Increased serum calcium levels are associated with carotid atherosclerotic plaque in normocalcaemic individuals with type 2 diabetes

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Abstract

Background: Patients with type 2 diabetes mellitus (T2DM) have an elevated risk of atherosclerotic cardiovascular disease. Although previous data have suggested that serum calcium levels could be involved in T2DM and cardiovascular disease, whether this applies in T2DM patients with atherosclerosis remains unclear. This study therefore aimed to investigate the relationship between serum calcium levels within the physiological ranges and carotid atherosclerotic plaque in T2DM patients.

Methods: A total of 594 normocalcaemic in-patients with T2DM were recruited, of whom 231 had carotid atherosclerotic plaque. Serum calcium levels were measured and carotid ultrasonography was performed.

Results: Patients with plaque had significantly higher serum albumin-corrected calcium than those without plaque [9.02 (8.78–9.34) mg/dL *versus* 8.86 (8.66–9.06) mg/dL, $p < 0.001$]. As serum albumin-corrected calcium levels increased across tertiles, the percentage of plaque increased (27.6%, 35.5%, and 55.7%; $p < 0.001$). Logistic regression showed that serum albumin-corrected calcium levels were independently and positively correlated with the presence of plaque, but not parathyroid hormone levels. Compared with patients in the lowest serum calcium tertiles, the odds ratio for plaque in patients in the upper quartile was 2.47 (95% confidence interval 1.51–4.03, $p < 0.001$) after adjustment for potential confounders.

Conclusion: Serum albumin-corrected calcium levels are elevated in patients with T2DM and carotid atherosclerotic plaques.

Keywords: atherosclerosis, calcium, carotid plaques, type 2 diabetes

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Introduction

Approximately 350 million people worldwide are living with type 2 diabetes mellitus (T2DM). Cardiovascular disease (CVD) is the major cause of morbidity and mortality in patients with T2DM. Patients with diabetes have a 2- to 4-fold higher risk of a CVD event than patients without diabetes, in particular, various types of atherosclerotic CVD.[1] Studies have even suggested that prediabetes was an independent risk factor for cardiovascular outcomes.[2,3] As is well known, long-term chronic hyperglycaemia can induce advanced glycation end-product formation, resulting in vascular endothelial damage and decreased arterial wall elasticity. In addition, chronic hyperglycaemia can lead to lipid metabolic disorders, oxidative stress, and inflammation, which are the pathological basis of atherosclerosis, ending with the occlusion of arterial stenosis or obstruction. In recent years, interest in the relationship between calcium and mineral metabolism with T2DM and cardiovascular health has burgeoned.

Calcium is the most abundant mineral in the human body. More than 99% of the calcium in the body is used as a structural component of

bones. Although less than 1% of the total body calcium is in the blood and is under tight hormonal control, the circulating ionized component is essential for normal functioning of the cardiovascular system, muscles, and nerves. Multiple studies have shown that serum calcium concentrations within the normal range were positively associated with carotid plaque thickness in participants who underwent a general health screening.[4,5] A meta-analysis of eight prospective studies found that an increase of one standard deviation in the calcium concentration was associated with an 8% increased risk of myocardial infarction/coronary heart disease (summary risk ratio (RR) 1.08, 95% confidence interval (CI) 1.04–1.13).[6] Another prospective study of atherosclerosis risk in the community, involving 15,732 participants followed for an average of 12.6 years, showed that baseline serum calcium levels were modifiable risk factors for stroke and death.[7] Similar to the observational data, the association between serum calcium levels and adverse cardiovascular risk has been confirmed in several intervention studies. A randomized double-blind placebo-controlled study reported that the hypocalcaemia agent EDTA appropriately reduced the risk of adverse cardiovascular outcomes.[8] Furthermore, calcium plays a key role in the regulation of insulin secretion and maintenance of blood glucose homeostasis. Many epidemiologic and clinical studies have suggested that serum calcium levels are increased in T2DM patients compared with those without the condition.[9,10] Serum calcium levels can be used to predict the incidence of T2DM.[11] Individuals with calcium levels $\geqslant$9.5 mg/dL had an approximately 79% higher risk of T2DM development than those with calcium levels $<$9.5 mg/dL.[12] Therefore, we hypothesize that an alteration in serum calcium may be involved in the occurrence and development of atherosclerosis in patients with T2DM.

Carotid atherosclerosis detected by ultrasonography was reported to be an accepted subclinical atherosclerotic marker and a strong predictor of future clinical cardiovascular events.[13] Based on the above these findings, we conducted a cross-sectional study to explore whether patients with T2DM and carotid atherosclerosis have a high-normal level of serum calcium and examine the association between serum calcium levels, obesity, and other related variables.

