ARIC Manuscript Proposal # 1824

PC Reviewed: 8/9/11	Status: A	Priority: 2
SC Reviewed: _____	Status: _____	Priority: ____

1.a. Full Title: Nonalcoholic fatty liver disease and myocardial subclinical cardiac disease
b. Abbreviated Title (Length 26 characters): Fatty liver disease and cTnT

2. Writing Group:
Writing group members:
Mariana Lazo; Jonathan Rubin; Jeanne M. Clark; Frederick Brancati; Andrea Christman; Chiadi Ndumele; Ron Hoogeveen; Christie M. Ballantyne; Elizabeth Selvin; others welcome.

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

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3. Timeline: We expect the liver enzyme assays from Visit 4 to be complete by the summer of 2011. We aim to submit this manuscript to the ARIC publications committee in <6 months from the date we receive the liver enzyme data.

4. Rationale:

Hepatic steatosis, or fatty liver, is characterized by the excessive accumulation of triglycerides in the form of lipid droplets in the liver. This, in the absence of excessive alcohol consumption, is termed nonalcoholic fatty liver disease (NAFLD), the most common liver abnormality in the western countries. Besides obesity, NAFLD is associated with type 2 diabetes, dyslipidemia, and hypertension[1, 2]. NAFLD encompasses a wide spectrum of disease ranging from steatosis to nonalcoholic steatohepatitis (NASH), fibrosis and cirrhosis [3-10]. While NAFLD is known to lead to liver related complications [5, 6],the role of NAFLD in the development of cardiovascular disease is controversial. In addition to shared risk factors, the liver is thought to be an important contributor to systemic inflammatory changes, and people with NAFLD have shown overexpression of genes involved in monocyte and macrophage recruitment and coagulation[11-15], key mechanisms in atherosclerosis. In spite of these studies, previous epidemiologic studies of the association between NAFLD and cardiovascular disease have been inconsistent [16-28], and limited by the use of small, highly selected samples (e.g. patients with liver biopsy) and therefore the question remains controversial.

Newly identified biomarkers have improved the accuracy in the diagnosis of subclinical cardiovascular damage. Cardiac Troponin-T (cTnT) is associated with cardiovascular disease risk and adverse outcomes in both the general population and in high-risk groups[29]. Newer highly sensitive cardiac troponin-T (hs-cTnT) assays have greater sensitivity as compared to earlier cTnT assays [30] and have also been shown to improve the prediction of cardiovascular morbidity and mortality in subjects with stable coronary artery disease [31] and in persons without clinically evident cardiovascular disease in ARIC [32]and other population-based cohorts[33, 34].

B-type natriuretic peptide is closely associated with left ventricular mass index[35] and accurately detects heart failure [36]. N-terminal pro-brain natriuretic peptide (NT-proBNP) is also associated with cardiovascular risk [37] and mortality[38, 39]. When compared to BNP, NT-proBNP was superior in the prediction of death in the general population [40]. NT-proBNP is elevated in patients with diabetes [41, 42] and has been demonstrated to detect subclinical left ventricular dysfunction [42]. It is also a reliable marker of future cardiac and all cause mortality in persons with diabetes [43].

Strictly speaking, the diagnosis of NAFLD remains clinico-pathological with well defined criteria for the patterns of liver injury. Liver biopsy remains the best available method to confirm, diagnose and stage NAFLD[44]. However, due to the invasive nature, liver biopsies are not feasible in large epidemiological studies, and are still not routinely performed in all patients with NAFLD in the clinical setting. For operational purposes, the majority of epidemiological studies define NAFLD using surrogates indicators such as elevated liver enzymes: aspartate aminotransferase –AST-, alanine aminotranferase –ALT- and gamma-glutamyl tranferase –GGT- [45]. Using theses test alone or in combination, a number of studies have shown strong associations with liver outcomes[46-51].

The upcoming availability of liver enzymes (AST, ALT, and GGT), and hs-cTnT and NT-proBNP measurements from all participants who attended the fourth ARIC visit provides a population-based sample in which to assess the relationship of NAFLD to markers of myocardial subclinical disease. To our knowledge the association between NAFLD and myocardial damage,

as measured by cTnT or NT-proBNP has not been studied before. We therefore propose to test the hypothesis that NAFLD--as defined by elevated liver enzymes in the absence of elevated alcohol consumption—is associated with subclinical myocardial damage indicated by elevated hs-cTnT and NT-proBNP values, after controlling for covariates of interest.

5. Main Hypothesis/Study Questions:

Hypothesis 1: NAFLD, as defined by elevated liver enzymes, will be associated with higher hs-cTnT levels and will be more likely to have detectable hs-cTnT levels.

a. These associations will be present both in persons with and without obesity.
b. These associations will be present both in persons with and without diabetes.
c. These associations will be present independent of known cardiovascular risk factors (Smoking, blood pressure and dyslipidemia).

Hypothesis 2: NAFLD will be positively associated with higher NT-proBNP levels.

a. The association will be present both in persons with and without obesity.
b. The association will be present both in persons with and without diabetes.
c. The association will be present independent of known cardiovascular 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).

Study design:
Cross-sectional study of liver enzymes, hs-TnT, and NT-pro BNP measured in all participants at Visit 4.

Exposure:
Elevated ALT, AST or GGT in the absence of elevated alcohol consumption.
Liver enzymes will be analyzed both as continuous variable and by clinical cut-points given by the laboratory performing the assays.

We will define elevated alcohol consumption as usual alcohol intake > 20 g/day (roughly equivalent to 2 drinks per day). Usual alcohol consumption will be derived from variable ETHANL41/7 (assessed at visit 4).
This cutoff level is well below the traditional threshold for alcohol-inducde liver disease [2]
Preliminary analyses suggest that ~9% of ARIC participants at Visit 4 have elevated alcohol consumption and thus would be excluded from the analyses.

