1.a. Full Title: Association of plasma oxysterols with MRI-detectable carotid wall and plaque characteristics: the ARIC Carotid MRI study

b. Abbreviated Title (Length 26 characters):

Oxysterols and carotid MRI-plaque characteristics

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

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3. **Timeline:** Plasma oxysterol measurements in the ARIC MRI cohort have recently been completed and data will be submitted to the ARIC CC by end of September, 2009. Data analyses will be started immediately following approval of this manuscript proposal. Anticipated date for completion of the manuscript is November of 2009.

4. **Rationale:**

   To date, there are only a few relatively small studies that have investigated the association of plasma oxysterols and risk for cardiovascular disease. Furthermore, there are no large epidemiological studies that have investigated the relationship between plasma levels of oxysterols and atherosclerotic plaque characteristics. Therefore, we propose to measure plasma oxysterols in 1200 individuals with high (>85th percentile) carotid artery wall thickness documented by B-mode ultrasound and 800 individuals sampled from the remainder of the carotid artery wall thickness distribution (<85th percentile) who received a contrast-enhanced carotid MRI examination.

   Oxysterols are cholesterol oxidation products that can be derived from the diet, endogeneous enzymatic reactions, or non-enzymatic autoxidation due to oxidative stress (1-4). Oxidized low-density lipoproteins (ox-LDL) are enriched in oxysterols and are believed to play a critical role in the development of atherosclerotic lesions. In particular, 7β-hydroxycholesterol (7βOH) and 7-ketocholesterol (7keto) are present at high levels in ox-LDL as well as in the lipid core of atherosclerotic plaques (5-7). A recent study demonstrated that administration of ezetimibe (10 mg daily for 1 month), a drug that inhibits cholesterol absorption in the small intestine by blocking the sterol transporter Niemann-Pick C1-like 1, markedly reduced the levels of oxidized cholesterol in the serum by 50% after feeding a test meal containing either α-epoxy cholesterol or 7-keto cholesterol (8).

   Numerous *in vitro* studies have clearly demonstrated that oxysterols present in ox-LDL are toxic to a variety of cell types found in the vascular wall, including monocyte-
derived macrophages, smooth muscle cells and endothelial cells (9-11). A recent in vitro study found that while 7βOH and 7keto demonstrate synergistic cytotoxic effects, 25-hydroxy cholesterol (25OH) and 27-hydroxy cholesterol (27OH) may actually have anti-apoptotic effects in monocytic cells, when examined separately (12). However, in this study the combination of all four oxysterols was proapoptotic. These data indicate that the combined effect of a physiologically relevant oxysterol mixture is capable of inducing pathological processes attributable to the development and instability of atherosclerotic plaques.

At present, only a limited number of small studies have examined the relationship between plasma oxysterol levels and established risk factors for heart disease with disparate results. Plasma 27OH levels were positively associated with plasma cholesterol levels, but were not different between patients with angiographically proven atherosclerosis and apparently healthy subjects (13). A different study, comparing plasma levels of oxysterols in 80 patients undergoing a first coronary angiography and 79 control subjects found no significant differences (14). In contrast, a Finnish study found that plasma level of 7βOH was the strongest predictor of a 3-year increase in carotid wall thickness of more than 30 variables tested in a multivariate analysis (15). In a study that compared cardiovascular risk factors between Swedish men and their Lithuanian counterparts, increased plasma levels of 7βOH were found in the Lithuanians, who have a 4-fold higher risk for heart disease compared to the Swedes (16). A more recent study found that plasma levels of 7keto, 7βOH and 5β,6β–epoxide cholesterol (β-epoxide) were higher in patients with stable coronary artery disease (CAD) compared to a control group with similar atherogenic risk profile and angiographically normal coronary arteries (17). Interestingly, cell culture studies by the same investigators showed that the β-isomers of several different oxysterols exhibited increased cytotoxic effects compared to their respective α-isomers, suggesting that oxysterol stereospecificity may be important in risk prediction for coronary artery disease.

In summary, these data suggest that elevated circulating levels of oxysterols may be associated with an increased risk for cardiovascular disease and that more specifically; the stereospecificity and ratios of different oxysterols may be important in relation to the development and characteristics of atherosclerotic plaque. If a clear association were shown between oxysterols levels or ratios and MRI measures of atherosclerosis in a population study such as ARIC, this would provide the scientific rationale to examine
whether pharmacological therapies which lower levels of plasma oxysterols could modify the progression of carotid atherosclerosis as measured by MRI.

**Literature References**

5. Main Hypothesis/Study Questions:

Primary Hypothesis:

Elevated plasma 7-keto cholesterol levels are correlated with increased carotid artery wall thickness, after adjusting for known plasma lipid risk factors.

Secondary Hypotheses:

1) Elevated plasma oxysterol levels or “composite oxysterol score”\(^1\) are associated with increased carotid artery wall thickness, after adjusting for known plasma lipid risk factors.

