ARIC Manuscript Proposal # 1789

PC Reviewed: 5/10/11	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Elevated Liver Enzymes and Risk of Diabetes

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

2. Writing Group:
Writing group members: Christman; Lazo; Ndumele; Clark; Coresh; Selvin; Pankow; others welcome

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. ALC **[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:

Nonalcoholic Fatty Liver Disease (NAFLD) includes a spectrum of disease characterized by ectopic fat accumulation in liver tissue in the absence of excess alcohol consumption (1, 2). NAFLD can result in inflammation and can eventually lead to cirrhosis (3-5) and even death (6, 7). In the U.S. adults, prevalence estimates of NAFLD typically range from 10% to 24% (8, 9), but is much more common in the presence of obesity and/or type 2 diabetes where prevalence estimates range from 57% to 75% (10, 11). Although the direction of the association is not clearly elucidated, NAFLD and type 2 diabetes commonly co-occur and insulin resistance is the main proposed mechanism linking NAFLD and type 2 diabetes, leading both to increased fat accumulation in the liver and hyperglycemia due to the inability of insulin to decrease hepatic glucose production (12). Most individuals with NAFLD are asymptomatic and are diagnosed following detection of mildly elevated liver enzyme tests (13, 14). Commonly performed liver enzyme tests include: alanine amino-transferase (ALT), aspartate amino-transferase (AST), and gamma glutamyl-amino-transferase (GGT). Typical abnormalities found on these tests in the setting of NAFLD are mild elevations in ALT, AST, and GGT, with the ratio of ALT/AST >1. Elevated ALT and GGT, as surrogates for NAFLD, are related to incident type 2 diabetes, with studies suggesting that the risk of diabetes is 2-3 times higher in individuals with elevated liver enzymes as compared to those persons with normal levels (15), but the majority of these studies have included a small number of participants, been of short follow-up duration, and only included white participants. Less is known about the relationship of elevated AST with incident type 2 diabetes. Little is known about the use of combinations of liver enzymes for diabetes risk. Recent work has suggested racial differences in NAFLD and elevated liver enzymes. Although blacks have a higher prevalence of obesity and are known to be at higher risk of diabetes and its complications as compared to whites, blacks have a paradoxically lower prevalence of NAFLD (16-18). The lower prevalence of NAFLD in blacks is consistent with a lower prevalence of visceral adiposity and dyslipidemia in this population. Very little is known about the association between elevated liver enzymes and diabetes in blacks. The proposed manuscript will test the following overarching hypothesis: NAFLD, as assessed by elevated liver enzymes (ALT, AST, and GGT), will be independently associated with the development of type 2 diabetes in a community-based bi-ethnic population.

5. Main Hypothesis/Study Questions:

Aim 1: To characterize the prospective association of elevated levels of ALT, AST, and GGT with incident self-reported type 2 diabetes during 10-12 years of follow-up in the community-based ARIC Study. We will specifically focus on how race might modify this relationship.

Hypothesis 1a: Elevated levels of ALT, AST, and GGT are independently associated with incident type 2 diabetes.

Hypothesis 1b: The relationship between liver enzymes and incident type 2 diabetes differs by race.

Aim 2: To assess the performance of different combinations of liver enzymes (ALT, AST, and GGT) to determine optimal combinations for prediction of diabetes risk.

Hypothesis 2: Combinations of liver enzymes will be better predictors of diabetes risk than any single enzyme alone. The combination of three liver enzymes may perform optimally.

Aim 3: To perform a validation study of diabetes case status in the ARIC Study comparing self-reported diabetes to data collected during an in-person visit (fasting glucose, hemoglobin A1c, and recorded medication bottles).

