ARIC Manuscript Proposal # 1305

PC Reviewed: _11/13/07 **Status: __A_** **Priority: __2__**
SC Reviewed: _____ Status: _____ Priority: ____

1.a. Full Title:
Interaction between *ANGPTL4* and Dietary Fat and Carbohydrate in relation to Triglycerides and HDL-cholesterol in the Atherosclerosis Risk in Communities (ARIC) Study

1.b. Abbreviated Title:
ANGPTL4, diet, and lipids

2. Writing Group:
Writing group members: Jennifer A. Nettleton, Kelly Volcik, Eric Boerwinkle… others 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]

Corresponding/senior author: Jennifer A. Nettleton
Data analyst: Jennifer A. Nettleton

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 writing group members within 4 months of proposal approval.

4. Background & Rationale:

Adipokines are proteins secreted by the adipose tissue that play a role in the regulation of glucose and lipid metabolism [1]. One such adipokine, angiopoietin-like 4 (ANGPTL4), is thought to regulate fatty acid transport among tissues via inhibition of lipoprotein lipase, a key enzyme in HDL-C and triglyceride metabolism [2-4]. One nonsynonymous sequence variant in *ANGPTL4* (*ANGPTL*4[E40K]) results in a lysine for glutamic acid substitution and a loss of function of the ANGPTL4 gene [5]. The *ANGPTL*4[E40K] mutation has been associated with lower triglyceride levels [5], and some data suggest *ANGPTL*4[E40K] may also influence other metabolic parameters such as HDL-C [5].

Dietary intake, particularly the relative amount of dietary carbohydrate and fat and specific types of fat such as ω-3 fatty acids of marine origin, influence plasma triglyceride concentrations [6-8]. However, the association between dietary intake and triglycerides may be influenced by other lifestyle and genetic factors [9]. Analogously, associations between plasma triglycerides and genetic polymorphisms in genes involved in the regulation of triglyceride concentrations may vary as a function of dietary intake. Although no studies have evaluated this possibility with respect to *ANGPTL*4, it is plausible that dietary intake may modify the biological impact of *ANGPTL*4.

Therefore we plan to explore the influence of dietary intake, particularly the percentage of calories from carbohydrate and fat, intake of ω-3 fatty acids, and intake of food sources ω-3 fatty acids, on the relation

between *ANGPTL*4[E40K] and TG and HDL-C in a well-characterized population-based sample of European Americans in the Atherosclerosis Risk in Communities (ARIC) study.

References
1. Rondinone CM. Adipocyte-derived hormones, cytokines, and mediators. *Endocrine.* Feb 2006;29(1):81-90.
2. Li C. Genetics and regulation of angiopoietin-like proteins 3 and 4. *Curr Opin Lipidol.* Apr 2006;17(2):152-156.
3. Merkel M, Eckel RH, Goldberg IJ. Lipoprotein lipase: genetics, lipid uptake, and regulation. *J Lipid Res.* Dec 2002;43(12):1997-2006.
4. Yoshida K, Shimizugawa T, Ono M, et al. Angiopoietin-like protein 4 is a potent hyperlipidemia-inducing factor in mice and inhibitor of lipoprotein lipase. *J Lipid Res.* Nov 2002;43(11):1770-1772.
5. Romeo S, Pennacchio LA, Fu Y, et al. Population-based resequencing of ANGPTL4 uncovers variations that reduce triglycerides and increase HDL. *Nat Genet.* Apr 2007;39(4):513-516.
6. Harris WS. n-3 fatty acids and serum lipoproteins: human studies. *Am J Clin Nutr.* May 1997;65(5 Suppl):1645S-1654S.
7. Mensink RP, Katan MB. Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. *Arterioscler Thromb.* Aug 1992;12(8):911-919.
8. Roche HM, Gibney MJ. Effect of long-chain n-3 polyunsaturated fatty acids on fasting and postprandial triacylglycerol metabolism. *Am J Clin Nutr.* Jan 2000;71(1 Suppl):232S-237S.
9. Ordovas JM. Genetic interactions with diet influence the risk of cardiovascular disease. *Am J Clin Nutr.* Feb 2006;83(2):443S-446S.

