



# Superficial Femoral Artery Calcification Is a Novel Risk Factor of Microvascular Complications in T2DM Patients

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## Abstract

Microvascular complications are prevalent in patients with type 2 diabetes mellitus (T2DM), resulting in increased risk of cardiovascular mortality. However, it is unclear whether above-knee artery calcification relates to microvascular complications. This study was aimed to investigate the role of calcification in superficial femoral arteries (SFA), the major above-knee artery, compared with anterior tibial arteries (ATA) and posterior tibial arteries (PTA), in T2DM-related microvascular complications and explore its risk factors. A single-center and observational study involving 359 T2DM patients was conducted. Clinical and laboratory data were collected. SFA calcification was evaluated by ultrasonography. Compared with ATA and PTA calcification, operating characteristics curve analysis showed that SFA calcification was the strongest predictor (63.1% sensitivity and 69.2% specificity) for T2DM-related microvascular complications (diabetic neuropathy, diabetic nephropathy and diabetic retinopathy). With the severity of SFA calcification increased, age, duration of T2DM, and SBP were significantly elevated, but triglyceride and glucose index and estimated glomerular filtration rate (eGFR) were significantly reduced (all  $P < 0.05$ ). Multivariate logistic analysis showed that eGFR (OR 0.953; 95% CI 0.931–0.976;  $P < 0.001$ ) was an independent risk factor of SFA calcification, especially in young patients with HbA1c  $> 7.0$ . We identified SFA calcification as a good predictor of microvascular complications in T2DM patients. Reduced eGFR was significantly associated with increased SFA calcification prevalence, especially in young T2DM patients with bad controlled hyperglycemia.

**Keywords** Type 2 diabetes mellitus · Superficial femoral artery calcification · Microvascular complications · Estimated glomerular filtration rate · Hyperglycemia

## Abbreviations

ATA Anterior tibial arteries  
BMI Body mass index

DBP Diastolic blood pressure  
eGFR Estimated glomerular filtration rate  
FPG Fasting plasma glucose  
HbA1c Hemoglobin A1c  
HDL-C High-density lipoprotein cholesterol  
LDL-C Low-density lipoprotein cholesterol  
MAC Medial arterial calcification  
PAD Peripheral arterial disease  
PTA Posterior tibial arteries  
ROC Receiver-operating characteristics curve  
SBP Systolic blood pressure  
SFA Superficial femoral arteries  
TC Total cholesterol  
T2DM Type 2 diabetes mellitus  
TG Triglycerides  
TyG index Triglyceride and glucose index  
WC Waist circumference

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## Background

Microvascular complications (neuropathy, nephropathy and retinopathy) affect hundreds of millions of patients with type 2 diabetes mellitus (T2DM) [1–3]. The progression of these complications can lead to loss of visual, renal, and neurologic functions, impaired mobility and cognition, poor quality of life, limitations for employment and productivity, and increased costs for the patient and society [4, 5]. If left uncontrolled or untreated, they lead to irreversible damage and even death. Thus, it is of great importance to investigate the risk factors and carry early intervention [6].

Arterial calcification in the lower extremity, particularly in the setting of T2DM, doubles cardiovascular mortality and quadruples the risk of amputation [7]. It is classified as two forms: intimal calcification is a marker of atherosclerotic disease and is associated with arterial stenosis; while T2DM patients usually have medial arterial calcification (MAC) [8]. MAC is a condition that leads to stiffening of the elastic layer of the arterial wall, resulting in a series of cardiovascular events [9]. Ultrasonography is widely used to assess arterial intimal abnormalities such as intima-medial thickness [10] and endothelial dysfunction [11]. Compared with conventional radiography, ultrasound can directly aid visualization of the arterial lumen of the peripheral arterial tree and has its advantages in detecting MAC accurately [12]. Previous studies mostly focused on the association between MAC in lower limb arteries and various diabetic macrovascular complications including peripheral arterial diseases (PAD) [13, 14]. Lower limb arteries mainly consist of above-knee arteries [superficial femoral arteries (SFA)], and below-knee arteries [anterior tibial arteries (ATA) and posterior tibial arteries (PTA)]. However, it remains a debate on whether below- or above-knee calcification plays a role in T2DM-related microvascular complications. Guzman RJ et al. showed that tibial artery calcification was associated with foot ulcer in T2DM [15]. By contrast, the results from a recent study indicated an association between SFA calcification and T2DM-related microvascular complications [16]. These studies were limited for two reasons. On one hand, they used computed tomography measurement, which may underestimate MAC severity; on the other hand, they just measured one segment of lower limb arteries and did not explore the related risk factors either.

