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The association between diabetes status, HbA1c, diabetes duration, microvascular disease, and bone quality of the distal radius and tibia as measured with high-resolution peripheral quantitative computed tomography—The Maastricht Study

E. A. C. de Waard [1,2] · J. J. A. de Jong [1,2] · A. Koster [3,4] · H. H. C. M. Savelberg [2,5] · T. A. van Geel [3,6] · A. J. H. M. Houben [7,8] · M. T. Schram [7,8] · P. C. Dagnelie [3,8,9] · C. J. van der Kallen [7,8] · S. J. S. Sep [3,7,8,10] · C. D. A. Stehouwer [7,8] · N. C. Schaper [3,7,8] · T. T. J. M. Berendschot [11] · J. S. A. G. Schouten [11] · P. P. M. M. Geusens [3,12,13] · J. P. W. van den Bergh [2,12,14]

Abstract

Summary In this small cross-sectional study of predominantly well-treated participants with relatively short-term type 2 diabetes duration, HbA1c > 7% (53 mmol/mol) was associated with lower cortical density and thickness and higher cortical porosity at the distal radius, lower trabecular thickness at the distal tibia, and higher trabecular number at both sites.

Introduction To examine the association between diabetes status and volumetric bone mineral density (vBMD), bone microarchitecture and strength of the distal radius and tibia as assessed with HR-pQCT. Additionally—in participants with type 2 diabetes (T2DM), to examine the association between HbA1c, diabetes duration, and microvascular disease (MVD) and bone parameters.

Methods Cross-sectional data from 410 (radius) and 198 (tibia) participants of The Maastricht Study (mean age 58 year, 51% female). Diabetes status (normal glucose metabolism, prediabetes, or T2DM) was based on an oral glucose tolerance test and medication history.

Results After full adjustment, prediabetes and T2DM were not associated with vBMD, bone microarchitecture, and strength of the radius and tibia, except for lower trabecular number (Tb.N) of the tibia (− 4%) in prediabetes and smaller cross-sectional area of the tibia (− 7%) in T2DM. In T2DM, HbA1c > 7% was associated with lower cortical vBMD (− 5%), cortical thickness (− 16%), higher cortical porosity (+ 20%) and Tb.N (+ 9%) of the radius, and higher Tb.N (+ 9%) and lower trabecular thickness (−

✉ E. A. C. de Waard
e.dewaard@maastrichtuniversity.nl

1   Department of Internal Medicine, Subdivision of Rheumatology, Maastricht University, Maastricht, the Netherlands

2   NUTRIM School for Nutrition and Translational Research in Metabolism, Maastricht University, Universiteitssingel 40, room C5.535, P.O. Box 5800, 6202 AZ Maastricht, the Netherlands

3   CAPHRI Care and Public Health Research Institute, Maastricht University, Maastricht, the Netherlands

4   Department of Social Medicine, Maastricht University, Maastricht, the Netherlands

5   Department of Human Movement Science, Maastricht University, Maastricht, the Netherlands

6   Department of Family Medicine, Maastricht University, Maastricht, the Netherlands

7   Department of Internal Medicine, Maastricht University Medical Center, Maastricht, the Netherlands

8   CARIM School for Cardiovascular diseases, Maastricht University, Maastricht, the Netherlands

9   Department of Epidemiology, Maastricht University, Maastricht, the Netherlands

10 Department of Rehabilitation Medicine, Maastricht University, Maastricht, the Netherlands

11 University Eye Clinic Maastricht, Maastricht, the Netherlands

12 Department of Internal Medicine, Subdivision of Rheumatology, Maastricht University Medical Centre, Maastricht, the Netherlands

13 Biomedical Research Institute, University of Hasselt, Hasselt, Belgium

14 Department of Internal Medicine, Subdivision of Endocrinology, VieCuri Medical Center, Venlo, the Netherlands

13%) of the tibia. Diabetes duration > 5 years was associated with higher Tb.N (+ 6%) of the radius. The presence of MVD was not associated with any bone parameters.

Conclusions In this study with predominantly well-treated T2DM participants with relatively short-term diabetes duration, inadequate blood glucose control was negatively associated with cortical bone measures of the radius. In contrast, trabecular number was increased at both sites. Studies of larger sample size are warranted for more detailed investigations of bone density and bone quality in patients with T2DM.

Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease, characterized by elevated blood glucose levels and insulin resistance. Over time, elevated blood glucose levels lead to organ damage, such as microvascular disease (retinopathy, nephropathy, and neuropathy) and macrovascular disease (cardiovascular disease). While participants with prediabetes (PDM) and newly diagnosed diabetes are reported to have a normal to decreased fracture risk [1–3], increasing evidence is showing that patients with established T2DM have an increased fracture risk, despite a normal to increased areal bone mineral density (aBMD) [4–6]. As aBMD is only one of the contributors to bone strength, the increased fracture risk may be caused by other factors that are not captured by aBMD measurements, such as changes in the bone microarchitecture and geometry.

High-resolution peripheral quantitative computed tomography (HR-pQCT) is a non-invasive three-dimensional imaging modality, which can be used to measure volumetric bone mineral density (vBMD) and microarchitecture of the cortical and trabecular region of the distal radius and tibia [7]. Additionally, HR-pQCT images can be used in micro-finite element analyses (μFEA) to calculate bone strength indices [8]. It has been shown that bone microarchitecture as measured with HR-pQCT is an independent predictor of fracture risk [9].

In previous small studies, HR-pQCT was used to examine the association of T2DM with vBMD, bone microarchitecture, and bone strength [10–17], while the association of PDM with HR-pQCT-derived bone parameters has never been studied before. The results of these studies varied from no differences between participants with and without diabetes [13–16], to unfavorable changes of the cortical compartment in patients with T2DM [10–12], and to better bone microarchitecture in patients with T2DM [17]. Additionally, a study of Shanboque et al. showed that unfavorable changes in microarchitecture were only present in subjects with T2DM and microvascular disease (MVD) [15]. As diabetes characteristics (e.g., glycemic control, diabetes duration, the number of patients with MVD) were often lacking in the other studies, the conflicting results of the studies may be the result of inclusion of diabetes populations with varying diabetes characteristics.

In this cross-sectional study, we examined the association between diabetes status and vBMD, bone microarchitecture, and bone strength. Additionally, within participants with T2DM, the association between glycemic control, diabetes duration, MVD, and HR-pQCT-derived bone parameters was examined. We hypothesized that unfavorable changes in vBMD, bone microarchitecture, and bone strength will only be present in T2DM participants with an HbA1c level above 7% (53 mmol/mol, the treatment target value according to the Dutch guideline [18]), a diabetes duration > 5 years, and/or the presence of MVD.

