

Serum magnesium, bone–mineral metabolism markers and their interactions with kidney function on subsequent risk of peripheral artery disease: the Atherosclerosis Risk in Communities Study

Steven Menez ^{1,2,3}, Ning Ding^{2,3}, Morgan E. Grams^{1,2,3}, Pamela L. Lutsey ⁴, Gerardo Heiss⁵, Aaron R. Folsom⁴, Elizabeth Selvin^{1,2,3}, Josef Coresh^{1,2,3}, Bernard G. Jaar^{1,2,3,6} and Kunihiro Matsushita^{1,2,3}

¹Johns Hopkins Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA, ²Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, ³Welch Center for Prevention, Epidemiology, and Clinical Research, Johns Hopkins School of Medicine, Baltimore, MD, USA, ⁴Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, Minneapolis, MN, USA, ⁵Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA and ⁶Nephrology Center of Maryland, Baltimore, MD, USA

Correspondence to: Kunihiro Matsushita; E-mail: kmatus5@jhmi.edu

ABSTRACT

Background. Few studies have investigated the association of magnesium levels with incident peripheral artery disease (PAD) despite emerging evidence of magnesium contributing to vascular calcification. Moreover, no data are available on whether the magnesium–PAD relationship is independent of or modified by kidney function.

Methods. A cohort of 11 839 participants free of PAD in the Atherosclerosis Risk in Communities Study at Visit 2 (1990–92) was studied. We investigated the association of serum magnesium and other bone–mineral metabolism markers [calcium, phosphorus, intact parathyroid hormone (iPTH) and intact fibroblast growth factor-23] with incident PAD using multivariable Cox proportional hazards regression.

Results. Over a median of 23 years, there were 471 cases of incident PAD. The hazard ratio for incident PAD in Quartile 1 (<1.5 mEq/L) versus Quartile 4 (>1.7 mEq/L) of magnesium was 1.96 (95% confidence interval 1.40–2.74) after adjustment for potential confounders. Lower magnesium levels were associated with greater incidence of PAD, particularly in those with estimated glomerular filtration rate (eGFR) ≥ 60 mL/min/1.73 m² ($n = 11\,606$). In contrast, the association was largely flat in those with eGFR <60 mL/min/1.73 m² ($n = 233$) with P-for-interaction 0.03. Among bone–mineral metabolism markers, only higher iPTH showed an interaction with kidney function (P-for-interaction 0.01) and iPTH >65 pg/mL was significantly related to PAD only in those with eGFR <60 mL/min/1.73 m².

Conclusions. Lower magnesium was independently associated with incident PAD, but this association was significantly weaker in

those with reduced kidney function. In contrast, higher iPTH levels were particularly related to PAD risk in this clinical population.

Keywords: bone–mineral metabolism, chronic kidney disease, epidemiology, peripheral artery disease

INTRODUCTION

Peripheral artery disease (PAD) affects >200 million individuals around the world [1]. PAD is especially important in persons with chronic kidney disease (CKD) since it occurs more frequently than myocardial infarction or stroke in this population [2]. There is a growing interest in studying risk factors of PAD since the contributions of risk factors to the development of PAD compared with other atherosclerotic diseases are known to be different (e.g. diabetes is more closely related to PAD than coronary artery disease) [3, 4].

Serum magnesium is a promising potential risk factor of PAD for several reasons. A number of cellular pathways have been elucidated by which magnesium may play a role in the inhibition of vascular calcification [5–7]. Magnesium can bind to phosphate and protect against the formation of calcium–phosphate nanocrystals, which can contribute to vascular calcification, particularly in the setting of reduced kidney function. *In vitro* and animal studies have demonstrated a strong association between magnesium deficiency and systemic inflammation, a condition known to promote atherosclerosis [8, 9]. In patients with Type 2 diabetes mellitus, hypomagnesemia has been associated with more rapid kidney function decline including progression to end-stage kidney disease [10, 11].

