1.a. Full Title: The Relation of Lp-PLA2 to PAD in Middle-Aged Men and Women

b. Abbreviated Title (Length 26 characters): Lp-PLA2 and risk for PAD

2. Writing Group:
   Writing group members: Christie M. Ballantyne, Ron Hoogeveen, LE Chambless, Aaron Folsom, Gerardo Heiss, Hanyu Ni, Eric Boerwinkle, Richey Sharrett, Josef Coresh, Vijay Nambi

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

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3. Timeline: The manuscript will be completed in 2007.

4. Rationale:
   Lipoprotein-associated phospholipase A2 (Lp-PLA2) has been associated with incident coronary heart disease (CHD) and stroke in the ARIC population. There are currently limited data on the relationship of Lp-PLA2 to peripheral artery disease (PAD) (Santos et al., 2004). We postulate that elevated plasma levels of Lp-PLA2 mass and activity are associated with PAD.

   The hypothesis that atherosclerosis is an inflammatory disease is supported by both the discovery of inflammatory cells in the cap of atherosclerotic plaques and recent reports that elevated levels of plasma markers of inflammation are associated with incidence of CHD (Ross, 1999). The oxidative modification of low-density lipoprotein (LDL) within the arterial wall is a
key early event in the development of atherosclerosis (Witztum and Steinberg, 1991). Therefore, numerous studies have focused on enzymes that are involved in the oxidation of LDL and, as a result, alter the proinflammatory activities of oxidized LDL (ox-LDL). The LDL oxidation process involves the oxidation of the polyunsaturated fatty acid component of phospholipids and ultimately leads to the conversion of phosphatidylcholine (PC) to lyso-PC (Parthasarathy et al., 1985). The increased lyso-PC content of oxLDL is a chemoattractant for human monocytes and induces endothelial dysfunction (Quinn et al., 1988; Kume et al., 1992). Lp-PLA₂, also known as platelet-activating factor acetylhydrolase, is a serine-dependent lipase that has been shown to hydrolyze oxidatively modified PC to release oxidized fatty acids and lyso-PC (Stremler et al., 1991). Lp-PLA₂ co-purifies with LDL and is responsible for >95% of the phospholipase activity associated with LDL (Tew et al., 1996). Its expression is regulated by mediators of inflammation, and inhibition of Lp-PLA₂ activity results in a significant decrease in both lyso-PC content and monocyte chemoattractant ability of ox-LDL (Cao et al., 1998; Tew et al., 1996). Although the majority of Lp-PLA₂ is associated with LDL in the circulation, there are conflicting data in the scientific literature regarding the relative distribution of Lp-PLA₂ among other lipoprotein subclasses, such as high-density lipoprotein (HDL) and very low density lipoprotein (VLDL). Furthermore, it has been reported that Lp-PLA₂ associated with HDL has reduced phospholipase activity compared with LDL-associated Lp-PLA₂ (Stafforini et al., 1987; Tselepsis and Chapman, 2002). Although the ARIC study has previously shown an association of Lp-PLA₂ mass to incident CHD and stroke, other studies such as the Pravastatin or Atorvastatin Evaluation and Infection Therapy–Thrombolysis in Myocardial Infarction trial (PROVE IT–TIMI 22) found stronger associations between Lp-PLA₂ activity and cardiovascular events (O’Donoghue et al., 2006).

5. Main Hypothesis/Study Questions:
Elevated levels of Lp-PLA₂ mass and activity are associated with increased risk for PAD in both men and women, and the increased risk is independent of C-reactive protein (CRP) and traditional risk factors, including baseline levels of total cholesterol.

Secondary hypothesis: Lp-PLA₂ activity is a stronger independent predictor of risk for PAD than Lp-PLA₂ mass after adjustment for CRP and traditional risk factors.

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).
Case–control design using stored plasma. PAD was defined by ankle–brachial index (ABI), which was not measured at the second exam; ABI measurement and ultrasound exams were performed on a sample of persons at the third exam, and those not measured at the third exam were measured at the fourth exam. Therefore, both exams 3 and 4 were needed, to increase the number of cases. To determine eligibility for the PAD case–control study participants, were divided into 2 groups: those with valid ABI data at exam 3, and those without valid data at exam 3 but with valid data at exam 4. Others were excluded. Persons in the exam 3 group were excluded if they had CHD or stroke prior to exam 3 or did not fast at least 8 hours before exam 3, and exam 3 values of covariates of interest were included. Persons in the exam 4 group were excluded if they had CHD or stroke prior to exam 4 or did not fast at least 8 hours before exam 4, and exam 4 values of covariates of interest were included. Persons who were not black or white were excluded, as were nonwhites at the Minnesota and Washington County centers. Persons in
the exam 3 group were classified as PAD cases if their ABI was below the sex-specific cutpoint at exam 3. Persons in the exam 4 group were classified as PAD cases if their ABI was below the sex-specific cutpoint at exam 4. For each exam group, an equal number of noncases were chosen as controls, stratified by field center. All persons with PAD at exam 3 were considered potential cases. Persons with PAD at exam 4 who did not have valid ABI data at visit 3 were also considered as potential cases. Actual exam 3 cases excluded those who had CHD or stroke, with an equal number of noncases at each exam chosen as controls.

Based on a 1:1 case:control ratio, with 148 PAD cases at visit 3 and 234 new PAD cases at visit 4, the sample size selected is 296 at visit 3 and 468 at visit 4 (total 764).

Lp-PLA₂ mass was measured by ELISA, and Lp-PLA₂ activity was measured by colorimetric activity method assay in EDTA plasma obtained at the time of ABI measurement (i.e., visit 3 or visit 4). CRP was measured by automated method on the Olympus AU400, which has been correlated to the previously performed measurements run on the Hitachi 911.

Comparisons of both Lp-PLA₂ mass and activity between PAD cases and controls will be examined by ANCOVA after adjustment for age, sex, and race. In addition, we will examine the correlations between Lp-PLA₂ mass, Lp-PLA₂ activity, LDL-C, HDL-C, and other traditional risk factors. Our test of the primary hypothesis will be with logistic regression of the probability of PAD as a function of Lp-PLA₂ mass or Lp-PLA₂ activity, adjusted only for age, sex, and race, or additionally adjusted for traditional risk factors, or still further adjusted for CRP. We will model Lp-PLA₂ mass and Lp-PLA₂ activity as continuous linear variables (in standard deviations of each) and as tertiles. For the secondary hypothesis we will test the difference in the size of the associations of Lp-PLA₂ mass and Lp-PLA₂ activity with PAD by bootstrapping. Note that for cases and controls chosen from Visit 3, covariates will be from Visit 3, while for cases and controls chosen from Visit 4, covariates will be from Visit 4.

Instead of analyzing the data as a cross-sectional case–control study, we could also analyze as a stratified random sample, with field center and high/low ABI defining the strata. With this approach we could analyze ABI as a continuous function of Lp-PLA₂.

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?  ____ 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 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.csec.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)?
"Lipoprotein-associated phospholipase A2, high-sensitivity C-reactive protein and risk for incident coronary heart disease in middle-aged men and women in Atherosclerosis Risk in Communities Study" (MS 889), "Lipoprotein-associated phospholipase A2, high sensitivity C-reactive protein, and risk for ischemic stroke (MS 940)," "Lp-PLA2 and hs-CRP as Predictors of Ischemic Stroke" (MS 1172)

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* _2004.09_)
   ___   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.

References: