1a. Full Title: Genetic predictors of Metabolic Responses to Dairy Foods

b. Abbreviated Title: Genetic predictors of Metabolic Responses to Dairy Foods

2. Writing Group:

Mariaelisa Graff, Anne Justice, Kristin Young, Kari E. North, other Authors for the CHARGE consortium

Other investigators welcome

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

MG

First author: Mariaelisa Graff

Address: Department of Epidemiology
University of North Carolina at Chapel Hill
137 E. Franklin St, Suite 306
CB #8050
Chapel Hill, NC 27514
Phone: 919-966-8491 Fax: 919-966-9800
E-mail: migraff@unc.edu

Corresponding/senior author (if different from first author correspondence will be sent to both the first author & the corresponding author): Kari North

Address: Department of Epidemiology
University of North Carolina at Chapel Hill
137 E. Franklin St, Suite 306
CB #8050
Chapel Hill, NC 27514
Phone: 919-966-2148 Fax: 919-966-9800
E-mail: kari_north@unc.edu

3. Timeline (The following are estimates. Please see project phases and timeline below for more details):
4. Rationale:

Obesity affects over a third of US adults and more than 400 million people worldwide, and its contribution to chronic diseases such as diabetes is a growing global concern. Environmental (eg, dietary) factors play an undeniable role in the rise in obesity and its complications, but a sizable minority of individuals seem resistant to weight gain despite exposure to a similar obesogenic environment. Some genetic associations with obesity risk have been well-replicated, but protection against adiposity and its complications have also been attributed to consumption of certain foods. Notably, dairy foods have been variably linked to adiposity, showing protective, detrimental or no differences in body weight in a range of observational and intervention studies that continue to accumulate (Bowen, 2005; Berkey, 2005; Gunther, 2005; Thompson, 2005; Rajpathak, 2006; Zemel, 2005; Beydoun, 2008; Dove, 2009; Astrup, 2010; Faghih, 2011; Chen, 2012; Gilbert, 2011; Gilbert, 2011; Josse, 2011; Weaver, 2011; van Meijl, 2011; Abargouei, 2012; Lamri, 2013; Kratz, 2013). Associations between dairy intake and glycemic traits are more consistently but not completely favorable, and appear, in some cases, to be independent of body weight (Choi, 2005; Hoyt, 2005; Lawlor, 2005; Liu, 2006; Liu, 2009; Drouillet, 2007; Louie, 2011; Arnberg, 2012; Sluijs, 2012; Grantham, 2013; Kratz, 2013; Mozaffarian, 2013). For both body weight and glycemic traits, exploration of genetic variability in conjunction with dairy food intake may explain a portion of the variability in responses to these foods.

The rationale for investigating the impact of genotype on responses to dairy foods is especially compelling in light of the evolutionary history of dairy intake. Most people (and non-human mammals) are unable to digest lactose post-weaning, but mutations near the lactase locus conferred sustained lactose tolerance in some groups. Evidence of recent positive selection (~8000 years ago) appears to coincide with dairy farming, and reflects reproductive advantages associated with lifelong dairy consumption (Bersaglieri, 2004). Although the strongest selection signal appears near the lactase locus, additional genetic loci were probably affected. Reproductive advantages provided by lifelong dairy consumption may have affected growth, development, body size and body composition, implicating a wide range of biological and genetic pathways. In spite of the large number of potential loci that may have been shaped by the introduction of dairy foods to the human diet, only a few studies so far have examined dairy intake in conjunction with genetic variability (Dedoussis, 2010; Corella, 2011).

Identifying the dairy constituents and genetic modulators that interact to influence health outcomes is challenging because of the chemical complexity and multiple forms of dairy foods. Key nutrients supplied by dairy foods (calcium, magnesium, vitamin D (if fortified), and good quality proteins) are well-recognized. However, additional unique and potentially bioactive components (phytanic acid (3,7,11,15-tetramethyl hexadecanoic acid), palmitoleic acid (C16:1n-7), oligosaccharides, branched chain amino acids and others) may interact with genetic regulators of energy homeostasis, including satiety mechanisms, to modulate obesity risk in unanticipated ways (Shah, 2000; Ward, 2004; German, 2008; Astrup, 2010; Gilbert, 2011). A genome-wide analysis of dairy x SNP interactions (GWIS) may detect novel loci and pathways through which this important food group may be acting to optimize health,
and enable identification of those who stand to benefit most from additional consumption. At the same time, these analyses will pave the way for functional studies that increase biological understanding of dairy benefits.

5. Main Hypotheses/Study Questions:

Aim: To combine into a meta-analysis of data from CHARGE cohorts with genome-wide arrays, including Cardio-MetaboChip, we will test interactions in the ARIC between genotype and i) total dairy foods ii) low-fat dairy foods and iii) high-fat dairy foods for the outcomes of BMI, fasting glucose and fasting insulin. We will also report associations between total dairy foods, high-fat dairy foods and low-fat dairy foods for the same outcomes, without considering genotypes. We will consider all European American and African American ARIC participants that have both GWAS Array data and also the dairy dietary data.

