Increased Serum Calcium Levels and Risk of Type 2 Diabetes in Individuals at High Cardiovascular Risk

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OBJECTIVE

Insulin resistance and secretion depend on calcium homeostasis. Cross-sectional studies have associated elevated serum calcium levels with markers of impaired glucose metabolism. However, only one prospective cohort study has demonstrated an increased risk of diabetes in individuals with increased serum calcium concentrations. The aim of the current study was to prospectively investigate the association between albumin-adjusted serum calcium concentrations and type 2 diabetes in subjects at high cardiovascular risk.

RESEARCH DESIGN AND METHODS

Prospective assessment of participants from two Spanish PREDIMED study centers where serum calcium levels were measured at baseline and yearly during follow-up. Multivariate-adjusted Cox regression models were fitted to assess associations between baseline and changes during follow-up in serum calcium levels and relative risk of diabetes incidence.

RESULTS

After a median follow-up of 4.78 years, 77 new cases of type 2 diabetes occurred. An increase in serum calcium levels during follow-up was related to an increased risk of diabetes. In comparison with individuals in the lowest tertile (−0.78 ± 0.29 mg/dL), the hazard ratio (HR) and 95% CI for diabetes incidence in individuals in the higher tertile of change (0.52 ± 0.13 mg/dL) during follow-up was 3.48 (95% CI 1.48–8.17; *P* for trend = 0.01). When albumin-adjusted serum calcium was analyzed as a continuous variable, per 1 mg/dL increase, the HR of diabetes incidence was 2.87 (95% CI 1.18–6.96; *P* value = 0.02). These associations remained significant after individuals taking calcium supplements or having calcium levels out of normal range had been excluded.

CONCLUSIONS

An increase in serum calcium concentrations is associated with an increased risk of type 2 diabetes in individuals at high cardiovascular risk.

Type 2 diabetes is an important health problem worldwide. In the last three decades, the number of individuals with type 2 diabetes has doubled (1). Diabetes is associated with such complications as blindness, renal failure, and lower-limb amputation (2) and increases the risk of premature cardiovascular disease (3).

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Calcium is an element that plays an important role not only in skeletal mineralization but also in a wide range of biological functions (4). In recent decades, insulin resistance and secretion have been shown to depend on calcium homeostasis. The secretion of insulin in response to an elevated concentration of plasma glucose is a Ca^{2+}-dependent process. Alterations in insulin secretion have also been involved with disorders in blood glucose homeostasis (5), and increasing cytosolic calcium has been associated with an increase in the expression of GLUT4 transporters in the myocyte, which, in turn, increases the insulin-stimulated glucose transport activity in these cells (6). Because both defects in insulin secretion and insulin action are related to type 2 diabetes (7), it is expected that abnormal calcium homeostasis could play an important role in the development of type 2 diabetes.

Previous studies have reported that serum total calcium levels are higher in individuals with diabetes than in those without (8,9). Cross-sectional studies have associated elevated serum calcium levels with *1*) fasting plasma glucose, insulin, or insulin resistance in men with type 2 diabetes (10) or in both sexes (11); *2*) a decrease in insulin sensitivity but not in insulin secretion (12); and *3*) impaired glucose tolerance but not with markers of insulin resistance or secretion (13). Although there are some contradictions between these studies, the results suggest that calcium could be involved in the development and maintenance of type 2 diabetes. Furthermore, an increased risk of diabetes prevalence was observed in middle-aged and elderly Koreans with increased serum calcium concentrations (14).

To the best of our knowledge, only two prospective studies have recently evaluated this association in adults (15,16). They both showed that elevated serum calcium concentrations were associated with increased risk of developing diabetes. Therefore, the aim of the current study was to evaluate the associations between serum calcium levels and risk of developing type 2 diabetes in an elderly Mediterranean population at high cardiovascular risk in the frame of the PREDIMED cohort.