Although several studies have reported the associations of hypomagnesemia with cardiovascular phenotypes such as heart failure [12], atrial fibrillation [13] and sudden cardiac death [14], data on the prospective associations of magnesium with PAD are limited. To our knowledge, a single study reported hypomagnesemia associated with greater incident PAD in the general population [15]. However, despite the tight pathophysiological link of magnesium to kidney function and calcium-phosphate metabolism, the study did not fully account for bone–mineral metabolism markers and explore potential interaction with kidney function in understanding the etiological association of magnesium and PAD. Furthermore, magnesium at replete or high levels can inhibit intact parathyroid hormone (iPTH), whereas several rodent models have demonstrated hypomagnesemia as a driver of intact fibroblast growth factor-23 (FGF-23) upregulation.

Identifying biomarkers of PAD can be particularly important since most individuals with PAD are asymptomatic or have atypical leg symptoms and thus are often diagnosed later in the disease course. In some patients, ischemic ulcers or gangrene can be the first manifestation of PAD in its most severe form, a condition known as critical limb ischemia (CLI). The prognosis of CLI is devastating, with half of the patients either dying or losing their limbs within a year from the time of diagnosis [16].

Therefore, we sought to investigate comprehensively the prospective association of serum magnesium levels with incidence of clinical PAD in a large African American and Caucasian community-based study, the Atherosclerosis Risk in Communities (ARIC) Study. We explored whether the adjustment for several bone–mineral metabolism markers (e.g. calcium, phosphorus, iPTH and FGF-23) attenuated the association of magnesium and PAD risk and whether the magnesium–PAD relationship was modified by kidney function. We also investigated the associations of bone–mineral metabolism markers with incident PAD risk by kidney function. We

hypothesized that hypomagnesemia would be independently, inversely associated with incident PAD and CLI, with potential interaction by kidney function. We further hypothesized that bone–mineral metabolism markers would be independently associated with incident PAD in participants with estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m².

MATERIALS AND METHODS

Study design and population

The ARIC Study is an ongoing prospective cohort study of 15 792 individuals aged between 45 and 64 years at Visit 1 (between 1987 and 1989) from four communities in the USA (Forsyth County, NC, USA; Jackson, MS, USA; suburban Minneapolis, MN, USA; and Washington County, MD, USA) [17].

We analyzed data from Visit 2 in this study due to the availability of relevant variables including magnesium and bone–mineral metabolism markers, with a starting population of 14 348 participants. We excluded Visit 2 participants of non-black or nonwhite race ($n = 42$), those with prevalent PAD ($n = 641$) and those with missing other covariates ($n = 1826$) for a final analytic population of 11 839 (Figure 1). Prevalent PAD was defined as self-reported leg pain with ambulation, self-reported leg artery revascularization or an ankle-brachial index ≤ 0.9 .

Magnesium

Serum magnesium was measured by the Gindler and Heth procedure, using metallochromic dye calmagite [1-(1-hydroxy-4-methyl-2-phenylazo)-2-naphthol-4-sulfonic acid]. When samples were split and measured a week apart, within-participant laboratory coefficient of variation (CV) was 3.6% [12].

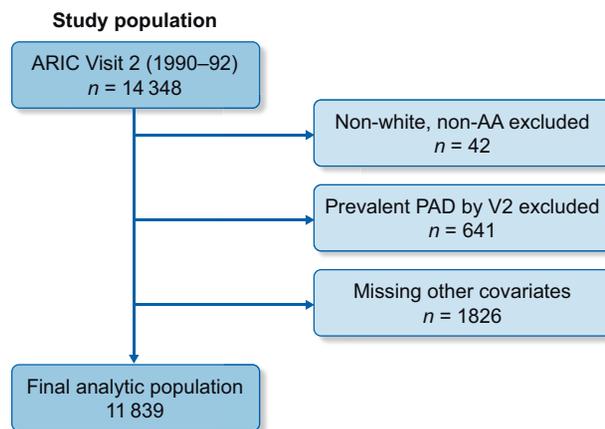


FIGURE 1: Flowchart of ARIC Study Visit 2 analytic population. Covariates: education, biochemical markers, BMI, smoking status, alcohol status, medical comorbidities. AA, African American; V2, Visit 2.

