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Original Articles

Blood and dietary magnesium levels are not linked with lower prostate cancer risk in black or white men

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ABSTRACT

Recent studies suggest a diet low in dietary magnesium intake or lower blood magnesium levels is linked with increased prostate cancer risk. This study investigates the race-specific link between blood magnesium and calcium levels, or dietary magnesium intake, and the diagnosis of low-grade and high-grade prostate cancer. The study included 637 prostate cancer cases and 715 biopsy-negative controls (50% black) recruited from Nashville, TN or Durham, NC. Blood was collected at the time of recruitment, and dietary intake was assessed by food frequency questionnaire. Percent genetic African ancestry was determined as a compliment to self-reported race. Blood magnesium levels and dietary magnesium intake were significantly lower in black compared to white men. However, magnesium levels or intake were not associated with risk of total prostate cancer or aggressive prostate cancer. Indeed, a higher calcium-to-magnesium diet intake was significantly protective for high-grade prostate cancer in black (OR = 0.66 (0.45, 0.96), p = 0.03) but not white (OR = 1.00 (0.79, 1.26), p = 0.99) men. In summary, there was a statistically significant difference in magnesium intake between black and white men, but the biological impact was unclear, and we did not confirm a lower prostate cancer risk associated with magnesium levels.

1. Introduction

Prostate cancer is the leading non-skin cancer and second leading cause of cancer death among men in the U.S [1]. There are substantive differences in prostate cancer incidence and mortality associated with race, with a 63% higher incidence and a 2.4-fold higher mortality overall among black men compared to white men [2–5], and a shorter time period of disease-free survival [6]. Further understanding potential contributing causes for these race differences in prostate cancer detection and survival is a priority area in prostate cancer research.

Magnesium deficiency is common, with analyses of data from National Health and Nutrition Examination Survey finding that 79% of U.S. adults have magnesium intake below the Recommended Dietary Allowance (males: 420 mg/d) [7]. Our prior study of predominately white men found that lower blood magnesium levels were associated with an increased risk of high-grade prostate cancer [8]. We also reported that the subset of black men in that study had lower blood magnesium levels than white men (2.03 vs. 2.16 ng/ml, respectively, $p < 0.01$, n = 51 black, n = 441 white) [8]. This is consistent with an analysis of data from the Atherosclerosis Risk in Communities (ARIC) study, which found that black vs. white men had significantly lower dietary magnesium intake (143 vs. 159 mg/1000 kcal, respectively, $p < 0.001$) and lower serum magnesium levels (1.92 vs. 2.02 ng/ml, respectively, $p < 0.001$) [9,10].

Calcium has long held an interest in cancer progression with potential roles as an essential messenger regulating cell cycle proliferation and apoptosis [11]. Prior human studies have reported an increased risk of aggressive prostate cancer associated with higher calcium levels

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[12], however studies have also reported an increased risk of non-aggressive prostate cancer [13] or a protective association with prostate cancer risk [14,15]. Such inconsistencies may reflect the range and biological complexity of these minerals in the body. Indeed, calcium and magnesium biologically interact through competition for intestinal absorption, transport, and renal reabsorption [16–18], and magnesium deficiency increases calcium retention and activity [19–21]. Further understanding the roles of magnesium in prostate cancer may require inclusion of calcium as one biological element that substantially affects magnesium action and availability.

This case-control analysis investigated the link between magnesium and prostate cancer in black and white men, and also investigated the potential interaction between magnesium and calcium levels. To address the challenge of magnesium and calcium measurement, our analysis included blood levels of magnesium and calcium as well as dietary intake estimates from food frequency questionnaires. Analysis of genetic markers of African ancestry was used as a complement to self-reported race in the analysis that begins to separate the inherited and non-inherited components of self-reported race. As prior data suggest that black men will have lower magnesium intake and lower blood magnesium levels than white men, we hypothesized that the protective association between magnesium measures and prostate cancer will be stronger within black men compared to white men. Analyses may provide evidence that magnesium deficiency contributes to race differences in prostate cancer risk, and lead to future investigations of mechanisms of magnesium in prostate cancer or dietary interventions to support the intake of magnesium-rich foods.

