ARIC Manuscript Proposal # 1101

PC Reviewed: 09/20/05	**Status: __A__**	**Priority: _2__**
SC Reviewed: __09/21/05_	Status: __A__	Priority: __2_

1.a. Full Title:
LIPC POLYMORPHISMS, DIETARY FAT, AND PLASMA HDL CHOLESTEROL IN ADULTS WITH AND WITHOUT TYPE II DIABETES

1.b. Abbreviated Title:
LIPC, dietary fat, and HDL

2. Writing Group:
Writing group members: Jennifer A. Nettleton, Lyn M. Steffen, Eric Boerwinkle, Aaron Folsom, Christie Ballantyne (other interested investigators welcome)

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

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3. Timeline:

Data preparation and analysis will begin upon approval, and manuscript drafting will commence once suitable analytical models are finalized.
Initial drafts will be circulated among the writing group members within four months of proposal approval.

4. Rationale:

Low plasma HDL-cholesterol is associated with increased cardiovascular disease risk. Diet, in addition to various other factors, such as gender, menopausal status, exercise, and alcohol intake can influence HDL-cholesterol concentrations. However, genetic variation among individuals may result in inconsistent relations between diet and cholesterol changes (1). Moreover, it is possible that interactions between dietary factors and polymorphisms in genes involved in HDL cholesterol synthesis and metabolism are partly responsible (2, 3).

Hepatic lipase hydrolyzes triglycerides and phospholipids, is involved in lipoprotein uptake, and therefore, plays an important role in HDL metabolism (4-6). Hepatic lipase deficiency results in increased HDL concentrations (7), and polymorphisms in the hepatic lipase gene (LIPC) have been identified and shown to influence HDL-cholesterol concentrations (4). Persons with LIPC -514C→T polymorphisms show reduced hepatic lipase activity, greater HDL-cholesterol concentrations (6, 8, 9), and greater concentrations of large HDL particles (10, 11). However, not all studies have found this relation (12-14), which could be due to insufficient sample size or the existence of gene-environment interactions that were not investigated. Several studies have shown that the effect of the -514C→T polymorphism is modified by dietary fat intake, although the nature of this interaction has varied among studies (15-17). Studies by Tai *et al.* (16) and Ordovas *et al.* (15) found that persons with the *TT* genotype had greater HDL concentrations only when fat intake was <30% total energy; whereas if fat intake was >30%, HDL concentrations were significantly lower in *TT* variants than in those with the *CC* or *CT* genotype. In contrast, Zhang *et al*. (17) found the -514C→T polymorphism to be associated with greater HDL concentrations only when fat intake was >32%. These contradictory findings could be due to differences in the population studied, smaller sample size, or inclusion of *CT* heterozygotes (18). The Zhang study was conducted in a relatively small group of 752 predominantly white, diabetic men and combined *CT* and *TT* genotypes in analyses because of the low number of participants expressing the *TT* polymorphism (17). Neither the larger Tai nor Ordovas studies combined *CT* with *TT* variants in their analyses, and in fact, in both studies the effect of *CT* genotype was opposite that of *TT* genotype (15, 16). In addition, these studies included both type II diabetics and non-diabetics and simply adjusted for diabetes status in their models rather than conducting analyses in exclusively one group. Lastly, Tai *et al*. found the interaction between *TT* genotype and dietary fat only in the Asian Indian participants but not other ethnicities (16), suggesting racial differences in gene expression may affect findings. All of these subtle differences deserve further attention and may have importantly influenced researchers' conclusions.

Subclasses of dietary fat may also be important. Ordovas *et al*. found interactions between LIPC genotype and saturated fat and monounsaturated fat but not polyunsaturated fat (15), but this finding was not replicated in the Tai study (16). Although a significant relation was reported for saturated fat in the Zhang study, the finding was again in contrast to that of

Ordovas et al. where the *TT* genotype was associated with *higher* HDL concentrations when saturated fat was *greater than* 11% of energy (17).

Additional study in other populations is needed in order to better understand the nature of the interaction between LIPC polymorphisms and dietary fat. Further, the Zhang study raises the interesting possibility that the effect of polymorphisms in the hepatic lipase gene on HDL concentrations depends not only on dietary fat but might also be influenced by diabetes status. It is important to determine if these findings can be replicated in another larger, diabetic population with greater ethnic diversity, such as that found in the ARIC cohort.

(For references see page 7)

5a. Main Hypotheses/Study Questions:

* Initial analyses will be conducted to determine whether LIPC genotype is significantly related to HDL-cholesterol concentrations in the whole cohort (after exclusions), and secondly, whether dietary fat modifies the relation between LIPC genotype and HDL-cholesterol.

5b. Secondary hypotheses/Study Questions:

(Because statistical power will be importantly compromised, the following are to be considered secondary aims of this study.)

* Previous LIPC*dietary fat investigations have shown conflicting results based on diabetes status, therefore, analyses will also be conducted separately in non-diabetics and diabetics to address the following hypotheses/study questions:

1. ARIC participants with no history of stroke or coronary heart disease (CAD) expressing the *TT* genotype will have higher total HDL and HDL2 concentrations compared to those with *CC* or *CT* genotype.
    * This will be true in both the sample excluding participants with type II diabetes and the sample composed only of participants with type II diabetes.

