ARIC Manuscript Proposal # 1248r

1.a.   **Full Title:** Does the UCP2 Ala55Val Polymorphism Influence the Relation between Dairy Consumption and Weight and Diabetes Risk in a Bi-Ethnic Sample of Adults from the Atherosclerosis Risk in Communities (ARIC) Study

b.  **Abbreviated Title (Length 26 characters):** UCP2 Ala55Val Polymorphism, dairy consumption, weight and diabetes risk

2. **Writing Group:** Drs. Goldy C. George, Deanna M. Hoelscher, Kelly A. Volcik, June Stevens, Kari North, Eric Boerwinkle, and other authors as desired.

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

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3. **Timeline:**  
Analyses: 10 months (Jan ‘08 – Oct ‘08)  
Manuscript writing: 6-8 months (Nov ‘08 – Jun ’09)
4. Rationale:

Studies have not been consistent in the relation between dairy consumption and weight status and diabetes risk. Although multiple studies in humans have shown that consumption of dairy foods / calcium is associated with lower body weight / adiposity, and diabetes risk, there have been other studies where these relations could not be confirmed. One possible explanation for this apparent discrepancy is the variation in the genetic make-up of individuals. A polymorphism in the uncoupling protein 2 (UCP 2) gene (a C/T polymorphism resulting in an Ala55Val substitution in exon 4) has been associated with higher body mass index (BMI) and greater risk of diabetes mellitus.12 The relation between the UCP2 Ala55Val polymorphism and the weight and diabetes health outcomes may be due to the role of UCP2 in regulating 24-hour energy expenditure, glucose metabolism, and fat oxidation. Also, it has been reported that dairy components, such as calcium and conjugated linoleic acid, induce expression of adipose tissue mitochondrial uncoupling protein 2 (UCP2) and stimulate adipose tissue apoptosis, in animal models. The proposed research will examine the main effect of dairy intake on weight and diabetes risk, and will investigate whether the UCP2 Ala55Val polymorphism modulates the association between dairy consumption and weight status, and between dairy consumption and diabetes / glucose tolerance in a biethnic sample of adults in the ARIC study.

5. Main Hypothesis/Study Questions:

(1) The main effect of dairy intake on impaired glucose tolerance and diabetes risk.
(2) The main effect of dairy intake on weight status
(3) The interaction between the UCP2 Ala55Val polymorphism and dairy intake in relation to diabetes/impaired glucose tolerance risk.
(4) The interaction between the UCP2 Ala55Val polymorphism and dairy intake in relation to multiple measures of body size.

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 other anticipated methodologic limitations or challenges if present).

Analyses will be performed utilizing STATA software. Participants will be excluded from analyses if they had restricted use of their DNA, if their ethnicities were not African-American or White, if they were pregnant at the time of examination, or if they had missing data. Analyses will be stratified by gender and ethnicity. All analyses will utilize data collected at baseline, and will require information on participant identification, ARIC field center, and visit date. The statistical tests outlined below for aims 1– 4 are intended to be a model and are subject to revision based on the needs of analyses following consultation with statistical experts.
**Statistical Analyses for Aim 1: The main effect of dairy intake on diabetes risk**

The outcome variable for aim 1 is diabetes-related [e.g., presence or absence of diabetes, derived glucose value in mg/dl, fasting and non-fasting blood glucose levels in mmol/L, and insulin in pmol/L; a measure of insulin resistance, namely Homeostasis Model Assessment (HOMA) will be derived using the formula: HOMA = fasting insulin concentration (µU/mL) x fasting glucose concentration (mmol/L) / 22.5]. The independent variable for aim 1 will be dairy-related. Potential confounding variables include other nutrients or foods, use of supplements, physical activity related variables, age, education, income, BMI, smoking, prevalent CHD, cancer, hypertension, and dyslipidemia. Multivariable logistic or linear regression tests will be used for these analyses.

**Statistical Analyses for Aim 2: The main effect of dairy intake on weight status**

The outcome variable for aim 2 will be weight-related [e.g., BMI (kg/m²), present weight, waist circumference, waist-to-hip ratio, weight-at the age of 25, plasma leptin levels, and triceps and subscapular skinfolds to calculate percent body fatness]. The independent variable for aim 2 will be dairy-related. Potential confounding variables include other nutrients or foods, use of supplements, physical activity related variables, age, education, income, smoking, and the prevalence of chronic conditions, such as CHD, diabetes, stroke or cancer. Multivariable linear or logistic regression tests will be used for these analyses.