2. Materials and methods

Participants came from two separate, but similar pre-existing studies located at Vanderbilt University Medical Center (Nashville, TN, USA) and the Veterans Administrations Hospital in Durham, NC, and Duke University Medical Center. Details of each protocol were previously reported [15,22]. Recruitment for this project targeted men over age 40 years and who were obtaining a diagnostic prostate biopsy. Biospecimen and data collection occurred prior to prostate biopsy, such that patients, medical staff, and research staff had no knowledge of the patient prostate cancer status at time of recruitment and data collection. All recruitment and data collection protocols were approved by the respective IRBs, with additional review from the Department of Defense Human Subjects Research Panel, and all participants provided written informed consent.

Fasting blood was collected from most men at the Durham site, while fasting collection was not feasible from community urology clinics in Nashville. Blood processing and storage were similar under both protocols. Whole blood was refrigerated immediately after collection, and serum was processed within 4–6 h of collection, aliquoted into labeled cryovials, and stored at −80 °C. DNA was extracted from blood at institutional genetic core laboratories, quantified, and stored at −80 °C.

Weight (kg) (no shoes, light clothes or hospital gown) and height were measured under both protocols at recruitment by trained staff. Study personnel were trained to review all medical charts systematically using a medical abstraction form. Chart review also ascertained cancer stage (TNM) and ISUP Grade group [23]. PSA levels at diagnosis were also collected during chart review at the Vanderbilt site. Vanderbilt participants completed the Diet History Questionnaire from the NCI, while participants from the Duke/Durham VA site completed the Willett questionnaire from Harvard University. Participants were asked to estimate their usual portion size for each reported foot item. Dietary supplement use was also reported on the food frequency questionnaires, and magnesium or calcium intake from supplements was added to the total magnesium or calcium scores used in the diet analysis.

The study targeted 750 prostate cancer cases (50% black, 50% white), with 450 cases from Vanderbilt and 300 from the Durham VA. A case was defined as a man diagnosed with prostate cancer at biopsy. We excluded cases with evidence of metastasis because of concerns that bone metastasis may affect serum calcium and magnesium. Controls included 750 men (50% black, 50% white) without prostate cancer at biopsy. Controls were randomly selected from over 2500 and 500 eligible biopsy-negative men at Vanderbilt and the Durham VA, respectively, and frequency matched to cases to have a similar distribution on age (5 years), race, and institution. Men with atypia, high-grade prostatic intraepithelial neoplasia (HGPIN), or other findings at biopsy that put men at greater risk for prostate cancer were excluded to further reduce the potential for a null-bias due to latent prostate cancer in the control series.

We assayed serum samples without evidence of hemolysis and without a prior thaw. We used inductively coupled plasma optical emission spectroscopy (ICP-OES) with simultaneous detection of multiple wavelengths, in radial and axial plasma view to measure total serum calcium and magnesium levels. Serum from both cohorts were assayed at the Vanderbilt Clinical and Pathology core laboratories, where these assays are routinely run. The method has coefficient of variations (%CV) of 2.29%–3.28% for calcium and 2.17%–2.34% for magnesium. Repeated blood magnesium levels over 3 years apart are correlated at $r = 0.46$ [10] indicting a single measure provides information toward a persistent exposure. The calcium-to-magnesium ratio (Ca/Mg) was calculated from these results. DNA extracted from blood, and % genetic African ancestry was estimated from 23 ancestry informative markers for each subject using the program STRUCTURE [24–27].

The final analysis included 1359 men with calcium and magnesium blood levels. Wilcoxon rank sum tests or Kruskal-Wallis rank sum tests were used to compare continuous participant characteristics between race groups, while chi-square tests were used to compare categorical variables between race groups. Distributions for serum magnesium and calcium levels were approximately normal, and calcium and magnesium levels were not transformed. Pearson correlation coefficients were calculated for the correlation between magnesium and calcium from the diet against blood levels. Prostate-specific antigen (PSA) level at diagnosis was available only in the Vanderbilt study sample, and Pearson correlations between PSA and magnesium or calcium were also calculated. We analyzed magnesium and calcium levels across quartile categories of % genetic African ancestry within black men to explore non-dietary factors potentially involved in blood magnesium and calcium levels.

