ARIC Manuscript Proposal #3150

PC Reviewed: 4/10/2018	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Serum Magnesium Levels and Subsequent Risk of Peripheral Artery Disease (PAD) in the Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters):
Magnesium & PAD

2. Writing Group:
Writing group members: Steven Menez, Ning Ding, Morgan Grams, Pamela Lutsey, Gerardo Heiss, Aaron Folsom, Elizabeth Selvin, Josef Coresh, Bernard Jaar, Kunihiro Matsushita

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

First author: Steven Menez, MD
Address: Department of Medicine, Division of Nephrology
Johns Hopkins School of Medicine
1830 E. Monument Street, Suite 416, Baltimore, MD 21287
Phone: 304-376-4752
Fax: 443-367-2258
E-mail: smenez1@jhmi.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: Kunihiro Matsushita, MD, PhD
Address: Department of Epidemiology
Johns Hopkins Bloomberg School of Public Health
Welch Center for Prevention, Epidemiology, and Clinical Research
2024 E Monument Street, 2-600
Phone: (410)502-2051
Fax: (410) 367-2384
E-mail: kmatsush@jhsph.edu

3. Timeline:
The analyses will use existing ARIC data, and manuscript preparation will be performed in the following 6 months.

4. Rationale:
Peripheral artery disease (PAD) is the third leading cause of atherosclerotic vascular morbidity after coronary heart disease and stroke, and this disorder affects more than 200 million individuals around the world.[1] Although traditional atherosclerotic risk factors (i.e. smoking, diabetes, hypertension and dyslipidemia) are considered to play important roles in the development of PAD, there is a growing interest in identifying nontraditional biomarkers of PAD since the contributions of risk factors to the development of PAD vs. other atherosclerotic diseases are known to be different.[2, 3] Also, it is expected that some biomarkers may help identify persons at high risk of PAD. This is an important aspect since most PAD patients are asymptomatic or have atypical leg symptoms and the diagnosis of PAD usually requires referral to vascular specialists for specific evaluation including ankle-brachial index.

In this context, we are interested in the potential role of magnesium in the development and progression of PAD for several reasons. First, hypomagnesemia as defined by serum magnesium < 1.6 mg/dL has been associated with a number of cardiovascular phenotypes such as heart failure [4] and sudden cardiac disease (SCD) [5]. Second, magnesium plays an important role in the synthesis of ATP, DNA, and proteins among other cellular functions. Indeed, various animal and *in vitro* studies have demonstrated the strong association between magnesium deficiency and systemic inflammation, and primary drivers of the inflammatory response include tumor necrosis factor-alpha, interleukin-1, interkleukin-6, among others.[6, 7] Third, magnesium may protect against vascular calcification through its interplay with calcium and phosphorous.[8] More specifically, magnesium can bind to phosphate and protect against the formation of calcium-phosphate nanocrystals[9, 10]. Several observational studies predominantly analyzing patients with end-stage renal disease (ESRD) have shown that hypomagnesemia may be associated with vascular calcification or the development of PAD. Finally, a few interventional trials reported protective effects of dietary magnesium administration on various outcomes including carotid intimal thickness.[11, 12]. However, those trials focused on ESRD patients.

To our knowledge, however, no large community-based study has explored serum magnesium levels as a predictor of incident PAD. Using data from the ARIC Study, we will quantify the associations of serum magnesium levels with the risk of PAD. In addition, with a follow-up over 25 years, the ARIC Study will allow us to explore uniquely the relationship of magnesium to critical limb ischemia (CLI), the most severe form of PAD as well.

5. Main Hypothesis/Study Questions:
Are serum magnesium levels independently associated with risk of incident PAD?

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).
Study design:
Multi-center population-based prospective cohort study[14]

Inclusion criteria:

All African American and white participants in the ARIC Study free of prevalent PAD at Visit 1 and with a serum creatinine (sCr) with data on serum magnesium (Mg) levels as well as possible confounding covariates, and incident PAD.

Exclusion criteria:
- Participants who identified themselves as non-white/non-black.
- Participants with prevalent PAD at baseline (i.e., ABI<0.9, self-reported peripheral revascularization, intermittent claudication based on the Rose questionnaire).
- Participants with missing data on serum magnesium and other covariates of interest, and PAD

Exposure:
- Serum magnesium level drawn at study visit 1 and visit 2 if available, measured by the Gindler and Heth procedure by using the metallochromic dye calmagite [1-(1-hydroxy-4-methyl-2-phenylazo)-2-napthol-4sulfonic acid].[15]
- Due to longer follow-up and a larger number of PAD cases, we will primarily analyze visit 1 magnesium and try to replicate the results using visit 2 magnesium.
- We will also evaluate the mean Mg level at visits 1 and 2 as well.
- We considered the possibility to explore dietary intake of magnesium, but prior work in the ARIC study has demonstrated a weak correlation between dietary intake of magnesium, as defined by food frequency questionnaires, with serum magnesium (r=0.053),[13] potentially due to limitations of evaluating magnesium intake from food frequency questionnaires. Thus, in this proposal, we will focus on serum magnesium levels as the exposure of interest. Also, this approach will be consistent with our original motivation of exploring a biomarker for PAD as described in Rationale above.