Thus, in this present study, we improved these flaws and adapted a sensitive method (ultrasonography) to evaluate MAC in the whole lower limb artery branches (SFA, ATA and PTA) of T2DM patients. We aimed to assess which lower limb artery segment calcification was associated with diabetes-related complications and to evaluate its risk factors.

## Methods

### Study Participants

This was a retrospective, single-center study consisting of T2DM patients admitted in our unit between March 2015 and December 2017. The demographic, biomedical and US profiles were extracted from medical database. The main inclusion criteria were based on the T2DM diagnostic guideline on the 1999 World Health Organization [17]. The main exclusion criteria were (1) type 1 diabetes mellitus; (2) recent infection inflammatory disorders, serious cardiovascular diseases, renal dysfunction or hepatic diseases; (3) recent receiving treatments of several agents affecting mineral metabolism such as steroid hormones and anti-osteoporosis drugs within three months; (4) malignancy or disability to complete required measurement. This study protocol conformed to the ethical guidelines of the Declaration of Helsinki as reflected in a priori approval by the Ethics Committee of Sun Yat-sen University.

### Measurement of Blood Pressure and Body Mass Index (BMI)

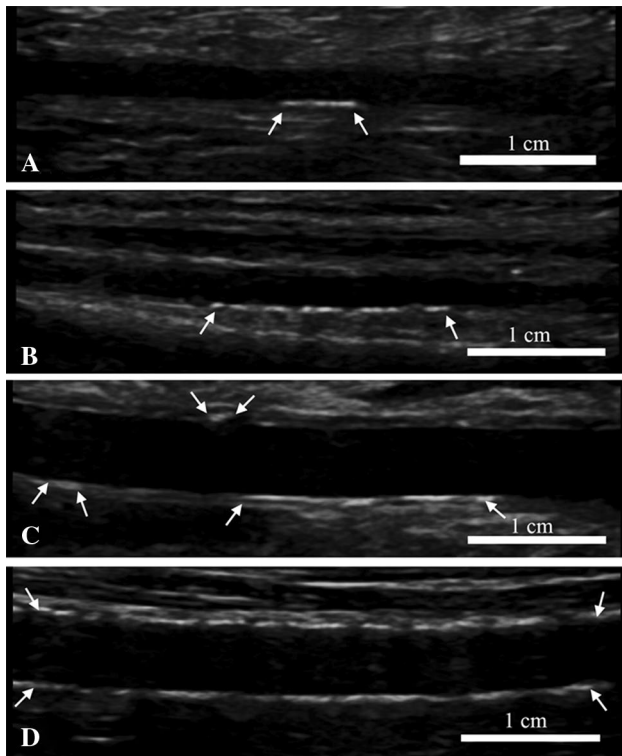
Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured three times using digital sphygmomanometers (OMRON Healthcare, Hoofddorp, The Netherlands) and the mean values of measurements were used as SBP and DBP in the analysis. BMI was calculated by  $\text{weight/height}^2$  ( $\text{kg/m}^2$ ).

### Biochemical Measurements

Blood samples were collected after an overnight fast for at least 8 h. Fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), creatinine, calcium and phosphorus were measured on a standardized and certified TBA-120 auto-analyzer (Toshiba Medical Systems, Japan) in the central laboratory of our unit. Notably, the triglyceride and glucose (TyG) index ( $\text{Ln} [\text{TG} (\text{mg/mL}) \times \text{FPG} (\text{mg/mL})/2]$ ) and estimated glomerular filtration rate (eGFR) were calculated with established formulas:  $141 \times \min(\text{Scr}/\kappa, 1)^\alpha \times \max(\text{Scr}/\kappa, 1) - 1.209 \times 0.993^{\text{Age}} \times 1.018$  [if female]  $_1.159$  [if black], where  $\kappa$  is 0.7 for females and 0.9 for males,  $\alpha$  is  $-0.329$  for females and  $-0.411$  for males,  $\min$  indicates the minimum of  $\text{Scr}/\kappa$  or 1, and  $\max$  indicates the maximum of  $\text{Scr}/\kappa$  or 1 [18].

## Ultrasonography Detection of MAC

All the T2DM patients underwent ultrasonography examination of bilateral superficial femoral arteries for the presence of MAC. An ultrasonography unit (LOGIQ E9, GE, USA) was used with an L5-12 MHz or L9 MHz transducer. The examinations were performed and interpreted by two sonographers, who were blinded to the clinical data. The SFA, ATA and PTA of both thighs were scanned in the cross-sectional plane from their origins at the groin to their distal parts at the entrance of the adductor canal to have an overall view of the blood vessels (Fig. 1). The MAC was assigned a score from 0 to 4 according to severity of the calcification within a 4-cm scanned area (which was usually the approximate size of the ultrasonography transducer). The definitions of unilateral artery were as follows: score 0, no MAC; score 1, linear MAC with a length of less than 1 cm; score 2, linear MAC with a length between 1 and 2 cm; score 3, more