Methods

Study population and design

Data from The Maastricht Study, an observational prospective population-based cohort study, was used. The rationale and methodology have been described previously [19]. In brief, the study focuses on the etiology, pathophysiology, complications, and comorbidities of T2DM and is characterized by an extensive phenotyping approach. Eligible for participation were all individuals aged between 40 and 75 years and living in the southern part of the Netherlands. Participants were recruited through mass media campaigns and from the municipal registries and the regional Diabetes Patient Registry via mailings. Recruitment was stratified according to known T2DM status, with an oversampling of individuals with T2DM for reasons of efficiency. The present report includes cross-sectional data from participants who completed the baseline survey between November 2010 and September 2013 and returned to the research center between March 2015 and September 2016 for the HR-pQCT scan ($n = 468$, mean ± SD time between baseline survey and HR-pQCT scan 42.5 ± 8.7 (radius) and 36.9 ± 8.4 months (tibia)). Clearance by the Dutch Ministry of Health for carrying out the HR-

pQCT scans of the distal tibia was granted later than clearance for HR-pQCT scans of the distal radius. Therefore, participants that returned to the research center between March 2015 and September 2015 underwent an HR-pQCT scan of the distal radius only. From September 2015 on, participants underwent an HR-pQCT scan of their distal radius as well as their distal tibia. In total, scans of the distal radius from 458 participants and from the distal tibia of 210 participants were performed. Scans with an inadequate position of the reference line or with selection of the wrong scan protocol (radius $n = 1$; tibia $n = 3$), scans of insufficient quality due to severe or extreme motion artifacts [20] (radius $n = 42$; tibia $n = 7$), and scans with extreme outliers of HR-pQCT-derived bone parameters (radius $n = 4$; tibia $n = 1$) were excluded. Additionally, participants with type 1 diabetes mellitus were excluded (radius $n = 1$; tibia $n = 1$), resulting in 410 participants with a radius scan and 198 participants with a tibia scan for further processing and data analysis (Supplemental Figure 1). The study has been approved by the institutional medical ethical committee (NL31329.068.10) and the Minister of Health, Welfare and Sports of the Netherlands (Permit 131088-105234-PG). All participants gave written informed consent.

Measures

Diabetes status

To determine diabetes status, all participants, except those who used insulin, underwent a standardized 7-point oral glucose tolerance test (OGTT) after an overnight fast [19]. Blood samples were taken at baseline, and 15, 30, 45, 60, 90, and 120 min after ingestion of a 75-g glucose drink. For safety reasons, participants with a fasting glucose level > 11.0 mmol/L (> 200.0 mg/dL), as determined by a finger prick, did not undergo the OGTT. Fasting glucose level, 2-h plasma glucose level, and information about diabetes medication were used to determine diabetes status. Glucose metabolism was defined according to the WHO 2006 criteria into normal glucose metabolism (NGM) (fasting plasma glucose < 6.1 mmol/L (< 110.0 mg/dL) and 2-h plasma glucose < 7.8 mmol/L (< 140.0 mg/dL) and no diabetes medication), impaired fasting glucose (IFG) (fasting plasma glucose 6.1–6.9 mmol/L (110.0–125.0 mg/dL) and 2-h plasma glucose < 7.8 mmol/L (< 140.0 mg/dL) and no diabetes medication), impaired glucose tolerance (IGT) (fasting plasma glucose < 7.0 mmol/L (<126.0 mg/dL) and 2-h plasma glucose ≥ 7.8 and < 11.1 mmol/L (≥ 140.0 and < 200.0 mg/dL) and no diabetes medication), and T2DM (fasting plasma glucose ≥ 7.0 mmol/L (≥ 126.0 mg/dL) or 2-h plasma glucose ≥ 11.1 mmol/L (≥ 200.0 mg/dL) or diabetes medication) [21]. Individuals without type 1 diabetes who used diabetes medication were classified as having T2DM. For this study, we defined having either IFG or IGT as having PDM.

T2DM subgroups

Participants with T2DM were divided into those with an HbA1c level ≤ 7% (≤ 53 mmol/mol) or > 7% (> 53 mmol/mol); a diabetes duration ≤ 5 years or > 5 years; and the absence of MVD or the presence of MVD to be able to examine the association between glycemic control, diabetes duration, MVD, and HR-pQCT-derived bone parameters. Diabetes duration (questionnaire), HbA1c level, and the presence of MVD were assessed at baseline [19]. Participants with newly diagnosed T2DM had a diabetes duration of 0 year. MVD was defined as the presence of diabetic retinopathy, nephropathy, and/or an impaired vibration sensation. Diabetic retinopathy was defined as the presence of diabetic retinopathy at the fundus photograph of one or both eyes. Nephropathy was defined as an estimated glomerular filtration rate (eGFR, estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula with serum creatinine and cystatin C (eGFR$_{crcys}$) [22]) < 60 mL/min and/or (micro)albuminuria ≥ 30 mg/24 h (based on the average albumin excretion of two 24-h collections). Serum creatinine, serum cystatin C, and urinary albumin excretion were measured as described elsewhere [23]. Impaired vibration sensation was assessed in triplo with a neurothesiometer (Horwell Scientific, London, UK). The sensor of the neurothesiometer was placed in a 90° angle on the plantar surface of the distal phalanx of the first toe of both feet in the supine participant with eyes closed. The vibration frequency was gradually increased; the voltage at time of the first vibration sensation was the vibration perception threshold (VPT). Impaired vibration sensation was defined as having a VPT at one or both toes above the predicted 97.5 percentile. The predicted 97.5 percentile was based on the VPT of the right toe of a healthy reference population (NGM, an ankle-brachial index ≥ 0.9, no kidney dysfunction (eGFR$_{crcys}$ > 45 mL/min/1.73m^2, no history of kidney transplantation or dialysis), no alcoholism (< 21 units of alcohol per week), no severe movement limitation (no severe impairment in walking up the stairs and/or walking a distance of 500 m), < 2 SD difference in VPT of the left and right toe, and no neuropathic pain according to the Douleur Neuropathique 4 (DN4) questionnaire [24]).

HR-pQCT imaging

The non-dominant radius and ipsilateral tibia were scanned on an HR-pQCT scanner (Xtreme-CT; Scanco Medical AG, Brüttisellen, Switzerland) using the standard in vivo protocol as described in literature [7, 25]. If the participants had a previously sustained distal radius or distal tibia fracture at the non-dominant side, the dominant side was scanned (~ 10% of all scans of the distal radius and tibia). An anteroposterior scout projection of the scan side was acquired for positioning of the tomographic acquisition. A reference line was placed on the

radial joint surface and the endplate of the distal tibia. The scan volume spanned 9.02 mm in length and started 9.5 mm and 22.5 mm (for the radius and tibia, respectively) from the reference line in the proximal direction. Images were reconstructed using an isotropic voxel size of 82 μm, resulting in 110 consecutive slices. Total scan time was 2.8 min, with each acquisition resulting in an effective dose of approximately 3 μSv. All scans were graded with regard to motion and scans with quality grade 4 (severe motion artifacts) or 5 (extreme motion artifacts) were repeated once [20]. Only scans with quality grades 1 to 3 (no, minor, or moderate motion artifacts) were used for subsequent image analysis [26].

Image analysis of HR-pQCT scans

All scans were evaluated using the standard patient evaluation protocol that was provided by the manufacturer and that has been described previously in detail [27–29]. First, the periosteal contour was automatically derived and manually modified when contours visually deviated from the periosteal boundary [30]. The images were automatically segmented and the following bone parameters were calculated from the images: cross-sectional area (CSA), total vBMD (Tt.BMD), trabecular vBMD (Tb.BMD), cortical vBMD (Ct.BMD), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), and cortical thickness (Ct.Th). In addition, extended analysis of the cortical compartment was performed to obtain cortical pore volume (Ct.PoV), cortical porosity (Ct.Po), and cortical pore diameter (Ct.PoDm) [31].

Micro-finite element analysis was performed in Scanco Finite Element Software v1.15 by creating micro-finite element models directly from the segmented HR-pQCT images as described previously [32, 33]. In short, all voxels representing bone tissue were converted into brick elements of the same size. A Young modulus of 10 GPa and a Poisson ratio of 0.3 were assigned to every element. Compression stiffness and estimated failure load were estimated by applying a virtual "high-friction" compression test in the axial direction [33].