RESEARCH DESIGN AND METHODS

Study Design

The PREDIMED (Prevención con Dieta Mediterránea) study was a randomized, multicenter parallel-group clinical trial conducted in Spain by primary care centers affiliated with 11 hospital centers or university hospitals between October 2003 and December 2010. The aim of the PREDIMED study was to evaluate the effectiveness of the Mediterranean diet (MedDiet) in the primary prevention of cardiovascular disease in subjects at high cardiovascular risk. The cohort, design, and protocol of study have previously been described (17).

The study included 7,447 participants (men aged 55–80 years and women aged 60–80 years) who had not previously reported any cardiovascular events but who were at high cardiovascular risk. They were eligible if they had either type 2 diabetes or at least three of the following cardiovascular risk factors: family history of premature coronary heart disease (before the age of 55 years in men or 65 in women) in first-degree relatives, current smoking, overweight/obesity (BMI $\geq$25 kg/m^2), hypertension (systolic blood pressure $\geq$140 mmHg or diastolic blood pressure $\geq$90 mmHg or on antihypertensive medication), dyslipidemia, and at least one of the following: hypercholesterolemia (high LDL cholesterol [$\geq$160 mg/dL]), low HDL cholesterol ($\leq$40 mg/dL in men; $\leq$50 mg/dL in women), or treatment with lipid-modulating agents. The exclusion criteria included presence of BMI $\geq$40 kg/m^2, alcohol or drug abuse, severe chronic illness, and allergy or intolerance to olive oil or nuts.

Participants were randomized to one of three different intervention groups: MedDiet supplemented with extra virgin olive oil, MedDiet supplemented with mixed nuts, or control diet (advice on a low fat-diet following the American Heart Association guidelines).

The protocol was approved by the institutional review boards of the recruiting centers. All participants provided written informed consent.

In the current study, data were analyzed as in an observational prospective cohort in those participants recruited from Reus-Tarragona and Barcelona centers in whom serum calcium and albumin were determined. Participants with prevalent diabetes were excluded from the present analysis.

Assessment of Serum Calcium Levels and Other Covariates

At baseline, all the participants completed a 47-item questionnaire about lifestyle, education, medical history, and drug treatments. Leisure physical activity was assessed by a validated Spanish version of the Minnesota Leisure Time Physical Activity Questionnaire (18).

Baseline food and alcohol intake was assessed by a validated semiquantitative food frequency questionnaire, with 137 items, completed by trained dietitians (19). Spanish food composition tables were used to estimate energy and nutrient intake (20,21).

Height (centimeters) and weight (kilograms) were measured with participants in light clothing and no shoes. Waist circumference (centimeters) was measured midway between the lowest rib and the iliac crest. BMI was calculated weight in kilograms divided by the square of height in meters. Blood pressure was measured using a validated oscillometer (HEM705CP; Omron) in triplicate with a 5-min interval between each measurement.

Centralized laboratory biochemical analyses were performed on blood samples obtained in fasting conditions. Plasma glucose, serum cholesterol, HDL cholesterol and triglyceride concentrations were measured using standard enzymatic automated methods. In patients with triglyceride concentrations $<$400 mg/dL, LDL cholesterol concentrations were estimated using the Friedewald formula (22). Serum calcium levels and albumin concentrations were also measured by automated techniques using COBAS integra reagents (Cobas Integra, Roche Diagnostics, Switzerland). Laboratory technicians were blinded to the intervention group.

When participants presented with hypoalbuminemia (albumin $<$4 g/dL), albumin-adjusted serum calcium was calculated using the following formula: albumin-adjusted serum calcium (mg/dL) = serum calcium (mg/dL) + [0.8 $\times$ (4 $-$ albumin [g/dL])] (23–25).

Ascertainment of Type 2 Diabetes

Diabetes was a prespecified secondary outcome of the PREDIMED trial. It was considered to be present at baseline by clinical diagnosis and/or use of antidiabetes medication. New-onset diabetes during follow-up was diagnosed by using the American Diabetes Association criteria—namely, fasting plasma glucose $\geq$126.0 mg/dL (7 mmol/L) or 2-h plasma glucose $\geq$200 mg/dL (11.1 mmol/L)—after a 75-g oral glucose load. All the medical records of the participants in the

PREDIMED trial were reviewed yearly in each center by a team of physician investigators who were blinded to the intervention. When cases of new-onset diabetes were identified on the basis of a medical diagnosis reported in the medical charts or on a glucose test during routine biochemical analyses (performed at least once per year), these reports were sent to the PREDIMED Clinical Events Committee, whose members were also blinded to treatment allocation. Only when a second test using the same criteria and repeated within the following 3 months confirmed the new diabetes case was the end point definitively confirmed by the adjudication committee. Only confirmed diabetes events that occurred between 1 October 2003 and 1 December 2010 were included in the analyses.

