

Circulating calcium levels and the risk of type 2 diabetes: a systematic review and meta-analysis

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Abstract

Abnormal Ca homeostasis has been associated with impaired glucose metabolism. However, the epidemiological evidence is controversial. We aimed to assess the association between circulating Ca levels and the risk of type 2 diabetes mellitus (T2DM) or abnormal glucose homeostasis through conducting a systematic review and meta-analysis. Eligible studies were identified by searching electronic database (PubMed, Embase and Google Scholar) and related references with *de novo* results from primary studies up to December 2018. A random-effects meta-analysis was performed to estimate the weighted relative risks (RR) and 95% CI for the associations. The search yielded twenty eligible publications with eight cohort studies identified for the meta-analysis, which included a total of 89 165 participants. Comparing the highest with the lowest category of albumin-adjusted serum Ca, the pooled RR was 1.14 (95% CI 1.05, 1.24) for T2DM (*n* 51 489). Similarly, serum total Ca was associated with incident T2DM (RR 1.25; 95% CI 1.10, 1.42) (*n* 64 502). Additionally, the adjusted RR for 1 mg/dl increments in albumin-adjusted serum Ca or serum total Ca levels was 1.16 (95% CI 1.07, 1.27) and 1.19 (95% CI 1.11, 1.28), respectively. The observed associations remained with the inclusion of a cohort study with ionised Ca as the exposure. However, data pooled from neither case–control (*n* 4) nor cross-sectional (*n* 8) studies manifested a significant correlation between circulating Ca and glucose homeostasis. In conclusion, accumulated data from the cohort studies suggest that higher circulating Ca levels are associated with an augmented risk of T2DM.

Key words: Blood calcium: Serum calcium: Diabetes: Insulin: Glucose

Type 2 diabetes mellitus (T2DM), a major metabolic disorder characterised by hyperglycaemia, is associated with an increased risk of multiple chronic conditions^(1,2), such as CVD⁽³⁾ and nephropathy^(4,5). T2DM has been recognised as a leading cause of morbidity and mortality^(1,6).

Ca, an essential mineral, exerts a wide range of biological functions, including bone and tooth mineralisation, blood coagulation, muscle contraction, nerve impulse transmission and cellular signalling transduction^(4,6–9). Additionally, Ca plays a pivotal role in insulin secretion and glucose homeostasis^(4,10,11). Glucose-dependent insulin secretion is a Ca-regulated process,

which depends on intracellular Ca concentration in pancreatic β -cells^(10,12). Moreover, increased cytosolic Ca also affects glucose uptake in the myocyte^(10,13,14). Consequently, abnormal Ca homeostasis could potentially be involved with defects in insulin action and disorders in glucose homeostasis, contributing to T2DM development^(4,10,11,15,16).

Findings from epidemiological studies are inconsistent. Some observational studies have indicated that increased serum Ca concentration may be directly associated with the risk of developing T2DM^(3,4,6,7,17,18–21), whereas others reported the null correlation between circulating Ca and prevalent T2DM^(7,10,22).

Abbreviations: NOS, Newcastle–Ottawa Scale; PTH, parathyroid hormone; RR, relative risk; T2DM, type 2 diabetes mellitus.

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Also, it has been observed that elevated serum Ca levels were related to insulin resistance^(4,20,21), reduced insulin sensitivity^(4,18,23,24) and impaired glucose tolerance^(4,16,17,23,24), but not a decrease in insulin secretion^(4,23,24). These contradictory results may be partially attributable to diverse Ca assessments, for example, total serum Ca and albumin-adjusted serum Ca, which are different biomarkers of Ca status^(10,23). Because approximate 40% of Ca in the serum is bound to albumin⁽⁸⁾, it is vital to know the serum albumin level when evaluating the total serum Ca. Moreover, circulating Ca homeostasis is exquisitely regulated by multiple negative feedback loops which involve several integrated hormonal responses and target organs⁽⁸⁾. Thus, each of these components may mediate the possible Ca-related risk of diabetes. Interestingly, two double-blind, placebo-controlled, randomised clinical trials reported non-significant effects of Ca supplementation on insulin resistance in obese adults⁽²⁵⁾, as well as insulin secretion, insulin sensitivity and glycaemia in adults at risk of T2DM⁽²⁶⁾. Of note, these studies have relatively short follow-up periods, insufficient numbers of participants and heterogeneous characteristics between populations^(25,26).

One early meta-analysis⁽⁹⁾ was conducted by Sing *et al.* based only on four cohort studies^(3,4,9,17), which reported a positive association of both serum total Ca and albumin-adjusted Ca with risk of diabetes. Since then, four additional cohort studies^(6,10,22,27) with larger sample sizes have been published. Moreover, the linear association between circulating Ca levels and the T2DM risk has not been investigated.

Therefore, in the present study, we aimed to quantitatively assess the overall association between circulating Ca levels and the risk of T2DM or abnormal glucose homeostasis by performing an up-to-date systematic review and meta-analysis.

Methods

The present study was conducted according to the guidelines of Meta-analysis of Observational Studies in Epidemiology (MOOSE)⁽²⁸⁾. The present study was registered with the International Prospective Register of Systematic Reviews (PROSPERO) (<https://www.crd.york.ac.uk/PROSPERO/>) with the registration number CRD42018092835.

Study search strategy

We performed a systematic search of published studies in English in PubMed from inception to December 2018 using the terms 'calcium or serum calcium or blood calcium', 'diabetes or insulin resistance or impaired glucose tolerance or impaired fasting glucose', 'epidemiological studies', 'cohort/prospective longitudinal/follow-up/cross-sectional/case-control studies' and 'survival analysis or proportional hazard model or hazard ratio Cox or hazards ratio or odds ratio'. In addition, we searched Embase, Google Scholar and reference lists of narrative and systematic reviews to identify missing studies. Importantly, to standardise results specifically for this meta-analysis and to obtain additional information, we requested *de novo* data from two original studies^(22,27).

