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Serum calcium and incident type 2 diabetes: the Atherosclerosis Risk in Communities (ARIC) study[1,2]

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ABSTRACT

Background: Elevated serum calcium has been associated with a variety of metabolic abnormalities and may be associated with a greater risk of diabetes.

Objective: The purpose of this study was to test the hypothesis that serum calcium concentration is positively and independently associated with the incidence of diabetes and to evaluate the association of calcium-sensing receptor (CaSR) gene single nucleotide polymorphism (SNP) rs1801725 with incident diabetes.

Design: Atherosclerosis Risk in Communities study participants free of diabetes at baseline (n = 12,800; mean age: 53.9 y; 22.6% black) were studied for incident diabetes. Serum calcium was measured at baseline and corrected for serum albumin. Diabetes was defined by use of glucose concentrations, self-report, or medication use. Cox proportional hazards regression was used.

Results: During a mean 8.8 y of follow-up, 1516 cases of diabetes were reported. Participants in the highest compared with lowest calcium quintile were at greater risk of incident diabetes after adjustment for demographic and lifestyle factors [HR (95% CI): 1.34 (1.14, 1.57); P-trend across quintiles <0.0001] and with further adjustment for waist circumference and body mass index [1.26 (1.07, 1.48); P-trend = 0.004]. Additional adjustment for biomarkers on the metabolic pathway (e.g., 25-hydroxyvitamin D, parathyroid hormone, phosphorus) had little impact. The calcium-diabetes association was statistically significant in blacks [1.48 (1.11, 1.98); P-trend = 0.002] but not whites [1.17 (0.96, 1.43); P-trend = 0.17] after adjustment for adiposity. In whites, CaSR gene SNP rs1801725 was associated with serum calcium but not with risk of diabetes.

Conclusions: Consistent with 3 previous cohort studies, elevated serum calcium was found to be associated with a greater risk of type 2 diabetes. Further research is needed to understand the role, if any, that calcium plays in the pathogenesis of diabetes. *Am J Clin Nutr* 2016;104:1023–9.

Keywords: calcium-sensing receptor polymorphism, cohort study, diabetes mellitus, race, serum calcium

INTRODUCTION

In 2012, >20 million US adults had type 2 diabetes, a condition that is characterized by abnormal glucose metabolism and is a major cause of morbidity and mortality (1). Calcium is a mineral obtained primarily through dietary intake of dairy products, dark green leafy vegetables, calcium-fortified foods, or supplementation. In the body, the majority of calcium is located within the skeleton (99%), where it provides structural support and helps maintain calcium balance through extraskeletal exchange. Serum calcium is tightly regulated by 1,25-dihydroxyvitamin D [1,25$(OH)_2$D],[8] parathyroid hormone (PTH), and ionized calcium (2).

Calcium has been reviewed traditionally in relation to bone health; however, contemporary epidemiologic studies have suggested that elevated serum calcium concentration may play a role in the development of diabetes (3–11). A positive association between elevated serum calcium and diabetes has been observed in several cross-sectional studies (3–6), 2 case-control studies (7, 8), and 3 prospective cohorts (9–11). The cohort studies did not report information on 25-hydroxyvitamin D [25(OH)D] or PTH, both of which are involved in calcium homeostasis (2) and have been associated with diabetes (12–14). In addition, the earlier studies had limited racial-ethnic diversity (9, 11) or may not have had sufficient power to detect race

[1] The research was supported by grants from the NIH National Heart, Lung, and Blood Institute (R01 HL103706 to PLL), the NIH Office of Dietary Supplements (R01 HL103706-S1 to PLL), and the National Institute of Diabetes and Digestive and Kidney Diseases (R01 DK089174 to ES). The Atherosclerosis Risk in Communities study is a collaborative study supported by National Heart, Lung, and Blood Institute contracts HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C. Reagents for the serum albumin assays were donated by the manufacturers. Genotyping was supported through NHLBI Candidate Gene Resource grant no. N01-HC-65226.

[2] Supplemental Figure 1 and Supplemental Tables 1–5 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[8] Abbreviations used: ARIC, Atherosclerosis Risk in Communities; CaSR, calcium-sensing receptor; eGFR, estimated glomerular filtration rate; PTH, parathyroid hormone; SNP, single nucleotide polymorphism; 1,25$(OH)_2$D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D.