**Fig. 1** Representative ultrasound images from T2DM population with different MAC severities in the lower limb arteries. **a** MAC with score 1 (linear calcification length of PTA is less than 1 cm in this scanning area). **b** MAC with score 2 (linear calcification length of ATA occupies approximately 1–2 cm in this scanning area). **c** MAC with score 3 (more extensive non-stenotic calcifications of PTA with approximately 2–3 cm in this scanning area). **d** MAC with score 4 (diffuse linear calcification of SFA with extent more than 3 cm in this scanning area). The white arrows indicate MAC. ATA, anterior tibial arteries; MAC, medial arterial calcification; PTA, posterior tibial arteries; SFA, superficial femoral arteries; T2DM, type 2 diabetes

extensive non-stenotic MAC with a length between 2 and 3 cm; and score 4, diffuse linear MAC with a length more than 3 cm (Fig. 1) [12].

## Clinical Assessment of Diabetic Complications

The included patients underwent comprehensive clinical evaluations. The definitions of various diabetic complications were consistent with recent guidelines [19–21]. The definition of diabetic neuropathy was as follows: if T2DM patients had clinical symptoms like pain, numbness, abnormal sensation, etc., accompanied with any one abnormality in examinations (ankle reflex, acupuncture pain, vibration, pressure and temperature sensation); or in the absence of clinical symptoms, 2 out of 5 tests were abnormal [19]. Diabetic nephropathy was defined as overt albuminuria (urinary albumin excretion rate > 300 mg/24 h) and eGFR < 60 mL/min per 1.73 m<sup>2</sup> (no end-stage renal disease or kidney transplantation) in T2DM, without any clinical or laboratory evidence of other kidney diseases [20]. Diabetic retinopathy was defined as the presence of dot and blot hemorrhages, hard exudates, cotton wool spots, neovascularization, laser scars, or a history of vitrectomy in T2DM [21].

## Statistical Analysis

Continuous variables with a normal distribution were reported as mean  $\pm$  standard deviation; skewed data as median (25–75th quartiles). Categorical variables were presented as numbers (percentages). Baseline variables among patients with different severity of SFA calcification groups were compared using variance or Kruskal–Wallis test followed by a LSD comparison test or Pearson chi-square according to the data types. Receiver-operating characteristics curve (ROC) analysis was used to quantify the assessing value of different lower limb artery calcification for diabetic complications. Univariate and multivariate logistic analysis were used to investigate the risk factors for SFA calcification, as well as in further subgroup analysis. Data were analyzed with SPSS version 20 (SPSS, Inc, Chicago, IL), and two-sided *P* values < 0.05 were considered statistically significant.

## Results

### Characteristics of Recruited T2DM Patients

A total of 359 consecutive patients with T2DM were finally included in this study. Baseline characteristics of all the T2DM patients were summarized in Table 1. The mean age was  $58.6 \pm 11.6$  years and 162 (45.1%) T2DM patients were male. The mean SBP and DBP

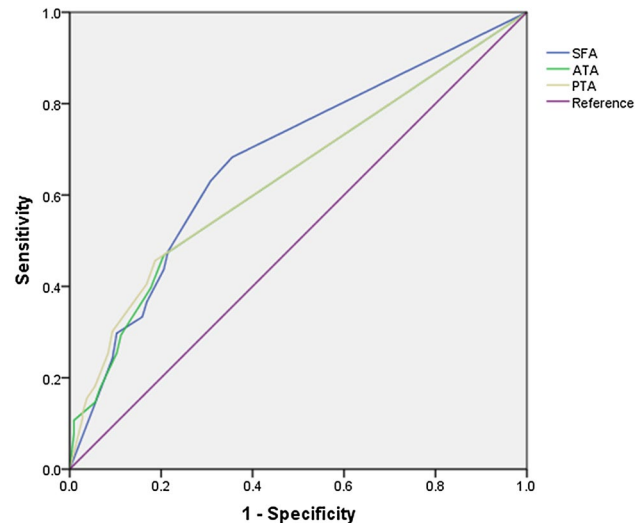
**Table 1** Baseline characteristics of included T2DM patients