**Overall genetic analyses for Aims (3) and (4):**

Genetic analyses for aims (3) and (4) will begin by estimating allele frequencies of UCP2 using gene-counting methods. Agreement of UCP2 allele frequencies with Hardy-Weinberg equilibrium (observed genotype frequencies minus expected genotype frequencies) will be tested in the two ethnic groups using the chi-square goodness of fit test.

**Statistical Analyses for Aim 3: UCP 2 Ala55Val polymorphism and dairy intake in relation to prevalent diabetes/impaired glucose tolerance risk**

The outcome variable for aim 3 will be diabetes-related [e.g., presence or absence of diabetes, derived glucose value in mg/dl, fasting and non-fasting blood glucose levels in mmol/L, and insulin in pmol/L; a measure of insulin resistance, namely Homeostasis Model Assessment (HOMA) will be derived using the formula: HOMA = fasting insulin concentration (µU/mL) x fasting glucose concentration (mmol/L) / 22.5]. The independent variables for aim 3 will be dairy-related variables and the UCP2 Ala55Val gene polymorphism. Potential confounding variables include other nutrients or foods, use of supplements, physical activity-related variables (ARIC physical activity questionnaire), age, education, income, BMI, smoking, prevalent CHD, cancer, hypertension and dyslipidemia. Variables may be categorized for analyses (e.g., categorization of dairy intake into tertiles or quartiles) as needed.
Analyses will be stratified by genotype, and the dairy – diabetes relation will be investigated for each genotype, after adjustment for confounding variables. Multivariable logistic or linear regression tests will be used for these analyses.

**Statistical Analyses for AIM 4: UCP 2 Ala55Val polymorphism and dairy intake in relation to weight status**

The outcome variable for aim 4 will be weight-related [e.g., BMI (kg/m²), present weight, waist circumference, waist-to-hip ratio, weight-at the age of 25, plasma leptin levels, and triceps and subscapular skinfolds to calculate percent body fatness]. The independent variables for aim 4 will be dairy-related variables and the UCP2 Ala55Val gene polymorphism. Potential confounding variables for these analyses include other nutrients or foods, use of supplements from visit 1 medication survey, age, education, income, smoking, physical activity-related variables (Baecke physical activity questionnaire – leisure time, sports and work physical activity), special diet/dieting, presence of hypertension, dyslipidemia, diabetes, and cancer. Variables may be categorized for analyses (e.g., classification of BMI based on World Health Organization (WHO) guidelines, categorization of dairy intake into tertiles or quartiles) as needed.

Analyses will be stratified by genotype, and the dairy – weight relation will be investigated for each genotype, after adjustment for confounding variables. Multivariable linear or logistic regression tests will be used for these analyses.

A limitation of the proposed study may be that the analytic strategy lists multiple related but not identical outcomes and multiple related but not identical measures of dairy consumption. In order to test the impact of multiple comparisons, the false discovery rate test may be used. We have also reduced the number of contrasts in the revised manuscript proposal to improve clarity.

Possible error in measurement of dietary intake is acknowledged as a potential limitation, as dietary data were self-reported. Measurement error will be minimized by excluding the highest and lowest 1% of the sex-specific total energy intake distributions. We will also report findings using data, adjusted and unadjusted for energy. Since dietary intake data do not usually follow a normal distribution, data may need to be log-transformed.

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

b. If Yes, is the author aware that the file ICTDER02 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? _X_ Yes  ____ No
(This file ICTDER02 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? _X_ 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 ICTDER02 must be used to exclude those with value RES_DNA = “No use/storage DNA”? _X_ 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

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)?

MS #1005, #1006 (Dr. June Stevens, the senior author on manuscript proposals (MS) #1005 and #1006 has approved formation of a new writing group, with Drs. George, Stevens, Hoelscher, and Boerwinkle on the present manuscript proposal #1248 also being included as writing group members on manuscript proposals #1005 and #1006. The current manuscript proposal (#1248) focuses on cross-sectional baseline analyses and includes genetic data. We intend that analyses for MS #1005 and #1006 will be longitudinal and will not include genetic data. Thus, manuscripts arising from the three proposals will be different. Dr. June Stevens, who was the senior author on manuscript proposals #1005 and #1006 has approved the current MS #1248 and will be a writing group member for all three proposals.)

MS #1116 – The focus of manuscript #1116 is different from that of the currently proposed study as MS#1116 examines UCP2 genetic variation and diabetes with possible effect modification of obesity.

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

   _X_ Yes  ____ No

11.b. If yes, is the proposal

   _X_  A. primarily the result of an ancillary study (list number* AS#1995.07)

   ___ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* __________ __________ __________)

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.

References