Selection criteria

Studies were considered for inclusion if they met the following criteria: (a) published in English; (b) had a cohort, case-control or cross-sectional design; (c) provided the exposure information of total serum Ca, albumin-adjusted serum Ca or serum ionised Ca and (d) had a relative risk (RR), hazard ratio (HR) or OR with 95% CI for T2DM in relation to Ca exposure or a correlation coefficient between Ca exposure and T2DM related variables (e.g. fasting glucose, fasting insulin, insulin sensitivity and insulin resistance), or such information could be derived from the published results.

Data extraction

Two investigators (J. Z. and P. C. X.) reviewed all relevant literature and assessed the eligibility of each study independently. Discrepancies were resolved with consensus reached by group discussion. From each retrieved study, the following information was extracted: author name, year of publication, study region, total number of participants and events (or total number of cases and controls), proportion of males, age of participants, exposure evaluation method, exposure classification, adjusted variables and HR, RR or OR estimates with corresponding 95% CI for all corresponding Ca exposure categories and/or for continuous exposure, compared with the lowest exposure group. OR reported in a cohort study⁽¹⁷⁾ was transformed to RR by adopting the following formula: $OR = ((1 - P_0) \times RR / (1 - RR \times P_0))$, where P_0 is the incidence rate in the reference group, according to the published methods⁽⁹⁾.

Quality assessment

The Newcastle-Ottawa Scale (NOS) system⁽²⁹⁾ was employed to evaluate the quality of each selected cohort study or case-control study. The NOS system has a maximum score of nine points. Studies with a sum score of 0–4, 5–7 and 8–9 were considered as low, moderate and high quality, respectively. Also, the original version of the NOS system was modified for assessing the quality of the included cross-sectional studies for the systematic review based on the existing literature^(30–32). The modified NOS system has a maximum score of eight points. Studies with a sum score of 0–4, 5–7 and 8 were considered as low, moderate and high quality, respectively. Two co-authors (J. Z. and P. C. X.) independently conducted the quality assessment. Any unconformity was resolved with consensus reached by group discussion with the involvement of a third investigator.

Statistical analysis

We pooled the cohort studies' data for each outcome of interest using a random-effects model in Stata 13.0 (STATA Corp). *P* values ≤ 0.05 were considered statistically significant if not otherwise specified. For the comparisons, we used the multivariable-adjusted associations (RR) for the highest *v.* lowest category of albumin-adjusted serum Ca or total Ca levels, and for the linear associations, we used standardised Ca levels with 1 mg/dl increments. Because of an insufficient number of studies measuring ionised Ca, we did not pool the data separately for this subgroup. Heterogeneity was estimated quantitatively using I^2



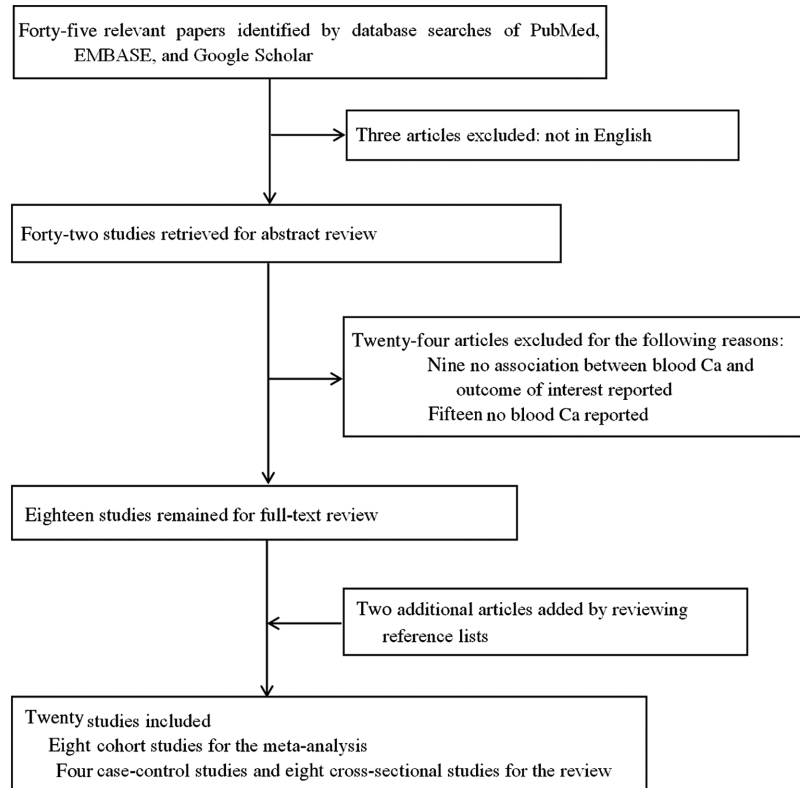


Fig. 1. Flow chart of study selection.

and tested by Cochran's Q -test. An I^2 value of >75 , 51–75, 26–50 or 0–25% corresponds to high, moderate, low or very low heterogeneity, respectively. We evaluated publication bias through both Egger's asymmetry test and Begg's non-parametric test with the α level set as 0.10. The Duval and Tweedie non-parametric 'trim and fill' method was adopted, if publication bias existed⁽³³⁾.

We also conducted sensitivity analyses to assess the effect of replacing a random-effects model with a fixed-effects model and the influence of an individual study on the overall association by excluding one study each time from the analysis. We also evaluated the impact of one study measuring ionised Ca on the overall association by combining it with other studies.

Results

Literature search

The literature selection process is shown in Fig. 1. A total of forty-five related articles were retrieved from PubMed, Embase and Google Scholar. Of these, three articles were excluded since they were not issued in English; nine publications were further excluded because they did not associate blood Ca with an outcome of interest and additional fifteen articles were excluded because they did not report blood Ca as either a categorical variable or a continuous variable on the original scale. Furthermore, we also identified two articles by searching the reference lists of relevant publications. Thus, the search revealed eight cohort studies^(3,4,6,9,10,17,22,27)

for the meta-analysis and another twelve eligible studies (four case-control studies^(7,18,34,35) and eight cross-sectional studies^(19–21,23,24,36–38)) for the systematic review.