Received January 25, 2016. Accepted for publication July 6, 2016.

First published online August 10, 2016; doi: 10.3945/ajcn.115.130021.

differences for the relation between calcium and the risk of incident diabetes (10). Because diabetes is more highly prevalent in blacks than in whites (1) and racial differences in calcium metabolism may exist (15–18), it is possible that the association between serum calcium and incident diabetes may vary by race.

The calcium-sensing receptor (CaSR) is expressed primarily in the parathyroid glands and kidneys, where it helps regulate serum calcium concentration through PTH secretion and calcium reabsorption (19). In genomic-wide association studies, a single nucleotide polymorphism (SNP; rs1801725) within the CaSR gene was associated with serum calcium concentration in individuals of European ancestry (20, 21). It is not yet known whether this gene variant also is related to serum calcium concentration in blacks, or whether this SNP is associated with risk of diabetes.

The purpose of this study was to examine associations of serum calcium with risk of diabetes during an average of 9 y of follow-up among black and white participants enrolled in the ARIC (Atherosclerosis Risk in Communities) study. In addition, we evaluated the impact of adjusting for biomarkers involved in the regulation of calcium, race interactions, and whether the CaSR SNP rs1801725 was associated with incident diabetes or modified the association between serum calcium and incident diabetes. We hypothesized that high serum calcium would be independently associated with an increased risk of incident diabetes, that the association would be attenuated with adjustment for biomarkers on the pathway, and that the association may differ by race.

METHODS

Study population

The ARIC study was a multicenter prospective cohort (22). The 15,792 participants, who were 45–64 y old at baseline (visit 1) in 1987–1989, were recruited from 4 US centers: Washington County, Maryland; suburbs of Minneapolis, Minnesota; Jackson, Mississippi; and Forsyth County, North Carolina. Participants subsequently attended 4 clinic examinations occurring in 1990–1992, 1993–1995, 1996–1998, and 2011–2013. Participants provided informed consent, and local institutional review boards approved the study protocol.

For this analysis we excluded participants with prevalent diabetes [based on fasting (≥8 h) blood glucose ≥126 mg/dL, nonfasting glucose ≥200 mg/dL, diabetes diagnosis, or current medication use for diabetes] at visit 1 (n = 1,863) or with missing data on diabetes status (n = 147), as well as those who were neither black nor white (n = 48) and black participants at the Maryland and Minnesota centers as a result of small numbers (n = 55). In addition, we excluded participants without diabetes follow-up information (n = 879). Our final analytic sample included 12,800 participants (**Supplemental Figure 1**).

Measurements

Trained interviewers collected information on demographic characteristics, medical history, and lifestyle habits, including physical activity, smoking status, and alcohol use. Anthropometric variables were measured with the use of standard procedures including waist circumference to the nearest centimeter. BMI was calculated as weight (kg) divided by the square of height (m). Physical activity was queried with the validated Baecke questionnaire and ranked from 1 (low) to 5 (high) (23).

Blood samples were obtained at visit 1. Plasma and serum were frozen at −70°C until analysis. Measurement of serum calcium took place shortly after the blood draw (1987–1989), at the University of Minnesota central chemistry laboratory. Blind duplicate split samples were sent to the laboratory 1 wk apart to allow for evaluation of the within-person laboratory CV. Serum total calcium concentration was measured with the use of *o*-cresolphthalein complexone (CV: 1.1%), albumin with a bromocresol green colorimetric assay (CV: 2.8%), and serum phosphorus with the use of ammonium molybdate (CV: 7.6%). Magnesium (CV: 3.6%) was measured with the metallochromic dye calmagite [1-(1-hydroxy-4-methyl-2-phenylazo)-2-naphthol-4-sulfonic acid] based on the Gindler and Heth procedure; sodium (CV: 0.6%) and potassium (CV: 5.4%) were measured with a direct ion-selective electrode (Coulter Diagnostics) (24). Approximately 40% of nonskeletal calcium is bound to proteins, primarily albumin and globulin (2). As such, calcium was corrected for serum albumin with the use of the following equation: measured total calcium (mg/dL) + 0.8 [4.0 − serum albumin (g/dL)] (25). Albumin-corrected calcium was used for all of the analyses.