Covariates

The following covariates were assessed: age, sex, BMI, level of education, smoking status, alcohol use, history of cardiovascular disease (CVD), moderate-to-vigorous physical activity (MVPA), a history of a fracture at or above the age of 50, and the use of antihyperglycemic medication. All covariates were determined at the baseline survey between November 2010 and September 2013 [19]. Level of education (low, intermediate, high), smoking behavior (never, former, current), alcohol consumption (no, low (≤ 7 glasses per week for women and ≤ 14 glasses per week for men), high (> 7 glasses per week for women and > 14 glasses per week for men)), history of CVD (myocardial infarction, and/or cerebrovascular infarction or

hemorrhage, and/or percutaneous artery angioplasty or vascular surgery on the coronary, abdominal, peripheral, or carotid arteries), and history of a fracture at or above the age of 50 were assessed by questionnaire. MVPA was calculated from the modified Community Health Activities Model Program for Seniors (CHAMPS) questionnaire [34]. Use of antihyperglycemic medication was assessed during a medication interview where generic name, dose, and frequency were registered.

Statistical analyses

All statistical analyses were performed using SPSS (version 22.0; IBM Corp., Armonk, NY, USA). Descriptives are provided as mean (±SD) for normally distributed variables, median [interquartile range (IQR), 25–75%] for skewed variables, or number (%) for categorical variables. General characteristics and mean HR-pQCT parameters were compared between participants with NGM, PDM, and T2DM using one-way ANOVA for continuous variables and a chi-square test for categorical variables. General characteristics and mean HR-pQCT parameters were compared between the T2DM subgroups (HbA1c $\leq 7\%$ (≤ 53 mmol/mol) vs. HbA1c $> 7\%$ (> 53 mmol/mol), diabetes duration ≤ 5 years vs. diabetes duration > 5 years, and MVD− vs. MVD+) using Student's t test for continuous variables and a chi-square test or—in case of an expected count ≥ 5 in less than 80% of cells and/or expected count < 1 in any cell—Fisher exact test for categorical variables. To examine the association between diabetes status and HR-pQCT parameters, between T2DM subgroups and HR-pQCT parameters, and between HbA1c and HR-pQCT parameters, linear regression analyses were used, yielding regression coefficients (B) and 95% confidence intervals (CI). The regression analyses with diabetes status and with T2DM subgroups were discrete; both PDM and T2DM were compared to NGM (the reference group), HbA1c $> 7\%$ was compared to HbA1c $\leq 7\%$, diabetes duration > 5 years was compared to diabetes duration ≤ 5 years, and MVD+ was compared to MVD−. As a result, the regression coefficient only reflects the difference in bone parameters between the examined group and the reference group. The regression analyses were adjusted for age, sex, BMI, MVPA (only for the association between diabetes status and HR-pQCT parameters), a history of CVD (only for the association between diabetes status and HR-pQCT parameters), and time gap (time in months between date of baseline visit and date of HR-pQCT scan). A p value < 0.05 was considered statistically significant.

Results

General characteristics of the study population with a usable HR-pQCT scan of the radius are shown in Table 1. In total, 279 (68.0%) participants had NGM, 66 (16.1%) PDM, and 65

Table 1 General characteristics of the study population (radius scans) according to diabetes status

	NGM ($n = 279$)	PDM ($n = 66$)	T2DM ($n = 65$)	p value
Age in years	56.8 (8.3)	59.9 (8.0)	62.5 (7.1)	< 0.001*
Female sex	152 (54.5)	34 (51.5)	24 (36.9)	0.04*
BMI, kg/m^2	25.8 (3.6)	28.6 (4.2)	30.0 (5.5)	< 0.001*
Level of education ($n = 404$)				
- Low	67 (24.1)	25 (40.3)	29 (45.3)	0.002*
- Medium	86 (30.9)	18 (29.0)	19 (29.7)	
- High	125 (45.0)	19 (30.6)	16 (25.0)	
Smoking status ($n = 407$)				
- Never	113 (40.5)	17 (26.2)	21 (33.3)	0.12
- Former	134 (48.0)	42 (64.6)	37 (58.7)	
- Current	32 (11.5)	6 (9.2)	5 (7.9)	
Alcohol use ($n = 407$)				
- None	38 (13.6)	9 (13.8)	14 (22.2)	0.51
- Low	161 (57.7)	37 (56.9)	31 (49.2)	
- High	80 (28.7)	19 (29.2)	18 (28.6)	
History of CVD ($n = 400$)	31 (11.3)	12 (18.5)	19 (31.7)	< 0.001*
MVPA in hours per week ($n = 363$)	6.9 (4.9)	5.3 (3.3)	4.3 (4.2)	< 0.001*
Fracture ≥ 50 years ($n = 340$)	15 (6.0)	6 (10.5)	1 (3.0)	0.32
Antihyperglycemic drugs	n/a	n/a	41 (63.1)	n/a
Insulin	n/a	n/a	12 (18.5)	n/a
Thiazolidinedione	n/a	n/a	0	n/a
HbA1c in mmol/mol ($n = 408$)	36.3 (3.9)	38.8 (4.6)	50.9 (8.5)	< 0.001*
HbA1c in % ($n = 408$)	5.5 (0.4)	5.7 (0.4)	6.8 (0.8)	< 0.001*
Diabetes duration in years	n/a	n/a	3.0 [0.0–8.0]	n/a
Retinopathy ($n = 382$)	0	0	5 (7.7)	n/a
Impaired vibration sensation ($n = 359$)	12 (4.8)	2 (3.6)	9 (16.7)	0.004*
Nephropathy ($n = 349$)	17 (7.1)	6 (9.8)	8 (16.3)	0.11
eGFR < 60 mL/min (402)	5 (1.8)	3 (4.5)	7 (11.1)	0.002*
Micro-albumin in urine > 30 mg/24 h ($n = 356$)	13 (5.3)	5 (8.2)	6 (12.0)	0.20
MVD ($n = 297$)	27 (13.8)	8 (16.3)	19 (36.5)	0.001*
Quality grade scan radius				
- 1	49 (17.6)	9 (13.6)	13 (20.0)	0.58
- 2	130 (46.6)	30 (45.5)	34 (52.3)	
- 3	100 (35.8)	27 (40.9)	18 (27.7)	

Continues variables are presented as mean (SD) or median [25–75%], categorical variables are presented as number of participants (%). *Statistically significant. *BMI*, body mass index; *CVD*, cardiovascular disease; *eGFR*, estimated glomerular filtration rate; *HbA1c*, glycated hemoglobin; *MVD*, microvascular disease; *MVPA*, moderate-to-vigorous physical activity; *NGM*, normal glucose metabolism; *PDM*, prediabetes; *T2DM*, type 2 diabetes

(15.8%) T2DM. Participants with T2DM were the oldest, were the least likely to be female, and had the highest BMI. The mean $N \pm$ SD HbA1c level of participants with T2DM was $6.8 \pm 0.8\%$ (50.9 ± 8.5 mmol/mol), the median [IQR] diabetes duration was 3.0 [0.0–8.0] year, and 63.1% used antihyperglycemic drugs, including insulin in 18.5%. Of the participants with T2DM, 36.5% had any form of MVD (7.7% retinopathy, 16.3% nephropathy, 16.7% impaired vibration sensation). The general characteristics of the study population with a usable HR-pQCT scan of the tibia (NGM $n = 140$, PDM $n = 25$, T2DM $n = 33$) were comparable to those of the study population with a usable HR-pQCT scan of the radius (data not shown).

