ARIC Manuscript Proposal # 1844

PC Reviewed: 9/1311	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Ceruloplasmin and the risk of Cardiovascular Disease in the Atherosclerosis Risk In Communities (ARIC) study

b. Abbreviated Title (Length 26 characters): **Ceruloplasmin and CVD in ARIC**

2. Writing Group:

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I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. RTD

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3. Timeline: All laboratory measurements of plasma ceruloplasmin on the entire ARIC Visit 4 cohort have been completed and data was submitted to the ARIC coordinating center. Analysis will start as soon as the manuscript proposal has been approved. We anticipate journal submission of the completed manuscript within 1 year after manuscript proposal approval.

4. Rationale:

The pathophysiology of coronary artery disease (CAD) is associated with an imbalance between damage caused by oxidative damage and protection offered by antioxidative processes.(1) It is likely that the oxidative effects of ceruloplasmin (CP) on serum lipids, in combination with decreased antioxidant protection, predominate in CAD patients.(2) The purpose of this study is to investigate the association between CP and cardiovascular disease in the ARIC study. We will determine the association of CP plasma concentration and incident cardiovascular disease events, heart failure hospitalizations and all cause mortality.

Background:

After much debate it has now become widely accepted that elevated plasma levels of low density lipoproteins (LDL) is a key pathogenic factor in the development of atherosclerosis. It is also well know that oxidative modification of LDL promotes its atherogenicity. The balance between oxidant production and antioxidant defense systems maintains physiological homeostasis and counteracts the oxidative damage of proteins, lipids and DNA. (3, 4) The pathophysiology of CAD is associated with an imbalance between oxidative damage and antioxidative protection (5, 6). CP is an acute phase reactant that has oxidative capabilities and was found to be increased in patients with atherosclerotic disorders. (7, 8, 9, 10)

Ceruloplasmin is a copper-containing alpha-2-glycoprotein with a molecular weight of approximately 132 kDa and it has diverse functions (11). The known functions of CP include copper transportation, iron metabolism, antioxidant defense, and involvement in angiogenesis and coagulation (12). While it was studied for years as an antioxidant, in the 1990's it was discovered that CP could also act as a potent oxidant of LDL. It was found that LDL exposed to CP exhibited many characteristics of LDL oxidized in the presence of free cupric ion. CP was added to cultures in which endothelial cell or smooth muscle cells were exposed to LDL in RPMI 1640, a cell culture medium without transition metal ion additives. CP markedly enhanced LDL oxidation by both cell types.(13) Among the candidates are, for example, O_2^- and lipoxygenase, both of which are increased by activation of monocyte-macrophage-related cells. The role of O_2^- may be to reduce the CP-bound "oxidant" copper. It is believed that LDL oxidation does not take place in the circulation, and it occurs in the arterial wall because serum lipoprotein lipids are well protected from oxidation by the robust antioxidant defenses and LDL itself is the major

transport vehicle for alpha-tocopherol, an antioxidant vitamin. LDL may be exposed to cell-derived oxidants in the subendothelial space of artery (14, 15, 16, 17,18). Minimally oxidized LDL has a low affinity to macrophages scavenger receptors, and thereby, minimally oxidized LDL can be recycled into blood circulation and can be detected as a serum oxidized LDL. The minimally oxidized LDL also stimulates adhesion molecules and chemokines. Extensively oxidized LDL can be taken up by macrophages through the scavenger receptors, leading to the formation of foam cells. These extensively oxidized LDL and minimally oxidized LDL enhance macrophage scavenger receptors with various modulations of cytokines. Oxidized LDL exerts a number of proatherogenic effects: promotion of foam cell formation, chemotaxis and activation of leucocytes, stimulation of monocytes and neutrophil adhesion to endothelial cells, impairment of endothelium-derived nitric oxide release and stimulation of endothelin-1 secretion by endothelial cells. (19)

Multiple studies found that CP levels also positively correlated with lipid peroxidation products (2). Boero L et al found that increased level of ceruloplasmin activity showed a direct association with oxidized LDL in patients with active acromegaly. (20) There is evidence that CP might have a role in CAD however there is controversy regarding its ability as a marker to predict CAD. Mänttäri M et al found that patients with angiographically proven CAD have significantly increased level of CP when compared with patients without CAD. His findings are supported by Fox et al and McMurray et al (21, 22). Grobusch et all found an increased risk for myocardial infarction in patients with high ceruloplamin level after adjustment for other risk factors. (23) Other investigators like Enbergs et al and Halevy et al did not find changes in plasma CP levels in angiographically detected CAD patients. (24, 25) Engstrom et al found that plasma levels of inflammatory markers (ISPs, fibrinogen, ceruloplasmin, haptoglobin, orosomucoid and alfa1-antitripsin) are associated with long-term incidence of hospitalizations due to HF in middle-aged men. (26)