Study characteristics

The information from the eligible studies was extracted (Tables 1–3). The total number of participants of all the included studies was 89 165 (48.6% males). For eight cohort studies^(3,4,6,9,10,17,22,27), there were 80 359 individuals (49.9% males) with 6447 incidents of diabetes. For four case-control studies^(7,18,34,35), there were 858 individuals (35.9% males) with 324 diabetes cases and 534 controls. For eight cross-sectional studies^(19–21,23,24,36–38), there were 7948 participants (37.2% males) including 740 diabetes cases. The mean age of participants across primary studies was from 25.7 to 80 years.

Regarding the quality of the included eight cohort studies^(3,4,6,9,10,17,22,27), six studies^(3,4,6,17,22,27) were assessed as high quality and two studies^(9,10) as moderate quality (online Supplementary Table S1). Of the included four case-control studies^(7,18,34,35), two studies^(18,34) were assessed as moderate quality and two studies^(7,35) as low quality. For the included eight cross-sectional studies^(19–21,23,24,36–38), two studies^(19,23) were assessed as high quality and six studies^(20,21,24,36–38) as moderate quality.

Meta-analysis of the cohort studies

A significant association between albumin-adjusted serum Ca levels and T2DM incidence was found (RR 1.14; 95% CI

Table 1. Characteristics of eight included cohort studies on the association between blood calcium concentrations and incidence of type 2 diabetes mellitus (T2DM)

Source	Participants (n)	Age (years)	Males (%)	Duration of follow-up (years)	Exposure assessment and categories	Number of cases	Case identification methods	Adjusted variables
Jorde <i>et al.</i> ⁽⁸⁾ , The Tromsø Study, Norway	25 657	Range 31.5–62.4	48.2	13	Non-fasting blood Ca (mmol/l): 2.20–2.29; 2.30–2.39; 2.40–2.49; 2.50–2.60	705	Self-reported T2DM in questionnaires and the record linkage to the national hospital discharge registry	Age, sex, BMI, smoking status, SBP and serum cholesterol
Becerra-Tomás <i>et al.</i> ⁽⁴⁾ , The Prevencion con Dieta Mediterránea (PREMEDI) study, Spain	707	67 (sd 6)	40	4.78	Fasting albumin-adjusted serum Ca (mg/dl): 9.01 (sd 0.28); 9.60 (sd 0.13); 10.20 (sd 0.29)	77	Diagnosed by clinical examination according to ADA 2010 criteria	Age, sex, intervention group, BMI, smoking status, educational level, prevalence of hypertension, prevalence of hypercholesterolaemia, use of antihypertensive medication, use of statins, alcohol intake, leisure-time physical activity and FPG at baseline
Lorenzo <i>et al.</i> ⁽¹⁷⁾ , The Insulin Resistance Atherosclerosis Study (IRAS), USA	863	Non-DM, 54.0 (se 0.3) DM, 56.5 (se 0.4)	Non-DM, 39.3 DM, 45.8	5.2	Fasting serum Ca (mmol/l): 2.13–2.24; 2.25–2.37 2.38–2.49; ≥2.50	140	Diagnosed by clinical examination and defined as fasting glucose ≥7.0 mmol/l and/or 2 h glucose ≥11.1 mmol/l, and impaired glucose tolerance as 2 h glucose ≥7.8–11.0 mmol/l.	Age, sex, ethnicity, clinic site, BMI, family history of diabetes, fasting and 2-h glucose concentrations, log ₁₀ SI, log ₁₀ AIR, eGFR and use of diuretic drugs
Rooney <i>et al.</i> ⁽⁶⁾ , The Atherosclerosis Risk in Communities (ARIC) study, USA	12 800	53.9 (sd 5.7)	44.5	8.8	Albumin-adjusted serum Ca (mg/dl): 7.28–9.54; 9.56–9.76; 9.78–9.96; 9.98–10.20; 10.22–13.28	1516	Defined as fasting (≥8 h) blood glucose ≥126 mg/dl, non-fasting glucose ≥200 mg/dl; self-reported physician diagnosis of diabetes or 'sugar in the blood' or current medication use for diabetes	Age, sex, race, centre, education, physical activity, smoking status, alcohol use, waist circumference, BMI, parathyroid hormone, 25-hydroxyvitamin D and P
Zaccardi <i>et al.</i> ⁽¹⁰⁾ , The Kuopio Ischaemic Heart Disease Risk Factor (KIHDRF) study, Finland	2350	52.9 (sd 5.2)	100	23.1	Fasting serum ionised Ca (mmol/l): 0.88–1.15; 1.16–1.18; 1.19–1.21; 1.22–1.50	140	Defined as a self-reported physician-set diagnosis and/or FPG ≥7.0 mmol/l or 2-h oral glucose tolerance test plasma glucose ≥11.1 mmol/l, and by record linkage to the national hospital discharge registry and to the Social Insurance Institution of Finland register	Age, BMI, SBP, serum HDL-C, family history of T2DM, C-reactive protein, physical activity, serum TAG and FPG
Sing <i>et al.</i> ⁽⁹⁾ , The Hong Kong Osteoporosis Study (HKOS), China	6096	Non-DM, 51.4 (sd 16.3) DM, 62.1 (sd 11.2)	27	10.2	Albumin-adjusted serum Ca (mmol/l): <2.25; 2.25–2.29; 2.30–2.35; >2.35 Serum total Ca (mmol/l): <2.33; 2.33–2.38; 2.39–2.44; >2.44	631	Ascertained from the EMR in several ways: (1) having a diagnosis of diabetes (ICD-9 code 250); (2) having a prescription record of diabetic medications; (3) having a laboratory record of HbA1c ≥6.5 % or FPG >7.0 mmol/l and (4) having enrolled in a diabetic complication screening programme	Age, sex, BMI, smoking status, drinking status, physical activity, serum albumin, serum phosphate, parathyroid hormone, alkaline phosphatase, femoral neck BMD T-score and season

