ARIC Manuscript Proposal # 1716

PC Reviewed: 11/9/10	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: CHARGE Nutrition x Gene Working Group: Analysis of interactions between dietary magnesium and SNPs related to fasting glucose and insulin

b. Abbreviated Title (Length 26 characters): CHARGE Magnesium x SPNs

2. Writing Group Writing group members:

Nicola McKeown (project leader, working group member, Framingham representative)
Jennifer Nettleton* (project leader, working group chair, ARIC & MESA representative)
Stavroula Kanoni* (working group member, GHRAS representative)
Ioanna Ntalla* (working group member, GENDAI representative)
Frank van Rooij* (working group member, Rotterdam Study representative)
Toshiko Tanaka* (working group member, InCHIANTI representative)
Vera Mikkilä* (working group member, Young Finns Study representative)
Mary Wojczynski* (working group member, Family Heart Study representative)
Rozenn Lemaitre* (working group member, Cardiovascular Health Study representative)
Emily Sonestedt* (working group member, Malmo Study representative)
Julius Ngwa* (working group member, Framingham representative)
Ani Manichaikul* (working group member, MESA representative)
Adrienne Cupples (working group member, Framingham representative)
James Meigs (working group member, Framingham senior representative)
David Siscovick (working group member, Cardiovascular Health Study senior representative)
George Dedoussis (working group member, GHRAS &GENDAI senior representative)

*indicates data analyst

Additional authors are likely to be added. Other ARIC contributors are welcome to join this effort if they have interest or expertise related to the proposed work.

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

First author: **Nicola McKeown**
Address: Nutrition Epidemiology Department,
JM USDA HNRCA at Tufts University
Boston, MA 02111
Phone: 617 556 3367 Fax:617 556 3344
E-mail: Nicola.McKeown@tufts.edu

ARIC author to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).
Name: Jennifer Nettleton
Address: 1200 Herman-Pressler; RAS E-641; Houston, TX 77030
Phone: 713-500-9367
E-mail: jennifer.a.nettleton@uth.tmc.edu

3. Timeline: 4 months

4. Rationale:

Magnesium is an essential mineral derived from several dietary sources, including whole-grains, green leafy vegetables, legumes, and nuts [1] and is critical in normal magnesium-dependent cellular reactions in the human body [2]. Evidence from observational studies suggest that diets rich in magnesium foods are protective against type 2 diabetes [3-4], while in intervention studies, pharmacological doses of magnesium improve glucose metabolism [5]. Although yet to be considered, it is possible that the effects of magnesium on glucose metabolism may depend on variation in the genes associated with glucose and insulin metabolism.

Candidate genes studies and genome-wide association studies (GWAS) have identified about several dozen single nucleotide polymorphisms (SNPs) associated with continuous measures of glucose and insulin metabolism. Recently, the MAGIC Consortium, of which FHS SHARe is a founding member, reported that variants in the gene encoding the melatonin receptor *MTNR1B* is associated with levels of fasting glucose and risk of T2D [6]. Ongoing genome-wide meta-analyses have revealed 26 novel, independent loci associated with diabetes-related quantitative traits (see the Table 1 below). Each of these loci offers the opportunity to test diet-gene interactions associated with diabetes-related quantitative traits

In addition, recent studies suggest that transient receptor potential membrane melastatin 6 and 7 (TRPM6 and TRPM7), two members of the "transient receptor potential" (TRP) family of cation channels, may play a central role in the regulation of magnesium homeostasis [7] . Our proposed study will be the first to examine in human population data whether magnesium intake modifies any common genetic effect of TRPM6 and TRPM7 on measures of fasting glucose and insulin. A recent study [8] found that two common non-synonymous TRPM6 coding variants may interact with magnesium intake in determining the risk of type 2 DM.

5. Main Hypothesis/Study Questions:

The goal of this analysis plan is to test interactions between dietary magnesium intake (continuous) and X SNPs (additive model) in prediction of fasting glucose and insulin concentrations (continuous) using linear regression models and accounting for familial relationships as appropriate. We will use a single, minimally adjusted model to test the interaction between dietary magnesium intakes and a given SNP (including dietary exposure, SNP, and dietary exposure x SNP) terms in each model.

REFERENCES ON PAGE 6-7

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).