Outcomes:
hs-TnT concentrations were measured with a novel precommercial highly sensitive assay, Elecsys Troponin T (Roche Diagnostics), on an automated Cobas e411 analyzer with a lower limit of detection (LOD) of 3 ng/L. The between-assay coefficient of variation was 2.6% and 6.9% for control materials with mean cTnT concentrations of 2.378 μg/L and 0.029 μg/L,

respectively (approximately the 99th percentile of ARIC). Repeatability of measurements was assessed by using blinded split samples (n=418). The reliability coefficient was 0.98, and coefficient of measurement variation was 15% when excluding >3–standard deviation outliers (n=3). Assays on repeat samples drawn within 2–6 weeks also showed high reliability coefficients.[52]

hs-cTnT will be analysed both categorically and as a continuous.
For the categorical analyses, the 33.5% with undetectable levels will be the reference group (group 1). The remaining 66.5% will be split into approximate thirds: cTnT levels 0.003 to 0.005 μg/L (group 2), 0.006 to 0.008 μg/L (group 3), and higher levels divided at approximately the 90th percentile of the ARIC population (group 4: 0.009 to 0.013 μg/L; group 5: ≥0.014 μg/L), Elevated hs-cTnT will be defined as levels above the previously reported 99th percentile value (0.014 μg/L) in a healthy subpopulation aged 20–70 years (Roche Diagnostics, data on file). In addition, we will also model hs-cTnT as a continuous variable with undetectable levels of hs-cTnT assigned a value of 0.0015 μg/L (i.e., half the lower limit of detection).

N-terminal pro–B-type natriuretic peptide (NT-proBNP) was measured by using an electrochemiluminescent immunoassay on an automated Cobas e411 analyzer (Roche Diagnostics) with lower limit of detection ≤5 pg/mL4 and coefficient of variation 3.5–4.7%.

NT-proBNP will be analyzed both categorically and as a continuous variable.. For the categorical analyses it will be categorized into: undetected, quartile 1, 2, 3 and 4.

Inclusions
All black and white ARIC participants who attended Visit 4, with valid data on ALT, AST, GGT, alcohol consumption, data on NT-proBNP and hs-cTnT available and no missing data on important variables (body mass index, history of diabetes, smoking, HDL and LDL-cholesterol, blood pressure and kidney function (n ≈ 11,500).

Exclusions:
Ethnicity other than black or white, missing ALT, AST and GGT. Missing hs-cTnT, NT-proBNP or missing covariates of interest.

Covariates
Other variables of interest will include age, sex, race, education, center, smoking status, alcohol use (drinks per day), body mass index, history of myocardial infarction and heart failure, blood pressure, hypertensive medication use, triglycerides, HDL- and LDL- cholesterol, fasting glucose and kidney function (estimated GFR from serum creatinine).

Potential effect modifiers:
Race, sex, diabetes, history of myocardial infarction and history of heart failure.

Sensitivity analyses:

To further control for alcohol intake, sensitivity analyses will be conducted among people who report never drinking.

Statistical Analysis

We will use linear and logistic regression models to assess the cross-sectional association between liver enzymes (ALT, AST and GGT) and hs-cTnT and NT-proBNP (Visit 4). Multivariable logistic regression models will be used to estimate odds ratios and their 95% CIs for detectable hs-cTnT or NT-proBNP levels above the 99% percentile, respectively, by elevated liver enzymes. Separate models will be used to test separately each liver enzyme, using clinically cut points. In addition, we plan to model the association of hs-cTnT and NT-proBNP using piece-wise linear splines (with knots at clinical cut-points) and restricted cubic splines to better characterize the shape of the potential associations.

Limitations

Only single measurements of liver enzymes, hs-TnT, and pro-BNP are available and intra-individual variability has been reported[52, 53] In addition, liver enzymes, are surrogates markers of liver disease with known limited sensitivity and specificity.[54, 55], however these represent the only available data on subclinical liver disease in this large community based study and are clinically relevant measures.

Despite adjustment for known risk factors for cardiovascular disease, we will also not be able to rule out the possibility of residual confounding in the interpretation of our results. Due to the cross-sectional nature of this investigation, the temporality of any observed associations cannot be established.

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

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)?

MP # 1808: The utility high sensitivity cardiac troponin t in the prediction of heart failure risk
MP #1564: Correlation of High Sensitivity Troponin-T (hs-cTnT) and Amino Terminal pro-Brain Natriuretic Peptide (NT-proBNP) with Renal Function Parameters; and Association with Mortality and Adverse Cardiovascular Events
MP #1563: Novel highly sensitive cardiac Troponin-T (hs-cTnT) assay, mortality, and major adverse cardiovascular events in the ARIC Study.
MP # 1734: Biomarker, anthropometric parameters associated with highly sensitive cardiac troponin T
MP # 1757: The association of high sensitivity troponin with heart failure, mortality and recurrent coronary heart disease (CHD) in individuals with prevalent CHD
MP # 1758: Chronic Hyperglycemia and Arterial Stiffness: the Atherosclerosis Risk in the Communities Study
MP # 1759: Associations of traditional cardiovascular risk factors and high-sensitivity cardiac troponin T.
MP # 1596: Hyperglycemia and risk of subsequent elevation of NT-proBNP and hs-cTnT

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? __X__ Yes ____ No
ARIC Ancillary Study #2008.10, "Measurement of NT-pro-BNP and troponin T at visit 4 for the full ARIC cohort"

11.b. If yes, is the proposal
_X_ A. primarily the result of an ancillary study (list number #2006.15 and #2008.10)
___ 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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