Table 1. (Continued)

Source	Participants (n)	Age (years)	Males (%)	Duration of follow-up (years)	Exposure assessment and categories	Number of cases	Case identification methods	Adjusted variables
Suh <i>et al.</i> ⁽²⁷⁾ , A retrospective longitudinal cohort study, Korea	23 086	50.7 (sd 8.2)	57.4	4.8	Albumin-adjusted serum Ca (mg/dl): 7.58–8.54; 8.56–8.76; 8.78–9.00; 9.02–10.40 Serum total Ca (mg/dl): 8.20–8.70; 8.80–8.90; 9.00–9.20; 9.30–10.40	1922	Determined as FPG \geq 126 mg/dl or HbA1c \geq 6.5 %; subjects who reported a history of diabetes or current use of insulin or oral antidiabetic drugs	Age, sex, BMI, LDL, TAG, SBP and smoking status
Kim <i>et al.</i> ⁽²²⁾ , The Korean Genome and Epidemiology Study (KoGES), Korea	8800	51.8 (sd 8.8)	47	10	Albumin-adjusted serum Ca (mg/dl): 5.64–9.10; 9.12–9.48; 9.48–9.76; 9.78–13.64 Serum total Ca (mg/dl): 5.80–9.20; 9.30–9.60; 9.70–9.90; 10.00–13.80	1316	Defined as fasting glucose concentration \geq 126 mg/dl, post-load 2-h glucose concentration \geq 200 mg/dl or antidiabetic medication use	Age, sex, residential area, monthly family income, tobacco smoking, alcohol intake, physical activity, BMI, SBP, DBP, serum creatinine level and dietary Ca intake

SBP, systolic blood pressure; ADA, American Diabetes Association; FPG, fasting plasma glucose; DM, diabetes mellitus; SI, insulin sensitivity index; AIR, acute insulin response; eGFR, estimated glomerular filtration rate; HDL-C, HDL-cholesterol; EMF, electronic medical record; HbA1c, glycated Hb; BMD, bone mineral density; LDL, LDL-cholesterol; DBP, diastolic blood pressure.

1.05, 1.24) (Fig. 2), comparing the highest with the lowest Ca levels based on available data from five cohort studies^(4,6,9,22,27). Neither heterogeneity ($I^2 = 0.0\%$, $P = 0.550$) nor publication bias (Egger's test: $P = 0.631$; Begg's test: $P = 0.462$) existed. Consistently, a significant linear association was found (RR 1.16; 95 % CI 1.07, 1.27) with 1 mg/dl increments in albumin-adjusted serum Ca levels. Low heterogeneity ($I^2 = 33.8\%$, $P = 0.183$) was observed, but no publication bias (Egger's test: $P = 0.104$; Begg's test: $P = 0.452$) was evidenced. When adding the cohort study with ionised Ca as the exposure⁽¹⁰⁾, the aforementioned results of pooled RR remained (the highest *v.* the lowest: 1.13 (95 % CI 1.04, 1.22); per 1 mg/dl increment: 1.16 (95 % CI 1.06, 1.27)).

Similar results (Fig. 3) were found when the pooled analysis was restricted to studies^(3,9,17,22,27) that measured serum total Ca (RR 1.25; 95 % CI 1.10, 1.42), comparing the highest with the lowest serum total Ca levels. However, low heterogeneity ($I^2 = 33.3\%$, $P = 0.199$) and potential publication bias (Egger's test: $P = 0.008$; Begg's test: $P = 0.027$) were observed. We then used the Duval and Tweedie non-parametric 'trim and fill' method and observed the overall RR was 1.21 (95 % CI 1.06, 1.38). A significant linear association was also found (RR 1.19 (95 % CI 1.11, 1.28) with 1 mg/dl increments in serum total Ca levels). No heterogeneity ($I^2 = 0\%$, $P = 0.694$) was observed, but potential publication bias (Egger's test: $P = 0.031$; Begg's test: $P = 0.221$) was evidenced. After using the Duval and Tweedie non-parametric 'trim and fill' method, we observed the overall RR was 1.18 (95 % CI 1.10, 1.26). When further including the study that assayed ionised Ca⁽¹⁰⁾, the results of pooled RR persisted (the highest *v.* the lowest: 1.21 (95 % CI 1.06, 1.39) and 1.19 (95 % CI 1.11, 1.27) with 1 mg/dl increments).

The sensitivity analyses revealed that replacing the random-effects model with a fixed-effects model or any single study did not appreciably influence the pooled results or conclusions (online Supplementary Tables S4–S7).

Systematic review of the case-control and cross-sectional studies

The included case-control studies reported inconsistent results on the association between Ca homeostasis and diabetic risk. Higher serum total Ca levels⁽⁷⁾ or higher plasma Ca levels⁽¹⁸⁾ were reported in the middle-aged and elderly diabetic patients. Contrarily, normal serum total Ca⁽³⁴⁾ and ionised Ca levels^(7,34) were observed, respectively, in the middle-aged and elderly diabetic patients, and decreased serum ionised Ca concentrations⁽³⁵⁾ were also observed in the young diabetic patients, respectively, compared with the non-diabetic individuals (Table 2).

The included cross-sectional studies reported that serum Ca was directly correlated to glucose intolerance^(21,23,36), insulin resistance⁽²¹⁾, impaired glucose metabolism⁽²⁰⁾, early phase insulin secretion⁽³⁷⁾ and diabetes^(19,38) mainly in middle-aged and elderly individuals. However, serum Ca was found inversely correlated to β -cell function in women⁽²¹⁾ and insulin sensitivity in older men⁽²⁴⁾ (Table 3).