Outcome:
- PAD-related hospitalizations will be identified with the following ICD codes based on previous literature: atherosclerosis of native arteries of the extremities, unspecified (440.20); atherosclerosis of native arteries of the extremities with intermittent claudication (440.21); atherosclerosis of native arteries of the extremities with rest pain (440.22); atherosclerosis of native arteries of the extremities with ulceration (440.23); atherosclerosis of native arteries of the extremities with gangrene (440.24); other atherosclerosis of native arteries of the extremities (440.29); atherosclerosis of bypass graft of the extremities (440.3); atherosclerosis of other specified arteries (440.8); leg artery revascularization (38.18, 39.25, 39.29, 39.50).
- Of PAD cases described above, those with 440.22, 440.23, and 440.24 as well as any cases with the coexisting code of leg amputation (84.1x), lower extremity ulcer (707.1x), and gangrene (785.4) will be considered as Critical limb ischemia (CLI).

Covariates (not all covariates listed below are available at both visits 1 and 2, and for visit 1 and visit 2 magnesium analyses, covariates at visit 1 and visit 2 will be used, respectively):
- Sociodemographic data: age, race, gender, education level, alcohol use
- Physical measurements: body mass index, systolic and diastolic blood pressure
- Serum electrolyte measurement: potassium, calcium, phosphorus
- Other biochemical data of interest: FGF-23, PTH, total cholesterol, HDL cholesterol
- Associated medical comorbidities:

- Diabetes, defined as fasting glucose level >126 mg/dL (>7.0 mmol/L), non-fasting glucose level >200 mg/dL (>11.1 mmol/L), self-reported physician diagnosis, or use of antidiabetic medications
- Prevalent coronary heart disease, defined as cases adjudicated by physician-panel between visits 1 and 2 in addition to self-reported clinical history and evidence of prior myocardial infarction by electrocardiogram obtained at visit 1.

- Medications, by self-reported use (antihypertensive medications, antiplatelet, diuretics, and lipid lowering medications)
- Kidney function: eGFR, calculated using the CKD-EPI (CKD Epidemiology Collaboration) equations based on demographical variables, age, gender, race, and either or both filtration markers, serum creatinine and cystatin C. Serum creatinine was measured by a modified kinetic Jaff_e method, and serum cystatin C and B2M were measured by a particle-enhanced mmunonephelometric assay using a BNII nephelometer (Siemens Healthcare Diagnostics).[16-18]

Statistical analysis plan:
- Baseline characteristics will be compared among all participants with quartiles of magnesium levels available at visit 1.
- Cox proportional hazards modeling will be used to analyze the time to development of PAD based on magnesium levels, broken up into quartiles of serum Mg level
  - Model 1 will be adjusted for baseline variables of age, race-center, gender, and education level
  - Model 2 will be adjusted further for smoking, BMI, diabetes, hypertension, lipids, prevalent coronary heart disease, prevalent stroke, alcohol use, and medication use
  - Model 3 will be further adjusted for potassium, phosphorous, calcium, FGF-23, and PTH
  - Model 4 will be additionally adjusted for inflammatory markers available at relevant visits (e.g., white blood cell count at visit 1 and high-sensitivity C-reactive protein at visit 2)
- We will include a sub-group analysis of development of PAD across CKD stages and by key demographic and clinical subgroups (e.g., gender, race, diabetic status)

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 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 ICTDER 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? ____ Yes __X__ No

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"? ____ 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)?

There are a few proposals exploring serum magnesium and the risk of coronary heart disease (#2615) and stroke (MS1268), but to the best of our knowledge, there are no ARIC proposals tackling serum magnesium and PAD.

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

___ A. primarily the result of an ancillary study (list number* __2014.05___)

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

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

12a. 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.

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is **your responsibility to upload manuscripts to PubMed Central** whenever the journal does not and be in compliance with this policy. Four files about the public access policy from http://publicaccess.nih.gov/ are posted in http://www.cscc.unc.edu/aric/index.php, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to PubMed central.

