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Volumetric femoral BMD, bone geometry, and serum sclerostin levels differ between type 2 diabetic postmenopausal women with and without fragility fractures

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Abstract

Summary While type 2 diabetes (T2D) is associated with higher skeletal fragility, specific risk stratification remains incompletely understood. We found volumetric bone mineral density, geometry, and serum sclerostin differences between low-fracture risk and high-fracture risk T2D women. These features might help identify T2D individuals at high fracture risk in the future.

Introduction Diabetic bone disease, an increasingly recognized complication of type 2 diabetes mellitus (T2D), is associated with high skeletal fragility. Exactly which T2D individuals are at higher risk for fracture, however, remains incompletely understood. Here, we analyzed volumetric bone mineral density (vBMD), geometry, and serum sclerostin levels in two specific T2D subsets with different fracture risk profiles. We examined a T2D group with prior history of fragility fractures (DMFx, assigned high-risk group) and a

fracture-free T2D group (DM, assigned low-risk group) and compared their results to nondiabetic controls with (Fx) and without fragility fractures (Co).

Methods Eighty postmenopausal women ($n=20$ per group) underwent quantitative computed tomography (QCT) to compute vBMD and bone geometry of the proximal femur. Additionally, serum sclerostin, vitamin D, parathyroid hormone (PTH), HbA1c, and glomerular filtration rate (GFR) levels were measured. Statistical analyses employed linear regression models.

Results DMFx subjects exhibited up to 33 % lower femoral neck vBMD than DM subjects across all femoral sites ($-19 \% \leq \Delta vBMD \leq -33 \%$, $0.008 \leq p \leq 0.021$). Additionally, DMFx subjects showed significantly thinner cortices (-6% , $p=0.046$) and a trend toward larger bone volume ($+10 \%$, $p=0.055$) relative to DM women and higher serum sclerostin levels when compared to DM ($+31.4 \%$, $p=0.013$), Fx ($+25.2 \%$, $p=0.033$), and control ($+22.4 \%$, $p=0.028$) subjects.

Conclusion Our data suggest that volumetric bone parameters by QCT and serum sclerostin levels can identify T2D individuals at high risk of fracture and might therefore show promise as clinical tools for fracture risk assessment in T2D. However, future research is needed to establish diabetes-specific QCT- and sclerostin-reference databases.

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Introduction

Type 2 diabetes mellitus (T2D) afflicts 18 million people in the USA and epidemiologic projections estimate up to 500 million affected worldwide by 2030 [1]. Diabetes complications

involve nearly every organ [2]. Recently, diabetic skeletal manifestations, diabetes bone disease, were added to the list of secondary diabetic complications. Epidemiological studies demonstrate T2 diabetics exhibit higher fracture risk compared to nondiabetics [3–5]. Especially postmenopausal T2D women suffer high fracture rates at multiple skeletal sites including hip, humerus, ankle, foot, and spine [3–5]. However, fracture risk stratification in T2 diabetics remains inexact. The current clinical standards of fracture risk assessment, dual X-ray absorptiometry (DXA), and the WHO-FRAX score tend to underestimate fracture risk in T2D patients [6, 7]. High-resolution peripheral quantitative computed tomography (HR-pQCT) and bone marrow magnetic resonance spectroscopy show promise in characterizing T2D patients at high fracture risk [8, 9], but both devices remain investigational. Unlike these techniques, quantitative computed tomography (QCT) is widely available and can provide detailed information on trabecular and cortical bone and bone geometry beyond the capability of DXA [10]. As of yet, few studies exist examining volumetric bone mineral density (vBMD) in Type 2 diabetics, and of those, none of them considered diabetic fracture status in their analysis [11, 12].

Recent evidence suggests that the osteocyte-specific glycoprotein sclerostin may be involved in diabetic bone fragility [13]. Circulating sclerostin levels were associated with a larger number of vertebral compression fractures in T2D postmenopausal women [14]. Elevated serum sclerostin levels, therefore, may correlate with T2D fragility fracture risk and may hence become a viable clinical tool for fracture risk assessment. Current diabetic sclerostin studies restrict their measurements to areal BMD by DXA and only enrolled T2D individuals with vertebral fractures [13, 14]. This limits their ability to generalize their sclerostin results. None of the studies assessed vertebral fracture age. Given that serum sclerostin levels have been found increased in the course of fracture healing [15] the reported diabetic sclerostin levels may have been confounded by fracture repair effects.

Our study addresses these limitations by including diabetic fragility fractures at multiple skeletal sites and by aging those fractures. The patient population we enrolled was stratified in a high-risk group (DMFx) that was characterized by a positive history of one or more remote fragility fractures of any skeletal site, and a low-risk T2D group (DM) which was reportedly free of any fragility fractures after the onset of T2D. The patients were investigated with volumetric bone mineral density, bone geometry by QCT, and serum sclerostin levels. The diabetic subsets were compared to a group of nondiabetic controls with and without fragility fractures. We hypothesized that low-risk and high-risk T2D groups would differ in their volumetric bone parameters and bone geometry. We also hypothesized that serum sclerostin levels would be higher in the high-risk group although we controlled for fracture repair effects and only included T2D patients with remote fragility fractures.