Materials and methods

Subjects and design

Initial data were obtained from 2014 individuals with T2DM who were hospitalized for the first time in the Department of Endocrinology and Metabolism of Soochow University Affiliated First Hospital from January 2017 to September 2019. Ultimately, 594 patients were included in the present study (Figure 1). The diagnosis of T2DM was based on the criteria of the American Diabetes Association. The exclusion criteria were: type 1 diabetes mellitus, secondary diabetes, gestational diabetes, acute complications of diabetes such as diabetic ketoacidosis, hyperglycaemic hyperosmolar status, lactic acidosis and hypoglycaemia coma, acute or chronic viral hepatitis, severe hepatic dysfunction (alanine aminotransferase or aspartate aminotransferase $>$1.5 times the normal upper limit), renal dysfunction [estimated glomerular filtration rate (eGFR) $<$ 60 mL/min per 1.73 m^2], thyroid diseases, parathyroid disease, vitamin D-related disorders, symptomatic cardiovascular disease, acute infection, malignant tumour, psychiatric disease, taken dyslipidaemia medications or medications in the past 1 month that may affect calcium metabolism (vitamin D, bisphosphonate, oestrogen, or diuretics) or serum calcium levels beyond the laboratory's normal range (8.42–10.42 mg/dL, or 2.10–2.60 mmol/L). The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Soochow University Affiliated First Hospital (2017-162). Written consent was obtained from all participants included in the study.

Clinical parameter measurements

Professional nurses collected general clinical data including sex, age, diabetes duration, height, weight. Blood pressure, including systolic blood pressure (SBP) and diastolic blood pressure (DBP), were measured with a mercury sphygmomanometer after the subject had rested for at least 10 min. The body mass index (BMI) of each patient was calculated as weight in kilograms divided by the square of height in metres.

Venous blood samples were taken after overnight fasting for at least 10 h. Serum calcium, albumin, creatinine, total cholesterol (TC), triglyceride

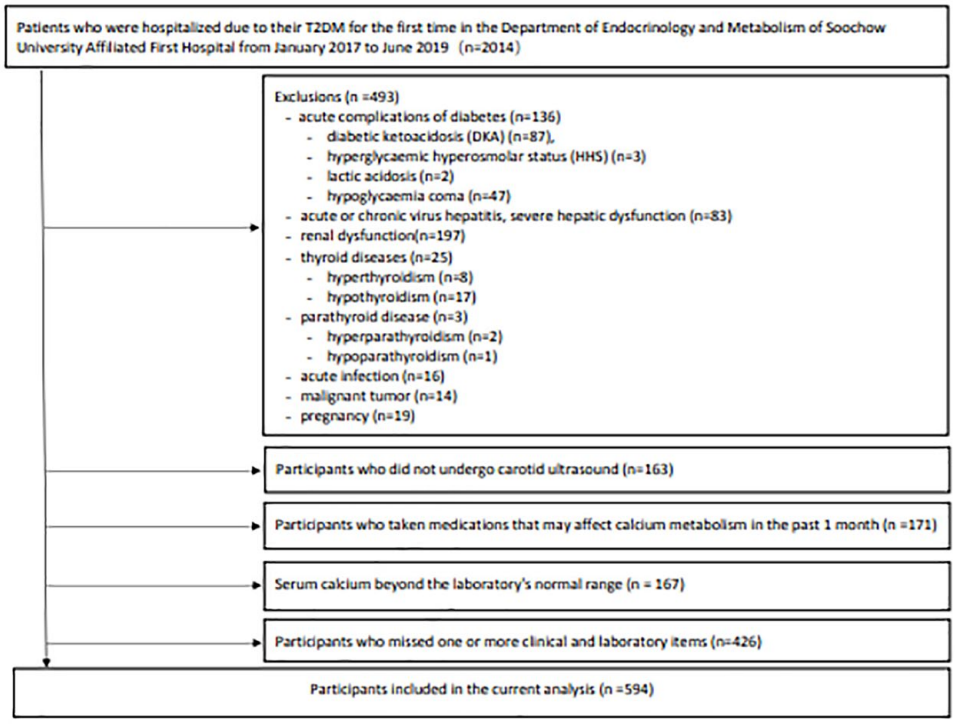

Figure 1. Flow diagram of the participant selection in the current analysis.
T2DM, type 2 diabetes mellitus.

(TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured on the Hitachi 7600 automatic analyser (Kyoto, Japan). Fasting plasma glucose (FPG) was immediately measured using the glucose oxidase technique. Glycosylated haemoglobin A1c (HbA1c) was measured using cation-exchange column chromatography on an automatic analyser (Bio-Rad Company, Hercules, CA, USA). Fasting insulin (FIns) and fasting C-peptide (FCp) were detected using ELISA kits (R&D company, USA). Electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Mannheim, Germany) was used to analyse the serum parathyroid hormone (PTH) and 25-hydroxyvitamin D (25OHD) concentrations. Approximately 40% of non-skeletal calcium is bound to proteins, primarily albumin and globulin. 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)].[14] Albumin-corrected calcium was used for all the analyses. Homeostasis model assessment of insulin resistance index (HOMA-IR) was used to evaluate the status of insulin resistance. The HOMA-IR formula = [fasting glucose (mmol/L) × fasting insulin (mIU/L)]/22.5. The eGFR was derived from the Chronic Kidney Disease Epidemiology Collaboration creatinine-based equation.[15]