Bone–mineral metabolism markers

Serum calcium was measured using *o*-cresolphthalein complexone with a CV of 1.1%. Serum phosphorus was measured using ammonium molybdate with CV 7.6%. iPTH was measured via sandwich immunoassay method using a Roche Elecsys 2010 Analyzer (Roche Diagnostics), with a reported CV of 9.7% [18]. FGF-23 was measured via a two-site enzyme-linked immunosorbent assay (FGF-23 ELISA Kit; Kainos Laboratories, Tokyo, Japan) with CV 16.6% from split paired samples and 8.8% from internal laboratory quality control samples at 41.4 pg/mL [18]. eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation based on age, gender, race and serum creatinine [19]. Serum creatinine was measured by a modified kinetic Jaffe method and then calibrated to isotope dilution mass spectrometry (IDMS) reference measurement [20].

Covariates

Information on demographic and medical history was obtained from in-person interviews at ARIC Visit 2. Self-reported data included age, race, smoking status and alcohol status (current, former or never). Body mass index (BMI) was calculated as weight (in kilograms) divided by height squared (in meters). Blood pressures were measured in triplicate after 5-min rest, for each participant in a seated position, with the mean of the last two measurements analyzed. Hypertension was defined as a systolic blood pressure ≥ 140 mmHg, a diastolic blood pressure ≥ 90 mmHg or the use of antihypertensive medications. Diabetes was defined as a fasting glucose level of ≥ 126 mg/dL, a nonfasting glucose level ≥ 200 mg/dL, self-reported physician diagnosis or the use of antidiabetic medications. Other biochemical data measured at Visit 2 included total and high-density lipoprotein (HDL) cholesterol [12, 21]. Prevalent coronary heart disease was defined by self-reported clinical history or evidence of prior myocardial infarction by electrocardiogram at Visit 1 as well as through chart adjudication by a physician panel for myocardial infarction between Visits 1 and 2. Similarly, prevalent stroke was defined by self-report at Visit 1 and additionally by chart adjudication for stroke between Visits 1 and 2.

Outcomes

Incident PAD was defined based on PAD-related hospitalizations identified with the following International Classification of Diseases (ICD) codes at any position based on previous literature [22, 23]: 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), chronic total occlusion of artery of the extremities (440.4), atherosclerosis of other specified arteries (440.8) and leg artery revascularization (38.18, 39.25, 39.29 and 39.50).

Leveraging a large sample size with long follow-up in ARIC, we also assessed CLI as a severe outcome of PAD. Among PAD

cases, those based on 440.22, 440.23 and 440.24 and those with coexisting codes of leg amputation (84.1x), lower extremity ulcer (707.1x) and gangrene (785.4) were considered CLI [23, 24]. Participants free of incident PAD were followed until the date of death, date of last contact or 30 September 2015, whichever came first.

Statistical analysis

We summarized baseline characteristics, including demographic information and medical comorbidities, across quartiles of magnesium level. Given the small differences in serum magnesium level by quartile, the number of participants within each quartile of magnesium was not uniform.

For our survival analysis, we used Cox proportional hazards regression to model the risk of incident PAD and CLI over time in relation to magnesium level, using Quartile 4 (>1.7 mEq/L) as a reference. We confirmed proportional hazards using Schoenfeld residuals. We ran a few models to assess the impact of potential confounders. Model 1 adjusted for baseline demographic data including age, sex, race and education level. Model 2 further accounted for other major atherosclerotic factors, including BMI, smoking status (current, former or never), total and HDL cholesterol, and a history of hypertension, diabetes, coronary heart disease and stroke. Model 3 additionally adjusted for eGFR and bone–mineral metabolism markers (calcium, phosphorus, potassium, iPTH and FGF-23). Given the potential impact of the competing risk of death, we conducted sensitivity analysis using Fine and Gray's proportional sub-hazards models [25].