2. The effect of *TT* genotype in participants without type II diabetes will be modified by dietary fat intake. *TT* variants with low total fat intake will have greater HDL concentrations, whereas *TT* variants with high total fat intake will have lower HDL concentrations than *CC* / *CT* variants.
    * The interaction between dietary fat and LIPC genotype will also be investigated in ARIC participants with type II diabetics to determine if the nature of the interaction differs from that found in participants without type II diabetes, i.e., will *higher* fat intake result in *greater* HDL concentrations in *TT* variants but lower HDL in *CC* and *CT* variants?

3. The nature of this interaction will also be investigated in relation to HDL particle size and dietary fat subclasses.

5c. Additional exploratory analyses:

Further exploratory analyses will also be conducted to determine whether similar interactions occur between dietary fat and other HDL-cholesterol metabolism-related genes. Genetic variations in cholesterol-esterase transfer protein (CETP) and lipoprotein lipase (LPL) have also been shown to partly explain phenotypic HDL variation (19-26), although the strength of the relation is not consistent in all study populations suggesting other factors may interplay with genetic expression of these proteins to further modify HDL concentrations. Factors such as, sex (27, 28), smoking status (19), body mass index (19, 29), and alcohol consumption (30) have each been hypothesized to alter the relation between HDL levels and the CETP gene polymorphism, *FaqIB*. Additionally, several dietary intervention studies involving dietary fat changes have studied whether the degree of cholesterol-responsiveness varies by LPL (*9N*, *S291*) or CETP (*FaqIB*) genotype, but due to insufficient samples sizes, results have been inconsistent (2, 3) and deserve further investigation in large cohorts. To our knowledge, a large epidemiological diet*CETP genotype or diet*LPL genotype investigation has not been undertaken.

(For references see page 7)

6. Data:

1. Population
   - ARIC participants (with and without type II diabetes) and no history of stroke or evidence of CAD at baseline, not taking lipid-lowering medications, and for whom adequate dietary and LIPC genotype (and other HDL-related genes as discussed above) data are available.

2. Dependent variables
   - Total HDL cholesterol
   - Additional analyses will also be conducted to evaluate effects on HDL2 and HDL3.

3. Independent variables
   - LIPC genotype (population will be first stratified into 3 genotypes: *CC*, *CT*, and *TT*, but data will likely be combined to form 2 contrasting groups: *TT* variants vs. *CC*/*CT* variants)
   - Other HDL-metabolism related genes:
     - CETP genotype (*FaqIB*)
       B1B1 vs. *B2B2* (*B2B2* variants expected to have higher HDL concentrations)
     - LPL genotypes
       D9N vs. *D/D* (*D9N* variants expected to have lower HDL concentrations)
       N291S vs. *N/N* (*N291S* variants expected to have lower HDL concentrations)
     - *S447X* vs. *S/S* (*S447X* variants expected to have higher HDL concentrations)

4. Gene environment interactions
   - Dietary total fat*LIPC genotype
     - Dietary fat will be studied as 1) a continuous variable— (energy-adjusted g/d or %energy) and as 2) a dichotomous variable— 'high' and 'low' as defined by population mean and/or median intake level.
     - Additional analyses will also be conducted to assess interactions between LIPC genotype and saturated fat, polyunsaturated fat, monounsaturated fat, and *trans* fat. Again dietary fat subclasses will be studied as continuous and dichotomous variables. Due to small ranges in intake for some dietary fat subclasses, these secondary investigations are exploratory and will likely suffer from insufficient statistical power.

   Other previously reported genotype*environment interactions will also be evaluated in secondary analysis.
   - Physical activity (31)
   - BMI (17)
   - Waist circumference (32)

5. Potential model covariates
   - Demographics- age, race, sex, (study center)
   - Education level
   - Lifestyle variables- physical activity, BMI, smoking status, energy intake, alcohol consumption, hormone use (women), other medications (beta-blockers)

Statistical Analysis

Participant characteristics will be determined across LIPC genotypes using generalized linear models. Multivariable adjusted models including demographics, education, and lifestyle covariates will be used to assess the relation between LIPC genotype and total-, LDL-, and HDL-cholesterol concentrations, (as well as HDL2 & HDL3), and when testing for interactions between genotype and dietary fat (total and subclasses). Cross-product terms will be used in these models to assess the significance and magnitude of interactions.

7.a. Will the data be used for non-CVD analysis in this manuscript? No

8.a. Will the DNA data be used in this manuscript? Yes

8.b. Is the author aware that either DNA data distributed by the Coordninating Center must be used, or the file ICTDER02 must be used to exclude those with value RES_DNA = "No use/storage DNA"? Yes, the author is aware of this issue.

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.
There is no overlap between this proposal and current proposals/published manuscripts.

10. What are the most related manuscript proposals in ARIC?
#560 1. Full title: Lipoprotein lipase gene variation predicts the occurrence of atherosclerosis, PAD and Incident CHD—E. Boerwinkle et al.

#214 Plasma CETP: Relationship to lipids, lipoproteins, apolipoproteins, and apoE genotype: the role of environmental factors—E. Boerwinkle et al.

#794 Dietary fat intake modulates the association between the Apolipoprotein E polymorphism and cardiovascular-related disease risk factors and outcomes— M. Bray et al.

#848 Physical activity and CETP polymorphisms as predictors of HDL cholesterol levels—M. Bray et al.

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or does it use any ancillary study data? No

12. 1-3 year completion expectation: Yes, the lead author is aware that manuscript preparation is expected to be completed in 1-3 years, and if this expectation is not met, the manuscript proposal will expire.

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