Variables	Total enrolled patients (N = 359)
Age (years)	58.6 ± 11.6
Sex (M/F)	162/197
Smoking [N (%)]	117 (32.6)
Duration of T2DM (years)	5.0 (0–12.0)
SBP (mmHg)	134.8 ± 21.3
DBP (mmHg)	78.5 ± 11.6
BMI (m <sup>2</sup> /kg)	24.0 ± 3.1
WC (cm)	88.4 ± 8.8
FPG (mmol/L)	8.6 ± 3.4
HbA1c (%)	9.6 ± 2.6
TG (mmol/L)	1.52 (1.10–2.28)
TC (mmol/L)	5.00 ± 1.60
HDL-C (mmol/L)	1.09 ± 0.34
LDL-C (mmol/L)	3.14 ± 1.03
Calcium (mmol/L)	2.24 ± 0.12
Phosphorus (mmol/L)	1.17 ± 0.21
Creatinine (mmol/L)	70.0 (81.1–99.0)
eGFR (mL/min per 1.73 m <sup>2</sup> )	75.83 ± 16.13
TyG index	9.27 ± 0.75
SFA calcification [N (%)]	210 (58.5)
ATA calcification [N (%)]	140 (39.0)
PTA calcification [N (%)]	135 (37.6)
Diabetic neuropathy [N (%)]	227 (63.2)
Diabetic nephropathy [N (%)]	105 (29.2)
Diabetic retinopathy [N (%)]	103 (28.7)
Metformin [N (%)]	155 (43.2)
Other hypoglycemic agents [N (%)]	190 (52.9)
Insulin [N (%)]	125 (34.8)

Values presented as mean ± SD or the median (25–75th quartiles)

ATA anterior tibial arteries, BMI body mass index, DBP diastolic blood pressure, eGFR estimated glomerular filtration rate, FPG fasting plasma glucose, HbA1c hemoglobin A1c, HDL-C high-density lipoprotein cholesterol, LDL-C low-density lipoprotein cholesterol, PTA posterior tibial arteries, SBP systolic blood pressure, SFA superficial femoral arteries, T2DM type 2 diabetes mellitus, TC total cholesterol, TyG index triglyceride and glucose index, TG triglycerides, WC waist circumference

were 134.8 ± 21.3 and 78.5 ± 11.6 mmHg, respectively. The median duration of T2DM was 5.0 (0–12.0) years. Moreover, the mean serum calcium, phosphorus, eGFR and TyG index in these patients were 2.24 ± 0.12 mmol/L, 1.17 ± 0.21 mmol/L, 75.83 ± 16.13 mL/min per 1.73 m<sup>2</sup> and 9.27 ± 0.75, respectively. The prevalence of calcification in different lower limb artery branches were: SFA calcification 210 (58.5%), ATA calcification 140 (39.0%) and PTA calcification 135 (37.6%). As regard to diabetic microvascular complications, 227 (63.2%) T2DM patients



**Fig. 2** Receiver-operating characteristic curve for SFA, ATA and PTA calcification in assessing T2DM-related microvascular complications, respectively. ATA anterior tibial arteries, PTA posterior tibial arteries, SFA superficial femoral arteries, T2DM type 2 diabetes

had diabetic neuropathy, 105 (29.2%) T2DM patients had diabetic nephropathy, and 103 (28.7%) T2DM patients had diabetic retinopathy.

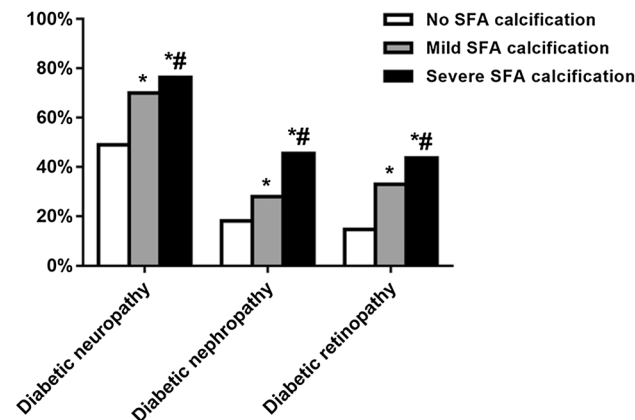
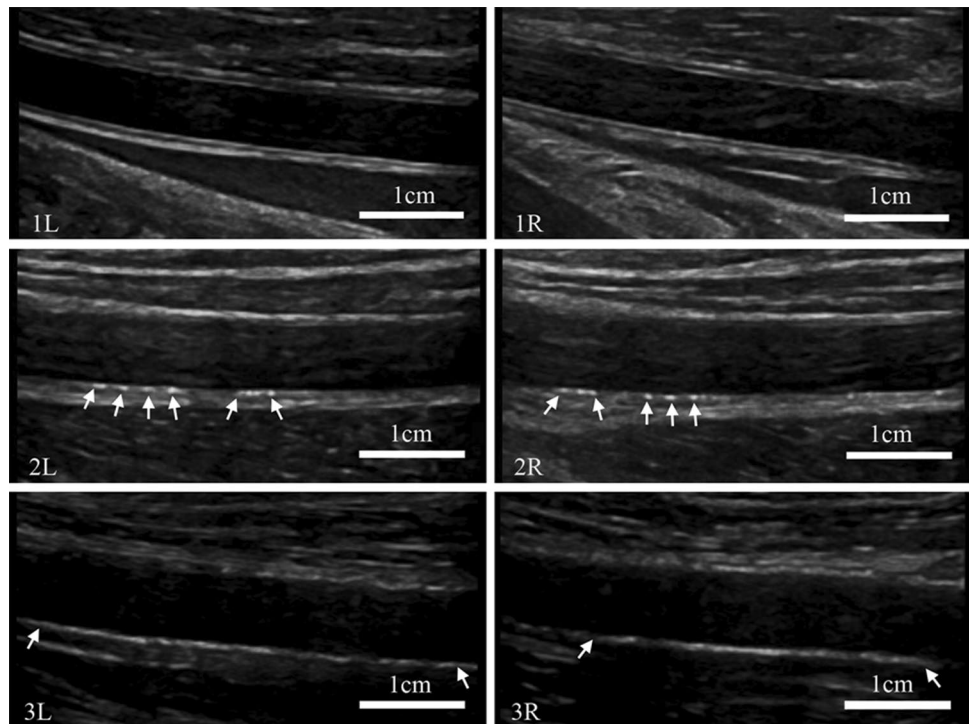
### SFA Calcification Is a Good Marker for Predicting Diabetic Complications