Bone parameters according to diabetes status

The mean unadjusted values of the bone parameters of the radius and tibia according to diabetes status are shown in Table 2. Ct.PoV (NGM, 14.6 ± 8.2 mm^3; PDM, 15.3 ± 6.2 mm^3; T2DM, 17.6 ± 8.1 mm^3; $p = 0.02$), Ct.Po (NGM,

Table 2 HR-pQCT parameters according to diabetes status, unadjusted

	Distal radius				Distal tibia			
	NGM ($n = 279$)	PDM ($n = 66$)	T2DM ($n = 65$)	p value	NGM ($n = 140$)	PDM ($n = 25$)	T2DM ($n = 33$)	p value
Cross-sectional area (mm^2)	328.0 (79.9)	333.6 (77.8)	340.4 (81.1)	0.51	777.5 (154.6)	798.2 (151.0)	828.9 (171.8)	0.23
Volumetric bone mineral density								
Total vBMD (mgHA/cm^3)	301.0 (62.1)	305.4 (58.2)	305.1 (71.6)	0.82	284.6 (51.7)	285.8 (51.6)	300.2 (50.3)	0.29
Trabecular vBMD (mgHA/cm^3)	163.7 (39.7)	165.3 (32.3)	166.8 (39.6)	0.82	175.9 (36.8)	176.8 (28.5)	185.4 (36.1)	0.38
Cortical vBMD (mgHA/cm^3)	852.1 (74.0)	850.3 (70.2)	836.5 (84.0)	0.32	834.5 (56.0)	828.1 (67.1)	838.2 (54.7)	0.80
Microarchitecture								
Trabecular number (1/mm)	1.93 (0.32)	2.00 (0.26)	1.96 (0.34)	0.27	1.94 (0.34)	1.86 (0.28)	2.08 (0.36)	0.03*
Trabecular thickness (mm)	0.07 (0.01)	0.07 (0.01)	0.07 (0.01)	0.53	0.08 (0.01)	0.08 (0.01)	0.07 (0.01)	0.26
Trabecular separation (mm)	0.47 (0.15)	0.44 (0.07)	0.46 (0.14)	0.32	0.46 (0.10)	0.47 (0.08)	0.42 (0.09)	0.10
Cortical thickness (mm)	0.75 (0.21)	0.77 (0.20)	0.77 (0.25)	0.60	1.13 (0.31)	1.15 (0.33)	1.24 (0.31)	0.21
Cortical pore volume (mm^3)	14.6 (8.2)	15.3 (6.2)	17.6 (8.1)	0.02*	79.4 (33.1)	83.3 (28.6)	94.4 (37.4)	0.07
Cortical porosity (%)	2.83 (1.45)	2.81 (0.94)	3.29 (1.29)	0.05*	7.63 (2.80)	7.92 (2.78)	8.00 (2.69)	0.74
Cortical pore diameter (mm)	0.17 (0.02)	0.17 (0.02)	0.18 (0.02)	0.01*	0.19 (0.02)	0.19 (0.02)	0.19 (0.02)	0.62
Bone strength								
Stiffness (kN/mm)	93.9 (28.0)	95.1 (24.8)	97.1 (25.7)	0.69	234.7 (63.2)	244.8 (56.7)	260.6 (64.6)	0.10
Failure load (N)	4480 (1318)	4556 (1174)	4643 (1206)	0.63	11,157 (2926)	11,587 (2648)	12,390 (3035)	0.09

Variables are presented as mean (SD). *Statistically significant using one-way ANOVA. *NGM*, normal glucose metabolism; *PDM*, prediabetes; *T2DM*, type 2 diabetes; *vBMD*, volumetric bone mineral density

2.83 ± 1.45%; PDM, 2.81 ± 0.84%; T2DM, 3.29 ± 1.29%; $p = 0.05$), and Ct.PoDm (NGM, 0.17 ± 0.02 mm; PDM, 0.17 ± 0.02 mm; T2DM, 0.18 ± 0.02 mm; $p = 0.01$) of the radius, but not the tibia, differed significantly between participants with NGM, PDM, and T2DM. The mean number of trabeculae of the tibia (NGM, 1.94 ± 0.34 mm^{-1}; PDM, 1.86 ± 0.28 mm^{-1}; T2DM, 2.08 ± 0.36 mm^{-1}; $p = 0.03$), but not of the radius, was significantly different between participants with NGM, PDM, and T2DM. There were no differences in vBMD, Tb.Th, Tb.Sp, Ct.Th, and bone strength according to micro-finite element analysis between participants with NGM, PDM, and T2DM (all $p > 0.05$).

The results of the linear regression analyses examining the association between diabetes status and bone parameters of the radius and tibia are shown in Table 3. After adjustment for age, sex, BMI, MVPA, a history of CVD, and time gap, PDM and T2DM were not associated with vBMD, microarchitecture, or bone strength of the distal radius or tibia when compared to participants with NGM, except for lower Tb.N (B, − 0.16 mm^{-1} (95% CI, − 0.30 to − 0.02 mm^{-1})) of the tibia in participants with PDM and a smaller CSA (B, − 72.0 mm^2 (95% CI, − 128.2 to − 15.8 mm^2) in participants with T2DM.

Bone parameters of type 2 diabetes subgroups

The general characteristics of the HbA1c, diabetes duration, and MVD subgroups are shown in Table 4. In the radius study population only, participants with an HbA1c > 7% (> 53 mmol/mol) had a significantly longer diabetes duration and were more often insulin users than those with an HbA1c ≤ 7% (≤ 53 mmol/mol), while the percentage of participants with MVD did not significantly differ. In both the radius and the tibia study population, participants with a diabetes duration > 5 years had a significantly higher HbA1c level and were more often insulin users than those with a diabetes duration ≤ 5 years, while the percentage of participants with MVD did not significantly differ. HbA1c level, diabetes duration, and the percentage of insulin users did not significantly differ between participants with and without MVD.

In the unadjusted analyses (Table 5), participants with an HbA1c level > 7% (> 53 mmol/mol) had a significantly lower Ct.BMD (806.9 ± 79.1 mgHA/cm^3 vs. 850.6 ± 83.4 mgHA/cm^3) and higher Tb.N (2.08 ± 0.31 mm^{-1} vs. 1.90 ± 0.34 mm^{-1}) of the radius, and a significantly lower Tb.Th (0.07 ± 0.01 mm vs. 0.08 ± 0.01 mm) of the tibia, compared to participants with an HbA1c level ≤ 7% (≤ 53 mmol/mol). Participants with a diabetes duration > 5 years had a significantly larger CSA (376.7 ± 83.0 mm^2 vs. 324.8 ± 78.2 mm^2) of the radius and no significant differences in HR-pQCT parameters of the tibia, compared to participants with a diabetes duration ≤ 5 years. Participants with MVD had a significantly higher Ct.PoV (20.4 ± 9.7 mm^3 vs. 15.5 ± 6.2 mm^3) and Ct.Po (3.69 ± 1.30% vs. 3.13 ± 1.30%) of the radius and a significantly larger CSA (932.2 ± 140.9 vs. 788.1 ± 139.1) of the tibia, compared participants without MVD.