Statistical Analysis

The baseline characteristics of participants were described using mean ± SD values for continuous variables and numbers and percentages for categorical variables. The ANOVA test for continuous variables and the χ^2 test for categorical variables were used to assess the baseline characteristics according to tertiles of albumin-adjusted serum calcium.

Multivariable time-dependent Cox proportional regression models were fitted to assess the relative risk of diabetes according to albumin-adjusted serum calcium tertiles at baseline (milligrams per deciliter) and also as a continuous variable per 1 mg/dL in the whole population. Additional Cox regression models were used to assess the associations between changes in albumin-adjusted serum calcium during the follow-up and the incidence of diabetes. Changes in albumin-adjusted serum calcium were calculated from baseline to the end of follow-up or until a year before the last date of diabetes diagnosis. Cox regression models were adjusted for several potential confounding factors by using three different models. The first model was adjusted for sex, age, and intervention group (and also for baseline serum calcium levels when analyzing the association between diabetes incidence and changes in serum calcium levels during the follow-up). The second model was also adjusted for BMI, smoking status (never, former, or current), prevalence of hypertension (yes/no), prevalence of hypercholesterolemia (yes/no), use of antihypertensive drugs (no antihypertensive drugs/thiazide diuretics/antihypertensive drugs other than thiazides), use of hypolipidemic drugs (yes/no), alcohol intake in g/day (adding a quadratic term), educational level (primary education, secondary education, or academic/graduate), and leisure physical activity (METs/day). Model 3 was also adjusted for baseline fasting plasma glucose concentrations in milligrams per deciliter. The time variable was the interval between randomization and the date of diabetes diagnosis or the date of the last visit for participants who were free of diabetes at the end of the study or when lost to follow-up. If a participant died after the last follow-up visit and had not been diagnosed with diabetes, the date of death was used.

Statistical interaction between tertiles of albumin-adjusted serum calcium and confounding variables, such as sex and intervention group, were checked by including the interaction terms in the models. Because no significant interactions were found, interaction terms were removed, and the models were checked again.

The median value of each tertile of albumin-adjusted serum calcium was assigned and used as a continuous variable to assess linear trend test in the Cox regression models.

We also conducted a series of sensitivity analyses to test the robustness of our primary results. These included additional exploratory analysis excluding participants with calcium and/or vitamin D supplementation at baseline and during follow-up when we analyze the associations between changes in serum calcium concentrations and diabetes incidence to avoid possible bias effects. Other analyses were also conducted excluding participants with serum calcium out of normal range (8.8–10.4 mg/dL) in order to minimize the possibility that some abnormal conditions (i.e., primary hyperparathyroidism) could influence the results. All statistical tests were two-tailed, and the significance level was set at $P \leq 0.05$. Analyses were performed with SPSS software (version 19.0; SPSS).

RESULTS

Albumin-Adjusted Serum Calcium at Baseline and Type 2 Diabetes

Of the total 1,551 randomized participants from the Reus-Tarragona and Barcelona centers, 813 were excluded because they were diagnosed with type 2 diabetes at baseline and 31 because we had no information about serum calcium concentrations. Finally, 707 participants were included in the present analysis of the association between baseline albumin-adjusted serum calcium levels and the incidence of type 2 diabetes.