Carotid ultrasound measurement

Carotid ultrasonography was performed using an Acuson Sequoia 512 scanner (Siemens Medical Solutions, Mountain View, CA, USA) equipped with a 5–13 MHz linear array transducer, and operated by an experienced vascular physician from the ultrasonic department. The patients were examined in the supine position with the head slightly extended to the opposite direction of the carotid artery being examined. Doppler recordings of the proximal segments of the common, internal, and external carotid arteries were recorded. A composite mean intima-media thickness (IMT) was calculated from the mean IMTs measured from three angles on both sides. The presence of carotid atherosclerotic plaque was defined as IMT ⩾1.5 mm, or a focal increase of either 0.5 mm or 50% compared with the surrounding IMT.[16]

Table 1. The clinical and laboratory characteristics of the diabetic patients with and without plaques.

Variables	Non-plaque	Plaque	*p* value
n	363	231	
Gender, M/F	212/151	122/109	0.203
Age, years	51 (43–57)	59 (54–66)	0.000
BMI, kg/m^2	23.49 (21.62–26.11)	25.45 (23.13–27.25)	0.000
WC, cm	87 (80–93)	89 (83–96)	0.003
Duration of diabetes, years	4 (1–10)	8 (3–12)	0.000
SBP, mmHg	120 (110–134)	130 (120–146)	0.000
DBP, mmHg	80 (70–82)	80 (70–90)	0.014
FPG, mmol/L	7.60 (6.00–9.50)	8.10 (6.60–10.20)	0.021
HbA1c, %	8.10 (6.80–10.00)	8.50 (7.40–9.90)	0.023
FIns, mU/L	10.44 (6.21–14.58)	12.72 (7.45–20.7)	0.000
FCp, ng/mL	1.80 (1.0–2.56)	2.00 (1.2–2.5)	0.245
HOMA-IR	3.54 (2.04–5.59)	4.60 (2.64–7.89)	0.000
TC, mmol/L	4.70 (4.00–5.32)	4.80 (4.20–5.50)	0.128
TG, mmol/L	1.34 (0.91–1.94)	1.45 (1.06–2.13)	0.018
LDL-C, mmol/L	2.92 (2.37–3.63)	3.07 (2.51–3.73)	0.036
HDL-C, mmol/L	1.09 (0.93–1.31)	1.06 (0.91–1.26)	0.249
eGFR, ml/min per 1.73 m^2	114.34 (104.77–231)	96.48 (86.76–103.06)	0.000
Corrected Ca, mg/dL	8.86 (8.66–9.06)	9.02 (8.78–9.34)	0.000
PTH, pg/mL	35.59 (26.91–45.39)	32.91 (26.75–43.5)	0.103
25OHD, ng/mL	17.69 (13.02–23.39)	16.79 (12.86–22)	0.262

Continuous variables are expressed as median (25th and 75th percentiles), while categorical variables are expressed as percentages.
25OHD, 25-hydroxyvitamin D; BMI, body mass index; Corrected Ca, albumin-corrected calcium; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FCp, fasting C-peptide; FIns, fasting insulin; FPG, fasting plasma glucose; HbA1c, glycosylated haemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; M/F, male/female; PTH, parathyroid hormone; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; WC, waist circumference.

Statistical analysis

SPSS version 21.0 (SPSS Inc., Chicago, IL, USA) was used for the data analyses. Continuous variables were presented as the median (interquartile range) and the Mann–Whitney *U* test was used to determine the statistical significance between the two groups. Categorical variables were expressed as counts and percentages (%), and significant differences between the two groups were assessed by the Chi-square test. Furthermore, the patients were divided into three groups based on the tertiles (Ts) of serum albumin-corrected calcium levels of the overall study population (T1: $<$8.78 mg/dL; T2: 8.78–9.06 mg/dL; T3: $\geqslant$9.06 mg/dL). For comparison among multiple groups, Kruskal–Wallis analysis of variance was performed and followed by the Dunn–Bonferroni test for post hoc comparisons. Multiple linear stepwise regression analysis was conducted to identify factors independently correlated with the serum albumin-corrected calcium

levels. Binary logistic regression analysis was performed to determine variables associated with carotid atherosclerotic plaque. To control for potential confounding factors, the multivariate logistic regression model was chosen to assess the association between serum albumin-corrected calcium (as a categorical variable) and carotid atherosclerotic plaque. The odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. Two-tailed p-values of <0.05 were considered statistically significant.

Results

Clinical and biochemical characteristics

A total of 594 T2DM participants were enrolled in the present study [median age: 54 (47–61) years], including 231 subjects with carotid atherosclerotic plaque and 363 subjects without carotid atherosclerotic plaque. As shown in Table 1, compared with those without plaque, the patients with plaque were older and had a longer duration of diabetes, higher BMI, waist circumference (WC), SBP, DBP, FPG, HbA1c, FIns, HOMA-IR, TG, LDL-C and serum creatinine (all $p<0.05$), along with a lower albumin and eGFR ($p<0.01$). Other variables did not differ significantly between the two groups (all $p>0.05$).