To explore the roles of different lower limb artery root calcification for predicting diabetic microvascular complications, we performed ROC curve analysis. As shown in Fig. 2, compared with ATA, PTA calcification, the area under the curve for SFA calcification was the largest [SFA vs. ATA vs. PTA: 0.679 (0.619, 0.739) vs. 0.636 (0.577, 0.696) vs. 0.641 (0.582, 0.700); all  $P < 0.001$ ]. SFA calcification with a threshold value of 1.5 provided 63.1% sensitivity and 69.2% specificity. In addition, we divided the SFA calcification into three levels (Fig. 3): no SFA calcification (score 0); mild SFA calcification (score 1–4); and severe SFA calcification (score 5–8). As shown in Fig. 4, with the severity SFA calcification elevated, the prevalence of diabetic microvascular complications was significantly increased [diabetic neuropathy: 73 (49.0%) vs. 70 (70.0%) vs. 84 (76.3%); diabetic nephropathy: 27 (18.1%) vs. 28 (28.0%) vs. 50 (45.5%); diabetic retinopathy: 22 (14.8%) vs. 33 (33.0%) vs. 48 (43.6%); all  $P < 0.05$ ].

### Characteristics of T2DM Patients According to Different SFA Calcification Severity

Then, we compared the characteristics among T2DM patients with different SFA calcification severity. As shown

**Fig. 3** Representative ultra-sound images of 3 levels of SFA calcification in T2DM patients. No SFA calcification (score 0): score 0 in the left SFA (1L) and score 0 in the right SFA (1R) from a 52-year-old man; Mild SFA calcification (score 2): score 1 in the left SFA (1L) and score 1 in the right SFA (1R) from a 54-year-old woman. Severe SFA calcification (score 8): score 4 in the left SFA (1L) and score 4 in the right SFA (1R) from a 64-year-old man. The white arrows indicate MAC. MAC medial arterial calcification, SFA superficial femoral arteries, T2DM type 2 diabetes



**Fig. 4** The prevalence of T2DM-related microvascular complications among different SFA calcification severity groups. SFA superficial femoral arteries, T2DM type 2 diabetes. \* $P < 0.05$  vs. no SFA calcification group; \*\* $P < 0.05$  vs. mild SFA calcification group

in Table 2, age, duration of T2DM, and SBP were significantly elevated with the severity of SFA calcification increased [ $53.0 \pm 10.9$  vs.  $61.2 \pm 9.3$  vs.  $63.8 \pm 11.3$  years; 0 (0, 8.0) vs. 6.0 (0, 12.8) vs. 10.0 (4.0, 15.3) years;  $129.7 \pm 17.7$  vs.  $136.2 \pm 22.3$  vs.  $140.6 \pm 23.4$  mmHg; all  $P < 0.05$ ]. As regard to the biomedical characteristics, T2DM patients with severe SFA calcification had significantly lower FPG, TyG index and eGFR than those with mild or no SFA calcification [ $7.8 \pm 2.6$  vs.  $8.8 \pm 3.9$  vs.  $9.1 \pm 3.4$  mmol/L;  $9.16 \pm 0.73$  vs.  $9.22 \pm 0.77$  vs.  $9.39 \pm 0.74$ ;  $64.77 \pm 14.14$

vs.  $76.10 \pm 12.55$  vs.  $83.81 \pm 14.89$  mL/min per  $1.73 \text{ m}^2$ ; all  $P < 0.05$ ]. There were no significant differences of sex, smoking, SBP, DBP, BMI, WC, serum lipids, calcium and phosphorus among three groups (all  $P > 0.05$ ).