Patients and methods

Subjects

Eighty postmenopausal women were recruited through diabetic and orthopedic clinics as well as media outlets and assigned to one of the following four study groups: healthy controls (i.e., nondiabetic, non-fracture) (Co, $n=20$), nondiabetics with fragility fractures (Fx, $n=20$), type 2 diabetics without any history of fragility fractures (DM, $n=20$), and type 2 diabetics with a positive history of fragility fractures (DMFx, $n=20$). The study was HIPAA compliant, approved by UCSF's Committee on Human Research, and all subjects provided written and informed consent prior to participation.

To be included in the study, all women had to be aged 50–75 years and have a body mass index between 18 and 37 kg/m². All subjects were required to be mobile and able to move without assistance. For T2D subjects, a minimum of a 3 years history of treatment for T2D by oral medication and/or insulin was required. Subjects with fractures were only included if the fractures were not acute (as determined by history, on radiographs, and by spinal MRI screening), caused by a low-energy trauma such as falls from standing height or less and if they had been sustained after menopause (DMFx and Fx), and subsequent to the onset of diabetes (DMFx). Patients with pathologic fractures (i.e. fractures caused by local tumors, tumor-like lesions, or focal demineralizations as visualized on radiographs) were excluded from the study.

Exclusion criteria comprised medical conditions affecting bone metabolism such as juvenile or premenopausal idiopathic osteoporosis, hyperthyroidism, hyperparathyroidism, a recent history of longer (>3 months) periods of immobilization, chronic drug use, alcoholism, chronic gastrointestinal disease, significant chronic renal impairment (CKD stages IV and V), significant chronic hepatic impairment, severe neuropathic disease, unstable cardiovascular disease, or uncontrolled hypertension. In addition, any chronic treatment over the last 6 months with adrenal or anabolic steroids, estrogens, antacids, anticonvulsants, anticoagulants, pharmacological doses of vitamin A, fluorides, bisphosphonates, calcitonin, tamoxifen or parathyroid hormone (PTH), or glitazones was considered an exclusion criterion.

Confirmation and characterization of fracture type and fracture age

For all subjects assigned to the fragility fracture groups (Fx or DMFx), fracture presence and location were verified on previous radiographs by a board-certified musculoskeletal radiologist (TML). Fracture age was calculated as the time that had elapsed from the radiologic fracture diagnosis to the

examination date. In order to screen for occult vertebral fractures, all study participants underwent MRI of the thoracolumbar spine during their study visit. MRI sequences included a sagittal T1-weighted fast spin-echo (FSE) sequence (TE/TR=10.34/575 ms, 40 cm field of view (FOV), 4 mm slice thickness) and a sagittal T2-weighted FSE sequence with fat saturation of the entire spine (TE/TR=84.22/5000 ms, 42 cm FOV, 4 mm slice thickness), followed by a sagittal T2-weighted FSE sequence of the lumbar spine (TE/TR=82.66/5000 ms, 22 cm FOV, 5 mm slice thickness). Images were evaluated for presence and acuity of vertebral fractures by a blinded board-certified radiologist (TML). Vertebral fractures were graded according to the standard semiquantitative score developed by Genant et al. [16]. By definition, a fracture was confirmed to be remote (>5 months) if only vertebral height loss was detected and bone marrow signal was found to be normal for age [17, 18]. In all four study groups, no occult acute vertebral fractures were detected on spinal MRI.

Laboratory analyses

Fasting blood samples were collected between 8 and 11 a.m. and sent for immediate blood workup. The test panel included measurements of blood glucose (mg/dL), HbA1c (%), total serum 25-hydroxyvitamin D (ng/mL), parathyroid hormone (PTH) (pg/mL), serum calcium (mg/dL), and serum creatinine (mg/dL). To calculate the estimated glomerular filtration rate (eGFR), the MDRD (Modification of Diet in Renal Disease) equation was used and corrected for race in African-American women [19, 20]. For each patient, remaining serum aliquots were frozen and stored at -70°C until further analysis.

For determination of serum sclerostin and serum bone remodeling marker levels, frozen serum samples were shipped to the Division of Endocrinology, Department of Medicine, Columbia University, NY, and analyzed. Serum sclerostin concentrations were measured using an enzyme-linked immunosorbent assay from TECOmedical Group, Quidel Corporation, Santa Clara, USA (TE 1023) [21]. For quantification of serum C-terminal telopeptide (CTX I) levels, an enzyme-linked immunosorbent assay was used (Serum CrossLaps[®], Immunodiagnostic systems, Scottsdale, AZ 85258, USA). Serum procollagen type 1 (P1NP) measurements were performed via radioimmunoassay (UniQ[™] P1NP, Immunodiagnostic systems, Scottsdale, AZ 85258, USA). All measurements were performed in 77 out of 80 patients. In three patients, blood collection yield was generally low and sera had been used up for basic blood workup, leaving no remaining material for sclerostin analysis. One patient for sclerostin measurements had to be excluded from the study in accordance with Chauvenet's criteria due to exceedingly high

sclerostin levels [22]. In total, serum sclerostin levels of 76 patients were used for analysis.