Subsequently, we modeled magnesium levels using piecewise linear spline models with knots at the 25th, 50th and 75th percentiles to evaluate potentially nonlinear relationships across the full spectrum of magnesium levels, evaluating effect modification between magnesium and kidney function (eGFR ≥ 60 versus <60 mL/min/1.73 m²) given the kidney's role in magnesium homeostasis. We also analyzed the association of bone–mineral metabolism markers with both incident PAD and CLI by kidney function. We analyzed calcium, phosphorus, iPTH and FGF-23 dichotomously (above and below median) given the limited size of comparison groups by quartile. Given the expected rise in levels of all these markers in the setting of CKD, we did further analysis dichotomizing calcium, phosphorus, iPTH and FGF-23 at the cut points used in previous literature, 10.2 mg/dL, 4.5 mg/dL, 65 pg/mL and 60.6 pg/mL, respectively [26, 27].

Finally, we evaluated whether the addition of magnesium level can improve model performance and risk prediction. Specifically, we assessed the Akaike information criterion, calibration plots (predicted versus observed risk across deciles of predicted risk), Harrell's C-statistics (a measure of discrimination) and categorical net reclassification improvement (NRI). For NRI, given the lack of established risk categories for PAD, we used risk thresholds in a previous study: 20-year risk of 10% and 20% for PAD and 3.5% and 7.0% for CLI [28]. As the base model, we used predictors in a PAD prediction model previously published: age, sex, race, hypertension, diabetes, smoking, total cholesterol and prevalent coronary heart disease [29].

Table 1. Baseline characteristics of participants at Visit 2 by magnesium quartile

Variable ^a	Totals	Q1 (<1.5 mEq/L)	Q2 (1.5–1.59 mEq/L)	Q3 (1.6–1.7 mEq/L)	Q4 (>1.7 mEq/L)
N	11 839	1587	2294	5805	2153
Age (years)	57.3 (5.7)	57.5 (5.8)	57.0 (5.9)	57.3 (5.6)	57.6 (5.7)
Male	5271 (44.5)	586 (36.9)	1019 (44.4)	2639 (45.5)	1027 (47.7)
African American	2891 (24.4)	685 (43.2)	676 (29.5)	1172 (20.2)	358 (16.6)
BMI	28.0 (5.4)	29.2 (6.2)	28.0 (5.5)	27.3 (4.9)	27.0 (4.6)
Education					
<HS graduate	2461 (20.8)	464 (29.2)	505 (22.0)	1093 (18.8)	399 (18.5)
HS graduate	4963 (41.9)	628 (39.6)	938 (40.9)	2481 (42.7)	916 (42.6)
>HS education	4415 (37.3)	495 (31.2)	851 (37.1)	2231 (38.4)	838 (38.9)
Cigarette use					
Current	2542 (21.4)	382 (24.1)	502 (21.9)	1196 (20.6)	462 (21.5)
Former	4493 (38.0)	541 (34.1)	877 (38.2)	2207 (38.0)	868 (40.3)
Never	4804 (40.6)	664 (41.8)	915 (39.9)	2402 (41.4)	823 (38.2)
Alcohol use					
Current	6763 (57.1)	762 (48.0)	1280 (55.8)	3412 (58.8)	1309 (60.8)
Former	2428 (20.5)	384 (24.2)	504 (22.0)	1145 (19.7)	395 (18.4)
Never	2648 (22.4)	441 (27.8)	510 (22.2)	1248 (21.5)	449 (20.8)
Hypertension	3439 (29.1)	709 (44.7)	719 (31.3)	1503 (25.9)	508 (23.6)
Diabetes	1338 (11.3)	438 (27.6)	329 (14.3)	448 (7.7)	123 (5.7)
Prevalent coronary heart disease	651 (5.5)	100 (6.3)	139 (6.1)	301 (5.2)	111 (5.2)
Prevalent stroke	205 (1.7)	51 (3.2)	43 (1.9)	81 (1.4)	30 (1.4)
Total cholesterol (mmol/L)	5.42 (1.02)	5.40 (1.08)	5.38 (1.02)	5.41 (0.991)	5.50 (1.03)
HDL cholesterol (mmol/L)	0.91 (0.28)	0.891 (0.31)	0.904 (0.29)	0.920 (0.28)	0.926 (0.28)
eGFR-Cr (mL/min/1.73 m ²)	96.4 (15.5)	100.3 (18.5)	98.0 (15.7)	95.9 (14.5)	93.1 (15.4)
Potassium (mEq/L)	4.18 (0.40)	4.06 (0.42)	4.15 (0.38)	4.20 (0.39)	4.24 (0.40)
Calcium (mg/dL)	9.35 (0.43)	9.37 (0.47)	9.32 (0.43)	9.34 (0.42)	9.37 (0.42)
Phosphorus (mg/dL)	3.53 (0.49)	3.56 (0.50)	3.51 (0.49)	3.52 (0.48)	3.53 (0.48)
iPTH (pg/mL)	42.5 (27.5)	42.5 (18.5)	42.7 (18.0)	42.2 (27.5)	43.3 (39.1)
FGF-23 (pg/mL)	58.1 (909)	50.5 (109)	45.2 (16.6)	58.7 (1088)	81.6 (1188)