Table 3 The association between diabetes status and HR-pQCT-derived parameters, adjusted for age, sex, BMI, MVPA, CVD, and time gap

	Distal radius			Distal tibia		
	NGM ($n = 246$)	PMD ($n = 56$) B (95% CI)	T2DM ($n = 52$) B (95% CI)	NGM ($n = 127$)	PDM ($n = 21$) B (95% CI)	T2DM ($n = 28$) B (95% CI)
Cross-sectional area (mm^2)	Ref	3.9 (−12.1 to 19.8)	−14.0 (−31.9 to 3.8)	Ref	−10.0 (−66.3 to 46.4)	−72.0* (−128.2 to −15.8)
Volumetric bone mineral density						
Total vBMD (mgHA/cm^3)	Ref	−5.3 (−23.2 to 12.6)	7.3 (−12.7 to 27.3)	Ref	−5.5 (−27.4 to 16.5)	4.2 (−17.7 to 26.0)
Trabecular vBMD (mgHA/cm^3)	Ref	−5.5 (−15.7 to 4.7)	−2.7 (−14.1 to 8.8)	Ref	−4.4 (−19.5 to 10.7)	−4.3 (−19.3 to 10.8)
Cortical vBMD (mgHA/cm^3)	Ref	−0.9 (−21.1 to 19.3)	12.9 (−9.7 to 35.5)	Ref	1.1 (−21.8 to 23.9)	12.4 (−10.3 to 35.2)
Microarchitecture						
Trabecular number (1/mm)	Ref	0.03 (−0.06 to 0.12)	−0.04 (−0.14 to 0.06)	Ref	−0.16* (−0.30 to −0.02)	−0.02 (−0.16 to 0.12)
Trabecular thickness (mm)	Ref	−0.003 (−0.007 to 0.000)	0.000 (−0.004 to 0.004)	Ref	0.004 (−0.001 to 0.010)	−0.001 (−0.007 to 0.004)
Trabecular separation (mm)	Ref	−0.01 (−0.05 to 0.03)	0.01 (−0.03 to 0.06)	Ref	0.04 (−0.00 to 0.08)	0.01 (−0.03 to 0.05)
Cortical thickness (mm)	Ref	−0.01 (−0.06 to 0.05)	0.02 (−0.04 to 0.09)	Ref	−0.04 (−0.17 to 0.09)	0.01 (−0.12 to 0.14)
Cortical pore volume (mm^3)	Ref	−1.32 (−3.40 to 0.77)	0.46 (−1.88 to 2.79)	Ref	−3.03 (−17.6 to 11.6)	−2.19 (−16.7 to 12.3)
Cortical porosity (%)	Ref	−0.29 (−0.67 to 0.09)	0.07 (−0.36 to 0.49)	Ref	0.04 (−1.19 to 1.27)	−0.01 (−1.23 to 1.21)
Cortical pore diameter (mm)	Ref	−0.005 (−0.011 to 0.001)	0.006 (−0.001 to 0.013)	Ref	0.009 (−0.001 to 0.018)	0.004 (−0.006 to 0.014)
Bone strength						
Stiffness (kN/mm)	Ref	−1.8 (−6.8 to 3.1)	−2.8 (−8.3 to 2.7)	Ref	−2.0 (−22.3 to 18.3)	−15.1 (−35.3 to 5.1)
Failure load (N)	Ref	−70.3 (−298.6 to 158.0)	−130.7 (−386.3 to 125.0)	Ref	−145.0 (−1067.6 to 777.6)	−732.6 (−1652.6 to 187.5)

The analyses are adjusted for age, sex BMI, MVPA, a history of CVD, and time gap (time in months between the baseline visit and the HR-pQCT scan). NGM is used as the reference group. *CVD*, cardiovascular disease; *MVPA*, moderate-to-vigorous physical activity; *NGM*, normal glucose metabolism; *PDM*, prediabetes; *T2DM*, type 2 diabetes; *vBMD*, volumetric bone mineral density. *p value < 0.05

Table 4 General characteristics of the type 2 diabetes subpopulations

Distal radius	HbA1c ≤ 7% (n = 44)	HbA1c > 7% (n = 21)	Diabetes duration ≤ 5 years (n = 40[#])	Diabetes duration > 5 years (n = 21[#])	MVD− (n = 33[#])	MVD+ (n = 19[#])
Age in years, mean (SD)	62.6 (7.3)	62.4 (7.0)	62.3 (7.4)	62.8 (6.6)	60.7 (7.7)	65.8 (5.1)[c]
Female sex, no. (%)	16 (36.4)	8 (38.1)	16 (41.0)	5 (23.8)	18 (54.5)	4 (21.1)[c]
BMI (kg/m^2), mean (SD)	29.9 (5.2)	30.2 (6.1)	30.4 (5.8)	29.6 (5.3)	29.4 (6.1)	30.8 (4.3)
HbA1c in %, mean (SD)	6.4 (0.4)	7.7 (0.6)[a]	6.6 (0.7)	7.1 (0.7)[b]	6.7 (0.6)	7.0 (0.9)
HbA1c in mmol/mol, mean (SD)	46.2 (4.6)	60.8 (6.1)[a]	48.5 (7.9)	54.4 (8.1)[b]	49.5 (6.6)	52.9 (10.1)
Diabetes duration in years, median [IQR]	1.5 [6.0]	7.0 [11.0][a]	1.0 [3.0]	11.0 [8.0][b]	2.0 [7.0]	3.5 [14.0]
Insulin use, no. (%)	4 (9.1)	8 (38.1)[a]	2 (5.0)	9 (42.9)[b]	4 (12.1)	5 (26.3)
MVD, no. (%)	10 (28.6)	9 (52.9)	10 (30.3)	8 (50.0)	n/a	n/a
Distal tibia	**HbA1c ≤ 7% (n = 22)**	**HbA1c > 7% (n = 11)**	**Diabetes duration ≤ 5 years (n = 20[#])**	**Diabetes duration > 5 years (n = 10[#])**	**MVD− (n = 14[#])**	**MVD+ (n = 12[#])**
Age in years, mean (SD)	64.4 (6.9)	62.5 (6.8)	63.8 (7.0)	65.2 (6.7)	61.4 (8.4)	66.5 (4.0)
Female sex, no. (%)	3 (13.6)	5 (45.5)	4 (20.0)	2 (20.0)	6 (42.9)	0 (0.0)[c]
BMI (kg/m^2), mean (SD)	29.7 (5.7)	27.7 (4.3)	29.4 (4.8)	27.0 (5.3)	29.2 (5.9)	29.6 (5.1)
HbA1c in %, mean (SD)	6.3 (0.4)	7.8 (0.6)[a]	6.5 (0.9)	7.2 (0.7)[b]	6.7 (0.6)	6.7 (1.0)
HbA1c in mmol/mol, mean (SD)	45.2 (4.7)	61.3 (7.0)[a]	48.1 (9.9)	55.5 (7.9)[b]	49.6 (6.5)	50.3 (10.5)
Diabetes duration in years, median [IQR]	2.0 [5.0]	7.0 [16.0]	1.0 [3.0]	10.0 [13.0][b]	3.5 [6.0]	1.5 [7.0]
Insulin use, no. (%)	2 (9.1)	3 (27.3)	1 (5.0)	4 (40.0)[b]	1 (7.1)	1 (8.3)
MVD, no. (%)	8 (47.1)	4 (44.4)	8 (47.1)	4 (57.1)	n/a	n/a