Serum creatinine (CV: 3.7%) was assessed with the use of a modified kinetic Jaffe–picric acid method (24). Estimated glomerular filtration rate (eGFR) was calculated by using serum creatinine (26) and categorized based on clinical cutoffs: <60, 60–89, and ≥90 $mL \cdot min^{-1} \cdot 1.73\ m^{-2}$.

Previously frozen serum samples from visit 2 were analyzed from 2012 to 2013 for numerous analytes, including 25(OH)D, PTH, calcium, and albumin. Concentrations of $25(OH)D_2$ (CV: 20.8%) and $25(OH)D_3$ (CV: 6.9%) were measured with liquid chromatography and high-sensitivity mass spectrometry (Waters Alliance e2795; Waters Corporation). Total 25(OH)D was calculated as the sum of $25(OH)D_2$ and $25(OH)D_3$ and was adjusted for seasonal variation with the use of a residuals approach (27). Intact PTH (CV: 9.7%) was assessed with a Roche Elecsys 2010 Analyzer by using a sandwich immunoassay method (Roche Diagnostics) (28). Serum total calcium concentration (visit 2) was measured with the use of *o*-cresolphthalein complexone (CV: 1.1%; Roche Diagnostics). Notably, raw means for serum calcium differed between visits 1 (9.86 mg/dL) and 2 (9.19 mg/dL). Visits 1 and 2 calcium were analyzed with the use of different methods and 25 y apart. We added a constant of 0.67 mg/dL to all visit 2 calcium values to achieve similar means between visits 1 and 2. Visit 2 serum albumin was measured with a bromocresol green colorimetric assay (CV: 2.2%; Asahi Kasei Corporation). Visit 2 serum calcium also was corrected for albumin concentration.

Data from the Affymetrix Genome-Wide Human SNP Array 6.0 were used to impute genotypes for rs1801725 using MACH version 1.0.16 (29). Genotyping, quality control procedures (30), and methods for imputation to ∼2.5 million SNPs in whites (31) and blacks (31) are described in detail elsewhere. Imputation quality was high ($r^2 > 0.99$).

Outcome assessment

At each visit blood samples were obtained. Glucose was measured in serum or plasma with the use of a modified hexokinase/glucose-6-phosphate dehydrogenase procedure. (33). In addition, participants were asked to bring all of their current medications to the visit; medication names were transcribed and coded. Incident type 2 diabetes was defined at the visit 2, visit 3, or visit 4 follow-up examinations as fasting (≥8 h) blood glucose ≥126 mg/dL, nonfasting glucose ≥200 mg/dL, self-report physician diagnosis of diabetes or "sugar in the blood," or current medication use for diabetes. Visit 5 information was not included because of the 15-y gap between visits 4 and 5.

Statistical analysis

All statistical analyses were performed with the use of SAS version 9.3 (SAS Institute). Baseline participant characteristics were described by proportions for categorical variables or means for continuous variables, stratified by serum calcium quintiles. Cox proportional hazards regression was used to evaluate the association between serum calcium and incident diabetes. Serum calcium was modeled as quintiles, and the linear trend across the quintiles was tested by including the serum calcium quintiles in the models as a continuous variable. Cubic spline models were used to illustrate the calcium-diabetes association. Serum calcium concentrations were truncated at the 1st and 99th percentiles of the distribution to enhance the interpretation of the spline figures. Person-time accrued from baseline until the visit in which diabetes was first ascertained or the date of the patient's last visit (visits 2–4).

Model 1 was adjusted for age, race (black compared with white), sex (male compared with female), center (Washington County, Maryland; suburbs of Minneapolis, Minnesota; Jackson, Mississippi; Forsyth County, North Carolina), and educational attainment (less than high school, high school graduate, or more than high school). Model 2 was adjusted for behaviors, including physical activity, smoking status (current, former, or never), and alcohol use (current, former, or never). Model 3 was further adjusted for waist circumference and BMI. In model 4, additional adjustment was made for PTH, 25(OH)D, and serum phosphorus. Effect modification for age, race, sex, eGFR, and the SNP within the CaSR gene (rs1801725) was tested. The proportional hazards assumption was evaluated by testing the interaction between calcium categories and ln(time).

We categorized CaSR SNP rs1801725 as *GG* compared with *GT* compared with *TT*. In race-stratified models, linear regression was used to evaluate the association between rs1801725 (both as continuous and categorical) and serum calcium, adjusted for age, sex, and center. Cox proportional hazards regression was used to assess the rs1801725-diabetes association in whites and was adjusted for age, sex, and center.