The baseline characteristics of the total study participants (n = 707) according to tertiles of albumin-adjusted serum calcium are presented in Table 1. Participants in the highest tertile had increased levels of fasting plasma glucose and total and HDL cholesterol. The means in the lowest and the highest tertile of albumin-adjusted serum calcium levels were 9.01 mg/dL and 10.20 mg/dL, respectively. At baseline, significant differences (P = 0.023) in albumin-adjusted serum calcium levels were observed between nonincident (9.60 ± 0.53 mg/dL) and incident diabetic subjects (9.74 ± 0.55 mg/dL).

After a median follow-up of 4.78 years (interquartile range 2.60–5.74), 77 new cases of type 2 diabetes occurred. The proportions of participants observed to develop diabetes increased across tertiles of albumin-adjusted serum calcium (9% in the first, 9.6% in the second, and 13.2% in the third).

Table 2 shows the hazard ratios (HRs) and 95% CIs for type 2 diabetes incidence according to the baseline tertiles of albumin-adjusted serum calcium levels and also as a continuous variable for each additional 1 mg/dL albumin-adjusted serum calcium in the whole population (n = 707). After adjustment for possible confounding factors (model 3), there was a nonsignificant increased risk of type 2 diabetes in those individuals in the highest tertile compared with those in the lowest (HR 1.12 [95% CI 0.62–2.03]; P for trend = 0.73). When we analyzed calcium as a continuous variable, in model 2, for every unit increase (in milligrams per deciliter) in the albumin-adjusted serum calcium, the risk of type 2 diabetes increased by 77%. However, this association disappeared after further adjustment for fasting plasma glucose.

In a sensitivity analysis that excluded those individuals (n = 92) who take calcium and/or vitamin D supplements at baseline (to avoid possible bias effects), participants in the highest tertile of

Table 1—Characteristics of the study population according to baseline albumin-adjusted serum calcium tertiles

	Tertiles of albumin-adjusted serum calcium (mg/dL)			
	1 (low)	2	3	*P*
n	222	250	235	
Albumin-adjusted serum calcium (mg/dL)	9.01 ± 0.28	9.60 ± 0.13	10.20 ± 0.29	
Age (years)	67 ± 6	67 ± 6	67 ± 6	0.37
MedDiet+EVOO/MedDiet+nuts/control, *n*	66/80/79	85/80/85	82/80/73	0.73
Women	133 (59.9)	156 (62.4)	135 (57.4)	0.53
Waist circumference (cm)	100.4 ± 9.2	99.9 ± 8.9	100.6 ± 9.4	0.70
BMI (kg/m^2)	29.5 ± 3.4	29.7 ± 3.2	29.4 ± 3.2	0.62
Leisure-time physical activity (METs/day)	280.7 ± 286.7	268.3 ± 232.8	266.2 ± 257.7	0.81
Smokers				0.89
Never	132 (59.5)	154 (61.6)	145 (61.7)	
Current	33 (14.9)	35 (14.0)	38 (16.2)	
Past	57 (25.7)	61 (24.4)	52 (22.1)	
Educational level				0.95
Primary education	161 (72.5)	181 (72.4)	177 (75.3)	
Secondary education	40 (18.0)	44 (17.6)	37 (15.7)	
Academic/graduate	21 (9.5)	25 (10.0)	21 (8.9)	
Fasting blood glucose (mg/dL)	95.42 ± 13.18	94.38 ± 13.03	97.66 ± 14.85	0.03
Hypertension	204 (91.9)	230 (92)	209 (88.9)	0.42
Hypercholesterolemia	187 (84.2)	219 (87.6)	193 (82.1)	0.23
Overweight or obesity	210 (94.6)	233 (93.2)	223 (94.9)	0.69
Metabolic syndrome	100 (45.0)	106 (42.4)	115 (48.9)	0.35
Current medication use				
Antihypertensive treatment	173 (77.9)	191 (76.4)	186 (79.1)	0.76
Thiazide diuretics	80 (36.0)	91 (36.4)	95 (40.4)	0.55
Statin drugs	103 (46.4)	125 (50.0)	111 (47.2)	0.71
Total cholesterol (mg/dL)	218.54 ± 35.06	222.65 ± 35.33	229.73 ± 39.13	0.04
HDL cholesterol (mg/dL)	55.37 ± 11.94	58.01 ± 14.50	58.64 ± 13.65	0.02
LDL cholesterol (mg/dL)	138.17 ± 31.71	140.37 ± 31.55	142.41 ± 32.82	0.37
PTH (pg/mL)*	62.63 ± 23.89	56.67 ± 22.70	57.95 ± 22.15	0.61
25-OH-vitamin D (nmol/L)†	25.17 ± 14.01	46.09 ± 57.28	40.01 ± 43.23	0.33
Alcohol (g/day)	10.34 ± 15.48	9.88 ± 14.49	9.31 ± 15.77	0.77