Our primary outcomes include prostate cancer, low-grade prostate cancer (Gleason 6), and high-grade prostate cancer (Gleason 7–10). The analytic approach in modeling was conceptually a stratified analysis by race, with a combined analysis for the investigation of race interactions. Primary analyses used logistic regression to estimate odds ratios summarizing the association between magnesium, calcium, or Ca/Mg with prostate cancer outcomes within race groups. Our approach determined the difference in prostate cancer risk that corresponds to change in magnesium or calcium from the 25th percentile to the 75th percentile of the distribution specific for each race group, providing a measure of effect that is within the range of the data and avoids analysis of extreme biomarker or reported outliers. Models controlled for age, BMI, and study source. Analysis of Ca/Mg included controlling for magnesium and calcium in the model to evaluate the relative calcium and magnesium amounts independent of magnesium and calcium levels. Analysis of dietary data also controlled for total energy intake. Interactions between race and magnesium, calcium, or the Ca/Mg ratio were estimated by including a cross-product term in the full dataset and using the Wald test to evaluate different effects between race groups.

3. Results

This analysis included 708 black and 651 white men recruited from urology clinics in Nashville, TN or Durham, NC. White men were older

Table 1
Description of black and white men in study population.

		Black	White	P[c]
		Median (25th, 75th)	Median (25th, 75th)	
Age	years	62.0 (57.8, 67.0)	65.0 (60.0, 69.0)	P < 0.001
BMI	kg/m^2	29.0 (25.8, 32.8)	29.1 (26.6, 32.2)	P = 0.760
Height	cm	175.9 (171.5, 181.6)	175.3 (170.4, 180.3)	P = 0.077
Weight	kg	90.0 (78.9, 103.0)	89.3 (80.3, 100.2)	P = 0.708
PSA[a]	ng/ml	5.6 (4.2, 8.2)	4.7 (3.7, 6.3)	P < 0.001
% genetic African Ancestry[b]		80% (70%, 90%)	0% (0.0%, 0.0%)	P < 0.001
% genetic African Ancestry (categories)				
		N (%)	N (%)	
	[0.0%,70%)	175 (25%)	646 (100%)	P < 0.001
	[70%, 83%)	170 (24%)	1 (0%)	
	[83%, 93%)	183 (26%)	0 (0%)	
	[93%,100%]	177 (25%)	0 (0%)	
Study Center	Duke/VA	307 (44%)	256 (40%)	P = 0.138
	Vanderbilt	398 (56%)	391 (60%)	
Prostate Cancer Status	Negative-Control	353 (50%)	362 (56%)	P = 0.060
	Low-Grade PC	183 (26%)	137 (21%)	
	High-Grade PC	169 (24%)	148 (23%)	

[a] PSA data were not collected from Duke/Durham VA site. Analysis of these variables is specific to Vanderbilt study subjects (n = 789).
[b] % genetic African Ancestry from analysis of ancestry informative markers, and evaluated as a continuous variable or as quartiles of the % genetic African ancestry distribution within black men.
[c] p-value from Wilcoxon rank sum test or chi-square test evaluating differences between black and white men.

than black men, but black and white men had a similar BMI, weight, and height (Table 1). Self-reported race tracked closely with genetic African ancestry markers, with the majority of white men having no evidence of genetic African ancestry. In contrast, median % genetic African ancestry was 80% within black men, with a wide distribution of % genetic African ancestry scores within black men. PSA levels were also higher among black vs. white men within the subset of 789 participants with PSA data.