Numerous sensitivity analyses were conducted. In separate analyses, we *1*) excluded those with possible primary hyperparathyroidism (PTH >65 pg/mL and calcium >10.2 mg/dL; $n = 248$), *2*) excluded diuretic users ($n = 1931$) because diuretic use has been shown to influence serum calcium concentration, *3*) analyzed the association with the use of the mean of visit 1 and visit 2 serum calcium concentrations, *4*) assessed the calcium–diabetes association by extending follow-up until 2012 to include incident diabetes (glucose concentrations, medication use for diabetes, or self-reported diabetes diagnosis), and *5*) added to model 4 biomarkers related to serum calcium, specifically serum magnesium, sodium, and potassium (modeled continuously). We also assessed the association between serum calcium and diabetes using complementary log-log analysis, an approach that assumes a constant hazard ratio within each interval but does not assume a constant hazard ratio between each interval (or visit) as does Cox proportional hazards regression. P values of <0.05 were considered statistically significant.

RESULTS

Our final analytic sample included 12,800 ARIC participants who were aged (mean ± SD) 53.9 ± 5.7 y at baseline. The sample was 55.5% women and 22.6% black. Mean ± SD albumin-corrected calcium was 9.87 ± 0.41 mg/dL, 25(OH)D was 24.7 ± 8.6 ng/mL, PTH was 41.9 ± 16.1 pg/mL, and phosphorus was 3.42 ± 0.48 mg/dL. Based on Spearman correlations, visit 1 and visit 2 serum calcium was correlated ($r = 0.28$, $P < 0.001$), and visit 1 calcium was weakly but significantly correlated with 25(OH)D (visit 2), PTH (visit 2), and phosphorus (visit 1): $r = -0.04$ ($P < 0.001$), $r = 0.02$ ($P = 0.01$), and $r = 0.19$ ($P < 0.001$), respectively. Correlations were similar when we compared visit 2 calcium with that visit's 25(OH)D, PTH, and phosphorus: $r = -0.07$, $r = 0.05$, and $r = 0.20$ ($P < 0.001$). Baseline characteristics stratified by quintiles of calcium are presented in **Table 1**. Overall, participants with higher serum calcium tended to be women, be black, and have higher PTH concentrations.

During the study period, 1516 incident diabetes events occurred during a median follow-up time of 8.8 y (maximum 11.9 y). After adjustment for demographic characteristics in model 1, participants in the highest calcium quintile had a significantly higher risk of incident diabetes than those in the lowest calcium quintile [HR Q5 compared with Q1 (95% CI), P-linear trend] [1.34 (1.14, 1.57), P-trend < 0.001] (**Table 2, Figure 1**). Results were similar with further adjustment for lifestyle factors in model 2 [1.34 (1.14, 1.57), P-trend < 0.001] but modestly attenuated after adjustment for waist circumference and BMI in model 3 [1.26 (1.07, 1.48), P-trend = 0.004]. The association remained significant after adjustment for 25(OH)D, PTH, and phosphorus [1.28 (1.08, 1.52), P-trend = 0.005]. There were no significant interactions of the calcium-diabetes association, regardless of the degree of adjustment, by race, age, sex, continuous eGFR, or eGFR clinical cutoffs. Although we did not find statistical evidence of a race interaction (P-interaction > 0.25 in all models), even after accounting for waist circumference and BMI (model 3), the association appeared somewhat stronger in blacks [1.48 (1.11, 1.98), P-trend = 0.002] than in whites [1.17 (0.96, 1.43), P-trend = 0.17] (Table 2).

In the sensitivity analysis, results were similar when we excluded patients with possible primary hyperparathyroidism (PTH >65 pg/mL and calcium >10.2 mg/dL; $n = 248$) (**Supplemental Table 1**). When users of diuretics ($n = 1931$) were excluded, the association of serum calcium with incident diabetes was slightly weakened but maintained a similar pattern [HR Q5 compared with Q1 (95% CI) model 1: 1.27 (1.06, 1.52), P-trend = 0.02; model 2: 1.28 (1.06, 1.53), P-trend = 0.02; model 3: 1.20 (1.00, 1.44), P-trend = 0.08] (**Supplemental Table 2**). The pattern of association was similar when we averaged visit 1 and visit 2