^aCategorical variables measured in *n* (%); continuous variables measured in mean (SD). Q, quartile; HS, high school; Cr, creatinine.

RESULTS

Baseline characteristics

Mean baseline age was 57.3 (SD 5.7) years. Compared with participants with higher serum magnesium levels, participants in Quartiles 1 and 2 were more likely to be female, be African American, to have a higher BMI and have less formal education (Table 1). They were also more likely to be current smokers and to have more baseline medical comorbidities overall, including hypertension, diabetes, coronary heart disease and prior strokes. There were no evident patterns in the levels of bone-mineral metabolism marker across serum magnesium, although FGF-23 was higher in Quartile 4 compared with the other three quartiles. We further reported characteristics of participants by baseline eGFR (Supplementary data, Table S1).

Association of magnesium with PAD

Over a median follow-up of 23.2 years, we identified 471 incident PAD cases and 175 cases of CLI. Table 2 displays the hazard ratio (HR) of incident PAD and CLI by magnesium quartile.

In Model 1, with adjustments for demographic data, those participants in either Quartile 1 or 2 of magnesium level had a significantly higher risk of incident PAD compared with those in Quartile 4. The higher risk of incident PAD persisted in Models 2 and 3. The HR for incident PAD for participants in Quartile 1 compared with Quartile 4 was 1.96 [95% confidence interval (CI) 1.40–2.74] after full adjustment for baseline

medical comorbidities and other potential confounders. Compared with PAD, the results for CLI were similar in Model 1 but weaker in Models 2 and 3.

Out of 11 839 total participants, 233 participants had an eGFR <60 mL/min/1.73 m² at baseline. Similar to the overall study population, lower magnesium levels were significantly associated with higher risk of incident PAD in participants with eGFR ≥60 mL/min/1.73 m², after full adjustment (Figure 2A). In contrast, the association between magnesium and incident PAD was largely flat in those with eGFR <60 mL/min/1.73 m² (Figure 2B; P-for-interaction 0.03). For CLI, weaker but similar patterns were observed (Supplementary data, Figure S1, P-for-interaction 0.003). A competing risk analysis for death showed similar results (Supplementary data, Table S2).

Association of bone-mineral metabolism markers with PAD

We evaluated whether the association between bone-mineral metabolism markers and incident PAD and CLI was modified by kidney function (Table 3; Supplementary data, Table S3). With bone-mineral metabolism markers categorized into above versus below their median values, notably, there was no interaction by kidney function, and no association of incident PAD with any of bone-mineral metabolism biomarkers regardless of kidney function. However, when we dichotomized the bone-mineral metabolism markers according to their clinical thresholds, we observed a significant interaction by kidney function only for iPTH (P-for-interaction 0.01), with greater risk

Table 2. HR of incident PAD and CLI by magnesium quartile, Visit 2

Outcome	Model	Serum magnesium			
		Q1 (<1.5 mEq/L)	Q2 (1.5 mEq/L)	Q3 (1.6–1.7 mEq/L)	Q4 (>1.7 mEq/L)
N		1587	2294	5805	2153
Events		105	107	199	60
PAD	Model 1	2.57 (1.86–3.55)	1.77 (1.29–2.44)	1.26 (0.95–1.68)	Ref.
	Model 2	1.64 (1.18–2.28)	1.48 (1.07–2.04)	1.23 (0.92–1.64)	Ref.
	Model 3	1.96 (1.40–2.74)	1.67 (1.20–2.31)	1.35 (1.01–1.80)	Ref.
CLI	Model 1	2.50 (1.54–4.06)	1.23 (0.73–2.06)	0.89 (0.56–1.41)	Ref.
	Model 2	1.25 (0.76–2.06)	0.89 (0.53–1.50)	0.82 (0.52–1.31)	Ref.
	Model 3	1.59 (0.95–2.66)	1.04 (0.61–1.78)	0.95 (0.58–1.53)	Ref.