Comparison of serum albumin-corrected calcium levels

Among the entire study population, the median (interquartile range) serum albumin-corrected calcium levels were 8.90 (8.70–9.14) mg/dL. No significant gender difference in the serum albumin-corrected calcium levels were detected (Figure 2). The patients with carotid atherosclerotic plaque had significantly higher serum albumin-corrected calcium levels compared with those without plaque (Table 1). The study population was divided into three groups based on BMI. As recommended by the World Health Organization, the BMI cut-offs for Chinese patients were used, with normal weight as BMI 18.5–25 kg/m^2, overweight as BMI 25.0–27.5 kg/m^2 and general obesity as BMI $\geqslant$27.5 kg/m^2.[17] No patients with a BMI lower than 18.5 kg/m^2 were included in the study. The serum albumin-corrected calcium levels in the overweight and obesity groups were significantly higher than in the normal-weight group (Figure 3). All patients were stratified into trisection according to albumin-corrected calcium tertiles of the overall study population (T1: <8.78 mg/dL; T2: 8.78–9.06 mg/dL; T3: $\geqslant$9.06 mg/dL). The patients with upper serum albumin-corrected calcium levels had a higher carotid atherosclerotic plaque formation rate than those with lower albumin-corrected calcium levels (Figure 4).

Multiple linear stepwise regression analysis for serum albumin-corrected calcium levels

The serum albumin-corrected calcium level was used as the dependent variable, and age, BMI, WC, SBP, DBP, FPG, HbA1c, HOMA-IR, FCp, TC, TG, HDL-C, LDL-C, eGFR, PTH, and 25OHD were used as independent variables. The multiple linear stepwise regression analysis demonstrated that BMI, HbA1c, and SBP were independently and positively correlated with the serum albumin-corrected calcium levels, while PTH and eGFR were independently and negatively associated with the serum albumin-corrected calcium levels (Table 2). No obvious collinearity among these predictors was detected.

Variables related to carotid atherosclerotic plaque

To determine the variables associated with carotid atherosclerotic plaque, binary logistic regression analysis was developed to include albumin-corrected calcium, age, gender, BMI, WC, SBP, DBP, FPG, HbA1c, HOMA-IR, FCp, TC, TG, HDL-C, LDL-C, eGFR, PTH, and 25OHD on the first step. The analysis identified serum albumin-corrected calcium levels as an independent and positive factor for carotid atherosclerotic plaque (OR 3.42, 95% CI 1.77–6.81, $p<0.001$), along with age, SBP, BMI, HbA1c, HOMA-IR, and diabetes duration (Table 3).

Serum albumin-corrected calcium levels and carotid atherosclerotic plaque

When albumin-corrected calcium was a categorical variable (tertiles), multinomial logistic regression analyses showed that subjects in the upper tertile of albumin-corrected calcium had a significantly greater risk for carotid atherosclerotic plaque compared with the lowest tertile (OR 3.31, 95% CI 2.18–5.03, $p<0.001$) (Table 4). Adjustment for age, sex, obesity (categorical variable), SBP, DBP, HbA1c, HOMA-IR, and diabetes duration did not change the association; however, subgroup analysis showed a stronger association between albumin-corrected calcium and risk of carotid atherosclerotic plaque among the female

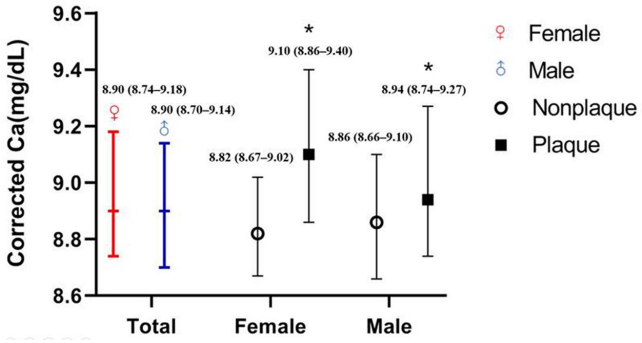

Figure 2. Serum albumin-corrected calcium levels [median (IQR) marked] in female and male with and without plaque.
*$p < 0.05$ *versus* non-plaque group.

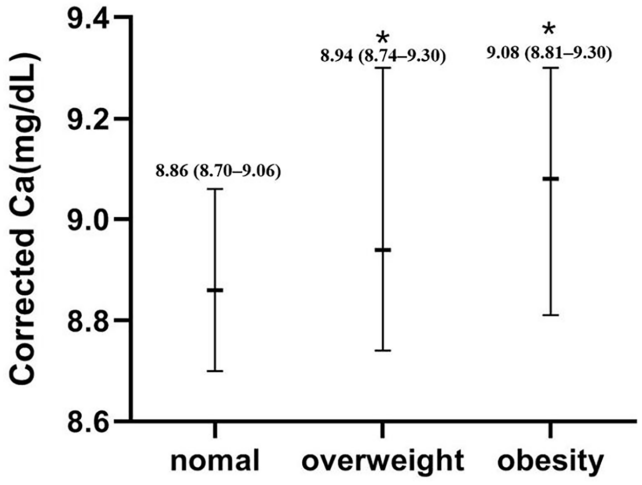

Figure 3. Serum albumin-corrected calcium levels based on body mass index.
*$p < 0.05$ *versus* normal weight group.
There were no patients with a BMI lower than 18.5 kg/m^2.