5. Hypotheses:

Classic associations between dietary macronutrient intake (dietary fat composition and percentage of energy intake from carbohydrate) and plasma TG and HDL-C and will differ by *ANGPTL4* genotype, with weaker diet-risk factor associations in heterozygotes (E/K) and homozygotes (K/K) compared to wild type homozygotes (E/E).

6. Data:

Participant exclusions:

- Non-fasting
- Non-white race (the *ANGPTL4* [E40K] snp is very rare in blacks—power would be insufficient for detecting diet x genotype interaction)
- Insufficient dietary data (extreme kcal intakes [upper and lower 1% of intake distribution, the ARIC precedent] or multiple missing responses)
- Diabetic (ADA fasting criteria)
- Heavy alcohol consumption
- Missing *ANGPTL4* [E40K] genotype information
- Lipid-lowering medication use

Outcomes:

- TG (primary)
- HDL-C

Exposures:

- Modeled continuously as % of energy intake (or dichotomized at median intake level)
    - Dietary carbohydrate
    - Dietary saturated fat
    - Dietary monounsaturated fat (MUFA)
    - Dietary polyunsaturated fat (PUFA)
    - Dietary total omega-3 PUFA
    - Dietary long-chain omega-3 PUFA

Diet is notoriously measured with error. We will explore the effects of bias due to this error. If we feel the degree of bias is large, we will explore methods to correct for some degree of the error in reported dietary intake.

STATISTICAL ANALYSIS:

SAS 9.1 will be used for all analyses.

NOTE: Main effects of ANGPTL4 genotype on TG and HDL-C have been reported (Romeo et al. 2007, ref 5). However, main effects of dietary macronutrients have not been reported per se. Thus, initial analyses will be conducted to establish criterion validity of classic dietary macronutrient–plasma lipid associations (see ref 7) before genotype stratified analyses are conducted.

Linear regression will be used to assess associations between dietary macronutrients (defined above) and TG, HDL-C. Beta regression coefficients will be presented to show the direction and magnitude of association between exposures and outcomes (expressed as predicted difference in risk factor concentration per 1-percentage energy change in dietary exposure). Due to anticipated skewed distribution of TG concentrations, TG will be transformed to the natural log scale for analysis. Analyses will be conducted in the combined sample (to demonstrate criterion validity of dietary intake measures) and stratified by *ANGPTL4* genotype.

To minimize the number of statistical tests performed, formal tests of interaction will be conducted only where stratified analyses indicate potential differences in direction and/or magnitude if diet-lipid associations. If an interaction achieves statistical significance, genotype-stratified results will be presented graphically or by comparison of mean risk factor concentrations in those participants below or above the median intake level for the macronutrient of interest. (method of presentation is to be determined based on author/analyst discretion)

CONFOUNDERS/MODEL COVARIATES:

Model 1 adjust for center, age, gender, education, physical activity, alcohol, smoking status, cigarette years, and energy.

Model 2 (carbohydrate = exposure) additionally adjust for % energy from protein.

Regression coefficients will then represent the association between carbohydrate and TG or HDL-C where total fat is decreased at the expense of greater carbohydrate (protein is held constant).

Model 2 (dietary saturated, monounsaturated, or polyunsaturated fat = exposure) additionally adjust for % energy from other types of dietary fat and protein.

Regression coefficients will then represent the association between saturated [or MUFA or PUFA] and TG or HDL-C, where carbohydrate is decreased at the expense of greater saturated [or MUFA or PUFA] fat (other fat types and protein are held constant).

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

7.b. NA

8.a. Will the DNA data be used in this manuscript? YES, and genotyping has been completed for the ANGPTL4 snp to be studied in this analysis.

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?

Romeo S, Pennacchio LA, Fu Y, et al. Population-based resequencing of ANGPTL4 uncovers variations that reduce triglycerides and increase HDL. *Nat Genet.* Apr 2007;39(4):513-516.

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

11.b. NA

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.