1. a. Full Title: Non-Alcoholic Fatty Liver Disease and the Risk of Incident Cardiovascular Disease: The Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): Liver Enzymes and Incident CVD

2. Writing Group: Chiadi E. Ndumele; Andrea Christman; Mariana Lazo; Jeanne Clark; Ron C. Hoogeveen; Roger S. Blumenthal; Josef Coresh; Elizabeth Selvin; others welcome

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

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3. Timeline: We expect all liver function test assays from Visit 4 to be complete by the summer of 2011. Analyses will be initiated once all data are cleaned. We aim to submit this manuscript to the ARIC publications committee in <6 months from this date.
4. **Rationale:**

Non-alcoholic fatty liver disease (NAFLD) is a highly prevalent condition, affecting 20-30% of the general U.S. adult population, with an even higher prevalence among individuals with obesity and insulin resistance. NAFLD is associated with each of the components of the metabolic syndrome, and it has been suggested that NAFLD be added to metabolic syndrome diagnostic criteria. Appreciation of the relationships between NAFLD and several metabolic risk factors has led to interest regarding whether NAFLD itself is a risk factor for incident cardiovascular disease (CVD).

NAFLD frequently results in elevated circulating levels of enzymes typically sequestered within hepatocytes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyl transpeptidase (GGT). NAFLD is the most common cause of elevated liver enzymes in adult ambulatory populations. Among individuals without known liver disease or excess alcohol use, elevated liver enzymes are overwhelmingly thought to represent fatty liver disease and, therefore, are typically used as a surrogate for NAFLD in the general population.

While some initial studies have shown an association of NAFLD with cardiovascular risk, there is a paucity of prospective data demonstrating an independent association between NAFLD and incident CVD. Furthermore, adjustment for known confounding variables in past studies has been inconsistent. There is also limited data examining whether the relationship between NAFLD and incident CVD varies by race/ethnicity. Additionally, while past analyses have separately examined the associations of AST and ALT (which are indicative of hepatocellular injury), and GGT (which reflects both liver injury and oxidative stress), with cardiovascular disease, none have yet examined the relationship of joint elevations in liver enzymes with CVD.

The Atherosclerosis Risk in Communities (ARIC) Study, with its long duration of follow-up for cardiovascular events, extensive evaluations for cardiovascular risk factors, inclusion of large numbers of racial minorities and measurement of the liver enzymes AST, ALT, and GGT from previously stored samples, is an ideal cohort for examining the association of NAFLD with incident CVD.

5. **Main Hypothesis/Study Questions:**

**Aims:**

1) To determine whether elevated levels of GGT, ALT, and AST are associated with incident CVD (coronary heart disease (CHD), ischemic stroke and congestive heart failure (CHF)), independent of traditional cardiovascular risk factors; and

2) To determine whether the association of elevated liver enzymes with incident CVD differs by race.

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:** We will assess the prospective associations of elevated liver enzymes with incident cardiovascular events, using ARIC Visit 4 as the baseline for all analyses. Visit 4 took place from 1996-1999, and is the first visit for which liver enzymes are being measured for all study participants.

**Exposures:** Plasma levels of the liver enzymes GGT, ALT and AST, measured with enzymatic assays in 2011 from thawed samples stored from Visit 4.

We will model the liver enzymes in three ways:

1) As dichotomous variables: elevated (outside of the gender-specific reference ranges for the laboratory) or normal (within the gender-specific reference range for the laboratory).
2) As a continuous variables: because liver enzymes are known to have skewed distributions, they will be natural log-transformed to assess their association with CVD as continuous variables, scaled by their respective standard deviations for comparability across liver enzymes. We will formally test the assumption of linearity in these models.
3) As quartiles: we will assess the association of increasing quartiles of natural log-transformed liver enzymes with incident CVD, with the lowest quartile being the reference category.

**Outcomes:** The primary outcome will be incident CHD (defined as fatal CHD, definite or probable nonfatal myocardial infarction, angioplasty/stenting, or coronary artery bypass graft surgery), occurring after Visit 4 through January 1, 2008 (or most current follow-up available). Secondary outcomes will include incident ischemic stroke, incident CHF, and cardiovascular and total mortality.

**Exclusions:** Because we are using elevated liver enzymes as a surrogate for NAFLD, we will exclude individuals with a self reported history of hepatitis, cirrhosis or other liver disease at Visit 3 (1993-95), those with a history of liver disease-related hospitalization, or those with reported excess alcohol consumption at Visit 4 (> 1 drink per day for women, and > 2 drinks per day for men). We will also exclude participants with known CVD prior to Visit 4 (self reported CVD or adjudicated CVD events at or prior to Visit 4). We will exclude the small number of participants at Visit 4 who are not black or white. We will also exclude participants missing covariates of interest at baseline.

**Covariates:** Age, sex, race, smoking status, systolic and diastolic blood pressures, blood pressure medication use, fasting glucose, BMI, waist circumference, diabetes, total-LDL-, and HDL-cholesterol, triglycerides, income, level of education, and alcohol consumption.
Main Analyses: Cox proportional hazards regression will be used to model the associations of GGT, ALT and AST with incident CVD, before and after adjusting for the covariates of interest above.

1) We will perform univariate comparisons of subjects with and without elevations in liver enzymes, with regards to demographic and cardiovascular risk factors
2) We will estimate the association between elevated measures of GGT, AST and ALT (modeled dichotomously, natural log-transformed, and by quartiles) and incident CVD using adjusted hazards ratios and their 95% CIs
3) We will also model the associations of joint elevations in any 1, 2, or 3 liver enzymes (each modeled as dichotomous variables) with incident outcomes. The reference group will be individuals with no elevated liver enzymes. We are particularly interested in assessing whether associations may differ across joint elevations in liver enzymes associated with hepatocellular injury (ALT or AST) and oxidative stress (GGT), versus isolated elevations in one of these subtypes of liver enzymes. We will use C-statistics to compare models with different patterns of liver enzyme elevation.
4) We will test for interactions by race and conduct stratified analyses to compare the association of elevated liver enzymes with the outcomes of interest in black and whites.

Subgroup Analyses:
- The hazard of incident CVD associated with elevated liver enzymes and with 1-SD increases in natural-log transformed liver enzymes, will be assessed in subgroups of individuals with and without obesity, and individuals with and without abdominal obesity (using AHA/NHLBI metabolic syndrome criteria). We will also assess the associations between elevated liver enzymes and incident CVD within the subgroup of non-drinkers.

Secondary Analyses: We will also compare the associations of abnormal liver enzymes with subsets of incident CHD events – CHD events excluding procedures, non-fatal MI, fatal MI/CHD and cardiac procedures.

Sensitivity Analyses: We will conduct sensitivity analyses comparing the associations of liver enzymes with different definitions of incident CHD (fatal CHD, non-fatal events, procedures).

Limitations:
- Elevated liver enzymes as surrogate for NAFLD: Elevated liver enzymes have a fairly high specificity for NAFLD (>90%), but poor sensitivity which may result in misclassification of cases as non-cases in these data, likely resulting in relative measures of association that are biased towards the null in the present study.
- Stored samples and reliability of liver enzyme assays: Duplicate assays will be performed on a subset of samples to assess the reliability of these measurements in long-term stored samples.
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 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)?

   ARIC Manuscript Proposal # 1789: Elevated Liver Enzymes and Risk of Diabetes  
   ARIC Manuscript Proposal # 977: Liver Enzyme Activity and Risk of Diabetes

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use  
     any ancillary study data?  ____ Yes  ____ 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.
Reference List