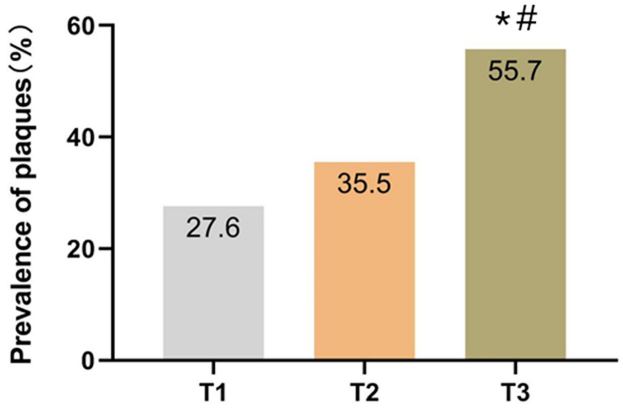

Figure 4. Prevalence of carotid atherosclerotic plaque according to albumin-corrected calcium tertiles.
*$p < 0.05$ *versus* T1 group, #$p < 0.05$ *versus* T2 group.
T, tertile.

participants after adjusting for potential confounding factors (OR 4.71, 95% CI 2.15–10.30, $p < 0.001$), but this correlation was not present among the males (OR 1.54, 95% CI 0.80–2.96, NS) (Table 4).

Discussion

In this cross-sectional study of patients with T2DM, we found a positive relationship between serum albumin-corrected calcium levels, within the normal range, and carotid atherosclerotic plaque. Based on the overall study population, the patients with upper serum calcium levels (T3 tertile: ⩾9.06 mg/dL) showed a greater prevalence of carotid atherosclerotic plaque than those with lower serum calcium levels (T1 tertile: <8.78 mg/dL). The association between albumin-corrected calcium and carotid atherosclerotic plaque persisted after adjustment for age, sex, obesity, SBP, DBP, HbA1c, HOMA-IR, and diabetes duration.

Diabetic macrovascular complications are the major causes of mortality and disability among patients with T2DM, of which the pathological basis is atherosclerosis. Recently, the potential role of serum calcium in the pathogenesis of metabolic-related cardiovascular complications has gained interest. Our study is the first to evaluate the association between serum albumin-corrected calcium levels within the normal range and carotid atherosclerosis in T2DM patients. Three mechanisms predominantly regulate calcium homeostasis in humans, including intestinal calcium absorption (dietary or supplemental), bone conversion, and glomerular calcium filtration/tubular calcium reabsorption. Due to the influence of renal function on calcium homeostasis, we included only patients with normal renal function (eGFR ⩾ 60 mL/min per 1.73 m^2) in this study. The results indicated that T2DM patients with carotid atherosclerotic plaque had significantly higher levels of serum albumin-corrected calcium than those without plaque. Additionally, patients in the top third of calcium concentration had a significantly greater percentage of carotid atherosclerotic plaque than those in the bottom third.

Ageing, obesity, insulin resistance, and chronic hypertension are known to be classic risk factors for atherosclerosis. Consistent with previous studies, in the present study, binary logistic regression analyses showed a significant association between

Table 2. Multiple linear stepwise regression analysis of independent influence factors associated with albumin-corrected calcium.

Independent factors	Unstandardized coefficient		Standardized coefficients Beta	*t*	*p* value	Collinearity statistics	
	β	Std. error				Tolerance	VIF
BMI	0.02	0.004	0.194	4.987	0.000	0.953	1.049
HbA1c	0.035	0.006	0.238	6.083	0.000	0.946	1.057
eGFR	−0.005	0.001	−0.212	−5.38	0.000	0.933	1.072
SBP	0.002	0.001	0.104	2.653	0.008	0.933	1.072
PTH	−0.002	0.001	−0.077	−2.024	0.043	0.993	1.007
Constant	8.463	0.164	–	51.705	0.000	–	–

Independent variables originally included: age, BMI, waist circumference, SBP, diastolic blood pressure, fasting plasma glucose, HbA1c, homeostasis model assessment of insulin resistance index, fasting C-peptide, total cholesterol, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, eGFR, PTH, 25-hydroxyvitamin D, diabetes duration.
BMI, body mass index; eGFR, estimated glomerular filtration rate; HbA1c, glycosylated haemoglobin; PTH, parathyroid hormone; SBP, systolic blood pressure; VIF, variance inflation factor.

Table 3. Independent factors for carotid atherosclerotic plaque identified by binary logistic regression analyses.