6. Design and Analysis:

Methods

Dependent Variables

1. BMI (kg/m^2)
2. Fasting glucose (SI units: mmol/L)
3. Fasting insulin (SI units: ln-pmol/L) (following natural-log transformation)

Exclusions

- Implausible dietary data
  - Women > 5500kcal or < 500kcal; Men > 6000kcal or <600 kcal.
- Non-white and non-African American (whites and African Americans will be analyzed separately)
- Missing genotype data
- Missing dependent variables
- Type 1 diabetes or type 2 diabetes (self-reported, anti-glycemic medication use, or fasting glucose ≥7 mmol/L (≥126 mg/dL))
- Filters to exclude SNPs based on low quality imputation, low MAF, etc., will be applied centrally.

Independent Dietary Variables

1. Total dairy (servings/day, evaluated continuously)
2. Low fat dairy (servings/day, evaluated continuously)
3. High fat dairy (servings/day, evaluated continuously)

Defining high and low fat dairy

Estimates of total, high-fat and low-fat dairy will be harmonized to the greatest extent possible given the number of questionnaires and differences in the food supply across countries. Proposed definitions of high and low fat dairy, serving size and milk equivalents are below:

High-fat dairy foods include whole milk, 2% milk, cheese, butter, and ice cream.
Low-fat dairy foods include 1% milk, skim milk, cottage cheese, and yogurt.

Definitions of high-fat and low-fat are based on:

Milk serving = 240 ml (1 cup or 8 fluid oz)
Milk serving equivalents*:
• 43 g (1½ oz) hard cheese (cheddar, mozzarella, Swiss, Parmesan)
• 480 ml (2 cups) cottage cheese, 57 g (2 oz) processed cheese
• 360 ml (1½ cups) ice cream
• Butter: 1 pat (1” sq, 1/3 “ high) or 1 teaspoon or 5 grams

Non-dairy milks (soy, nut, rice, coconut) are not included as dairy foods.
*from United States Department of Agriculture (USDA) food groups
http://www.choosemyplate.gov/food-groups/dairy.html# (Note that the dairy group is based on calcium but the genetic impact involves lactose).

Genetic Variables

We will include genome-wide (via GWAS array) genetic data that has been imputed to cover 2.5M SNPs based on Phase II HapMap. All non-autosomal SNPs will be excluded. SNPs are coded as dosages, ranging between 0 and 2.

MODELS
Phase 1 – BMI
Model 1 = Age, Sex, Field Center (as needed), Principal components (as needed to adjust for population substructure), Family structure (as needed)
Model 2 = Model 1 + total energy (kcal/day) + physical activity (sedentary vs. non-sedentary, if available) + CHARGE diet score

Phase 2 – Fasting glucose and fasting insulin
Model 3: Fasting Glucose = Age, Sex, Field center (as needed), Principal components (as needed for population substructure), Family structure (as needed) + total energy (kcal/day)
Model 4: Fasting Insulin = Same model as for Fasting Glucose + BMI

Note: When the trait of interest is insulin, analysis should also be adjusted for BMI.

ANALYSES

For the models above conduct genome-wide linear regression analyses or linear mixed effects models with indicated metabolic traits (BMI, fasting glucose or fasting insulin) as outcomes.

1. Association analyses

MODEL: METABOLIC TRAIT = Dietary exposure + covariates
2. Interaction analyses

The primary independent variables of interest will be the main effect of each SNP and its interaction with a dietary exposure. Output from analyses will include regression parameters, their standard errors and p values, and also covariance estimates. The covariance estimate will be used to conduct 2 df tests of the association of a SNP, taking possible interaction into account. The Model can be stated as follows:

MODEL: METABOLIC TRAIT = SNP + Dietary exposure + SNP*Dietary exposure + covariates

Genome-wide significance threshold will be results with p value < 5 x 10^{-8}. Each study will conduct analyses of its data and results will be meta analyzed with fixed effects inverse variance weighting of the regression coefficients.

Obtain robust standard errors and covariance estimate between the SNP beta and the Interaction beta. These will be run using ProbABEL version 0.1-3 (http://mga.bionet.nsc.ru/~yurii/ABEL/)

REFERENCES


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Lawlor DA, Ebrahim S, Timpson N et. al. Avoiding milk is associated with a reduced risk of insulin resistance and the metabolic syndrome: findings from the British Women's Heart and Health Study. Diabetic Medicine. 22(6):808-11, 2005 PMID: 16911634


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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 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

_ 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)?
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 (AS #2006.03 & 2007.02)
   ___ 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.