Data are means ± SD or *n* (%) unless otherwise indicated. *P* value for comparisons across tertiles of albumin-adjusted serum calcium was calculated with one-way ANOVA for continuous variables or the Pearson χ^2 test for categorical variables. Hypertension: systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or antihypertensive medication. Hypercholesterolemia: LDL cholesterol ≥160 mg/dL or hypocholesterolemic medication. MedDiet+nuts, MedDiet supplemented with nuts; MedDiet+EVOO, MedDiet supplemented with extra virgin olive oil. *Data available for a random sample of 106 individuals (20, 47, and 39 in first, second, and third calcium tertiles, respectively). †Data available for a random sample of 89 individuals (16, 37, and 36 in first, second, and third calcium tertiles, respectively).

albumin-adjusted serum calcium showed a nonsignificant increased risk of diabetes (HR 1.10 [95% CI 0.59–2.03]; *P* for trend = 0.80) compared with participants in the lowest tertile (fully adjusted model). With the albumin-adjusted serum calcium used as a continuous variable, the HR per unit increase in the albumin-adjusted serum calcium was 1.68 (95% CI 1.07–2.63), and the *P* value = 0.02 in multivariate model 2. This association disappeared after adjustment for fasting plasma glucose concentrations.

Changes in Albumin-Adjusted Serum Calcium During the Follow-up and Risk of Type 2 Diabetes

Of the 707 participants in the previous analysis, we excluded all individuals who developed diabetes before the first 6 months of follow-up and those for whom no information was available on serum calcium concentrations through the subsequent years of follow-up (*n* = 57 participants). Finally, 650 participants were included to analyze the association between changes in serum calcium levels and risk of developing diabetes.

Table 3 shows the HRs and 95% CI for type 2 diabetes incidence according to changes in albumin-adjusted serum calcium concentrations during the follow-up. After adjustment for potential confounders, in model 2, the risk of diabetes observed in subjects whose levels of albumin-adjusted serum calcium were in the highest tertile of change (+0.52 ± 0.13 mg/dL) during the follow-up was more than three times that of subjects whose levels were in the lower tertile (−0.78 ± 0.29 mg/dL) (HR 3.48 [95% CI 1.48–8.17]; *P* for trend <0.01). This association was slightly attenuated but remained significant after additional adjustment for fasting plasma glucose levels (model 3) (2.87 [1.18–6.96]; *P* for trend = 0.02). When the albumin-adjusted serum calcium was analyzed as a continuous variable, per 1 mg/dL increase, the HR of diabetes incidence was 3.52 (95% CI 1.84–6.75), *P* value <0.01, in the fully adjusted model that also included fasting plasma glucose. The results remained significant (the

Table 2—HRs (95% CI) for type 2 diabetes incidence according to baseline albumin-adjusted serum calcium

	Tertiles of albumin-adjusted serum calcium, mg/dL				
	1 (low)	2	3	*P* for trend	Continuous variable HR (95% CI)
n	222	250	235		
Mean albumin-adjusted serum calcium	9.01 ± 0.28	9.60 ± 0.13	10.20 ± 0.29		
Diabetes, *n* (%)	20 (9.0)	26 (10.4)	31 (13.2)		77 (10.90)
Crude model	1 (ref.)	1.21 (0.67–2.17)	1.40 (0.79–2.46)	0.24	1.51 (1.01–2.27)
Multivariate model 1	1 (ref.)	1.24 (0.69–2.23)	1.42 (0.81–2.50)	0.22	1.52 (1.01–2.30)
Multivariate model 2	1 (ref.)	1.20 (0.67–2.18)	1.69 (0.94–3.04)	0.07	1.77 (1.15–2.73)
Multivariate model 3	1 (ref.)	1.17 (0.64–2.11)	1.12 (0.62–2.03)	0.73	1.37 (0.88–2.14)

