1.a. Full Title: Association of circulating levels of oxidized LDL and MPO, with CETP activity and risk for Peripheral Arterial Disease: the Atherosclerosis Risk In Communities Study

b. Abbreviated Title (Length 26 characters): Oxidative stress and CETP and risk for PAD

2. Writing Group:
   Writing group members: Ron C. Hoogeveen, Vijay Nambi, Woody Chambless, Christie M. Ballantyne. Others are welcome.

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: All Atherosclerosis Laboratory analyses and data submission to the ARIC Data Coordinating Center was completed in March of 2007. Statistical analysis and manuscript preparation will start immediately upon approval of the manuscript proposal.
and will be completed by August 2007. Manuscript submission to a peer-reviewed journal is anticipated by October 2007.

4. Rationale:

Oxidative stress is thought to play a key role in the pathogenesis of many human diseases, including diabetes, atherosclerosis, stroke and kidney disease. Oxidative stress can be mediated by specific enzymes, which can exert either pro- or anti-oxidant effects, and host anti-oxidant defense systems. When host anti-oxidant defense systems are insufficient to prevent a state of systemic oxidant stress, generation of oxidant species leads to the formation of modified lipids, proteins, and DNA bases.

Myeloperoxidase (MPO), an abundant heme enzyme released by activated leukocytes, catalyzes the formation of reactive intermediates that promote lipid peroxidation. Catalytically active MPO has been found in human atherosclerotic tissue, predominantly colocalized with lipid-laden foam cells (1). Furthermore, elevated circulating levels of MPO are significantly associated with increased incidence of CHD (2). One major target of MPO-mediated oxidation is LDL, and exposure of LDL to the MPO–H₂O₂–NO₂⁻ system of monocytes leads to LDL lipid peroxidation and conversion into a more atherogenic form of LDL (3). Experimental studies in cultured cells and animals have shown that oxidized LDL (ox-LDL) is more atherogenic than native LDL because it is internalized via scavenger receptors on monocyte derived macrophages, which leads to lipid accumulation and formation of lipid-laden foam cells (4). Plasma levels of oxidized LDL have been shown to be significantly higher in patients with CHD compared with normal controls (5).

Cholesteryl ester transfer protein (CETP) is a key enzyme involved in the remodeling of lipoproteins and plays a central role in reverse cholesterol transport (RCT), the major pathway for excretion of free cholesterol from the body. However, the role of CETP in atherogenesis remains controversial. Although CETP deficiency generally is associated with increased HDL-C levels, epidemiologic studies investigating CETP deficiency have produced contradicting results with regards to risk for atherosclerosis in Japanese and Caucasian populations (6-9). Furthermore, a recent study in CHD patients showed that a CETP inhibitor did not significantly decrease the progression of coronary atherosclerosis (10). These data clearly demonstrate that the role of CETP in the development of atherosclerosis is not fully understood. In vitro studies have demonstrated that CETP-mediated cholesteryl ester (CE) transfer between HDL and apo-B-containing lipoproteins such as VLDL and LDL is bidirectional (11,12). Furthermore, oxidation of LDL enhances CETP-mediated CE transfer rate to HDL, which results in a diminished net transfer of CE from HDL to ox-LDL (13). These findings emphasize the complex role of CETP in atherogenesis and may in part explain the seemingly controversial findings from epidemiology and intervention studies.

In summary, although there is increasing evidence from both basic research as well as clinical studies of a role for oxidative stress in the pathogenesis of atherosclerosis, there is only very limited data available from large population based studies. Therefore, we propose to investigate the association of the oxidative stress markers ox-LDL and MPO with CETP activity and risk for PAD in the large biracial cohort of the ARIC study.

References:
13. Castilho LN, Oliveira HCF, Cazita PM, de Oliveira AC, Sesso A, Quintao ECR. Oxidation of LDL enhances the cholesteryl ester transfer protein (CETP)-mediated cholesteryl ester transfer rate to HDL, bringing on a diminished net transfer of cholesteryl ester from HDL to oxidized LDL. *Clin Chim Acta* 2001;304:99-106.
5. **Main Hypothesis/Study Questions:**

Elevated plasma levels of ox-LDL, MPO and CETP activity are associated with increased risk for PAD in both men and women.

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 uses a cross-sectional design to investigate the relation between plasma levels of ox-LDL, MPO and CETP activity and prevalence of PAD. Ox-LDL, MPO and CETP activity measurements were made on Visit 3 and Visit 4 plasma samples of PAD cases and controls. Groups were defined as participants with prevalent PAD (n=382) and a noncase comparison group (n=362) consisting of a random sample from the ARIC cohort, stratified on the basis of ultrasound examination of carotid arteries, age, sex, and field center. PAD prevalence was determined on the initial visit and each subsequent visit by ankle–brachial index (ABI; measured on one leg) ≤0.90 for men and ≤0.85 for women.

Data will include PAD status and plasma levels of ox-LDL, MPO and CETP activity. Multivariable logistic regression models will be used to evaluate the relation between PAD status and plasma ox-LDL, MPO and CETP activity levels, modeled both as continuous variables and in tertiles. Consideration will be given to the effect of measurement error on the results of the analyses.

SUDAAN software will be used to adjust for sampling strategy in the logistic regression models. The established cardiovascular risk factors evaluated as potential confounders in the logistic regression models will include age, gender, race, center, BMI, years of cigarette smoking, incident CHD, incident diabetes, lipids (triglycerides, total cholesterol, LDL-C, HDL-C), and inflammatory markers (WBC, ICAM-1, VCAM-1, fibrinogen, vWF, CRP, selectins, MCP-1). Covariates will be assessed for statistical significance in the models by the Wald $\chi^2$ statistic. P<0.05 will be considered statistically significant.

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 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?  
_X__ Yes  

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.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?  
_____ 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.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.