Hypothesis 3: There will be under-ascertainment of diabetes status using self-reported data as compared to visit-based data. We will observe similar patterns in the association of liver

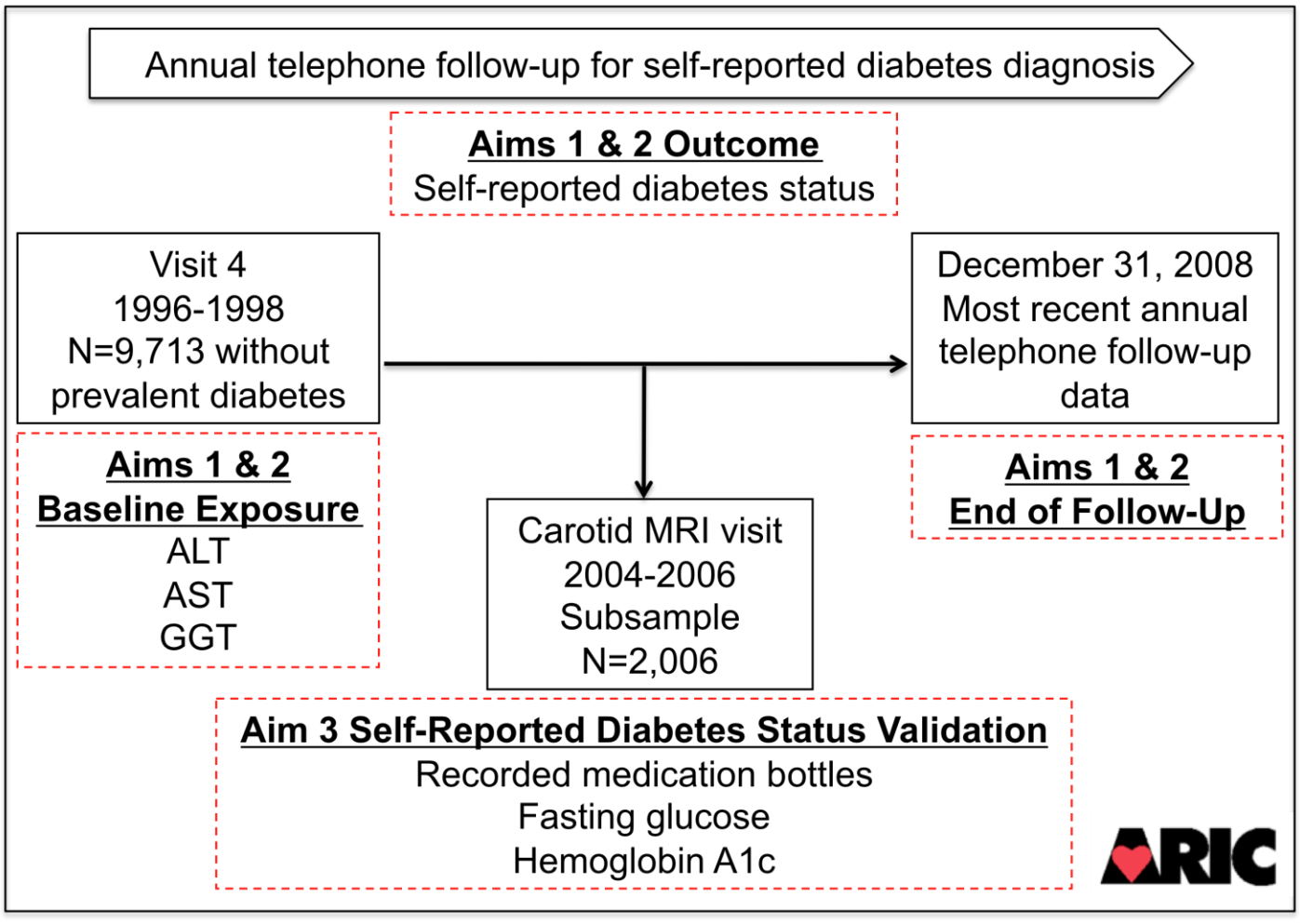
enzymes with self-reported diabetes as compared to the association with diabetes cases confirmed using information collected at the in-person visit.