TABLE 1
Unadjusted baseline characteristics by serum calcium quintiles: the ARIC study, 1987–1989 (n = 12,800)[1]

	Quintiles of serum total calcium					
	1 (n = 2742)	2 (n = 2516)	3 (n = 2607)	4 (n = 2495)	5 (n = 2440)	P^2
Calcium, mg/dL	9.34 (7.28–9.54)	9.66 (9.56–9.76)	9.87 (9.78–9.96)	10.08 (9.98–10.20)	10.46 (10.22–13.28)	
Age, y	53.3 ± 5.7	53.5 ± 5.7	54.1 ± 5.8	54.2 ± 5.7	54.5 ± 5.6	<0.001
Female sex	1250 (45.6)	1294 (51.4)	1431 (54.9)	1500 (60.1)	1630 (66.8)	<0.001
Black race	487 (17.8)	496 (19.7)	542 (20.8)	589 (23.6)	783 (32.1)	<0.001
Education						<0.001
<High school	489 (17.9)	470 (18.7)	511 (19.6)	524 (21.0)	610 (25.0)	
High school graduate	1164 (42.6)	1036 (41.2)	1090 (41.9)	1053 (42.3)	1005 (41.2)	
>High school	1082 (39.6)	1007 (40.1)	1002 (38.5)	915 (36.7)	825 (33.8)	
Sport index	2.50 ± 0.80	2.50 ± 0.83	2.48 ± 0.80	2.45 ± 0.78	2.39 ± 0.77	<0.001
Smoking status						<0.001
Current	564 (20.6)	599 (23.8)	652 (25.0)	707 (28.4)	700 (28.7)	
Former	961 (35.1)	850 (33.8)	860 (33.0)	778 (31.2)	737 (30.2)	
Never	1215 (44.3)	1065 (42.4)	1094 (42.0)	1008 (40.4)	1002 (41.1)	
Alcohol use status						<0.001
Current	1690 (62.0)	1525 (60.9)	1559 (59.9)	1483 (59.5)	1287 (52.9)	
Former	429 (15.7)	419 (16.7)	451 (17.3)	422 (16.9)	463 (19.1)	
Never	608 (22.3)	561 (22.4)	591 (22.7)	586 (23.5)	681 (28.0)	
Family history of diabetes	552 (22.2)	532 (23.7)	564 (24.1)	542 (24.2)	517 (24.1)	0.10
BMI, kg/m^2	27.1 ± 4.6	27.0 ± 4.8	27.2 ± 5.0	27.1 ± 5.1	27.7 ± 5.6	<0.001
Waist circumference, cm	95.9 ± 12.3	95.6 ± 12.9	95.5 ± 13.2	95.2 ± 13.9	96.1 ± 14.1	0.95
Estimated glomerular filtration rate, mL · min^{-1} · 1.73 m^{-2}	103.0 ± 13.9	103.2 ± 14.5	102.2 ± 14.4	102.0 ± 14.8	102.0 ± 15.9	<0.001
Estimated glomerular filtration rate category						<0.001
≥90 mL · min^{-1} · 1.73 m^{-2}	2345 (85.5)	2192 (87.1)	2202 (84.5)	2083 (83.5)	1993 (81.7)	
60–89 mL · min^{-1} · 1.73 m^{-2}	383 (14.0)	310 (12.3)	386 (14.8)	386 (15.5)	414 (17.0)	
<60 mL · min^{-1} · 1.73 m^{-2}	14 (0.5)	14 (0.6)	19 (0.7)	26 (1.0)	33 (1.4)	
Parathyroid hormone, pg/mL	41.63 ± 14.99	41.34 ± 15.68	41.10 ± 14.94	41.19 ± 15.5	44.18 ± 19.18	<0.001
25-hydroxyvitamin D, ng/mL	25.00 ± 8.34	24.92 ± 8.38	24.61 ± 8.43	24.76 ± 8.54	23.94 ± 9.03	<0.001
Phosphorus, mg/dL	3.29 ± 0.47	3.37 ± 0.47	3.42 ± 0.46	3.50 ± 0.47	3.54 ± 0.50	<0.001
Diuretic use	283 (10.3)	311 (12.4)	395 (15.2)	402 (16.1)	540 (22.1)	<0.001

[1]Values are medians (ranges), means ± SDs for continuous variables, and n (%) for categorical variables. Serum calcium was corrected for serum albumin. ARIC, Atherosclerosis Risk in Communities.