Table 2. Characteristics of four case-control studies on the association between blood calcium concentrations and incidence of diabetes

Source	Participants (n)	Age (years)	Males (%)	Exposure assessment	Cases		Controls		Case identification methods	Adjusted variables
					n	Blood Ca	n	Blood Ca		
Heath <i>et al.</i> (34), USA	122	Untreated, 58 (SE 1) Treated A*, 43 (SE 5); Treated B†, 51 (SE 4); Control, 44 (SE 2)	53.3	Fasting serum total Ca	Total, 82, Untreated, 47 Treated A*, 19 Treated B†, 16	Untreated, 9.4 (SE 0.1) mg/dl Treated A*, 9.2 (SE 0.1) mg/dl Treated B†, 9.4 (SE 0.1) mg/dl	40	9.4 (SE 0.1) mg/dl	All were judged by endocrinologist	—
McNair <i>et al.</i> (35), Denmark	48	Case, 28.5 (range 16–41); Control, 25.7 (range 16–40)	Case, 47.8; Control, 64.0	Fasting serum ionised Ca Fasting pH-adjusted serum ionic Ca	7 25 insulin-treated DM	Untreated, 4.22 (SE 0.6) mg/dl 1.16 (SE 0.01) mmol/l	11	4.2 (SE 0.1) mg/dl 1.20 (SE 0.01) mmol/l	—	—
Levy <i>et al.</i> (18), Israel	184	Case, 48 (SD 4); Control 48 (SD 4)‡	Case, 52.2; Control, 52.2‡	Fasting plasma Ca	92	2.48 (SE 0.004) mmol/l	92‡	2.38 (SE 0.006) mmol/l	—	Age, sex and mode of treatment
Sorva & Tilvis (7), Finland	504	80 (SD 9)	Case, 28.8; Control, 19.0	Fasting serum total/ionised Ca concentrations	125	CaT, 2.31 mmol/l/Cal, 1.24 mmol/l	379	CaT, 2.27 mmol/l Cal, 1.23 mmol/l	Use antidiabetic medication or fasting plasma glucose exceeded 7 mmol/l	Albumin

DM, diabetes mellitus; CaT, total Ca; Cal, ionised, actual Ca.

* Treated A: fasting plasma glucose > 200 mg/dl.

† Treated B: fasting plasma glucose < 200 mg/dl.

‡ Matched with the case group.

Table 3. Characteristics of eight cross-sectional studies on the association between blood calcium concentrations and incidence of diabetes or impaired glucose tolerance

Source	Participants (n)	Age (years)	Males (%)	Exposure categories	No. of cases	Case identification methods	Adjusted variables	OR*	95 % CI	r/β
Wareham <i>et al.</i> ⁽²³⁾ , UK	1071	40–65	42.8	Fasting albumin-adjusted serum Ca (mmol/l): <2.20; 2.20–2.25; 2.25–2.31; 2.31–2.38; >2.38	188 with IGT	IGT by WHO criteria	Age, BMI, sex, season and 25(OH)D	1.63	1.42, 1.88	–
Sun <i>et al.</i> ⁽²¹⁾ , Canada	1182	Men, 39.47 (sd 12.65) Women, 42.93 (sd 9.98)	18.4–20.1	Fasting albumin-adjusted serum Ca (mmol/l): men, 2.31 (sd 0.12); women, 2.31 (sd 0.12) Fasting total serum Ca (mmol/l): men, 2.36 (sd 0.12); women, 2.33 (sd 0.12)	–	–	Age, trunk fat percentage, P and Mg	–	–	Correlation between serum Ca and fasting glucose†: r = 0.31 for women; r = 0.22 for men
Hagström <i>et al.</i> ⁽²⁴⁾ , Sweden	961	71.0 (sd 0.58)	100	Albumin-adjusted serum Ca (mmol/l): 1.62–2.28; 2.29–2.33; 2.34–2.39; 2.40–2.85	–	DM was diagnosed as FPG ≥7.0 mmol/l, 2-h post-load glucose level ≥11.1 mmol/l, or by the use of oral hypoglycaemic agents or insulin	BMI, physical activity, smoking, consumption of tea, alcohol, coffee and dietary Ca, serum phosphate and serum creatinine	–	–	Linear regression analysis on MI against serum Ca levels in total cohort‡: β = –0.17
Kim <i>et al.</i> ⁽¹⁹⁾ , Korea	1329	65.8	38.1	Fasting albumin-adjusted serum Ca (mmol/l): ≤2.24; 2.24–2.30; 2.30–2.36; 2.37–2.44; ≥2.45	260 with DM	FPG ≥7.0 mmol/l and a 2-h post-load glucose level ≥11.1 mmol/l	Age, sex, BMI, season of blood sampling, and serum creatinine, P, PTH, 25(OH)D levels, smoking, alcohol drinking, exercise and total energy, Ca and Na intake	3.32	1.87, 5.88	–
Yamaguchi <i>et al.</i> ⁽²⁰⁾ , Japan	480	Men, 60.8 (sd 13.0); Women, 65.5 (sd 11.4)	56.5	Fasting albumin-adjusted serum Ca (mg/dl): men, 9.2 (sd 0.4); women, 9.2 (sd 0.4)	480 with T2DM	–	Age, body weight, height, creatinine, albumin, phosphate, intact PTH, BAP, osteocalcin, UNTX and oestradiol	–	–	Correlations between serum Ca and FPG: r = 0.215 for men; 0.118 for women Correlations between serum Ca and HOMA-IR: r = 0.184 for men; –0.026 for women Correlations between serum Ca and fasting glucose: r = 0.148
Cho <i>et al.</i> ⁽²⁶⁾ , Korea	1941	65–16 (sd 4.58)	0	Fasting serum Ca (mg/dl) 8.40–< 9.00; 9.00–< 9.20; 9.20–< 9.40; 9.40–10.20	648 with high fasting glucose	The criteria for high glucose by ADA 2010 criteria	Age, BMI, alcohol intake, cigarette smoking and exercise	2.77	2.07, 3.72	Correlations between serum Ca and fasting glucose: r = 0.148

Table 3. (Continued)