Data are mean ± SD unless otherwise indicated. Cox regression models were used to assess the risk of diabetes by albumin-adjusted serum calcium at baseline and as a continuous variable (1 mg/dL). Model 1: adjusted for age in years, sex, and intervention group. Model 2: additionally adjusted for BMI (kg/m^2), smoking status (never, former, and current), educational level (primary education, secondary education, and academic/graduate), prevalence of hypertension (yes and no), prevalence of hypercholesterolemia (yes and no), use of antihypertensive medication (no antihypertensive use, thiazide diuretics, or antihypertensive drugs other than thiazides), use of statins (yes and no), alcohol intake in g/day (continuous and adding a quadratic term) and leisure-time physical activity (METs/day). Model 3: additionally adjusted for fasting plasma glucose at baseline (mg/dL).

HRs were 3.06 [95% CI 1.22–7.65], *P* for trend = 0.02, for higher tertile of calcium changes and 3.70 [1.91–7.15], *P* value <0.001, when calcium was analyzed as a continuous variable) even after adjustment of model 3 for calcium intake at baseline and changes in calcium consumption during follow-up (both were adjusted for energy intake using the residual method). We repeated the analysis for the three intervention groups and observed a nonsignificant trend to increased risk of diabetes in the three groups (data not shown).

Our results remained robust in several sensitivity analyses. When we repeated all of our analyses and additionally excluded subjects who were taking calcium and/or vitamin D supplements at baseline or during the follow-up (*n* = 144 participants), individuals in the higher albumin-adjusted serum calcium tertile of change during the follow-up had a significantly greater risk of diabetes than subjects in the lower tertile (HR 3.23 [95% CI 1.16–8.97]; *P* for trend = 0.02) after full adjustment for potential confounders (model 3). Similarly, a statistically significant positive relationship was observed when the changes in albumin-adjusted serum calcium were used as a continuous variable (3.92 [1.85–8.30]; *P* value <0.01). The magnitude of the association between changes in albumin-adjusted serum calcium and incident diabetes was similar when we also excluded subjects who had serum calcium concentrations outside the reference range (8.8–10.4 mg/dL) (3.28 [1.31–8.18]; *P* value = 0.01).

CONCLUSIONS

To the best of our knowledge, the present longitudinal study is the first to have studied the association between changes in albumin-adjusted serum calcium concentrations and the development of diabetes. Our results showed that an increase in albumin-adjusted serum calcium levels during follow-up was associated with an increased risk of diabetes in elderly Mediterranean individuals at high cardiovascular risk. The increased risk is maintained after exclusion of participants with calcium serum concentrations out of the normal range or who took calcium and/or vitamin D supplements at baseline or during follow-up.

In our study, individuals with diabetes incidence during follow-up had higher albumin-adjusted serum calcium at

Table 3—HRs (95% CI) for type 2 diabetes incidence according to changes in albumin-adjusted serum calcium

	Tertiles of changes in albumin-adjusted serum calcium, mg/dL				Continuous variable
	1 (low)	2	3	*P* for trend	HR (95% CI)
n	205	227	218		
Mean changes of albumin-adjusted serum calcium	−0.78 ± 0.29	−0.17 ± 0.14	0.52 ± 0.13		
Diabetes, *n* (%)	18 (8.8)	20 (8.8)	20 (9.2)		58 (8.9)
Crude model	1 (ref.)	1.07 (0.56–2.02)	1.12 (0.59–2.13)	0.72	1.26 (0.80–2.01)
Multivariate model 1	1 (ref.)	1.72 (0.85–3.45)	3.40 (1.43–8.12)	<0.01	2.81 (1.56–5.04)
Multivariate model 2	1 (ref.)	1.80 (0.88–3.68)	3.48 (1.48–8.17)	<0.01	3.09 (1.71–5.61)
Multivariate model 3	1 (ref.)	1.96 (0.94–4.09)	2.86 (1.18–6.96)	0.02	3.52 (1.84–6.75)