[#] Data on diabetes duration was missing in 4 out of 65 T2DM participants (radius) and 3 out of 33 T2DM participants (radius) and 7 out of 33 T2DM participants (tibia). Data on MVD was missing in 13 out of 65 T2DM participants (radius) and 3 out of 33 T2DM participants (tibia). [a] p value of the independent t test HbA1c subgroups < 0.05, [b] p value of the independent t test diabetes duration subgroups < 0.05, [c] p value of the independent t test MVD subgroups < 0.05. IQR, interquartile range; MVD, microvascular disease

Table 5 HR-pQCT parameters in subgroups of participants with T2DM, unadjusted

	Distal radius						Distal tibia					
	HbA1c ≤7% (n = 44)	HbA1c >7% (n = 21)	DD ≤5 years (n = 40)	DD >5 years (n = 21)	MVD− (n = 33)	MVD+ (n = 19)	HbA1c ≤7% (n = 22)	HbA1c >7% (n = 11)	DD ≤5 years (n = 20)	DD >5 years (n = 10)	MVD− (n = 14)	MVD+ (n = 12)
Cross-sectional area (mm²)	334.9 (76.7)	352.0 (90.6)	324.8 (78.2)	376.7[b] (83.0)	314.3 (68.7)	381.0[c] (89.4)	845.8 (164.4)	795.0 (189.1)	842.0 (162.1)	849.9 (188.5)	788.1 (139.1)	923.2[c] (140.9)
vBMD												
Total vBMD (mgHA/cm³)	311.9 (77.2)	290.9 (57.3)	307.6 (66.6)	309.4 (76.5)	303.5 (75.6)	292.3 (73.2)	304.4 (50.2)	291.9 (52.0)	291.5 (56.8)	314.0 (34.3)	300.6 (58.2)	298.8 (44.3)
Trabecular vBMD (mgHA/cm³)	163.7 (42.5)	173.5 (32.7)	165.6 (40.7)	173.0 (38.3)	164.2 (45.7)	171.4 (35.9)	187.1 (39.0)	182.2 (30.8)	182.4 (38.7)	195.1 (28.9)	189.4 (33.5)	191.1 (35.8)
Cortical vBMD (mgHA/cm³)	850.6 (83.4)	806.9[a] (79.1)	847.5 (61.8)	833.0 (86.2)	842.5 (83.8)	806.1 (92.0)	846.1 (51.1)	822.5 (60.6)	827.3 (57.8)	850.8 (50.3)	825.0 (63.9)	832.3 (40.6)
Microarchitecture												
Trabecular number (1/mm)	1.90 (0.34)	2.08[a] (0.31)	1.92 (0.36)	2.04 (0.31)	1.91 (0.36)	2.00 (0.32)	2.02 (0.40)	2.19 (0.26)	2.08 (0.38)	2.13 (0.35)	2.09 (0.32)	2.16 (0.38)
Trabecular thickness (mm)	0.07 (0.01)	0.07 (0.01)	0.07 (0.01)	0.07 (0.01)	0.07 (0.01)	0.07 (0.01)	0.08 (0.01)	0.07[a] (0.01)	0.07 (0.01)	0.08 (0.01)	0.08 (0.01)	0.07 (0.01)
Trabecular separation (mm)	0.48 (0.16)	0.42 (0.09)	0.48 (0.16)	0.43 (0.08)	0.48 (0.18)	0.44 (0.09)	0.44 (0.10)	0.39 (0.06)	0.42 (0.09)	0.41 (0.10)	0.41 (0.07)	0.40 (0.09)
Cortical thickness (mm)	0.81 (0.26)	0.68 (0.21)	0.77 (0.20)	0.80 (0.30)	0.74 (0.24)	0.72 (0.26)	1.28 (0.31)	1.15 (0.31)	1.21 (0.38)	1.29 (0.21)	1.20 (0.39)	1.26 (0.27)
Cortical pore volume (mm³)	17.4 (8.4)	18.2 (7.4)	18.2 (7.8)	17.8 (8.6)	15.5 (6.2)	20.4[c] (9.7)	96.1 (39.3)	91.1 (34.7)	98.9 (39.6)	94.2 (35.8)	93.6 (34.4)	108.1 (39.4)
Cortical porosity (%)	3.08 (1.22)	3.71 (1.38)	3.43 (1.31)	3.06 (1.25)	3.13 (1.30)	3.69[c] (1.30)	7.86 (2.77)	8.28 (2.58)	8.43 (2.76)	7.73 (2.62)	8.49 (2.78)	8.46 (2.54)
Cortical pore diameter (mm)	0.18 (0.02)	0.17 (0.02)	0.18 (0.02)	0.17 (0.02)	0.18 (0.02)	0.18 (0.02)	0.19 (0.03)	0.18 (0.02)	0.18 (0.02)	0.20 (0.04)	0.19 (0.02)	0.19 (0.03)
Bone strength												
Stiffness (kN/mm)	98.7 (27.0)	93.7 (22.7)	94.6 (23.5)	105.8 (26.9)	91.0 (25.8)	102.8 (22.5)	271.9 (62.6)	237.9 (65.4)	257.8 (71.1)	278.7 (56.5)	250.4 (63.2)	284.5 (52.7)
Failure load (N)	4701 (1269)	4519 (1079)	4515 (1107)	5072 (1257)	4353 (1219)	4927 (1047)	12,883 (2973)	11,402 (3050)	12,292 (3342)	13,190 (2658)	11,902 (2962)	13,548 (2455)

Variables are presented as mean (SD). [a] p value of the independent t test HbA1c subgroups < 0.05, [b] p value of the independent t test diabetes duration subgroups < 0.05, [c] p value of the independent t test MVD subgroups < 0.05. DD, diabetes duration; MVD, microvascular disease; vBMD, volumetric bone mineral density

After adjustment for age, sex, BMI, and time gap, an HbA1c > 7% (> 53 mmol/mol) was significantly associated with a lower Ct.BMD (B, − 44.4 mgHA/cm^3 (95% CI, − 85.4 to − 3.4 mgHA/cm^3)), lower Ct.Th (B, − 0.13 mm (95% CI, − 0.26 to − 0.00 mm)), higher Tb.N (B, 0.19 mm^{-1} (95% CI, 0.03 to 0.34 mm^{-1})), and higher Ct.Po (B, 0.65% (95% CI, 0.06 to 1.23%)) of the radius, and with a higher Tb.N (B, 0.27 mm^{-1} (95% CI, 0.01 to 0.53 mm^{-1})) and lower Tb.Th (B, − 0.010 mm (95% CI, − 0.018 to − 0.002 mm)) of the tibia (Table 6). Additionally, HbA1c (in %) was significantly associated with lower Ct.BMD (B, − 28.3 mgHA/cm^3 (95% CI, − 53.3 to − 3.2 mgHA/cm^3)), higher Tb.N (B, 0.15 mm^{-1} (95% CI, 0.06 to 0.24 mm^{-1})), and lower Tb.Sp (B, − 0.005 mm (95% CI, − 0.009 to 0.001 mm)) of the radius. HbA1c was not significantly associated with any of the bone parameters of the tibia (data not tabulated). Diabetes duration > 5 years was significantly associated with higher Tb.N (B, 0.17 mm^{-1} (95% CI, 0.03 to 0.34 mm^{-1})) of the radius, but not with HR-pQCT parameters of the tibia. The presence of MVD was not statistically significantly associated with any of the bone parameters of the radius or tibia.