Source	Participants (n)	Age (years)	Males (%)	Exposure categories	No. of cases	Case identification methods	Adjusted variables	OR*	95 % CI	r/β
Guasch et al. ⁽³⁸⁾ , Spain	316	46.85 (95 % CI 45.44, 48.26)†	24.1	Fasting albumin-adjusted plasma Ca (mmol/l) tertile (with no specific data)	—	From the medical records of T2DM	PTH, 25(OH)D, age, sex, season and current smoking, BMI, uCRP and leucocyte count	2.17	0.92, 5.26	—
Shimodaira et al. ⁽³⁷⁾ , Japan	668	NGT men, 48.8 (sp 9.1); NGT women, 51.6 (sp 6.7); pre-DM men, 52.5 (sp 8.6); pre-DM women, 54.2 (sp 8.0)	66.8	Fasting albumin-adjusted serum Ca (mg/dl): NGT men, 9.0 (sp 0.3); NGT women, 9.1 (sp 0.3); pre-DM men, 9.0 (sp 0.3); pre-DM women, 9.1 (sp 0.3)	316 with pre-DM	Based on the criteria of ADA (2014): NGT = FPG, < 100 mg/dl and 2-h PG, < 140 mg/dl and pre-DM = FPG, 100–125 mg/dl or 2-h PG, 140–199 mg/dl	Age, BMI, HDL-C and HbA1c	—	—	Multiple linear regression analyses on IGI against adjusted Ca: β = 0.236 for NGT men; 0.128 for pre-DM men

IGI, insulinogenic index; 25(OH)D, 25-hydroxyvitamin D₃; DM, diabetes mellitus; FPG, fasting plasma glucose; MI, glucose disposal rate [M] divided by mean insulin concentration [I]; PTH, parathyroid hormone; T2DM, type 2 diabetes mellitus; BAP, bone-specific alkaline phosphatase; uNTX, urinary N-terminal cross-linked telopeptide of type-1 collagen; HOMA-IR, homeostatic model assessment for insulin resistance; ADA, American Diabetes Association; uCRP, ultrasensitive C-reactive protein; NGT, normal glucose tolerance; Pre-DM, pre-diabetes mellitus; PG, plasma glucose; HDL-C, HDL-cholesterol; HbA1c, glycated Hb; IGI, impaired glucose tolerance.

* The highest quintile of blood Ca was compared with the lowest quintile.

† Partial correlations between total serum Ca and fasting serum glucose.

‡ Partial correlations between total serum Ca and insulin resistance.

§ Standard deviation increase in serum Ca was associated with 0.17 mg·kg⁻¹·min⁻¹ (mU/l)⁻¹ × 100 (0.024 mg·kg⁻¹·min⁻¹ (pmol/l) × 100) decrease in MI.

Discussion

The accumulated evidence from the cohort studies suggests that either albumin-adjusted serum Ca or serum total Ca was directly associated with T2DM risk, though data from case-control and cross-sectional studies were inconsistent and inconclusive. The present study provided updated and robust data to the literature by including additional and *de novo* results from a few newly published large cohort studies. Also, the present study investigated both categorical and linear associations between circulating Ca and T2DM incidence, which strengthened our conclusion.

The results in case-control and cross-sectional studies were not consistent, which may be partially explained by the different Ca measurements, such as total serum, albumin-corrected serum, serum ionised or plasma levels or heterogeneous study populations with various outcomes.

The results of the present study are inconsistent with findings from studies on dietary Ca intake and the T2DM risk^(22,39–42). One possible explanation is that dietary estimation and blood Ca are different assessments, which are subject to different measurement errors. Another explanation is that the dietary Ca and diabetes association may be confounded by Mg intake, which is highly correlated with Ca intake and associated with diabetes risk⁽⁴³⁾. Moreover, circulating Ca concentrations could reflect not only the exogenous Ca intake but also the endogenous capability of maintaining homeostasis⁽²²⁾, which is tightly regulated by multiple negative feedback loops involving several target organs and hormones^(3,6,8,22). Ca intake may lead to an elevation of serum Ca that activates the Ca-sensing receptor (CaR) in the parathyroid glands to reduce parathyroid hormone (PTH) secretion⁽⁸⁾. The reduced PTH inactivates the PTH receptor (PTHr) in kidney to decrease tubular Ca reabsorption, and PTHR in bone to decrease net bone Ca resorption⁽⁸⁾. The reduced PTH also results in a decreased secretion of 1,25-dihydroxyvitamin D (1,25(OH)₂D), which inactivates the vitamin D receptor in intestine to reduce Ca absorption, in the parathyroid glands to augment PTH secretion and in bone to reduce Ca resorption⁽⁸⁾. The rise in serum Ca may also activate the CaR in kidney to decrease Ca reabsorption⁽⁸⁾. When a decrease in serum Ca occurs, this integrated hormonal response is reversed with serum Ca increased, which helps to maintain total serum Ca levels within a physiological range of approximately 10%⁽⁸⁾. However, emerging evidence showed that hormones (such as PTH, 1,25(OH)₂D) that participate in this complex homeostatic system have themselves been related to diabetes^(44,45). Thus, abnormalities of these pivotal physiological factors may affect the Ca-diabetes association, which warrants further investigation.