Data are mean ± SD unless otherwise indicated. Cox regression models were used to assess the risk of diabetes by changes in albumin-adjusted serum calcium and as a continuous variable (1 mg/dL). Model 1: adjusted for age in years, sex, intervention group, and albumin-adjusted serum calcium at baseline. Model 2: additionally adjusted for BMI (kg/m^2), smoking status (never, former, and current), educational level (primary education, secondary education, and academic/graduate), prevalence of hypertension (yes and no), prevalence of hypercholesterolemia (yes and no), use of antihypertensive medication (no antihypertensive use, thiazide diuretics, or antihypertensive drugs other than thiazides), use of statins (yes and no), alcohol intake in g/day (continuous and adding a quadratic term), and leisure-time physical activity (METs/day). Model 3: additionally adjusted for fasting plasma glucose at baseline (mg/dL).

baseline than those who did not develop diabetes. These results are in line with previous cross-sectional studies, in which patients with diabetes showed higher serum calcium levels than nondiabetic individuals (8,9).

Our results are also in agreement with cross-sectional (14) and prospective (15,16) studies that show a direct association between serum calcium levels and risk of diabetes. In the Chungju Metabolic Disease Cohort study conducted in 1,064 Korean individuals of more than 40 years of age without hypo- or hypercalcemia, an increased risk of diabetes prevalence was observed in individuals in the fourth and fifth quintile of albumin-adjusted serum calcium compared with those in the first quintile (14). Also, in the Tromsø 4 study (15) conducted in 25,657 men and women, participants with albumin-unadjusted serum calcium concentrations between 2.50 and 2.60 mmol/L had a 49% greater risk of diabetes than the reference group (2.20–2.29 mmol/L) after adjustment for age, sex, BMI, and smoking. Similarly, in the recent prospective Insulin Resistance Atherosclerosis Study (IRAS), individuals with calcium concentrations $\geq$2.38 mmol/L (9.5 mg/dL) had a 79% higher risk of developing diabetes than those with calcium concentrations $<$2.38 mmol/L (16). In our study, when we analyzed diabetes incidence according to tertiles of serum calcium levels at baseline, fasting plasma glucose seems to be the best predictor of diabetes during follow-up because when it was included in model 3 the association disappeared, suggesting that serum calcium levels may play a marginal role in the increased risk. However, associations between changes in serum calcium concentrations and diabetes incidence remained significant when model 3 was adjusted for fasting glucose at baseline, suggesting that serum calcium can be viewed as an independent factor associated with the incidence of diabetes.

We can speculate about the mechanisms that would explain the association between increased serum calcium levels and diabetes risk. High calcium concentrations could induce a decrease in insulin secretion from pancreatic β-cells. It is well-known that the release of insulin is a calcium-dependent process (5). The release of insulin, which is stored in secretory granules inside the pancreatic β-cells, depends on the influx of calcium through voltage-gated calcium channels (26). In fact, it was reported that β-cell function assessed by homeostasis model assessment was negatively associated with total serum calcium levels (11,14). Because insulin plays an important role in the regulation of blood glucose (27), alterations in the calcium flux can have adverse effects on β-cell secretory function and increase the risk of diabetes development. However, no associations between high serum calcium and defective insulin secretion have been reported in two cross-sectional epidemiological studies (12,13). Calcium can also modulate insulin sensitivity through other mechanisms. An increase in cytosolic calcium concentrations in L6 myotubes was related to an activation of GLUT4 transporter expression and an increase in insulin-stimulated glucose transport activity (6). However, it has been reported that calcium regulates GLUT4 expression in a time- and dose-dependent manner in C2C12 myotubes (28) and that chronic exposure to elevated cytosolic calcium concentration blocks AMPK-induced GLUT4 expression in skeletal muscle (28). Moreover, an increase in intracellular calcium levels has been shown to decrease the effect of insulin in adipocytes due to the reduced number of glucose transporters (GLUT4) and a decrease in insulin receptor activity (29–31). Consequently, increased calcium levels can decrease the expression of GLUT4 transporters and, consequently, decrease glucose uptake and, as a result, increase glucose plasma concentrations. Therefore, further studies are warranted to understand the mechanisms involved between alterations in serum calcium homeostasis, insulin, and glucose metabolism.