Discussion

In this study, we examined the association between diabetes status and vBMD, bone microarchitecture, and calculated bone strength at the distal radius and tibia. We observed that after adjustment for age, sex, BMI, MVPA, history of CVD, and time gap, PDM was only associated with lower Tb.N of the tibia (− 4%) and T2DM was only associated with a smaller CSA (− 7%) of the tibia. The results of previous studies that examined the association between T2DM and HR-pQCT-derived bone parameters varied between no association [13–16], a positive association [17], and a negative association [10–12], but an association between T2DM and a smaller CSA of the tibia has not been described before.

We hypothesized that differences in the T2DM study populations explain these varying results, as most of the studies did not report on characteristics related to diabetes, such as HbA1c level [10–12, 17], diabetes duration [10–13], insulin use [10–13, 15, 16], and the presence of micro- or macrovascular disease [10–14, 17]. Since it has been thought that the increased fracture risk is another long-term complication [35] of T2DM, we hypothesized that unfavorable bone microarchitecture will only be present in T2DM participants with HbA1c levels above the treatment target value, those with longer disease duration, and those who already have a form of MVD. In our study, the total group of participants with T2DM had a relative short-term diabetes duration (median 3 years) and a mean HbA1c level below the treatment target value (6.8% (51 mmol/mol)) and only 36.5% had a form of MVD. This may explain the absence of significant

associations other than a smaller CSA of the tibia between T2DM and bone parameters. In contrast to a study by Shanboque et al. [15], we did not observe a significant association between the presence of MVD and bone parameters in participants with T2DM. Additionally, a diabetes duration > 5 years was not significantly associated with vBMD, microarchitecture, and bone strength, except for higher Tb.N (+ 6%) of the distal radius. However, glycation patterns in patients with T2DM showed some significant association with bone properties. For the radius, but not the tibia, poorer control of HbA1c was associated with some deteriorated cortical bone properties, while some trabecular measures were improved. In line with these findings, in a previous study, we showed significantly lower vBMD, Tb.Th, Ct.Th, Ct.PoV, bone stiffness, and failure load in insulin users, when compared to non-insulin users after adjustment for age, sex, BMI, HbA1c level, and diabetes duration [36].

We hypothesized that alterations in bone parameters would be comparable between T2DM participants with an HbA1c > 7% (> 53 mmol/mol) and a diabetes duration > 5 years and those with MVD, as we believe that these are all indicators of having an increased risk of long-term complications of the disease. Interestingly, when looking at the general characteristics of the T2DM subpopulations, those with an HbA1c > 7% (> 53 mmol/mol) also had a significantly longer diabetes duration, used insulin more often, but did not have MVD more often. In contrast, those with MVD did not have a significantly higher HbA1c, disease duration, or increased use of insulin. Additionally, although those with a diabetes duration > 5 years had a significantly higher HbA1c, it was still around the treatment target. Therefore, the contrasts between the T2DM subpopulations may not have been large enough to observe significant differences in bone parameters. Furthermore, in this study, we defined MVD as having diabetic retinopathy, nephropathy, and/or an impaired vibration sensation. As the presence of nephropathy and an impaired vibration sensation can be caused by other diseases (for example hypertension) than T2DM, it would be interestingly to examine the association between diabetic retinopathy and bone parameters. Larger studies, including more T2DM participants, are needed to realize this. Additionally, larger studies can be used to examine differences in between several HbA1c (e.g., < 6, 6–7, 7–8, 8–9, > 9) and diabetes duration (e.g., newly diagnosed, 1–5 years, 5–10 years, 10–15 years, > 15 years) subgroups to gain more insight whether alterations in bone parameters are indeed only present in those with higher HbA1c levels, or also in those with long duration of diabetes and MVD.

In T2DM participants with an HbA1c > 7% (53 mmol/mol), a significant increase in Tb.N of the distal radius and tibia was observed, and in T2DM participants with a diabetes duration > 5 years, a significant increase in Tb.N of the distal radius was observed. Additionally, in the T2DM group, HbA1c was associated with an increase in Tb.N and a

Table 6 The association between HbA1c, diabetes duration, MVD, and HR-pQCT-derived parameters, adjusted for age, sex, BMI, and time gap

	Distal radius			Distal tibia		
	HbA1c >7% (n = 21) B (95% CI)	Diabetes duration > 5 years (n = 21) B (95% CI)	MVD+ (n = 19) B (95% CI)	HbA1c >7% (n = 11) B (95% CI)	Diabetes duration > 5 years (n = 10) B (95% CI)	MVD+ (n = 12) B (95% CI)
Cross-sectional area (mm^2)	18.2 (−14.9 to 51.3)	33.3 (−2.5 to 69.1)	39.4 (−1.9 to 80.6)	12.9 (−106.4 to 132.1)	4.6 (−121.2 to 130.5)	96.2 (−36.0 to 228.5)
vBMD						
Total vBMD (mgHA/cm^3)	−21.0 (−58.3 to 16.9)	5.7 (−34.7 to 46.2)	−19.3 (−67.9 to 29.3)	−1.7 (−41.1 to 37.6)	34.1 (−2.0 to 70.2)	−7.4 (−51.2 to 36.4)
Trabecular vBMD (mgHA/cm^3)	10.6 (−8.5 to 29.8)	10.0 (−11.0 to 30.9)	−6.0 (−31.4 to 19.5)	−0.1 (−30.1–30.0)	19.0 (−9.1 to 47.1)	1.9 (−31.7 to 35.6)
Cortical vBMD (mgHA/cm^3)	−44.4* (−85.4 to −3.4)	−13.4 (−54.1 to 27.2)	−33.5 (−87.1 to 20.1)	−15.8 (−58.3 to 26.8)	26.2 (−15.4 to 67.8)	0.6 (−48.6 to 49.8)
Microarchitecture						
Trabecular number (1/mm)	0.19* (0.03 to 0.34)	0.17* (0.03 to 0.34)	0.09 (−0.12 to 0.29)	0.27* (0.01 to 0.53)	0.17 (−0.09 to 0.42)	0.10 (−0.23 to 0.42)
Trabecular thickness (mm)	−0.001 (−0.007 to 0.005)	−0.002 (−0.008 to 0.005)	−0.005 (−0.013 to 0.003)	−0.010* (−0.018 to −0.002)	0.002 (−0.007 to 0.011)	−0.002 (−0.014 to 0.009)
Trabecular separation (mm)	−0.06 (−0.12 to 0.01)	−0.06 (−0.13 to 0.01)	−0.03 (−0.12 to 0.06)	−0.06 (−0.13 to 0.00)	−0.04 (−0.11 to 0.02)	−0.02 (−0.09 to 0.06)
Cortical thickness (mm)	−0.13* (−0.26 to −0.00)	0.03 (−0.11 to 0.17)	−0.05 (−0.21 to 0.11)	−0.02 (−0.24 to 0.20)	0.14 (−0.07 to 0.35)	−0.05 (−0.31 to 0.21)
Cortical pore volume (mm^3)	0.94 (−3.01 to 4.90)	−0.57 (−4.77 to 3.63)	2.44 (−2.41 to 7.29)	9.89 (−19.8 to 39.5)	−3.34 (−34.3 to 27.7)	−4.89 (−41.7 to 31.9)
Cortical porosity (%)	0.65* (0.06 to 1.23)	−0.29 (−0.95 to 0.36)	0.29 (−0.45 to 1.04)	0.41 (−1.78 to 2.61)	−0.91 (−3.15 to 1.34)	−0.48 (−3.13 to 2.18)
Cortical pore diameter (mm)	−0.01 (−0.02 to 0.00)	−0.01 (−0.02 to 0.00)	0.00 (−0.01 to 0.01)	−0.02 (−0.04 to 0.00)	0.01 (−0.01 to 0.03)	−0.01 (−0.03 to 0.02)
Bone strength						
Stiffness (kN/mm)	−4.4 (−13.9 to 5.0)	5.9 (−4.4 to 16.1)	−0.9 (−12.0 to 10.2)	−10.4 (−51.2 to 30.4)	24.5 (−15.0 to 64.1)	15.8 (−27.3 to 58.9)
Failure load (N)	−158.6 (−596.9 to 279.6)	308.3 (−166.4 to 783.0)	−20.7 (−533.1 to 491.7)	−344.5 (−2261.0 to 1571.9)	1103.8 (−753.4 to 2961.1)	824.4 (−1186.6 to 2835.4)