The potential mechanisms underlying the role of circulating Ca in T2DM incidence remain unclear. However, growing evidence implies that Ca may mediate insulin secretion via activation of the CaR^(46,47), which is widely expressed in tissues, such as pancreatic islets of Langerhans⁽⁴⁶⁾. Glucose-dependent insulin secretion from the pancreatic β-cells is a Ca-regulated process^(11,12,48), which requires the influx of Ca through voltage-gated Ca channels to the secretory granules in β-cells^(49–51). Thus, high Ca concentration may induce β-cell dysfunction⁽²¹⁾. Moreover, abnormal Ca status impacts insulin

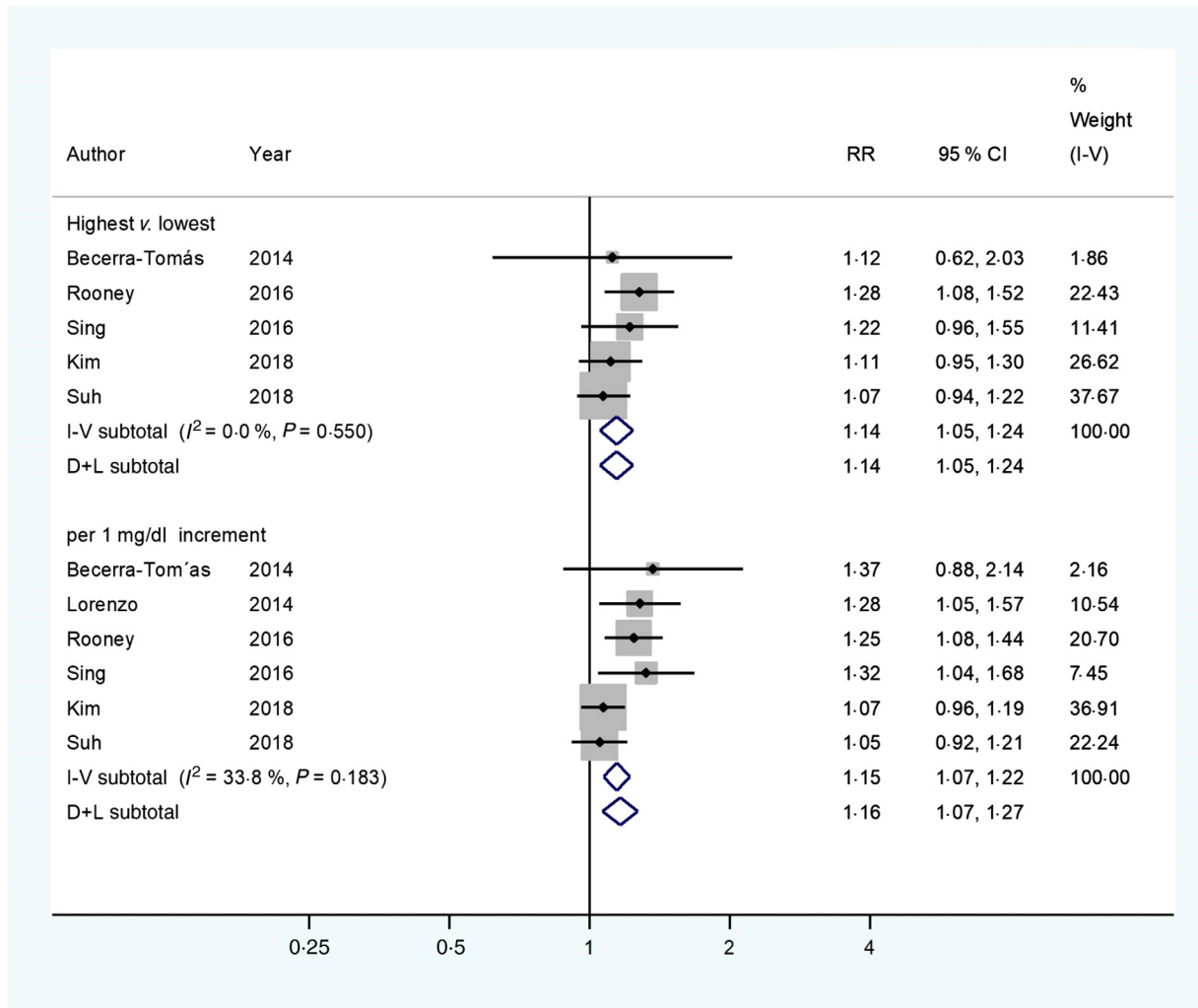


Fig. 2. Multivariable-adjusted relative risks (RR) (◆) and 95 % confidence intervals (—) of type 2 diabetes mellitus (T2DM) in relation to albumin-adjusted serum calcium levels. The summary assessments (◇) were obtained by adopting a random-effects model. Values are adjusted RR comparing the highest with the lowest category levels or per 1 mg/dl increase in albumin-adjusted serum calcium. The size of the shaded square is proportional to the weight of each study. We requested *de novo* data from the authors of Kim *et al.*⁽²²⁾ and Suh *et al.*⁽²⁷⁾, respectively.

sensitivity and glucose transport in adipocytes and skeletal muscle^(14,52,53) through regulating GLUT4 expression, which is a passive transporter necessary for glucose uptake in peripheral tissues^(13,14). Chronic exposure to increased cytosolic Ca levels has been shown to prohibit GLUT4 expression in skeletal muscle⁽⁵⁴⁾. Furthermore, elevated or sustained cytosolic Ca levels have been revealed to reduce GLUT4 expression and consequently decrease insulin receptor activity and reduce glucose uptake in adipocytes⁽⁴⁾. Because of the pivotal role of insulin in modulating blood glucose⁽⁵⁵⁾, abnormalities in circulating Ca levels could potentially impair β -cell secretory function, glucose intolerance and insulin sensitivity. Thus, it may consequently lead to T2DM development⁽¹⁷⁾. Notably, it has been observed that abnormal intracellular Ca may cause pancreatic β -cell apoptosis^(56,57). Intracellular Ca increases may accompany the activation of endoplasmic reticulum stress and dysfunction of organelles, including mitochondria and the nucleus, which may lead to destruction of pancreatic β -cells⁽⁵⁸⁾. Therefore, future studies investigating such targets might help elucidate

mechanisms relevant to the Ca–diabetes association and progression to T2DM.