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 and Study Population

For study aims 1 and 2, we will use ARIC visit 4 as baseline and will conduct prospective analyses assessing the relationship between elevated liver enzymes at visit 4 and incident type 2 diabetes with follow-up until December 31, 2008. Of the 11,656 visit 4 participants, 1,943 participants have diagnosed diabetes and will be excluded from our baseline population. In addition to excluding prevalent self-reported cases of diabetes, we will also exclude participants who report drinking in excess of 1 drink per day for females and 2 drinks per day for males to prevent misclassification of liver disease. We will also exclude participants who are taking medications that affect the liver. Other exclusions will be made for participants who have missing values for covariates of interest.

For Aim 3, we will validate our incident diabetes outcome in a subsample of ARIC participants who took part in the carotid MRI (CARMRI) visit from 2004-2006 (n=2,006). As a result of this visit, we will have recorded medication bottles, fasting glucose, and hemoglobin A1c on a subsample of our participants in which we can assess any misclassification of self-reported diabetes status. In particular, we will be assessing how many missed cases we obtain from the visit based definition as compared to the self-report definition.



Exposures: Liver Enzymes (ALT, AST, and GGT)

We will define NAFLD as elevated liver enzymes (ALT, AST, and GGT) in the absence of elevated alcohol consumption . The liver enzymes will be measured from stored frozen plasma samples collected from all participants at visit 4 (n=11,336) at Baylor University, Texas. Quality control and assay reliability will be performed. The ARIC Study stored plasma samples include masked duplicate plasma samples (3% of total vials) with IDs that are indistinguishable from the regular ARIC ID. These masked duplicate samples provide the opportunity for us to rigorously evaluate the reliability of each assay. Because we will be masked to ID linkage, the Coordinating Center will perform linkage and all quality control analyses of masked duplicate specimens. We will send assay results to the ARIC Study Coordinating Center for linkage of masked duplicates to the corresponding true ARIC ID. The quality control report will include coefficient of variation, kappa, percent agreement and other measures of method reliability. We expect high method reliability. The laboratory will also perform routine quality control analyses to ensure reliability of the assay over time.

Outcome: Incident Type 2 Diabetes

Self-Report Diabetes Status (Aims 1 and 2)

Self-reported diabetes status has been (and continues to be) obtained during ARIC annual telephone follow-up calls for all participants since Visit 4. Self-report incident diabetes diagnosis will be coded as "yes" at the time the participant first answers either or both of the following questions with a "yes":

1. Since we last contacted you has a doctor said you have diabetes or sugar in the blood?
2. Did you take any medications during the past two weeks for diabetes or high blood sugar?

The date of annual telephone follow-up call where self-report diabetes was reported will be used as a surrogate for the date of diabetes diagnosis.

Validation of Diabetes Status (Aim 3)

Self-reported diabetes is commonly used in epidemiologic studies and is highly specific, but has limited sensitivity. We will not be able to examine the association of NAFLD with incident undiagnosed diabetes in the present study. Nonetheless, in aim 3 we will perform an internal validation study comparing self-report diabetes status to visit-based diabetes status (including assessment of undiagnosed diabetes cases) using a subsample of the ARIC population.

Visit-based diabetes status will be available for a subsample (n=2,006) of ARIC participants who were selected for the carotid MRI visit. Information available for varying definitions of visit based diabetes includes: a fasting blood glucose value ≥ 126 mg/dl, HbA1c >=6.5%, and a diabetes medication among their recorded medication bottle list.

Statistical Analysis

In aim 1, Cox proportional hazard models stratified by race will be used to quantify the relationship between baseline liver enzyme levels and risk of type 2 diabetes. We will first fit a model using continuous levels of ln(ALT), ln(AST), and ln(GGT) and test to see if the associations are linear. If the associations are not linear, we will use spline models to characterize nonlinear relationships in these data. We will use natural log transformed values for the liver enzymes as suggested by the literature (19) and to normalize the distribution in the population. Levels of ALT, AST, and GGT will also be divided into quartiles and modeled categorically to assess the relationship with incident type 2 diabetes. Model 1 will be adjusted for demographic factors (age, gender, field center, education, and income). Model 2 will include variables in Model 1 + diabetes risk factors (body-mass index, smoking,

alcohol consumption, hypertension, triglycerides, total cholesterol, LDL and HDL cholesterol). In addition to stratification by race, we will also consider models stratified by obesity, represented by the body-mass-index categories of <25 kg/m^2, 25-<30 kg/m^2, and ≥30 kg/m^2.