The analyses are adjusted for age, sex, BMI, and time gap (time in months between the baseline visit and the HR-pQCT scan). HbA1c ≤7% (radius $n = 44$, tibia $n = 22$), DD ≤ 5 years (radius $n = 40$, tibia $n = 33$, tibia $n = 14$) are used as the reference categories. MVD− (radius $n = 20$, and MVD− (radius $n = 33$, tibia $n = 14$) are used as the reference categories. *MVD*, microvascular disease; *vBMD*, volumetric bone mineral density. *p value <0.05

decrease in Tb.Sp. Since all other observed alterations in bone microarchitecture have a negative influence on bone strength, this may feel counterintuitive at first. However, due to the deterioration of the predominantly cortical microarchitecture, the higher Tb.N may compensate for the loss in bone strength. This also explains why bone stiffness and failure load are not deteriorated in these subgroups. Second, T2DM participants with an HbA1c > 7% (> 53 mmol/mol) and with a diabetes duration > 5 years used metformin more often than T2DM participants with an HbA1c ≤ 7% (≤ 53 mmol/mol) and those with a diabetes duration ≤ 5 years (66.7% vs. 52.3% and 81.0% vs. 45.0%, respectively). As previous research showed that metformin may be osteogenic [37, 38], use of metformin may partly explain the increase in trabecular number. However, since we observed no difference in the number of trabeculae between the NGM and T2DM groups, metformin is a less likely explanation for the difference in trabecular number between the HbA1c and diabetes duration subgroups.

This is the first study that examined the association between PDM and HR-pQCT-derived bone parameters. In previous studies, a normal to decreased fracture risk was observed in subjects with PDM [3, 39], leading to our hypothesis that the bone parameters in PDM would be comparable to or favorable of those in NGM. Besides a significantly lower Tb.N of the tibia, no significant differences in bone parameters were observed between participants with PDM and NGM. As the general characteristics of these study populations—including the percentage of participants with a history of a fracture—were highly comparable, it may not be surprising that the bone parameters were also not different. Future studies are needed to confirm our results.

This study has several limitations. First, HbA1c was only measured once (at baseline), and that the mean time between the HR-pQCT scan and the baseline visit was approximately 3.5 years. Within this time gap, HbA1c levels of the participants may have changed and the results may thus have been different when HbA1c was assessed again at the moment of the scan. Additionally, although we adjusted the analyses for time gap, other covariates and diabetes status may have changed in the time gap between the baseline survey and the HR-pQCT scan. Due to logistical and financial reasons, it was not feasible to re-assess those parameters at the date of the HR-pQCT scan. Future studies that measure diabetes status, diabetes characteristics, and performing HR-pQCT scans at the same date are needed to confirm our results. Second, although being one of the largest HR-pQCT studies in participants with T2DM, the absolute number of scanned participants with T2DM was still small. Therefore, the analyses could only be adjusted for a limited number of potential confounders, the analyses could not be stratified to sex and the study did not have enough power to examine interaction with sex, and we could not correct for multiple testing. Additionally, the small sample size limits the power of this study and increases the risk of making type 2

errors. Therefore, the negative results (absence of associations) in this study should be interpreted with caution and larger studies are needed to confirm our results. Third, we used the uncorrected automatically generated endocortical contour for cortical bone analysis, which results in lower values of porosity-related parameters due to less inclusion of the transitional zone [40]. However, a study by Heilmeier et al. showed that differences in Ct.Po between patients with T2DM with and without fragility fractures were present in the periosteal and midcortical layer of the cortex, but not in the endosteal layer [41]. Therefore, they hypothesized that pores located closer to the marrow cavity have a less detrimental effect on bone mechanical properties than those located near the periosteal surface. Thus, although differences in Ct.Po of the endosteal layer between participants with and without diabetes in our study cannot be completely ruled out, the absence of a negative association between T2DM and porosity-related parameters suggests that the bone extrinsic material properties are not different between participants with and without diabetes. Fourth, we used fundus photography, eGFR, microalbuminuria, and the vibration sensation threshold to define MVD. As a decline in eGFR, the presence of microalbuminuria and an impaired vibration sensation can also be caused by other mechanisms than microvascular disease; misclassification can be present. Additionally, no DXA scans or bone turnover markers were available. Bone microarchitecture may be affected by changes in bone turnover; if higher HbA1c levels are associated with an increase in bone resorption markers and/or a decrease in bone formation markers, negative effects on bone structure can be expected. Additionally, higher blood glucose levels result in an increase in advanced glycations end products and may lead to damage to the microvasculature of the bone, both factors that may be associated with the observed alterations in bone microarchitecture. Future studies that perform HR-pQCT scans and measure bone turnover markers and levels of AGEs are warranted to reveal the mechanism underlying the alterations in bone microarchitecture in T2DM participants with HbA1c levels above the treatment target value. Finally, the reference line for the HR-pQCT scans was placed at a fixed reference point, which resulted in scanning a fixed region in every participant. However, bone morphology at that region differs between individual patients, where a higher amount of cortical bone will be present in participants with relatively short arms. A recent study suggests scanning at a percentage distance of the total length of the bone, or alternatively, the analyses should be adjusted for height [42]. Because of the clear difference in BMI between participants with NGM, PDM, and T2DM, we adjusted the analyses for BMI instead of height.

In conclusion, in this small cross-sectional study of predominantly well-treated T2DM participants with a relatively short-term diabetes duration, PDM was only associated with lower Tb.N of the tibia and T2DM was only associated with a smaller CSA of the tibia. Interestingly, within the group of

participants with T2DM, an HbA1c level > 7% (53 mmol/mol) was negatively associated with cortical bone parameters of the distal radius and trabecular bone parameters of the distal tibia. This suggests that alterations in bone microarchitecture may only be present in T2DM participants with inadequate blood glucose control. However, because of the limited sample size (65 and 33 participants with T2DM with an available HR-pQCT scan of the radius and tibia, respectively) and the time gap between the HbA1c measurement and the HR-pQCT scan, the results of our study should be interpreted with caution and future studies are needed to confirm our results and to unravel the mechanism underlying the changes in bone microarchitecture in T2DM patients with HbA1c levels above the treatment target.

Compliance with ethical standards

The study has been approved by the institutional medical ethical committee (NL31329.068.10) and the Minister of Health, Welfare and Sports of the Netherlands (Permit 131088-105234-PG). All participants gave written informed consent.

Conflicts of interest None.

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