Black men reported statistically significantly lower dietary magnesium intake than white men, and blood magnesium levels in black men were also statistically significantly lower than in whites (Table 2). Black men also reported less dietary calcium intake, however this did not correspond to lower blood calcium levels compared to white men. Blood magnesium or calcium levels were not significantly correlated with dietary intake estimates (magnesium $_{(\text{diet vs blood})}$: $r_{(\text{black})} = -0.07$, $p = 0.18$, $r_{(\text{white})} = -0.02$, $p = 0.58$; calcium$_{(\text{diet vs. blood})}$: $r_{(\text{black})} = -0.06$, $p = 0.31$, $r_{(\text{white})} = 0.03$, $p = 0.52$). Indeed, the blood Ca/Mg ratio was significantly higher in black men, while the dietary Ca/Mg level was significantly higher in white men.

Table 2
Blood levels and reported dietary intake of magnesium and calcium.

			n	Black	White	P[b]
				Median (25th, 75th)	Median (25th, 75th)	
Blood	Mg	ng/ml	1322	2.3 (2.1, 2.5)	2.4 (2.2, 2.6)	P < 0.001
	Ca	ng/ml	1322	9.8 (9.4, 10.6)	9.7 (9.3, 10.5)	P = 0.094
	Ca/Mg		1320	4.3 (4.0, 4.6)	4.1 (3.8, 4.3)	P < 0.001
Diet[a]	Mg	mg/d	903	261.0 (177.3, 378.2)	321.5 (234.1, 422.0)	P < 0.001
	Ca	mg/d	903	544.4 (363.8, 840.0)	681.0 (481.0, 990.5)	P < 0.001
	Ca/Mg		903	2.0 (1.7, 2.5)	2.2 (1.8, 2.8)	P = 0.018

[a] Food frequency questionnaires completed by 903 study participants with measured blood magnesium (Mg) and calcium (Ca) levels.
[b] P: Wilcoxon rank sum test evaluating differences in Mg, Ca, or Ca/Mg between black and white men.

Race differences in blood magnesium or calcium levels did not translate to differences in blood magnesium or calcium levels across categories of genetic African ancestry within black men (Table 3). There was marginally significant variation in dietary calcium and magnesium intake linked with % genetic African ancestry within black men, however no trend was evident and the rationale for why % genetic African ancestry would affect dietary intake in one specific category is unclear. There was insufficient variation in % genetic African ancestry in white men to support a comparable analysis within white men.

There was no evidence that increasing magnesium or calcium in blood was associated significantly with prostate cancer in either black or white men (Table 4). Similarly, dietary magnesium and calcium intakes were not significantly associated with prostate cancer (Table 5). In contrast, an increasing dietary Ca/Mg ratio was significantly associated with a lower risk of total prostate cancer and also high-grade prostate cancer risk, but only within black men. Analyses of prostate cancer with blood magnesium, calcium, or Ca/Mg levels or dietary intakes were similar across study centers, and tests for interaction with study center were not statistically significant (Supplemental Tables 1 and 2). We considered a potential detection bias if magnesium or calcium affects PSA levels used to screen men for possible prostate cancer. This analysis was limited to the subset of participants recruited at the Vanderbilt study site and with PSA data at diagnosis (Table 1). Pearson correlation coefficients between PSA and diet or blood magnesium, calcium, or Ca/Mg values ranged from 0.04 to 0.01, and all p-values were greater than 0.31.

4. Discussion

This study found a small but statistically significant difference in blood magnesium levels related to race, with lower levels in black men compared to white men. In contrast to our analysis of self-described race, there was no significant differences or suggestive trends between blood magnesium levels and increasing genetic African ancestry. Furthermore, we found no evidence that higher magnesium was associated with lower prostate cancer risk. Similarly, we found no association between blood calcium or the Ca/Mg ratio in blood with prostate cancer in either black or white men. Dietary estimates of Ca and Mg

Table 3
Difference in Blood Levels and Diet Intake of Magnesium, Calcium, and the Ca/Mg ratio with Level of % genetic African ancestry in self-reported Black Men.