Variable	β	SE	OR	95% CI	*p* value
Corrected Ca	1.231	0.336	3.424	1.774–6.81	0.000
Age	0.086	0.016	1.090	1.056–1.125	0.000
SBP	0.027	0.008	1.027	1.012–1.043	0.000
BMI	0.092	0.045	1.096	1.002–1.198	0.044
HbA1c	0.117	0.058	1.124	1.004–1.259	0.043
HOMA-IR	0.037	0.019	1.038	1.000–1.077	0.048
Duration of diabetes	0.046	0.019	1.047	1.009–1.087	0.016

Variables entered on first step: age, gender, BMI, waist circumference, SBP, diastolic blood pressure, HbA1c, fasting plasma glucose, homeostasis model assessment of insulin resistance index, fasting C-peptide, total cholesterol, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, estimated glomerular filtration rate, parathyroid hormone, 25-hydroxyvitamin D, diabetes duration.
BMI, body mass index; CI, confidence interval; HbA1c, glycosylated haemoglobin; HOMA-IR, homeostasis model assessment; OR, odds ratio; SBP, systolic blood pressure.

age, SBP, BMI, HOMA-IR, diabetes duration, and carotid atherosclerotic plaque. Adjusting for these factors, the correlation between calcium and carotid atherosclerosis remained robust.

Until now, the underlying mechanism *via* which serum calcium may contribute to carotid atherosclerosis in patients with T2DM remained unclear. Calcium-sensing receptors (CaSRs), the major sensor and regulator of extracellular calcium, is expressed on parathyroid cells and also commonly expressed in various tissues and cells. The serum calcium concentration influences CaSR activity.[18] Qi *et al.*[19] reported that CaSR expression was increased in vascular endothelial cells of STZ-diabetic rats. Increased expression

Table 4. OR (95% CI) of carotid atherosclerotic plaque according to albumin-corrected calcium.

		Tertiles of corrected Ca, mg/dL		
		T1 (<8.78)	**T2 (8.78–9.06)**	**T3 (>9.06)**
Overall	Non-adjusted	1.00 (ref)	1.448 (0.953–2.20), NS	3.308 (2.177–5.026), 0.000
	Adjusted[a]	1.00 (ref)	1.512 (0.937–2.44), NS	2.470 (1.514–4.029), 0.000
Male	Non-adjusted	1.00 (ref)	1.491 (0.862–2.578), NS	2.023 (1.164–3.514), 0.012
	Adjusted[b]	1.00 (ref)	1.685 (0.891–3.188), NS	1.540 (0.800–2.964), NS
Female	Non-adjusted	1.00 (ref)	1.41 (0.736–2.702), NS	6.291 (3.240–12.216), 0.000
	Adjusted[b]	1.00 (ref)	1.336 (0.634–2.812), NS	4.706 (2.151–10.297), 0.000

Values are OR (95% CI) and *p* value.
[a]Adjusted for age, sex, obesity (categorical variable), systolic blood pressure, diastolic blood pressure, glycosylated haemoglobin, homeostasis model assessment of insulin resistance index, diabetes duration.
[b]Adjusted for age, obesity (categorical variable), systolic blood pressure, diastolic blood pressure, glycosylated haemoglobin, homeostasis model assessment of insulin resistance index, diabetes duration.
CI, confidence interval; OR, odds ratio; T, tertile.

could induce endothelial cell apoptosis in diabetic atherosclerosis injury through increasing calcium influx, mitochondrial activation, and mitogen-activated protein kinase pathway stimulation. Both the mRNA and protein expression of CaSR were also demonstrated in vascular smooth muscle cells (VSMCs). *Ex vivo* experiments confirmed that elevated extracellular calcium levels promote VSMC proliferation, which had been considered an important process in diabetic atherosclerosis.[20] Additionally, calcium-dependent protein kinase C (PKC) activation responds within seconds to a calcium concentration elevation.[21] Among them, PKC-β activation promoted the production of inflammatory cytokines and cellular adhesion molecules that contribute to atherosclerotic plaque formation in diabetes.[22] Calcium is an essential cofactor in the coagulation pathway and regulates platelet function *via* the CaSR. Serum calcium may affect vascular risk *via* effects on blood coagulation. Furthermore, a complex system of mineralization inhibitors (such as pyrophosphate, fetuin-A, matrix GLA protein, etc.) exists in human soft tissues to prevent calcium deposition. When errors occur in the metabolism of circulating calcium and mineralization inhibitors, severe vascular calcification may happen.[23,24]

Similar to the investigations by Sabanayagam and Shankar,[25] Wu *et al.*,[26] and Chou *et al.*,[27] the stepwise multiple linear regression demonstrated that the serum albumin-corrected calcium levels were independently and positively associated with SBP in the current study. Serum calcium may participate in the regulation of blood pressure by regulating peripheral vascular resistance and controlling the contractility of VSMCs,[28] which may be another mechanism by which calcium and atherosclerosis are linked: however, Ahlström *et al.*[29] and Cho *et al.*[30] showed no significant correlation between serum calcium levels and blood pressure. Some studies reported that hypertensive subjects had significantly lower serum calcium.[31,32] The discrepancies among the studies might be attributed to ethnic differences of the study population and the influence of antihypertensive medications. Future research should verify and discuss the conflicting results.