[2]Continuous variables: P-trend; categorical variables: P value for χ^2 test of association.

calcium concentrations (**Supplemental Table 3**) and when we extended follow-up to 2012 (**Supplemental Table 4**). Results were largely similar to those of the primary analyses when serum magnesium, sodium, and potassium were included in model 4 [1.33 (1.12, 1.59), P-trend = 0.001]. The overall pattern of the serum calcium-diabetes association, when analyzed with complementary logistic-logistic regression, yielded results similar to those for Cox proportional hazards regression (**Supplemental Table 5**).

In linear regression, among white participants, the CaSR gene SNP rs1801725 (continuous) was associated with serum calcium ($t = -8.15$, $P < 0.001$) after adjustment for age, sex, and center. Relative to participants with the genotype *GG*, participants with the *GT* genotype had mean ± SE serum calcium concentrations −0.07 (0.009) mg/dL lower, and those with the *TT* genotype had concentrations on average −0.14 (0.009) mg/dL lower. rs1801725 accounted for 4.5% of the variation in serum calcium concentration among white participants, after adjustment for age, sex, and center; however, rs1801725 was not associated with incident diabetes [HR 0.94 (0.82, 1.07)], nor did it modify the calcium-diabetes association. In black participants there was no association between rs1801725 and serum calcium concentration.

DISCUSSION

Among the ARIC participants, higher concentrations of serum calcium were independently associated with risk of incident diabetes. The association remained even after adjustment for 25(OH)D, PTH, and phosphorus, which indicates that these physiologically related adjustment factors may not fully capture the calcium-diabetes relation. Although we found no statistical evidence for a multiplicative interaction by race, the association appeared to be modestly stronger among blacks than among whites. CaSR SNP rs1801725 was not associated with diabetes risk in whites.

Three cohort studies found similar positive associations of serum calcium with diabetes risk. In the Insulin Resistance Atherosclerosis Study (10), high serum calcium (per SD increment) was associated with an OR of 1.26 for incident diabetes, after extensive covariate adjustment. In the Tromsø study, conducted in northern Norway, participants in the highest calcium category had a 1.36 times greater risk of diabetes than those in the lowest category. Jorde et al. (9) also evaluated the CaSR gene SNP rs17251221 [in linkage disequilibrium (r^2 = 0.95) with rs1801725]. Similar to our ARIC findings, they found that rs17251221 was associated with serum calcium concentration

TABLE 2
Adjusted HRs (95% CIs) with the use of Cox proportional hazards regression for serum calcium and risk of type 2 diabetes: the ARIC study, 1987–1998 (n = 12,800)[1]