ANALYSIS 1a: Magnesium intake → Fasting glucose (continuous)
ANALYSIS 1b: SNP(s) → Fasting glucose (continuous)
ANALYSIS 1c: Magnesium intake x SNPs → Fasting Glucose (continuous)
ANALYSIS 2a: Magnesium intake → Fasting Insulin (continuous, with natural log transformation)
ANALYSIS 2b: SNP(s) → Fasting insulin (continuous)
ANALYSIS 2c: Magnesium intake x SNPs → Fasting Insulin (continuous, with natural log transformation)

The rationale behind this analysis plan is to test the main effect and interactions between magnesium intake (continuous) and X SNPs (additive model) in prediction of fasting glucose concentrations (continuous) and magnesium intake and X SNPs in prediction of ln-transformed fasting insulin concentrations using linear regression models and accounting for familial relationships, if appropriate. We will use a single, minimally adjusted model to test the interaction between dietary magnesium intake and a given SNP (including dietary magnesium intake, SNP, and dietary magnesium intake x SNP) terms in each model. (*SNP main effects have previously been estimated in MAGIC, and their achieved genome-wide significance was among the criteria for their inclusion in the current analysis*).

The specifics of the analysis plan are outlined below.

Exclusions:

- Prevalent type 2 diabetes
  - ✓ Self-reported diabetes
  - ✓ Taking medication for diabetes
  - ✓ Fasting glucose ≥126 mg/dL (≥7 mmol/L)
- Non-fasting status
- Implausible dietary data (cohort-specific definition)
- Non-white race

Model Covariates:

- sex
- age (continuous)
- field center (if needed)
- total energy intake (kcal intake per day, continuous)
- population/family substructure adjustment, as needed

Dependent Variable:
Analysis 1: Fasting glucose concentration (mmol/L, continuous, untransformed)
Analysis 2: Fasting insulin concentration (pmol/L, continuous, ln-transformed)

Main-effects Analysis (*cohort-specific results will be meta-analyzed*)**:**

Magnesium main effects →

Multivariable Model Analysis 1a:
fasting glucose = magnesium intake (mg/d, continuous), + model covariates listed above

Multivariable Model Analysis 2a:
ln fasting insulin = magnesium intake (mg/d, continuous) + model covariates listed above

SNP main effects →

Multivariable Model Analysis 1B:
fasting glucose = SNP + model covariates listed above* (*but w/o energy intake)

Multivariable Model Analysis 2B:
ln fasting insulin = SNP + model covariates listed above* (*but w/o energy intake)

Interaction Analysis (*cohort-specific results will be meta-analyzed*)**:**

Multivariable Model Analysis 1c:
fasting glucose = SNP (estimated copies of risk allele), magnesium intake (mg/d, continuous), **SNP*Magnesium Intake** + model covariates listed above

Multivariable Model Analysis 2c:
ln fasting insulin = SNP (estimated copies of risk allele), magnesium intake (mg/d, continuous), **SNP*Magnesium Intake** + model covariates listed above

*→Each cohort will provide beta regression coefficient, SE, and p value for A) the dietary magnesium intake (mg/d)*SNP product term, B) the dietary magnesium intake (mg/d) marginal term, C) the SNP marginal term, and D) the intercept β and SE using the single model specified above.*

Data Sharing

MAIN-EFFECTS ANALYSES: β, SE, *p*
- Cohort-specific magnesium term*
- Cohort-specific SNP term*

**two separate models*

INTERACTION ANALYSES: β, SE, *p* (from likelihood ratio tests)
- Cohort-specific magnesium x SNP interaction product term (*regression coefficient, SE, and p value*)
- Cohort-specific magnesium marginal effect term (*regression coefficient, SE, and p value*)
- Cohort-specific SNP marginal effect term (*regression coefficient, SE, and p value*)
- Cohort-specific intercept (*regression coefficient, SE*)

***single model for each Mg*SNP test (one for each SNP listed on page 4)*

Also, for descriptive purposes, please provide the following:
Mean and SE or %
- Cohort-specific sex distribution (%female)
- Cohort-specific age (years *mean ± SE*)
- Cohort-specific energy intake (kcal/day *mean ± SE*)
- Cohort-specific magnesium intake (mg/day *mean ± SE*)
- Cohort-specific fasting glucose concentrations (mmol/L *mean ± SE*)
- Cohort-specific fasting ln-transformed insulin concentrations (pmol/L *mean ± SE*)
- Cohort-specific glucose/insulin-raising allele frequency

An Excel file will be populated by each cohorts
When complete, the file will be emailed to Jennifer Nettleton (jennifer.a.nettleton@uth.tmc.edu).

Meta analyses: Meta analyses will be conducted on the regression coefficients for the magnesium x SNP interaction term.