Several limitations need to be acknowledged when interpreting the findings from this systematic review and meta-analysis. First, most observational studies adopted albumin-adjusted serum Ca as exposure assessment^(2,6,9,17,19–21,23,24,37,38), which is prone to errors because of the assumption that Ca binds to albumin steadily^(59,60). In fact, it may neglect other relevant ligands and Ca fractions⁽⁶¹⁾. Hence, the results of the risk prediction based on measuring albumin-adjusted serum Ca need to be interpreted with caution. Ionised Ca is considered as the physiologically active form⁽⁶¹⁾ and the ‘gold standard’ of Ca homeostasis assessment^(4,62,63). However, only one available cohort study measured ionised Ca and reported a null association with incident T2DM⁽¹⁰⁾, though it did not appreciably alter the overall association. The association of ionised Ca levels with T2DM risk merits further investigation. Second, serum Ca is highly modulated by some other factors, such as PTH or vitamin D. However, not all primary studies adjusted for these

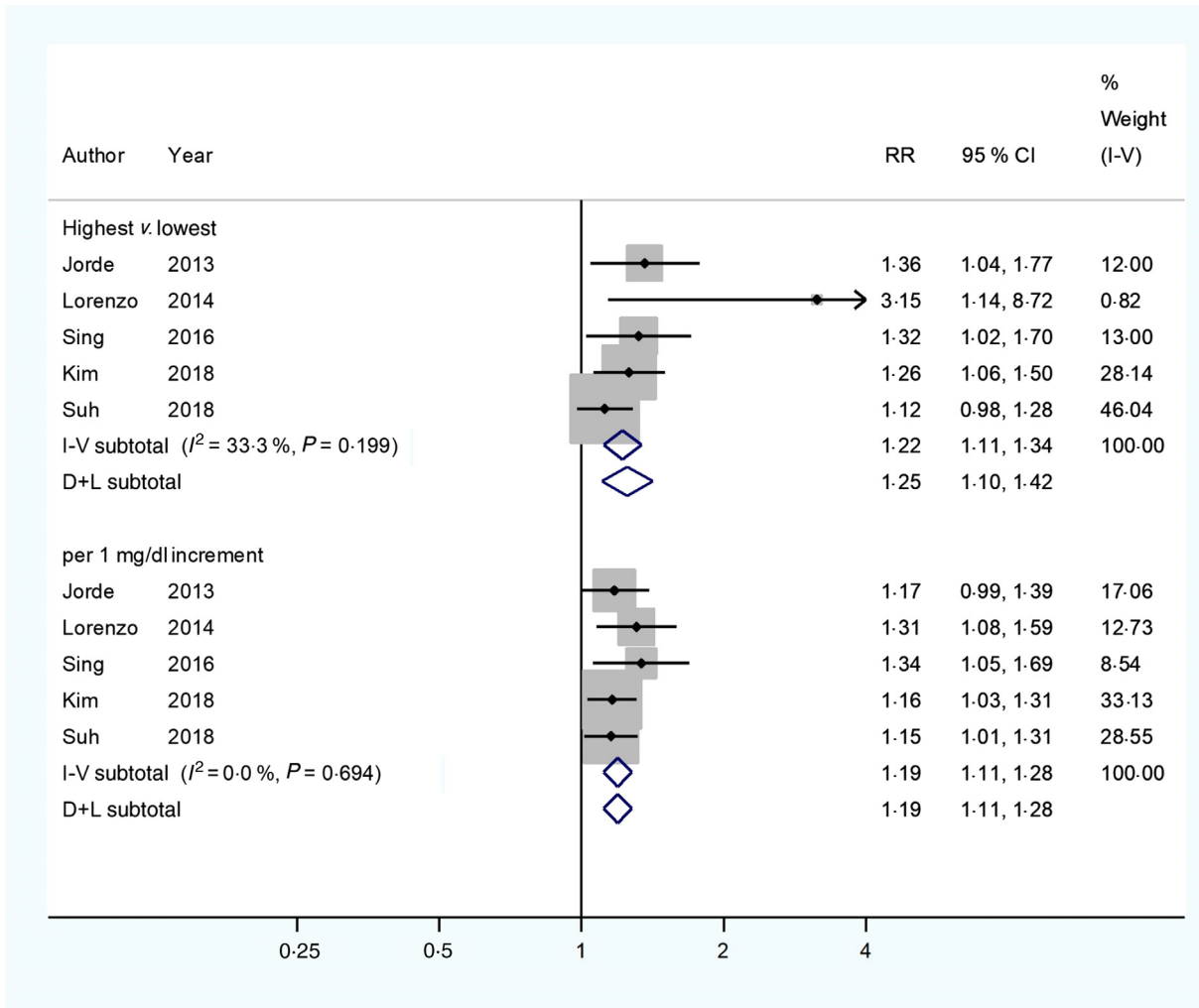


Fig. 3. Multivariable-adjusted relative risks (RR) (◆) and 95 % confidence intervals (—) of type 2 diabetes mellitus (T2DM) in relation to serum total calcium levels. The summary assessments (◇) were obtained by adopting a random-effects model. Values are adjusted RR comparing the highest with the lowest category levels or per 1 mg/dl increase in serum total calcium. The size of the shaded square is proportional to the weight of each study. We requested *de novo* data from the authors of Kim *et al.*⁽²²⁾ and Suh *et al.*⁽²⁷⁾, respectively.

potential confounding variables. This inherent limitation may somehow affect the combined association. Third, low heterogeneity was evidenced for studies with some serum Ca measurements. The impact should be reduced using a random-effects model in the analyses. Fourth, a potential publication bias could not be completely excluded in some pooled analyses. Nevertheless, the associations were not substantially changed after using the 'trim and fill' method to account for the publication bias. Thus, our conclusion should remain.

In summary, data from this systematic review and meta-analysis provide accumulated evidence supporting the conclusion that circulating Ca levels are associated with the incidence of T2DM. Further studies are needed to establish the causal inference and elucidate the underlying mechanism of action.

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The authors' contributions were as follows: K. H. conceived the study concept, designed the protocol and supervised the manuscript writing; J. Z. and P. C. X. conducted research, analysed data and wrote the manuscript; J. C. B., J. H. K., D. J. K. and K. F. Y. revised the manuscript. K. H. had primary responsibility for final content. All authors read and approved the final manuscript.

The authors declare that there are no conflicts of interest.

Supplementary material

For supplementary materials referred to in this article, please visit <https://doi.org/10.1017/S0007114519001430>

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