In aim 2, we will evaluate cut-points for each liver enzyme using: 1.) laboratory defined cut-points based on the continuous distributions of liver enzyme values, and 2.) "optimal" cut-points obtained by maximizing sensitivity and specificity to predict incident type 2 diabetes in our data. Cox proportional hazards models will be used to identify the best single enzyme predictor of diabetes risk in the ARIC cohort. Each liver enzyme will be modeled three ways: 1.) natural log transformed continuous, 2.) dichotomous using laboratory defined cut-points, and 3.) dichotomous using "optimal" cut-points. We will consider three models in this analysis. Model 1 will be univariate. Model 2 will be adjusted for the demographic factors of age, race, gender, field center, education, and income. Model 3 will add the diabetes risk factors of body-mass index, smoking, alcohol consumption, hypertension, triglycerides, total, LDL, and HDL cholesterol. We will compare C-statistics from the models with different liver enzymes in order to identify the best single enzyme predictor of diabetes risk.

The same three adjusted Cox proportional hazard models will be used to compare combinations of liver enzymes versus the one single best liver enzyme in the prediction of diabetes risk. Specifically we will be interested in comparing the C-statistic for models with all three liver enzymes together to the C-statistic for models with the single best liver enzyme. We will also perform a reclassification analysis comparing all three liver enzymes together to the single best liver enzyme using net reclassification indices and integrated discrimination improvement.

In Aim 3, validation of self reported diabetes status from annual telephone follow-up calls will be performed by calculating the proportion of the CARMRI subsample with diabetes according to different visit-based definitions (e.g., fasting blood glucose value ≥ 126 mg/dl, HbA1c >=6.5%, or recorded diabetes medications). We will compare the visit-based cases to the self-report cases in the CARMRI subsample to quantify the hypothesized under-ascertainment of undiagnosed cases using our self-report definition. We will also use logistic regression models to compare the associations of baseline liver enzymes with different definitions of incident diabetes (self report diabetes status versus visit based diabetes status) using the carotid MRI visit subsample. That is, we will run two models: model 1 will be adjusted for demographic factors (age, gender, field center, education, and income). Model 2 will include variables in Model 1 + diabetes risk factors (body-mass index, smoking, alcohol consumption, hypertension, triglycerides, total cholesterol, LDL and HDL cholesterol). As the CARMRI visit is a subsample of 2,066 participants, there is a possibility of imputing missing values for visit-based diabetes for the entire population based on the CARMRI data. This would allow us to assess the impact of our hypothesized under-ascertainment of undiagnosed cases using our self-report definition in the full ARIC cohort population.

Limitations

Intraindividual short-term variability of liver enzyme tests is significant, with variability significantly higher for ALT than for AST and GGT. In a NHANES III second examination substudy analysis, Lazo et al found that 36%, 31%, and 12% of adults with initially elevated AST, ALT, and GGT levels, respectively, had normal levels at the second examination (mean 17.5 days later). However, if normal at the first examination, 95% of tests remained normal at the second examination (20). The Intraindividual short-term variability of liver enzyme tests is similar to that for fasting plasma glucose. Using this information, we will be able to do sensitivity analyses to assess impact of possible misclassification due to only having one measurement of liver enzymes. One possibility to assess the effect of having only one measurement of liver enzymes is to incorporate measurement error estimates into Cox models using simulation extrapolation (SIMEX) methods. Additionally, we will not have information on viral hepatitis, which has been shown to be associated with incident diabetes and

elevated liver enzymes. However in a case-cohort analysis of ARIC data, the prevalence of hepatitis C was very low (0.8%) (21).

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

997 – Liver enzyme activity and risk of diabetes – J. Pankow
891 – Hepatitis C virus infection and incident type 2 diabetes – S.H. Mehta
1431 – Hemoglobin A1c, glucose, and incident diabetes – E. Selvin

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.

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