	% genetic African ancestry								P*
	0–69%		70%–82%		83%–92%		93%–100%		
	n	Median (25th, 75th)	n	Median (25th, 75th)	n	Median (25th, 75th)	n	Median (25th, 75th)	
Blood Mg	175	2.3 (2.2, 2.5)	170	2.3 (2.2, 2.6)	177	2.3 (2.1, 2.5)	173	2.3 (2.1, 2.5)	0.89
Ca	175	9.7 (9.3, 10.3)	168	9.9 (9.4, 10.8)	177	9.8 (9.3, 10.8)	173	9.7 (9.4, 10.4)	0.19
Ca/Mg	175	4.2 (3.9, 4.54)	167	4.3 (4.0,4.7)	177	4.3 (4.0.,4.7)	172	4.3 (4.0.4.7)	0.31
Diet Mg	102	262 (190,420)	100	289 (189,397)	92	230 (148,334)	89	260 (209,407)	0.09
Ca	102	573 (370,845)	100	569 (410,917)	92	475 (284,713)	89	534 (370,847)	0.051
Ca/Mg	102	2.1 (1.6, 2.5)	100	2.1 (1.7, 2.5)	92	1.9 (1.7, 2.4)	89	2.1 (1.7, 2.7)	0.41

p-value: Kruskal-Wallis test for differences in median values across % genetic African ancestry categories.

were also not linked with prostate cancer, although a higher Ca/Mg ratio as estimated by food frequency questionnaire was significantly associated with lower prostate cancer risk among black, but not white, men.

Dietary sources provide most needed magnesium, with high levels found in spinach, whole grains, beans, dairy foods, nuts, certain fish, and possibly water if there is a high mineral content. Recent data from National Health and Nutrition Examination Survey report that most U.S. adults are magnesium deficient, with 79% of U.S. adults reporting magnesium intake below the U.S. Recommended Daily Allowance [7]. Hypomagnesemia may be asymptomatic, but magnesium deficiency is linked severe morbidity associated with an inflammatory response or metabolic dysregulation, including insulin resistance [28,29], fasting glucose levels [30], Type 2 diabetes [31], cardiovascular disease [32,33], colorectal cancer [34–36] and colorectal adenomas [35,37]. Magnesium is also inversely linked with blood C-reactive protein (CRP) levels [38–40], while induced magnesium deficiency in rodents leads to increased plasma IL6 levels [40] and increased markers of oxidative stress [19,41]. Human intervention studies that administered magnesium supplements or magnesium replacement led to lower fasting glucose levels [42], increased high-density lipoprotein (HDL) [42], and improved insulin sensitivity [28,43–45]. Evidence that magnesium supplementation alters pro-inflammatory markers has been suggested in a limited number of small studies, but effects are less clear and requires further investigation [44,46].

Analysis of the Atherosclerosis Risk in Communities (ARIC) study found black vs. white men had significantly lower dietary magnesium intake (143 vs. 159 mg/1000 Kcal, $p < 0.001$, respectively) and serum magnesium levels (1.92 vs. 2.02 ng/ml, $p < 0.001$, respectively) [9,10]. Indeed, many of the conditions linked with magnesium deficiency also appear to be diseases in which blacks experience greater risk or a poorer prognosis [47–51]. We previously reported that black men had significantly lower blood magnesium levels compared to white men in a study of men who were seeking a diagnostic prostate biopsy (2.03 vs. 2.16 ng/ml, $p < 0.01$, $n = 51$ vs. 441, respectively) [8]. The present report confirms this prior observation. We cannot say with certainty why black men had lower blood magnesium levels. There was an absence of any trend between blood magnesium levels and % genetic African ancestry, however genetic variation between race groups not encompassed by the % genetic African ancestry marker panel could certainly play a role. Dietary magnesium intake was also significantly lower among black men, suggesting dietary differences between race groups may be involved. However, the correlation between diet and blood magnesium levels was weak. The observed race differences in blood magnesium were small and may thus be a consequence of differences in the intake of specific food items not analyzed here, perhaps combined with genetic differences or differences in exposure to other environmental sources of magnesium.

