and 68th percentiles of maximal IMT from Exam 4. A random sample of nearly 800 participants whose CIMT was approximately $<68^{\text{th}}$ percentile (though this again varied by field center) was also studied. The carotid MRI procedure included measurements of multiple variables (see below) using gadolinium-enhanced MRI on the thicker internal carotid artery with a 1.5 T

magnet. Similarly, plasma cholesterol levels, LDL-C levels, HDL-C levels, non HDL-C levels, triglycerides, total apolipoprotein B levels, apo A-1 levels are also available on these patients.

All individuals enrolled in the ARIC MRI study who have had plasma lipoprotein levels measured and have good quality MRI will be eligible for this analysis. Of all the carotid MRI performed, a total of 1769 have been reported to be of good quality.

Carotid MRI variables used for the analysis will include:

Carotid wall thickness

Total wall volume (GDISCA-TOTAL WALL VOLUME)
Maximal wall thickness (GDSICA-MAXWALLTHICK-MAXCORE)
Lumen area (LUMENAREA_MAXMEANWALL1)
Vessel wall area (VESSELWALL AREA_MAXMEANWALL1)

Lipid core

Total lipid core volume (GDSICA-TOTALLIPIDCOREVOLUME)
Max lipid core area (GDSICA-MAXLIPIDCOREAREA-NEW2)
Lipid core (present/absent) (LIPID_core)
Lipid core present in two adjacent slices (CORE_in_two)

Fibrous cap thickness

Mean cap thickness (MEAN-CAP-THICKNESS-2ADJACENT)
Mean minimum cap thickness (MEAN-MIN-CAP-THICKNESS-2ADJACENT)
(note: restricted to participants with lipid core present)

Lipoprotein Cholesterol variables used for the analysis will include:

Total cholesterol, calculated LDL cholesterol, HDL cholesterol, triglycerides, non-HDL cholesterol, total cholesterol /HDL cholesterol ratio, total apo B lipoprotein concentration, total apo A-1 lipoprotein concentration, Non HDL-C/HDL-C ratio, and apo B/ apo A-1 ratio for baseline visit (visit 1) and year 18 of the study. Plasma LDL-C levels would be calculated using Friedewald equation [total cholesterol-HDL cholesterol- VLDL cholesterol (estimated as triglycerides divided by 5)]. Non HDL-C levels would be derived using the formula: non-HDL-C = total cholesterol-HDL cholesterol.

STATISTICAL ANALYSIS [PLAN]:

For the purpose of analysis, we will have 3 adjustment models:

Model 1 (basic model): age, sex, race.

Model 2: Model 1 + BMI, history of hypertension, use of blood pressure-lowering medication, systolic and diastolic blood pressure, history of diabetes, diabetes medication use, smoking (never, current, and former), history of cardiovascular disease, lipid-lowering medication, aspirin, , and Hs CRP.

Model 3 (Only for aim 2): Model 2 + maximum wall thickness.

For each of the models above, a R squared value will be described showing its association with the MRI variables. Then, we will add the lipoprotein parameter or ratio of interest to each model and determine that addition of which lipid parameter or ratio leads to largest increase in R squared.

For the dichotomous MRI variables, we will exponentiate the beta coefficient of the logistic regression model and then present the data in terms of OR to see which lipid parameter or ratio is associated with the largest OR (per 1 SD increase in the lipid parameter or ratio of interest).

We will do exploratory analyses in participants aged ≤ 55 years and those >55 years on visit 1, and age ≤ 65 and > 65 in year 18 to ascertain if lipid parameters are predictive of measures of extent of atherosclerosis and plaque vulnerability in both of these age groups.

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All analyses would be based on methods appropriate for stratified random sample methods. In particular, all analyses would be weighted by the inverse of the sampling fractions in the 8 sampling strata (4 field centers X 2 IMT groups). The association between MRI variables and lipid fractions/ratios will be analyzed by linear regression for continuous MRI variables and logistic regression for categorical MRI variables, with the MRI variables as the dependent variables.

For adjustment for standard risk factors, outside of age, sex, and race, the analysis will consider both concurrent (cross-sectional) measures of risk factors as well as cumulative exposure or rate of change of exposure. The cumulative exposures will be determined for continuous variables as the area under the curve of exam-specific values plotted versus exam time, divided by time between first and last exam. This can be interpreted as the estimated mean daily value over the period. For dichotomous risk factors the cumulative indicator is the proportion of time exposed. For the continuous variables we will calculate the rate of change over the period as the person-specific slope from a random coefficients linear model.

LIMITATIONS ANTICIPATED:

The use of cholesterol lowering medications in the ARIC cohort increased from less than 3% at baseline to nearly 36% at the time of the carotid MRI study. Consequently, the levels of total cholesterol, LDL cholesterol and apo B will likely be lower in participants using cholesterol lowering medications, and may lose some predictive capability.

Similarly, the use of some cholesterol lowering medications i.e. statins may also alter plaque morphology. This may create a bias that would move our results towards null.

Keeping that in mind, we propose to first analyze all participants and then restrict the analysis to participants who were not on any cholesterol lowering medications on either baseline visit or year 18 of the study.

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 ICTDER02 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 ICTDER02 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 ICTDER02 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>

_____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)?

Wagenknecht LE, Wasserman BA, Chambless LE, Coresh J, Folsom AR, Heiss G, Mosley TH, Ballantyne CM, Sharrett R, Sanhueza A, Boerwinkle E. Correlates of carotid plaque presence and composition as measured by magnetic resonance imaging: the Atherosclerosis Risk in Communities (AIRC) Study. Under review.

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

11.b. If yes, is the proposal

_____ **A. primarily the result of an ancillary study (list number* _____)**

_____ **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 <http://www.csc.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.

REFERENCES:

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2. Cui Y, Blumenthal RS, Flaws JA, et al. Non-high-density lipoprotein cholesterol levels as a predictor of cardiovascular disease mortality. *Arch Intern Med* 2001;161:1413-1419.
3. Ridker PM, Rifai N, Cook NR, Bradwin G, Buring JE. Non-HDL cholesterol, apolipoproteins A-1 and B100, standard lipid measures, lipid ratios, and CRP as risk factors for cardiovascular disease in women. *JAMA* 2005;294:326-333.
4. Lamarche B, Moorjani S, Lupien PJ, et al. Apolipoprotein A-1 and B levels and the risk of ischemic heart disease during a five-year follow-up of men in Quebec Cardiovascular Study. *Circulation* 1996;94:273-278.
5. Walldius G, Jungner I, Holme I, Aastveit AH, Kolar W, Steiner E. High apolipoprotein B, Low apolipoprotein A-1, and improvement in prediction of fatal myocardial infarction (AMORIS study): a prospective study. *Lancet* 2001;358:2026-2033.
6. Wagenknecht LE, Wasserman BA, Chambless LE, Coresh J, Folsom AR, Heiss G, Mosley TH, Ballantyne CM, Sharrett R, Sanhueza A, Boerwinkle E. Correlates of carotid plaque presence and composition as measured by magnetic resonance imaging: the Atherosclerosis Risk in Communities (AIRC) Study. Under review.