	Quintiles of serum calcium					
	1	2	3	4	5	*P*-trend
Overall						
Median (range), mg/dL	9.34 (7.28–9.54)	9.66 (9.56–9.76)	9.87 (9.78–9.96)	10.08 (9.98–10.20)	10.46 (10.22–13.28)	
Events/total, *n*	309/2742	284/2516	288/2607	297/2495	338/2440	
Incidence rate[2]	14.27	14.42	14.13	15.4	18.7	
Model 1	1 (ref)	1.04 (0.89, 1.23)	1.03 (0.87, 1.21)	1.13 (0.96, 1.33)	1.34 (1.14, 1.57)	<0.001
Model 2	1 (ref)	1.05 (0.89, 1.23)	1.03 (0.88, 1.21)	1.13 (0.96, 1.33)	1.34 (1.14, 1.57)	<0.001
Model 3	1 (ref)	1.03 (0.87, 1.21)	1.01 (0.86, 1.19)	1.10 (0.94, 1.30)	1.26 (1.07, 1.48)	0.004
Model 4	1 (ref)	1.03 (0.86, 1.22)	1.01 (0.85, 1.20)	1.10 (0.93, 1.32)	1.28 (1.08, 1.52)	0.005
Blacks (n = 2897)						
Median (range), mg/dL	9.36 (8.20–9.54)	9.68 (9.56–9.76)	9.88 (9.78–9.96)	10.08 (9.98–10.20)	10.42 (10.22–13.28)	
Events/total, *n*	72/487	75/496	101/542	107/589	153/783	
Incidence rate[2]	19.96	20.95	25.98	25.52	28.72	
Model 1	1 (ref)	1.06 (0.77, 1.47)	1.35 (0.99, 1.83)	1.35 (1.00, 1.82)	1.56 (1.17, 2.07)	<0.001
Model 2	1 (ref)	1.08 (0.78, 1.51)	1.36 (1.00, 1.85)	1.34 (0.99, 1.83)	1.56 (1.17, 2.09)	<0.001
Model 3	1 (ref)	1.03 (0.74, 1.43)	1.24 (0.91, 1.69)	1.28 (0.94, 1.74)	1.48 (1.11, 1.98)	0.002
Model 4	1 (ref)	1.12 (0.79, 1.59)	1.26 (0.90, 1.76)	1.26 (0.90, 1.76)	1.58 (1.15, 2.18)	0.003
Whites (n = 9903)						
Median (range), mg/dL	9.38 (7.28–9.54)	9.66 (9.56–9.76)	9.86 (9.78–9.96)	10.08 (9.98–10.20)	10.38 (10.22–12.26)	
Events/total, *n*	237/2255	209/2020	187/2065	190/1906	185/1657	
Incidence rate[2]	13.13	12.97	11.34	12.59	14.51	
Model 1	1 (ref)	1.06 (0.88, 1.28)	0.95 (0.78, 1.15)	1.10 (0.91, 1.33)	1.29 (1.06, 1.57)	0.03
Model 2	1 (ref)	1.06 (0.88, 1.28)	0.96 (0.79, 1.16)	1.10 (0.90, 1.33)	1.27 (1.04, 1.55)	0.03
Model 3	1 (ref)	1.08 (0.89, 1.30)	0.98 (0.81, 1.19)	1.08 (0.89, 1.31)	1.17 (0.96, 1.43)	0.17
Model 4	1 (ref)	1.07 (0.88, 1.31)	1.01 (0.83, 1.24)	1.11 (0.91, 1.37)	1.20 (0.97, 1.48)	0.11

[1]Serum calcium was corrected for serum albumin. Model 1 was adjusted for age, sex, race, center, and education (not adjusted for race in stratified analyses). Model 2 was adjusted as for model 1 plus for physical activity, smoking status, and alcohol use. Model 3 was adjusted as for model 2 plus for waist circumference and BMI. Model 4 was adjusted as for model 3 plus for parathyroid hormone, 25-hydroxyvitamin D, and phosphorus. ARIC, Atherosclerosis Risk in Communities; ref, reference.

[2]Unadjusted incidence rate per 1000 person-years.

but not with incident diabetes. Becerra-Tomás et al. (11) also examined change in serum calcium from baseline and risk of diabetes in participants from 2 centers of the Spanish Prevención con Dieta Mediterránea study, which included older individuals with poor cardiovascular disease risk factor profiles. They found that individuals in the highest tertile of change (mean: 0.52 mg/dL) had a 3.48 (95% CI: 1.48, 8.17) times higher diabetes risk compared with those in the lowest tertile of change (mean: −0.78 mg/dL). Furthermore, 2 case-control studies found that individuals with type 2 diabetes had significantly higher serum calcium than did control subjects (7, 8), although ionized calcium concentrations were not significantly different between geriatric cases (n = 125) and control subjects (n = 379). Serum calcium has been associated cross-sectionally with prevalent diabetes, metabolic syndrome (3), insulin resistance, fasting plasma glucose (4, 5), glucose intolerance (6), and β-cell function (5).

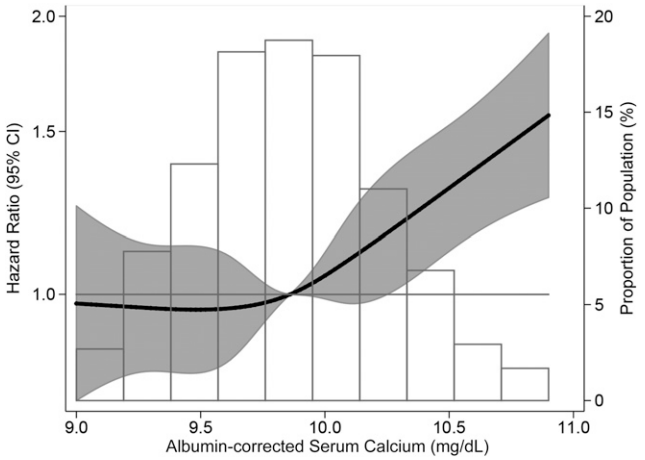

FIGURE 1 Association of serum calcium (corrected for serum albumin) with risk of type 2 diabetes: the Atherosclerosis Risk in Communities study, 1987–1998 (n = 12,800). Biomarkers modeled as restricted cubic splines with knots at the 5th, 27.5th, 50th, 72.5th, and 95th percentiles with adjustment for age, race, sex, education, and study center.