Model 1: adjusted for sex, race and education. Model 2: further adjustment for smoking status, drinking status, hypertension, diabetes mellitus, prevalent coronary heart disease, prevalent stroke, total cholesterol, HDL cholesterol. Model 3: further adjustment for serum electrolytes (potassium, calcium and phosphate), eGFR, iPTH and FGF-23. Q, quartile.

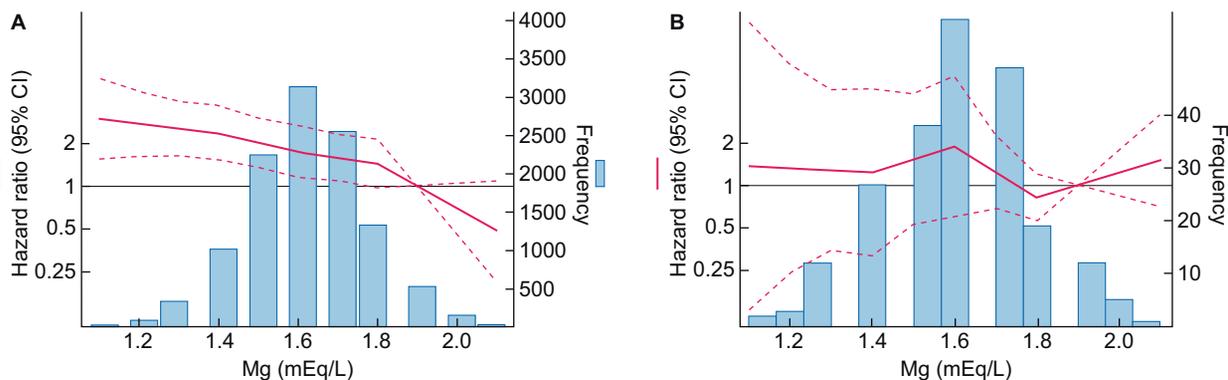


FIGURE 2: Risk of PAD by serum magnesium level, in (A) the 436/11 606 participants with eGFR ≥ 60 mL/min/1.73 m² and in (B) the 35/233 participants with eGFR < 60 mL/min/1.73 m². P-for-interaction 0.03.

Table 3. HR of incident PAD by eGFR, Visit 2

Variable	eGFR ≥ 60		eGFR < 60		P-for-interaction
	HR (95% CI)	P-value	HR (95% CI)	P-value	
N	11 606		233		
Events	436		35		
Higher calcium ^a	1.06 (0.86–1.29)	0.60	0.68 (0.31–1.48)	0.33	0.17
Higher phosphorus ^a	1.01 (0.83–1.24)	0.90	1.22 (0.53–2.78)	0.64	0.95
Higher iPTH ^a	1.07 (0.88–1.30)	0.52	0.51 (0.22–1.22)	0.13	0.93
Higher FGF-23 ^a	1.09 (0.90–1.32)	0.39	1.40 (0.42–4.73)	0.58	0.84
Higher calcium ^b	0.59 (0.33–1.07)	0.08	2.10 (0.44–9.97)	0.35	0.43
Higher phosphorus ^b	2.01 (1.36–2.95)	< 0.001	1.87 (0.64–5.49)	0.25	0.82
Higher iPTH ^b	1.09 (0.76–1.55)	0.64	2.24 (0.94–5.36)	0.07	0.01
Higher FGF-23 ^b	1.43 (1.10–1.86)	0.01	0.94 (0.37–2.39)	0.90	0.60

Adjusted for age, sex, race, education, smoking status, drinking status, hypertension, diabetes mellitus, prevalent coronary heart disease, total cholesterol and HDL cholesterol.