### Reduced eGFR Is an Independent Risk Factor of SFA Calcification

We further performed logistic analysis to explore the potential risk factors of SFA calcification. On the univariate logistic analysis, age (OR 1.090; 95% CI 1.064–1.117;  $P < 0.001$ ), duration of T2DM (OR 1.090; 95% CI 1.064–1.117;  $P < 0.001$ ), SBP (OR 1.021; 95% CI 1.010–1.032;  $P < 0.001$ ), TyG index (OR 0.688; 95% CI 0.517–0.917;  $P = 0.011$ ) and eGFR (OR 0.925; 95% CI 0.905–0.945;  $P < 0.001$ ) were significantly associated with the presence of SFA calcification. However, when we put these factors into the multivariate logistic analysis, only age (OR 1.053; 95% CI 1.025–1.082;  $P < 0.001$ ) and eGFR (OR 0.953; 95% CI 0.931–0.976;  $P < 0.001$ ) were independent risk factors of SFA calcification (Table 3).

### Identifying the T2DM Patients with High Risk of SFA Calcification

Since aging is an unmodifiable fact OR we then further investigated the association between eGFR and SFA stratified by traditional cardiovascular risk factors, including age, sex, smoking, SBP, BMI and HbA1c, respectively. As shown

**Table 2** Characteristics of T2DM patients among different SFA calcification severity groups

Variables	No SFA calcification N=149	Mild SFA calcification N=100	Severe SFA calcification N=110	P values
Age (years)	53.0±10.9	61.2±9.3*	63.8±11.3*	<0.001
Sex (M/F)	60/89	43/57	59/51	0.090
Smoking [N (%)]	54 (36.2)	36 (36.0)	27 (24.5)	0.097
Duration of T2DM (years)	0 (0, 8.0)	6.0 (0, 12.8)*	10.0 (4.0, 15.3)*#	<0.001
SBP (mmHg)	129.7±17.7	136.2±22.3*	140.6±23.4*	<0.001
DBP (mmHg)	79.1±10.8	77.5±13.2	78.5±11.2	0.550
BMI (g/m <sup>2</sup> )	24.3±3.3	23.9±2.8	23.6±3.2	0.183
WC (cm)	88.7±8.5	88.1±6.8	88.5±10.7	0.885
FPG (mmol/L)	9.1±3.4	8.8±3.9	7.8±2.6*#	0.006
HbA1c (%)	9.7±2.5	9.5±2.8	9.4±2.6	0.722
TG (mmol/L)	1.65 (1.21, 2.36)	1.51 (1.01, 2.34)	1.42 (1.09, 2.11)	0.262
TC (mmol/L)	5.12±1.73	5.01±1.36	4.78±1.56	0.231
HDL-C (mmol/L)	1.11±0.39	1.08±0.28	1.07±0.29	0.526
LDL-C (mmol/L)	3.20±1.02	3.20±0.97	3.00±1.08	0.230
Calcium (mmol/L)	2.25±0.12	2.24±0.12	2.22±0.13	0.160
Phosphorus (mmol/L)	1.19±0.21	1.17±0.19	1.18±0.21	0.620
TyG index	9.39±0.74	9.22±0.77	9.16±0.73*#	0.029
eGFR (mL/min·per 1.73 m <sup>2</sup> )	83.81±14.89	76.10±12.55*	64.77±14.14*#	<0.001
Metformin [N (%)]	62 (41.6)	49 (49.0)	44 (40.0)	0.371
Other hypoglycemic agents [N (%)]	73 (49.0)	59 (59.0)	58 (52.7)	0.300
Insulin [N (%)]	51 (34.2)	35 (35.0)	39 (35.4)	0.975

Values presented as mean ± standard deviation or the median (25-75th quartiles)

*BMI* body mass index, *DBP* diastolic blood pressure, *eGFR* estimated glomerular filtration rate, *FPG* fasting plasma glucose, *HbA1c* hemoglobin A1c, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *SBP* systolic blood pressure, *SFA* superficial femoral arteries, *T2DM* type 2 diabetes mellitus, *TC* total cholesterol, *TG* triglycerides, *TyG index* triglyceride and glucose index, *WC* waist circumference