2) Elevated plasma levels of 7-keto cholesterol are associated with plaque characteristics, including:

   (a) presence of lipid core,

   (b) lipid core volume,

   (c) decreased mean fibrous cap thickness.

3) Elevated plasma levels of oxysterols or “composite oxysterol score”\(^1\) are associated with plaque characteristics, including:

\(^1\) Defined as total plasma concentration of 7α-hydroxycholesterol, 7β-hydroxycholesterol, 7-ketocholesterol, 4β-hydroxycholesterol, 24S-hydroxycholesterol, 25-hydroxycholesterol, 27-hydroxycholesterol, 5α,6α-epoxide-cholesterol, 5β,6β-epoxide-cholesterol, and Cholestane-3β,5α,6β-triol in ng/mL.
(a) presence of lipid core,
(b) lipid core volume,
(c) decreased mean fibrous cap thickness.

4) Elevated plasma levels of oxysterol β-isomers or “composite β-isomers score”\(^2\), including 7β-hydroxycholesterol, 4β-hydroxycholesterol, and 5β,6β-epoxide-cholesterol, are associated with plaque characteristics, including:
(a) presence of lipid core,
(b) lipid core volume, and
(c) decreased mean fibrous cap thickness of carotid artery plaques.

**Exploratory Hypotheses:**

1) Some individual plasma oxysterol levels (taking multiplicity into account) are associated with increased carotid artery wall thickness.

2) Some individual plasma dietary oxysterol levels (taking multiplicity into account) are associated with plaque characteristics, including:
(a) presence of lipid core,
(b) lipid core volume, and
(c) decreased mean fibrous cap thickness of carotid artery plaques.

3) Elevated plasma “composite oxysterol scores” and “composite β-isomers scores”, are correlated with some traditional cardiovascular risk factors and measures of oxidative stress (e.g., homocysteine, hemoglobin A1c), taking multiplicity into account.

\(^2\) Defined as the total plasma concentration of 7β-hydroxycholesterol, 4β-hydroxycholesterol, and 5β,6β-epoxide-cholesterol expressed in either ng/mL or as a numerical composite rank score (3-12) based on interquartile ranking of each β-isomer. Q1 = 1, Q2 = 2, Q3 = 3, and Q4 = 4.
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 has a cross-sectional design, using a random sample recruited based on intima-media thickness (IMT) from a previous ultrasound examination and stratified by age, sex, and race (blacks and whites). Measurement of plasma oxysterols have been performed on BHT-treated plasma from 1200 individuals with high (>85th percentile) carotid artery wall thickness (“high IMT group”) documented by B-mode ultrasound and 800 individuals sampled from the remainder of the carotid artery wall thickness distribution (<85th percentile or “low IMT group”) who received a contrast-enhanced carotid MRI examination. Standardized MRI measures include carotid artery wall thickness, T2 signal intensity changes and percent contrast enhancement indicative of endothelial dysfunction, and for those with plaque, fibrous cap thickness, lipid core volume, and calcification.

**Statistical Methods**

All analysis is based on methods appropriate for stratified random sample methods. In particular, all analyses are 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 oxysterols 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, adjusted first for Model 1 (basic model): age, sex, and race, and then additionally for other covariates, including Model 2: Model 1 + total cholesterol, HDL-C, and triglycerides, and Model 3: Model 2 + smoking, BMI, blood glucose, blood pressure, use of blood pressure-lowering medication, lipid-lowering medication (statin and ezetimibe), diabetes medications, and CRP.

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.

After the laboratory measurements are transferred to the ARIC Coordinating Center (CC), the CC will look for potential problems, such as missing data, outliers, or replicate pairs with extreme differences, and will resolve these issues with the laboratory. If remeasurements or corrections are needed, they will be transferred to the CC. We will then assess lab repeatability, using both an intraclass correlation coefficient and a coefficient of laboratory variation. Methods for correction for measurement error will be applied to the linear and logistic models where appropriate. After applying any additional exclusions related to the particular analyte being studied, we will first implement a descriptive analysis, generally comparing “high IMT” subjects with “low IMT” subjects with respect to several variables of interest. In this and all analyses we account for the sampling scheme in analysis. The ARIC CC will work with the authors to perform all required statistical analyses for the manuscript, after the manuscript proposal is approved. In addition, the ARIC CC will assist in writing the manuscript. Statistical analysis results will be sent only to the PI of the ARIC Carotid MRI ancillary study. In addition, all computer printouts and logs will be made available to the PI, if desired. Reports on blind replicate repeatability will be listed on the ARIC website and be available to all ARIC investigators.

Methods

Levels of oxysterols (see Table 1) have been measured by stable isotope dilution-GC-mass spectrometry as described by Dzeletovic and Diczfalussy with modifications because of the smaller sample volume of 500 μL (Anal. Biochem. 1995; 225:73-80).

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  ____ 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)? N/A

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* 2007.09C)
____ 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.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.