^aCalcium, phosphorus, iPTH and FGF-23 above versus below their median values.

^bCalcium, phosphorus, iPTH and FGF-23, above versus below clinical cut-points.

of incident PAD only in participants with eGFR < 60 mL/min/1.73 m² [HR (95% CI) = 2.24 (0.94–5.36) versus 1.09 (0.76–1.55) in eGFR ≥ 60 mL/min/1.73 m²].

Model performance and prediction of PAD with and without magnesium

The base model with established predictors of PAD demonstrated decent calibration visually (Supplementary data, Figure

S2), although Hosmer–Lemeshow χ^2 test showed statistical significance ($P = 0.001$) for PAD. The addition of magnesium did not alter the overall calibration. Similarly, the risk discriminations of PAD and CLI were excellent in the base model (c -statistic 0.839 and 0.920, respectively), and the addition of magnesium did not improve it (Supplementary data, Table S4). NRIs for both PAD and CLI were not significant [0.004 (95% CI –0.022, 0.031) and 0.000 (–0.027, 0.028), respectively]

either. However, the overall model fit was better when we added magnesium (AIC with versus without magnesium, 8033 versus 8047 for PAD and 2841 versus 2842 for CLI).

DISCUSSION

In this community-based cohort study with more than two decades of follow-up, lower serum magnesium levels were independently associated with a greater risk of incident PAD. Specifically, those individuals in Quartile 1 with serum magnesium level <1.5 mEq/L had approximately two times higher risk of PAD compared with those in Quartile 4, even after accounting for several potential confounders including kidney function and bone-mineral metabolism markers. However, this association was not evident in the relatively small number of participants with $eGFR < 60$ mL/min/1.73 m², and the addition of magnesium did not necessarily improve risk prediction of PAD beyond a base model with traditional predictors. Among the bone-mineral metabolism markers, only higher iPTH dichotomized by a clinical cut point showed a significant interaction with kidney function, as a result of an elevated PAD risk only in those with $eGFR < 60$ mL/min/1.73 m².

These results extend the current literature of the magnesium-PAD relationship in several aspects. First, although there have been some cross-sectional studies [30, 31], our study is one of very few showing a prospective inverse association of serum magnesium with incident PAD [15, 32]. While the study by Sun *et al.* [15] also investigated the association of magnesium with incident PAD, we used a more specific definition of PAD and uniquely investigated CLI as an outcome. Second, we confirmed that the association is independent of kidney function and bone-mineral metabolism markers. Third, we observed that the inverse association is generally consistent for both overall PAD and CLI. Fourth, we uniquely observed that the magnesium-PAD relationship is significantly modified by kidney function, with a largely flat risk gradient in individuals with reduced eGFR. Finally, our results indicate that the predictive value of magnesium for PAD is limited.

There are several possible mechanisms by which lower magnesium levels may play a role in the development of PAD. Magnesium can inhibit formation of calcium-phosphate apatite by forming a more soluble compound as well as competitively binding with the calcium-sensing receptor similar to a calcimimetic. Furthermore, magnesium deficiency has been shown to inhibit endothelial proliferation, in the setting of increases in interleukin-1 and upregulation of both vascular cell adhesion molecule-1 and plasminogen activator inhibitor-1 [33]. Thus, lower magnesium levels may indicate a milieu prone to vascular calcification and systemic inflammation [5, 6, 8]. In multiple animal studies, augmentation of dietary magnesium has been associated with a decrease in vascular calcification both in central arteries such as the aorta as well as cardiac and renal arteries [34–36]. Similarly, low-magnesium diets increased the risk of vascular calcification in animal models [36].

We observed a lack of any inverse association between serum magnesium levels and incident PAD in participants with reduced eGFR. The kidneys serve as the primary regulator of

magnesium homeostasis in the body. Roughly 70% of serum magnesium is filtered by the glomerulus, of which the vast majority is reabsorbed and only ~3–5% excreted in the urine [37, 38]. Thus, reduced GFR can induce hypermagnesemia pathologically [35]. In the setting of reduced kidney function, other factors involved in vascular calcification such as calcium, phosphate, iPTH and FGF-23 may play a more important role than magnesium as drivers of vascular calcification [39]. However, since the number of study participants with reduced eGFR in our study was limited, further investigations into these associations of magnesium with PAD and other cardiovascular outcomes in CKD-enriched cohorts would be warranted.