Cohort studies that have evaluated the association between serum calcium and incident diabetes did not account for vitamin D or PTH, both of which are involved in the regulation of serum calcium (2) and may themselves be related to diabetes risk (12, 13). Our results were robust to additional adjustment for these biomarkers, which share a metabolic pathway with serum calcium. Moreover, previous cohort studies did not examine racial differences (9, 11) or were modestly powered to detect an interaction (10). Calcium metabolism may differ between races—blacks may be more efficient than whites at its metabolism (15–18). In our sample, calcium concentrations were higher in blacks, and although we did not find statistically

significant race interactions in the relation between serum calcium and incident diabetes, the association appeared somewhat stronger in blacks. It is noteworthy that the metabolism of 25(OH)D, phosphorus, and PTH, all of which are involved in calcium homeostasis, may vary by race (34–37). As such, it is unclear how potential racial differences within this intricate physiologic web might influence diabetes risk.

Calcium homeostasis involves a complex negative feedback loop that influences calcium transport in bone, kidneys, and intestine. Briefly, an increase in serum calcium activates the CaSR, which decreases PTH secretion. This triggers decreased calcium resorption and reabsorption within the bone and kidney, respectively, as well as decreased synthesis of $1,25(OH)_2D$, which then decreases dietary calcium absorption. When calcium concentrations are restored, the feedback loop ends (2).

Altered calcium homeostasis may contribute to abnormal β cell functioning (5), which could then contribute to altered glucose homeostasis (38). Although serum calcium may not necessarily be an index or reflection of this pathophysiology, in vitro studies have found that elevated or sustained cytosolic calcium contributes to insulin resistance in adipocytes and skeletal muscle (39–41). It has been suggested that there is a range of cytosolic calcium within which insulin sensitivity is optimal (42). Moreover, Levy (43) suggested the existence of a "vicious cycle" between cell calcium and glucose metabolism, by which abnormal cell calcium impairs glucose tolerance and poor glucose tolerance impairs cell calcium. As such, it is possible that abnormal calcium concentrations could play a role in the development of diabetes.

Our study has some limitations. First, this study did not measure ionized calcium, which is the physiologically relevant form of calcium. In most individuals, however, ionized calcium is highly correlated with total calcium (25). Second, the mineral metabolism pathway is incredibly complex; despite our results being robust with adjustment for other markers on the pathway, it remains possible that other markers may account for elevated diabetes risk among those with high serum calcium concentrations. Serum calcium concentrations were measured in visit 1 samples, whereas PTH and 25(OH)D were assessed in visit 2 serum samples. In the sensitivity analyses, results were similar when we averaged serum calcium concentrations from visits 1 and 2. Third, a causal effect of serum calcium on diabetes risk should not be inferred. Although our results were robust in sensitivity analyses, given the observational study design, residual confounding may be present. Approximately half of the subjects in quintile 5 have clinical hypercalcemia, which may reflect an underlying health problem. Lastly, were this association causal, as a result of the highly regulated nature of serum calcium it is unclear how or when to intervene on elevated serum calcium to minimize diabetes risk; however, treatment of the underlying cause of hypercalcemia may then influence diabetes risk.

Our analysis also has some important strengths. To our knowledge, this was the first cohort study that incorporated PTH and vitamin D into analyses of serum calcium and diabetes risk, thereby enhancing etiologic understanding. Other strengths include the racial diversity of the cohort, large number of events, objective exposure and outcome ascertainment, and wealth of covariate information available.

In conclusion, higher serum calcium concentration was associated with a greater risk of incident type 2 diabetes in ARIC participants. Further research is needed to understand the role, if any, that altered calcium homeostasis plays in the pathogenesis of diabetes and other metabolic abnormalities, as well as the potential racial differences of this association.

The authors' responsibilities were as follows—MRR, JSP, and PLL: designed the research; MRR: performed the statistical analysis and had primary responsibility for the final content; and all authors: wrote the manuscript and read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

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