In addition, our study observed a strong positive correlation between albumin-corrected calcium and BMI. The serum albumin-corrected calcium levels in the overweight and obesity groups were significantly higher than in the normal-weight group. Therefore, the degree of obesity should be taken into consideration when exploring abnormal serum calcium levels. Obesity induces the production of inflammatory cytokines which stimulate bone absorption by osteoclasts that might subsequently lead to a higher serum calcium level.[33] In the context of obesity, calcium influx and elevated intracellular calcium levels

can in turn trigger lipogenesis, suppress lipolysis,[34] and promote endoplasmic reticulum -mitochondrial dysfunction, oxidative stress, and an inflammatory response, which are important underlying factors in the pathogenesis of atherosclerosis.[35] Therefore, in how to best target calcium signalling and homeostasis for the treatment of obesity, T2DM and atherosclerosis may be important areas of future research.

Furthermore, because most of the female patients included in our study were postmenopausal women with an average age of 57 years, and given the influence of oestrogen on bone and mineral metabolism, we compared the gender differences in serum calcium levels. No significant difference was observed between female and male participants: however, after stratification by gender, we found a stronger association between serum calcium levels and the risk of carotid atherosclerotic plaque among the female participants in the fully adjusted model, but this correlation was lost among the males. We speculate that this may be attributed to the loss of the protective effects of oestrogen against cardiovascular events among postmenopausal female patients.[36]

Several limitations of the present study should be noted. First, because our research is a cross-sectional study and many patients were excluded due to missing values, the samples were relatively limited and the participants were Chinese, which should be evidenced in other ethnicities through prospective cohort studies with larger samples. Second, we did not specifically distinguish the types of carotid atherosclerotic plaques and the degree of their extension. Third, the results are based on single serum calcium measurements, using the subjective criteria of our laboratory; therefore, changes in calcium levels over time are available. Finally, calcium intake was not considered.

Conclusion

Our study supports the reported correlation between calcium and glycometabolism, and extends on previous findings of the association between serum calcium and cardiovascular disease, especially in T2DM patients with carotid atherosclerotic plaque. Increased serum albumin-corrected calcium levels, within the physiological ranges, are independently associated with prevalence of carotid atherosclerotic plaque in patients with T2DM.

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Author contributions

Conceptualization: Huijing Zhu and Xingbo Cheng. Methodology: Huijing Zhu, Huili Wang and Xingbo Cheng. Software: Huijing Zhu and Lin Cheng. Data Curation: Huijing Zhu and Xingbo Cheng. Formal Analysis: Huijing Zhu, Huili Wang, Yuqing Jia and Lin Cheng. Investigation: Huijing Zhu and Xingbo Cheng. Writing – Original Draft Preparation: Huijing Zhu. Writing – Review & Editing: Huijing Zhu, Huili Wang, Yuqing Jia and Xingbo Cheng. Visualization: Huijing Zhu. Supervision and Validation: Yuqing Jia and Xingbo Cheng. Funding Acquisition: Huijing Zhu and Xingbo Cheng.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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References

1. Gan W, Bragg F, Walters RG, *et al.* Genetic predisposition to type 2 diabetes and risk of subclinical atherosclerosis and cardiovascular diseases among 160,000 Chinese adults. *Diabetes* 2019; 68: 2155–2164.
2. Scicali R, Giral P, D'Erasmo L, *et al.* High TG toHDL ratio plays a significant role on atherosclerosis extension in prediabetes and newly diagnosed type 2 diabetes subjects. *Diabetes Metab Res Rev*. Epub ahead of print 18 June 2020. DOI: 10.1002/dmrr.3367.
3. Scicali R, Giral P, Gallo A, *et al.* HbA1c increase is associated with higher coronary and peripheral

atherosclerotic burden in non diabetic patients. *Atherosclerosis* 2016; 255: 102–108.