Calcium has long held an interest in cancer, as calcium plays essential messenger roles regulating cell cycle proliferation and apoptosis

Table 4
Associations between blood Mg, Ca, Ca/Mg Levels and prostate cancer, by race and PC grade.

		blacks				whites				p-int[a]
		N (case/ctl)	OR*	95% CI	p	N (case/ctl)	OR	95% CI	p	
All PC	Mg	342/341	1.14	0.96–1.35	0.14	282/357	1.07	0.92–1.24	0.41	0.27
	Ca	341/343	1.11	0.93–1.32	0.26	282/356	1.05	0.87–1.27	0.59	0.16
	Ca/Mg	341/341	0.98	0.92–1.04	0.52	282/356	0.98	0.82–1.16	0.81	0.96
	Ca/Mg#	341/341	0.99	0.94–1.04	0.71	282/356	1.24	0.77–1.98	0.38	0.68
Low-grade PC	Mg	176/341	1.19	0.95–1.48	0.13	135/357	1.06	0.87–1.30	0.54	0.21
	Ca	176/343	1.05	0.86–1.29	0.63	135/356	0.96	0.76–1.21	0.74	0.18
	Ca/Mg	176/341	0.93	0.76–1.13	0.45	135/356	0.90	0.71–1.14	0.38	0.77
	Ca/Mg#	176/341	0.99	0.92–1.07	0.81	135/356	1.02	0.55–1.89	0.94	0.87
High-grade PC	Mg	166/341	1.10	0.89–1.37	0.37	147/357	1.06	0.88–1.28	0.53	0.56
	Ca	165/343	1.19	0.94–1.51	0.15	147/356	1.15	0.91–1.46	0.25	0.34
	Ca/Mg	165/341	0.99	0.95–1.04	0.71	147/356	1.05	0.85–1.29	0.66	0.61
	Ca/Mg#	165/341	0.99	0.94–1.05	0.83	147/356	1.40	0.81–2.43	0.23	0.64

*OR is effect prostate cancer association for difference between 25th and 75th percentile of magnesium (Mg), calcium (Ca), or Ca/Mg ratio within controls for each race group. Analyses are run separately within each race group and adjusted for age, BMI, and study center. # Analysis of Ca/Mg that controls for Mg and Ca levels as well as age, BMI, and study center.

[a] P-int – p-interaction between Mg, Ca, or Ca/Mg with race group on prostate cancer risk in full dataset.

Table 5
Associations between diet Magnesium, Calcium, and Ca/Mg ratio Levels and prostate cancer, by race and prostate cancer grade.

		N (case/ctl)	blacks			N (case/ctl)	whites			p-int**
			OR*	95% CI	p		OR	95% CI	p	
All PC	Mg	175/187	1.04	0.64–1.69	0.89	236/305	0.91	0.61–1.36	0.64	0.36
	Ca	175/187	0.76	0.54–1.08	0.13	236/305	1.08	0.85–1.38	0.53	0.06
	Ca/Mg	175/187	0.72	0.55–0.95	0.02	236/305	1.08	0.89–1.30	0.44	0.03
Low-grade PC	Mg	101/187	0.87	0.47–1.60	0.65	113/305	0.91	0.56–1.47	0.69	0.08
	Ca	101/187	0.61	0.36–1.02	0.06	113/305	1.15	0.86–1.54	0.35	0.01
	Ca/Mg	101/187	0.75	0.55–1.04	0.08	113/305	1.16	0.91–1.48	0.23	0.04
High-grade PC	Mg	74/187	1.26	0.67–2.37	0.48	123/305	0.92	0.54–1.59	0.78	0.69
	Ca	74/187	0.84	0.57–1.26	0.40	123/305	1.00	0.70–1.42	0.98	0.79
	Ca/Mg	74/187	0.66	0.45–0.96	0.03	123/305	1.00	0.79–1.26	0.99	0.10

*OR is effect prostate cancer association for difference between 25th and 75th percentile of magnesium (Mg), calcium (Ca), or Ca/Mg within controls for each race group. Analyses are run separately within each race group and adjusted for age, BMI, study center, and total energy intake. ** P-int – p-interaction between Mg, Ca, or Ca/Mg with race group on prostate cancer risk in full dataset.