\**P* < 0.05 vs. no SFA calcification group and

#*P* < 0.05 vs. mild SFA calcification group

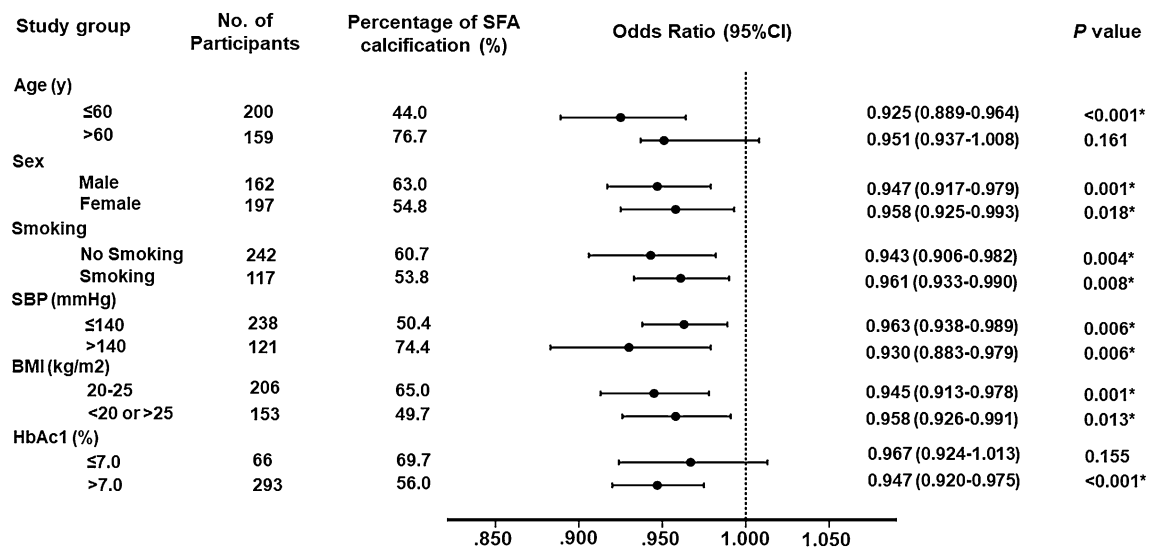
**Table 3** Logistic analysis for the risk factors of SFA calcification in T2DM

Variables	OR (95% CI) <sup>a</sup>	P value	OR (95% CI) <sup>b</sup>	P value
Age	1.090 (1.064–1.117)	<0.001	1.053 (1.025–1.082)	<0.001
Duration of T2DM	1.103 (1.066–1.141)	<0.001	1.037 (0.999–1.077)	0.059
Smoking	0.754 (0.483–1.177)	0.214	–	–
SBP	1.021 (1.010–1.032)	<0.001	1.010 (0.998–1.022)	0.106
BMI	0.943(0.881–1.009)	0.090	–	–
FPG	0.928 (0.871–0.989)	0.021	0.983 (0.901–1.073)	0.699
HbA1c (%)	0.968 (0.797–1.043)	0.434	–	–
TC	0.912 (1.047–1.408)	0.178	–	–
TG	0.944 (0.867–1.028)	0.944	–	–
LDL-C	0.905 (0.738–1.111)	0.342	–	–
TyG index	0.688 (0.517–0.917)	0.011	0.863 (0.575–1.296)	0.478
eGFR	0.925 (0.905–0.945)	<0.001	0.953 (0.931–0.976)	<0.001

*BMI* body mass index, *eGFR* estimated glomerular filtration rate, *FPG* fasting plasma glucose, *HbA1c* hemoglobin A1c, *LDL-C* low-density lipoprotein cholesterol, *SBP* systolic blood pressure, *SFA* superficial femoral arteries, *T2DM* type 2 diabetes, *TC* total cholesterol, *TG* triglycerides, *TyG index* triglyceride and glucose index

<sup>a</sup>Results from univariate logistic analysis

<sup>b</sup>Results from multivariate logistic analysis



**Fig. 5** Subgroup analysis of assessing the association between eGFR and SFA calcification in T2DM. Multivariate logistic analysis after adjustment for age, duration of T2DM, BMI, SBP, and TyG index was performed in subgroup according to age (≤60 or >60 years), sex (male or female), systolic blood pressure (SBP; ≤140

or >140 mmHg), and BMI (20–25 or >25 kg/m<sup>2</sup>). Data were expressed as the odds ratio and 95% confidence interval (CI). \**P* < 0.05. *BMI* body mass index, *eGFR* estimated glomerular filtration rate, *SBP* systolic blood pressure, *SFA* superficial femoral arteries, *T2DM* type 2 diabetes, *TyG index* triglyceride and glucose index

in Fig. 5, we found that the negative association between eGFR and SFA calcification was significant in PA patients with ≤60 years of age (OR 0.925; 95% CI 0.889–0.964; *P* < 0.001), HbA1c > 7.0 (OR 0.947; 95% CI 0.920–0.975; *P* < 0.001). However, the association between eGFR and SFA calcification became nonsignificant in T2DM patients with > 60 years of age and HbA1c ≤ 7.0 subgroups (all *P* > 0.05). Moreover, this association remained significant among sex, smoking and BMI subgroups, suggesting it was not influenced by sex, smoking and BMI difference (all *P* < 0.05).