4. Montalcini T, Gorgone G and Pujia A. Serum calcium level is related to both intima-media thickness and carotid atherosclerosis: a neglect risk factor in obese/overweight subjects. *J Transl Med* 2012; 10: 114.
5. Rubin MR, Rundek T, McMahon DJ, *et al*. Carotid artery plaque thickness is associated with increased serum calcium levels: the Northern Manhattan study. *Atherosclerosis* 2007; 194: 426–432.
6. Reid IR, Gamble GD and Bolland MJ. Circulating calcium concentrations, vascular disease and mortality: a systematic review. *J Intern Med* 2016; 279: 524–540.
7. Foley RN, Collins AJ, Ishani A, *et al*. Calcium-phosphate levels and cardiovascular disease in community-dwelling adults: the Atherosclerosis Risk in Communities (ARIC) study. *Am Heart J* 2008; 156: 556–563.
8. Lamas GA, Goertz C, Boineau R, *et al*. Effect of disodium EDTA chelation regimen on cardiovascular events in patients with previous myocardial infarction. *JAMA* 2013; 309: 1241.
9. Becerra-Tomás N, Estruch R, Bulló M, *et al*. Increased serum calcium levels and risk of type 2 diabetes in individuals at high cardiovascular risk. *Diabetes Care* 2014; 37: 3084–3091.
10. Rooney MR, Pankow JS, Sibley SD, *et al*. Serum calcium and incident type 2 diabetes: the Atherosclerosis Risk in Communities (ARIC) study. *Am J Clin Nutr* 2016; 104: 1023–1029.
11. Jorde R, Schirmer H, Njølstad I, *et al*. Serum calcium and the calcium-sensing receptor polymorphism rs17251221 in relation to coronary heart disease, type 2 diabetes, cancer and mortality: the Tromsø study. *Eur J Epidemiol* 2013; 28: 569–578.
12. Lorenzo C, Hanley AJ, Rewers MJ, *et al*. Calcium and phosphate concentrations and future development of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetologia* 2014; 57: 1366–1374.
13. Basili S, Loffredo L, Pastori D, *et al*. Carotid plaque detection improves the predictive value of CHA2DS2-VASc score in patients with non-valvular atrial fibrillation: the ARAPACIS study. *Int J Cardiol* 2017; 231: 143–149.
14. Bushinsky DA and Monk RD. Electrolyte quintet: Calcium. *Lancet* 1998; 352: 306–311.
15. Levey AS, Stevens LA, Schmid CH, *et al*. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; 150: 604.
16. Touboul PJ, Hennerici MG, Meairs S, *et al*. Mannheim Carotid intima-media thickness and plaque consensus (2004–2006–2011). *Cerebrovasc Dis* 2012; 34: 290–296.
17. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* 2004; 363: 157–163.
18. Cianferotti L, Gomes AR, Fabbri S, *et al*. The calcium-sensing receptor in bone metabolism: from bench to bedside and back. *Osteoporosis Int* 2015; 26: 2055–2071.
19. Qi H, Cao Y, Huang W, *et al*. Crucial role of calcium-sensing receptor activation in cardiac injury of diabetic rats. *PLoS One* 2013; 8: e65147.
20. Ping S, Li Y, Liu S, *et al*. Simultaneous increases in proliferation and apoptosis of vascular smooth muscle cells accelerate diabetic mouse venous atherosclerosis. *PLoS One* 2015; 10: e141375.
21. Oancea E and Meyer T. Protein kinase C as a molecular machine for decoding calcium and diacylglycerol signals. *Cell* 1998; 95: 307–318.
22. Durpès M, Morin C, Paquin-Veillet J, *et al*. PKC-β activation inhibits IL-18-binding protein causing endothelial dysfunction and diabetic atherosclerosis. *Cardiovasc Res* 2015; 106: 303–313.
23. Lomashvili KA, Narisawa S, Millán JL, *et al*. Vascular calcification is dependent on plasma levels of pyrophosphate. *Kidney Int* 2014; 85: 1351–1356.
24. Cai MMX, Smith ER and Holt SG. The role of fetuin-A in mineral trafficking and deposition. *Bonekey Rep* 2015; 4: 672.
25. Sabanayagam C and Shankar A. Serum calcium levels and hypertension among US adults. *J Clin Hypertens (Greenwich)* 2011; 13: 716–721.
26. Wu X, Han T, Gao J, *et al*. Association of serum calcium and insulin resistance with hypertension risk: a prospective population-based study. *J Am Heart Assoc* 2019; 8: e009585.
27. Chou C, Fang W, Chen Y, *et al*. Association between serum calcium and risk of cardiometabolic disease among community-dwelling adults in Taiwan. *Sci Rep* 2020; 10: 3192.
28. Zemel MB. Regulation of adiposity and obesity risk by dietary calcium: mechanisms and

implications. *J Am Coll Nutr* 2002; 21: 146S–151S.

29. Ahlström T, Hagström E, Larsson A, *et al*. Correlation between plasma calcium, parathyroid hormone (PTH) and the metabolic syndrome (MetS) in a community-based cohort of men and women. *Clin Endocrinol* 2009; 71: 673–678.
30. Cho GJ, Shin J, Yi KW, *et al*. Serum calcium level is associated with metabolic syndrome in elderly women. *Maturitas* 2011; 68: 382–386.
31. Zemel MB. Calcium modulation of hypertension and obesity: mechanisms and implications. *J Am Coll Nutr* 2001; 20(Suppl. 5): 428S–435S.
32. Behradmanesh S and Nasri H. Association of serum calcium with level of blood pressure in type 2 diabetic patients. *J Nephropathol* 2013; 2: 254–257.
33. Mundy GR. Osteoporosis and inflammation. *Nutr Rev* 2007; 65: S147–S151.
34. Zemel MB. Role of dietary calcium and dairy products in modulating adiposity. *Lipids* 2003; 38: 139–146.
35. Arruda AP and Hotamisligil GS. Calcium homeostasis and organelle function in the pathogenesis of obesity and diabetes. *Cell Metab* 2015; 22: 381–397.
36. Mendelsohn ME and Karas RH. The protective effects of estrogen on the cardiovascular system. *N Engl J Med* 1999; 340: 1801–1811.