The above mention studies have a few potential limitations. The only study that analyzed the association of ceruloplasmin and cardiovascular events is the study performed by Grobush et al on a small number of patients (N=210) in 1999 (23). The only outcome investigated is the myocardial infarction and its association with the ceruloplasmin level was examined by the use of a nested case control design. The association between ceruloplamin and incidence of heart failure was analyzed by Engstrom et al in a study performed mainly on men lacking generalizability in a population composed of both genders (26). We believe that a study on a larger and more heterogenous patient population with a main outcome consisting of cardiovascular events that not only includes myocardial infarction but also fatal cardiovascular death, and revascularization as well as ischemic strokes is going to help us understand more the association of ceruloplamin and CVD events. Oxidative stress has also been shown to be important in the pathogenesis of heart failure (27). By virtue of its association with oxidative stress, it is possible that elevated levels of ceruloplasmin could be associated with incident heart failure. Although this is possible, this has not been evaluated.

5. Main Hypothesis/Study Questions:

Hypothesis:

Elevated plasma ceruloplasmin levels in individuals who do not have clinical evidence of cardiovascular disease (CVD) in visit 4 in ARIC will be associated with increased risk of CVD events which includes CHD (fatal coronary heart disease, definite or probable myocardial infarction and coronary revascularizations) and ischemic strokes (thrombotic). It will also be associated with an increase in incidence of congestive heart failure (CHF) hospitalizations and all cause mortality

Study questions:

1. Is ceruloplasmin associated with CVD events?

2. Is ceruloplasmin associated with increased incidence of CHF hospitalizations?

3. Is ceruloplasmin associated with increased in all cause mortality?

4. In the case of a strong association between CP and CVD events, does the CP help improve the ARIC CVD risk prediction model?

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).

Overview: To test our hypotheses, we will utilize plasma samples from 11,490 participants from the Atherosclerosis Risk in Communities Study Visit 4.

Plasma levels of ceruloplasmin have been measured in the entire ARIC Visit 4 cohort. We request access to the ARIC data analysis files, and their periodic updates, for cohort data collected by the ARIC study on risk factors and incident CVD.

We are interested in the following variables in the ARIC database: age, gender, race, body mass index, smoking status, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, troponins, hs-CRP, NT-proBNP, creatinine, estimated glomerular filtration rate, the presence of left ventricular hypertrophy (calculated by ECG with the Cornell criteria), systolic blood pressure, presence of diabetes (fasting blood sugar $\geq$ 126 mg/dl or use of diabetes medication), use of antihypertensive medications, use of diabetes medications, use of aspirin, incident cases of coronary heart disease (CHD) events (fatal coronary heart disease, definite or probable myocardial infarction, and coronary

revascularizations), ischemic strokes (thrombotic strokes), and CHF hospitalizations and all cause mortality, occurring after ARIC V4

For analysis of the association between ceruloplasmin and incident cardiovascular events, CHF hospitalizations and all cause mortality after ARIC visit 4, we will evaluate the distribution of ceruloplasmin levels to determine normality. Log rank test and Cox regression analysis will be used to test our hypothesis. We will create 3 models:

- Model 1 will be a basic model adjusted for age gender, race and ARIC center
- Model 2 will be adjusted for: all variables in Model 1 plus total cholesterol, high density lipoprotein cholesterol, systolic blood pressure, antihypertensive medication use, smoking status and the presence of diabetes mellitus (fasting blood glucose> 126 mg/dl or diabetes medication use), BMI
- Model 3 will be adjusted for all the factors included in model 2 plus hs-CRP, NT-proBNP and estimated GFR, troponin, left ventricular hypertrophy (calculated by ECG with the Cornell criteria)

Depending on the strength of the associations found in the primary analysis we will perform stratified analysis by other variables such as: age, race, gender, body mass index, smoking status, blood pressure, presence of diabetes, total cholesterol, HDL cholesterol low density lipoprotein, hs-CRP lipoprotein (a) and antihypertensive medication use. There is also data in literature that suggests the statins may increase the ceruloplasmin levels (28) therefore we will also perform analysis stratified by statin use.

Depending on which outcome will be associated with CP we will test if it will CP adds to risk prediction of that outcome using the ARIC CHD, stroke or HF risk prediction model (29). We will examine the area under the curve (AUC) for the overall study population by methods that account for follow-up time. We will calculate the net reclassification index (NRI) and clinical NRI for 10 year follow-up using methods that account for follow up time to assess improvement in risk classification. We will also calculate the goodness of fit using the Gronnesby Borgan test.

Inclusion criteria:
Patients with no clinical evidence of CVD at ARIC visit 4.

Exclusion criteria
1. Patients who have missing covariate data.
2. Patients with prevalent CVD or history of heart failure hospitalization before ARIC visit 4 will be excluded.

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)?

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 ()
___ 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.

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