Significance: Need to include but appears to be 19 tests for glucose/5 insulin
(Bonferroni correction)

Fasting Glucose SNP list:
Please use the following SNPs identified in MAGIC as significant predictors of fasting glucose concentrations in GWA. We will use an additive model based on the estimated copies of the high-risk allele.

No.	SNP	Chr	Nearest gene	Effect/other allele
1	rs560887	2	*G6PC2*	C/T
2	rs10830963	11	*MTNR1B*	G/C
3	rs4607517	7	*GCK*	A/G
4	rs2191349	7	*DGKB/TMEM195*	T/G
5	rs780094	2	*GCKR*	C/T
6	rs11708067	3	*ADCY5*	A/G
7	rs7944584	11	*MADD*	A/T
8	rs11605924	11	*CRY2*	A/C
9	rs10885122	10	*ADRA2A*	G/T
10	rs174550	11	*FADS1*	T/C
11	rs340874	1	*PROX1*	C/T
12	rs11920090	3	*SLC2A2*	T/A
13	rs7034200	9	*GLIS3*	A/C
14	rs11558471	8	*SLC30A8*	A/G
15	rs11071657	15	FAM148B	A/G
16	rs4506565	10	*TCF7L2*	T/A

Fasting Insulin SNP list:
Please use the following SNPs identified in MAGIC as significant predictors of fasting insulin concentrations in GWA. We will use an additive model based on the estimated copies of the high-risk allele (with natural log transformation of Insulin).

No.	SNP	Chr	Nearest gene	Effect/other allele
5	rs780094	2	*GCKR*	C/T
17	Rs35767	12	*IGF1*	G/A

Biological SNP list:

No.	SNP	Chr	Nearest gene	Effect/other allele
18	rs3750425	9	*TRPM6*	G/A
19	rs2274924	9	*TRPM6*	A/G
20	rs8042919	15	*TRPM7*	G/A

7.a. Will the data be used for non-CVD analysis in this manuscript? _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? _x_ ***Yes***

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"?
_x_ ***Yes***

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)?

Other from the CHARGE Nutrition working group:

1534 *"Interactions between whole grain intake and genotype with respect to fasting glucose concentrations in multiple cohorts within the CHARGE & MAGIC consortia"*
1577 *"Interactions between zinc intake and SNPs and their impact on fasting blood glucose levels in multiple cohorts within the CHARGE and MAGIC consortia"*
1675 *"Low density lipoprotein receptor related protein 1, fatty acids and anthropometric traits"*
1656 *"Genome-wide association analysis of macronutrient intake"*

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

GWAS via STAMPEDE & GENEVA, #2006.03
Interactions between Diet and Genes Related to Risk of Type II Diabetes, #2007.12

11.b. If yes, is the proposal

A. primarily the result of an ancillary study (list number* *2007.12 & 2006.03)*

ARIC is one of several cohort studies contributing data to the CHARGE/MAGIC-based meta-analysis. Since this work is a product of CHARGE which utilizes GWA data, ancillaries related to STAMPEDE & GENVA are also acknowledged.

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.

1. Subar, A.F., et al., *Dietary sources of nutrients among US adults, 1989 to 1991.* J Am Diet Assoc, 1998. **98**(5): p. 537-47.
2. Elin, R.J., *Magnesium: the fifth but forgotten electrolyte.* Am J Clin Pathol, 1994. **102**(5): p. 616-22.
3. Kim, D.J., et al., *Magnesium Intake in Relation to Systemic Inflammation, Insulin Resistance, and the Incidence of Diabetes.* Diabetes Care, 2010.
4. Larsson, S.C. and A. Wolk, *Magnesium intake and risk of type 2 diabetes: a meta-analysis.* J Intern Med, 2007. **262**(2): p. 208-14.
5. Yokota, K., et al., *Clinical efficacy of magnesium supplementation in patients with type 2 diabetes.* J Am Coll Nutr, 2004. **23**(5): p. 506S-509S.
6. Prokopenko, I., et al., *Variants in MTNR1B influence fasting glucose levels.* Nat Genet, 2009. **41**(1): p. 77-81.

7. Touyz, R.M., *Transient receptor potential melastatin 6 and 7 channels, magnesium transport, and vascular biology: implications in hypertension.* Am J Physiol Heart Circ Physiol, 2008. **294**(3): p. H1103-18.
8. Song, Y., et al., *Common genetic variants of the ion channel transient receptor potential membrane melastatin 6 and 7 (TRPM6 and TRPM7), magnesium intake, and risk of type 2 diabetes in women.* BMC Med Genet, 2009. **10**: p. 4.