[11]. However, the literature is inconsistent, with dietary calcium associated with increased risk of aggressive prostate cancer [12] but also with increased risk of non-aggressive prostate cancer risk [13] or a reduced prostate cancer risk [14,15]. There is perhaps some greater consistency in reported foods rich in calcium such as dairy or milk intake [52–54]. Fewer studies have considered the opposing interaction between calcium and magnesium. Calcium and magnesium compete for intestinal absorption, transport, and renal reabsorption [16–18]. *In vitro* and *in vivo,* magnesium administration inhibits calcium activity while magnesium deficiency increases calcium retention and activity [19–21]. Increasing the ratio of calcium-to-magnesium (Ca/Mg) in the medium of cultured DU145 prostate cells increased proliferation, consistent with a biological interaction between calcium and magnesium [55]. Results of the current study are in contrast to our prior study, in that blood calcium levels or the blood Ca/Mg ratio in the current study were not significantly associated with prostate cancer in black or white men. A recent case-only analysis reported that black men diagnosed with aggressive prostate cancer had a higher dietary Ca/Mg ratio compared to black men diagnosed with less aggressive prostate cancer [52]. However, we found dietary Ca/Mg ratio was significantly protective for high-grade prostate cancer within black men. Reasons for this inconsistency with a prior study may involve the use biopsy negative controls compared to controls with non-aggressive prostate cancer, and further investigation is needed to understand the mechanisms underlying this effect.

Strengths of our analysis include a large sample of black and white men, inclusion of dietary and blood magnesium and calcium levels, and analysis of self-reported race with a genetic African ancestry score. Our controls included men without prostate cancer as determined by prostate biopsy. We considered using a general clinical population as a control group, however these patients will have a different comorbidity profile, may harbor latent prostate cancer leading to a null bias, and may differ with regard to healthcare access or screening. We found no correlation between magnesium or calcium with PSA levels, suggesting our analysis is unlikely to be biased due to any effect of magnesium or calcium on lowering PSA levels used to screen men for prostate cancer. Evaluation of blood markers of magnesium or calcium avoids dietary assessment errors inherent in estimates of dietary intake from food frequency questionnaires. Our single blood magnesium level would thus represent the balance of intake, absorption from the colon and kidney, and excretion. Blood magnesium levels would not address bone and muscle magnesium stores that may serve as a reserve in times of magnesium deficiency, other hormones (e.g., PTH) known to affect magnesium absorption, or variation attributable to genetic variants in TRMP6 or other proteins responsible for transporting magnesium through the cell. There are technical and financial challenges to analyzing magnesium within specific cellular or tissue compartments. Interestingly, use of a reported dietary intake estimate suggested the Ca/Mg ratio was linked with prostate cancer risk, while results were weaker using the blood biomarker, perhaps suggesting that dietary intake estimates provide a more stable estimate of sustained calcium and magnesium exposure despite potential reporting errors. Alternatively, dietary reporting of the Ca/Mg ratio is potentially non-specific and thus may be reflecting some other correlated factor linked with prostate cancer risk.

There are several limitations to address in this analysis. As previously discussed, food frequency questionnaires have known limitations [56]. Our case-control study design does not remove reverse causation as an alternative explanation. For example, the 'magnesium trap' hypothesis developed by Wolf and colleagues suggests cancer cells may pull magnesium from blood and retain high intracellular magnesium levels necessary for cell proliferation [57]. Since it is possible that metastasis to the bone could affect circulating calcium and magnesium levels, we excluded men with known metastatic disease from analyses. Alternatives to the analysis of blood levels include a 24-h urine sample or nails, however these types of biospecimens were not available for this analysis. We did not have data on nonprescription and magnesium-rich medications (e.g., milk of magnesia), and we did not measure ionized magnesium and calcium, vitamin D, insulin, blood cytokines, or other interesting biomarkers, but we may in the future [40]. Race differences in magnesium levels reached a level of statistical significance, but the biological relevance of these differences in the context of cardiovascular disease or other morbidity is unclear.

In summary, we found a small but statistically significant differences in magnesium levels associated with race, such that black men had lower magnesium intake and lower blood magnesium levels than comparable white men. However, we found no evidence of a protective association between magnesium and prostate cancer in either black or white men.

Conflicts of interest

No author has a conflict of interest or any financial interests to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.canlet.2019.02.023.

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