Although hypomagnesemia was not associated with PAD in persons with reduced kidney function, greater iPTH levels were associated with increased PAD risk in this clinical subgroup. While this may have been a chance finding as a result of multiple statistical testing in our study, there are plausible mechanisms to explain this interaction. As kidney function declines, iPTH is upregulated to maintain homeostasis of vitamin D, calcium and phosphorus [39]. Thus, levels of iPTH rise earlier and more rapidly than abnormalities seen in calcium or phosphorus in CKD [39]. Of note, elevations in iPTH level have been independently associated with higher levels of systemic inflammatory markers including C-reactive protein in the general population, as well as aortic stiffness and vascular calcification in individuals with primary hyperparathyroidism [40–42]. A randomized clinical trial in CKD patients demonstrated that the use of cinacalcet and vitamin D analogs for secondary hyperparathyroidism attenuated vascular and valvular calcification [43].

The results of this study may provide areas of increased focus for health-care providers managing patients at high risk for PAD. If our findings are confirmed in other, larger CKD cohorts, monitoring of magnesium may be less informative in this population. Closer monitoring of bone-mineral metabolism markers such as iPTH in the CKD population, instead, may provide added benefit. Our study generates a hypothesis that magnesium supplementation may provide benefit in reducing future PAD risk, especially in persons with hypomagnesemia and preserved kidney function, which could be evaluated in a clinical trial.

This study has several limitations. The measure of serum magnesium in this study may not accurately reflect ionized magnesium levels, which may be more biologically meaningful [44]. Also, the baseline magnesium level was based on a single blood test, which may be prone to misclassification and typically bias estimates to the null. This may be particularly relevant among those with reduced kidney function since magnesium levels may fluctuate as eGFR declines. The relatively high CV of FGF-23 indicates some misclassification, which might bias its associations with PAD and CLI toward null. Our use of ICD codes from hospital discharges to diagnose clinical PAD or CLI may not capture less severe cases of PAD that did not require hospitalization. Additionally, our study population only included Caucasian and African American participants, so our results may have limited generalizability to other racial/ethnic groups. While we have adjusted for many potential confounding variables, there is still the potential for residual confounding

in our results as in any observational study. The small sample size particularly in patients with an eGFR <60 mL/min/1.73 m² limits our statistical power. Furthermore, study participants were middle-aged (45–64 years) at the start of this study, and thus these results may not be simply generalizable to older adults where PAD and CLI are more common.

In summary, lower levels of magnesium were independently associated with increased PAD risk overall. However, this association was significantly weaker in participants with an eGFR <60 mL/min/1.73 m². In contrast, higher iPTH levels were particularly related to PAD risk in this clinical population. Our findings suggest the complex interplay among magnesium, bone–mineral metabolism markers and kidney function in their contributions to vascular health.

SUPPLEMENTARY DATA

Supplementary data are available at [ndt online](http://ndt.online).

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AUTHORS' CONTRIBUTIONS

S.M. and K.M. were involved in the research idea and study design. J.C. and K.M. were involved in data acquisition. S.M., N.D., M.E.G., P.L.L., G.H., A.R.F., E.S., J.C., B.G.J. and K.M. were involved in data analysis/interpretation. S.M., N.D. and K.M. were involved in statistical analysis. J.C., B.G.J. and K.M. were involved in supervision and mentorship. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST STATEMENT

K.M. received research funding and personal fee from Fukuda Denshi and Kyowa Kirin outside of the work. Other authors declare that they have no conflicts of interest. The results presented in this paper have not been published previously in whole or part, except in abstract format.

(See related article by de Borst and de Baaij. Low serum magnesium as a risk factor for peripheral artery disease in chronic kidney disease: an open verdict. *Nephrol Dial Transplant* 2020; 35: 1831–1833)

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