13. Per Data Use Agreement Addendum, approved manuscripts using CMS data shall be submitted by the Coordinating Center to CMS for informational purposes prior to publication. Approved manuscripts should be sent to Pingping Wu at CC, at pingping_wu@unc.edu. I will be using CMS data in my manuscript ____ Yes __X__ No.

REFERENCES

1. Fowkes FG, Rudan D, Rudan I, et al. Comparison of global estimates of prevalence and risk factors for peripheral artery disease in 2000 and 2010: a systematic review and analysis. *Lancet*. 2013;382(9901):1329-1340. doi: 10.1016/S0140-6736(13)61249-0 [doi].

2. Hazarika S, Annex BH. Biomarkers and Genetics in Peripheral Artery Disease. *Clin Chem*. 2017;63(1):236-244. doi: 10.1373/clinchem.2016.263798 [doi].

3. Benjamin EJ, Virani SS, Callaway CW, et al. Heart Disease and Stroke Statistics-2018 Update: A Report From the American Heart Association. *Circulation*. 2018;137(12):e67-e492. doi: 10.1161/CIR.0000000000000558 [doi].

4. Lutsey PL, Alonso A, Michos ED, et al. Serum magnesium, phosphorus, and calcium are associated with risk of incident heart failure: the Atherosclerosis Risk in Communities (ARIC) Study. *Am J Clin Nutr*. 2014;100(3):756-764. doi: 10.3945/ajcn.114.085167 [doi].

5. Peacock JM, Ohira T, Post W, Sotoodehnia N, Rosamond W, Folsom AR. Serum magnesium and risk of sudden cardiac death in the Atherosclerosis Risk in Communities (ARIC) Study. *Am Heart J*. 2010;160(3):464-470. doi: 10.1016/j.ahj.2010.06.012 [doi].

6. Mazur A, Maier JA, Rock E, Gueux E, Nowacki W, Rayssiguier Y. Magnesium and the inflammatory response: potential physiopathological implications. *Arch Biochem Biophys*. 2007;458(1):48-56. doi: S0003-9861(06)00136-6 [pii].

7. Nielsen FH. Magnesium deficiency and increased inflammation: current perspectives. *J Inflamm Res*. 2018;11:25-34. doi: 10.2147/JIR.S136742 [doi].

8. Massy ZA, Drueke TB. Magnesium and outcomes in patients with chronic kidney disease: focus on vascular calcification, atherosclerosis and survival. *Clin Kidney J*. 2012;5(Suppl 1):i52-i61. doi: 10.1093/ndtplus/sfr167 [doi].

9. LeGeros RZ, Contiguglia SR, Alfrey AC. Pathological calcifications associated with uremia: two types of calcium phosphate deposits. *Calcif Tissue Res*. 1973;13(3):173-185.

10. Boskey AL, Posner AS. Effect of magnesium on lipid-induced calcification: an in vitro model for bone mineralization. *Calcif Tissue Int*. 1980;32(2):139-143.

11. Spiegel DM, Farmer B. Long-term effects of magnesium carbonate on coronary artery calcification and bone mineral density in hemodialysis patients: a pilot study. *Hemodial Int*. 2009;13(4):453-459. doi: 10.1111/j.1542-4758.2009.00364.x [doi].

12. Turgut F, Kanbay M, Metin MR, Uz E, Akcay A, Covic A. Magnesium supplementation helps to improve carotid intima media thickness in patients on hemodialysis. *Int Urol Nephrol*. 2008;40(4):1075-1082. doi: 10.1007/s11255-008-9410-3 [doi].

13. Peacock JM, Folsom AR, Arnett DK, Eckfeldt JH, Szklo M. Relationship of serum and dietary magnesium to incident hypertension: the Atherosclerosis Risk in Communities (ARIC) Study. *Ann Epidemiol*. 1999;9(3):159-165. doi: S1047279798000404 [pii].

14. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. The ARIC investigators. *Am J Epidemiol*. 1989;129(4):687-702.

15. The ARIC Investigators. Atherosclerosis Risk in Communities Study: operations manual 10. Clinical chemistry determinations. 1987.

16. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150(9):604-612. doi: 150/9/604 [pii].

17. Stevens LA, Coresh J, Schmid CH, et al. Estimating GFR using serum cystatin C alone and in combination with serum creatinine: a pooled analysis of 3,418 individuals with CKD. *Am J Kidney Dis*. 2008;51(3):395-406. doi: 10.1053/j.ajkd.2007.11.018 [doi].

18. Levin A, Stevens P, Bilous R, et al. Kidney Disease: improving Global Outcomes (KDIGO) CKD Work Group: KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease . 2013;3:1-150.