### Discussion

Major findings from our study demonstrated that (1) SFA calcification had a strongest correlation with microvascular complications in patients with T2DM; (2) reduced eGFR was an independent risk factor for SFA calcification, especially in T2DM patients ≤60 years old with HbA1c > 7.0.

Microvascular complications are great threats to patients with T2DM, which reduces their life quality and increases the hospitalized duration [22]. MAC is a common condition in T2DM patients, which was shown to associate with various diabetic macrovascular complications [12, 13]. Chowdhury et al. showed that CT-based lower limb arterial calcification was strongly associated with cardiovascular events [23]. However, these studies were limited by radiology technique, which was inferior to US in evaluating MAC [12, 13]. In addition, it is under debate whether below- or above-knee

calcification plays a role in T2DM-related microvascular complications. Thus, we adapted ultrasonography measurement and compared the roles of MAC in above-knee arteries (SFA), and below-knee arteries (ATA and PTA) in predicting microvascular complications in T2DM. It was emphasized that compared with ATA and PTA, SFA lesion had a more important role in predicting prognosis. McDermott MM et al. showed that lipid-rich necrotic core in the SFA was associated with higher rates of clinical PAD events [24]. Another study demonstrated that greater plaque quantity and smaller lumen area in the proximal SFA were associated with higher mobility loss in people with PAD [25]. Consistently, our results showed that SFA calcification had the strongest association with various diabetic microvascular complications than ATA and PTA calcification. Thus, early diagnosis and appropriate control of the risk factors of SFA calcification was possibly useful to reduce T2DM-related complications.

Instead of a passive product of calcium and phosphate precipitation, vascular calcification is now regarded as an actively regulated process involving many factors [26]. Although there were a series of studies investigating the risk factors of larger-sized lumen vascular calcification like coronary artery calcification, few have been known about lower limb arterial calcification in T2DM. Insulin resistance and hyperglycemia are major characteristics of T2DM [27]. It was demonstrated that insulin resistance was associated with coronary artery calcification [28]. As a reliable indicator of insulin resistance, high-TyG index was reported to be independently associated with the presence of coronary artery calcification in healthy Korean

adults [29]. Although our data showed that neither high-TyG index nor FPG was an independent risk factor of SFA calcification, it indicated that under impaired kidney function condition, hyperglycemia may accelerate the development of SFA calcification. It was supported by the fact that the induction of in vitro T2DM calcification model required the high phosphate microenvironment (mimicked the reduced eGFR) [30–32]. Vascular calcification was thought to be a manifestation of vascular aging [33]. We consistently showed that older age was a strong risk factor of SFA calcification. Interestingly, the results of our subgroup analysis suggested that in T2DM patients with bad controlled hyperglycemia, downregulation of eGFR might trigger SFA calcification in the early stage. And this may explain why improving eGFR showed little effect on SFA calcification in older T2DM patients even with better control of blood glucose. However, further investigation is still required to verify our results.

This study had several limitations that should be highlighted. First, the number of T2DM patients enrolled in the study was relatively small. Therefore, further studies with larger sample size are required. Second, because of the cross-sectional design of the present study, causality between reduced eGFR and SFA calcification extent cannot be established. Third, some residual factors may confound our results despite careful adjustment, further studies are needed to verify our findings.

In summary, we provided clinical evidence that SFA calcification was a good predictor of microvascular complications in T2DM patients. Reduced eGFR was probably an important risk factor of SFA calcification in T2DM patients, especially in those young with bad controlled hyperglycemia.

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**Author contributions** JT, WBH, JWG designed the study. LY, ML and WYZ contributed to data acquisition. WBH and JWG performed the statistical analysis. JT, JWG, and LY contributed to the discussion. WBH and JWG drafted the manuscript. JT, BML and XLX edited the manuscript. All authors read and approved the final manuscript.

## Compliance with Ethical Standards

**Conflict of interest** Jing Tian, Wanbing He, Jingwei Gao, Li Yan, Ming Liang, Wenye Zhang, Xiaolin Xu and Baoming Luo confirms that they have no conflict of interest related to this study.

**Human and Animal Rights and Informed Consent** This study was approved by the Sun Yat-sen Memorial Hospital of Sun Yat-sen University Research Ethics Committee and all participants provided written informed consent.

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