In fact, resistance to insulin-stimulated glucose uptake is reported in individuals with impaired glucose tolerance (32), and in two cross-sectional studies, an association between serum calcium levels and insulin sensitivity or resistance has been demonstrated. In the Newfoundland population, a positive correlation between serum calcium levels, fasting serum glucose, and insulin resistance was observed in male and female individuals (11). The Uppsala Longitudinal Study of Adult Men, conducted in 961 elderly men, also reports an inverse association between serum calcium levels and insulin sensitivity measured by euglycemic-hyperinsulinemic clamp (12). Unfortunately, in our study no measurements of plasma insulin are available for most of the population, so the association between serum calcium levels and insulin resistance or secretion cannot be explored.

We also found significantly higher levels of total cholesterol and HDL cholesterol in higher tertiles of serum calcium, as has been previously described in other populations (33–36). Whether these results could be explained through high parathyroid hormone (PTH) levels (37) cannot be assessed because of lack of information regarding PTH levels in our study.

Our study has several limitations that must be taken into account when interpreting the results. First, although ionized calcium should be used whenever possible (23), because it is regarded as the goal of calcium homeostasis measurement (38), it was not measured for the current study. However, total calcium is highly correlated with ionized calcium in many patients (38), and for this reason we used serum calcium for the analysis. In addition, because the amount of total serum calcium varies with the level of serum albumin, a protein to which calcium is bound, we adjusted calcium levels to allow for the change in total calcium due to the change in albumin-bound calcium. Second, our study did not collect information about PTH and vitamin D for all participants. Therefore, it was impossible to determine which individuals were at high risk of primary hyperparathyroidism or secondary hyperparathyroidism due to vitamin D deficiency. Both of these conditions, which are relatively frequent in elderly people, have been related to an increased risk of abnormal glucose metabolism and diabetes (39,40). Because primary hyperparathyroidism is the first cause of hypercalcemia, the association between serum calcium and risk of diabetes observed in our study could be explained by the presence of individuals with established or incipient primary hyperparathyroidism. In contrast, secondary hyperparathyroidism due to vitamin D deficiency is associated with lower or low-normal serum calcium levels. Consequently, according to the current data, vitamin D deficiency does not account for the

higher incidence of new-onset diabetes among patients in the high calcium tertile. However, because of these limitations, to discard the effect of these conditions we conducted a sensitivity analysis, like the Tromsø Study did (15), which excluded individuals with serum calcium concentrations outside the reference range (8.8–10.4 mg/dL). The association remained significant. Third, the study sample consisted of older white Mediterranean individuals at high risk of coronary heart disease, which limits the generalizability of our results to other age-groups or ethnicities.

Among the strengths of our study are that the studied sample was large with a relatively large number of incident cases and a long follow-up period. Also, to the best of our knowledge, this is the first study conducted in a well-characterized group of old individuals at high cardiovascular risk to examine the association between changes in serum calcium levels and risk of type 2 diabetes.

In conclusion, our results support the notion that an increase in albumin-adjusted serum calcium increases the risk of diabetes in Mediterranean subjects at high cardiovascular risk. Further investigations are needed to establish a causal relationship.

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Author Contributions. N.B.-T. analyzed and interpreted data, drafted the manuscript, and performed statistical analysis. R.E., J.B., M.F., and L.S.-M. developed the study concept and design. M.B. analyzed and interpreted data and performed statistical analysis. R.C. managed the laboratory logistics and database. A.D.-L. analyzed and interpreted data and performed statistical analysis. J.S.-S. developed the study concept and design, analyzed and interpreted data, drafted the manuscript, and performed statistical analysis. All authors critically revised the manuscript for important intellectual content. N.B.-T. and J.S.-S. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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