Quantitative computed tomography (QCT)

Axial quantitative CT images of the hip were obtained on a multislice spiral CT scanner (LightSpeed VCT 64 CT scanner, GE Healthcare, Waukesha, WI, USA). Only one CT scanner was used with the same calibration phantom (INTable[™] Calibration Phantom, Image Analysis, Inc., Columbia, KY, USA) and the same analysis software for all 80 patients. The scanner BMD stability was monitored monthly using a QA phantom and software. Acquisition and density results were plotted via QA software, and eventual scanner drifts were corrected. Imaging parameters were as follows: table height 165 cm, scan field of view of 48 cm, 120 kVp, 140 mAs, 1.25 mm slice thickness, helical scan mode, and pitch=0.53. Each subject was scanned in supine position with both legs extended and secured in internal rotation. A scan region spanning from 1 cm superior to the acetabulum to 1 cm distal to the lesser trochanter was prescribed from a scout scan and imaged along with the calibration phantom visible in each image slice. Images with a spatial resolution of $0.94\text{ mm} \times 0.94\text{ mm}$ in-plane pixel size and 1.25 mm slice thickness were obtained using a standard reconstruction kernel with a 48 cm reconstruction field of view and a 512×512 matrix.

Image analysis

Analysis of volumetric bone mineral density (vBMD) and geometric parameters at the proximal femur

Volumetric bone mineral density (vBMD) and geometric measures of the left proximal femur were computed in 75 of 80 patients, using an in-house-developed, semiautomated QCT software [23] utilized in previous NIH-funded studies [24]. One scan of the DMFx group had to be excluded due to potential attenuation errors caused by contralateral total hip replacement; the results of four other subjects (Fx=2, DMFx=2) could not be obtained due to technical difficulties in BMD conversion and image post processing. In brief, the QCT images were first reformatted along the femoral neck axis, and a threshold-driven contour-tracking algorithm was applied to extract the entire bony proximal femoral envelope from the surrounding soft tissue. Three measurement regions were automatically defined encompassing the femoral neck, the lesser and greater trochanters, and the entire proximal femur. Within each region, cortical, trabecular, as well as integral volumetric BMD were computed. To evaluate femoral geometry, three parameters were computed: the proximal femoral bone volume, the minimal cross-sectional area (Min neck CSA)

as a measure of bone size [25], and the ratio of cortical to total tissue volume (cvol/ivol) as a measure of cortical thickness [26].

Statistical analysis

Normal distribution of each variable was explored via visualization of histograms, Q-Q plots, and Shapiro-Wilk tests. Continuous demographic parameters as well as metabolic and bone turnover markers were compared among groups using one-way analysis of variance (ANOVA) with subsequent post hoc Tukey-Kramer tests or independent samples *t*-tests as appropriate. Pearson's chi-squared test was used for categorical variables. To assess differences in (1) QCT-derived volumetric bone mineral density and geometry measures and (2) serum sclerostin levels among the four groups, linear regression models were performed with adjustments for age, weight, height, and race. The model for sclerostin was additionally adjusted for the confounding bone size and PTH levels [27–29]. All analyses were performed using IBM SPSS® Statistics 20.0 (IBM, Armonk, NY) besides the linear regression models which were carried out using Stata 11 software (StataCorp LP, College Station, TX, USA). Statistical significance was defined as $p < 0.05$.

Results

Subject characteristics

Descriptive characteristics of the four study groups are summarized in Table 1. Comparing all four groups, no significant differences in height, weight, racial composition, BMI, parathyroid hormone levels, and estimated glomerular filtration rates (eGFR) were found. Fx patients not only showed higher total serum 25-hydroxyvitamin D levels than controls ($p = 0.002$) and DM subjects ($p = 0.001$) but also had greater vitamin D supplement use. The diabetic groups (DM and DMFx) differed significantly in their mean T2D duration (DM: 8.4 ± 4.6 years; DMFx: 13.6 ± 8.9 years; $p = 0.032$) but otherwise exhibited similar antidiabetic medication use and comparable anthropometric and laboratory characteristics as indicated by similar age, height, BMI, HbA1c, serum glucose, eGFR, serum calcium, PTH, and total serum 25-hydroxyvitamin D levels. The racial composition within the DM and DMFx groups was also very similar (chi-squared test $p = 0.688$). With respect to bone turnover, both diabetic groups (DM and DMFx) exhibited significantly lower levels in serum CTX I and P1NP compared to nondiabetic Co subjects (CTX I: Co vs. DM $p = 0.041$; Co vs. DMFx $p = 0.003$; P1NP: Co vs. DM $p = 0.001$; Co vs. DMFx $p = 0.012$). No significant differences in bone turnover marker levels were found between the unfractured (DM) and fractured

diabetic groups (DMFx) (CTX I: DM vs. DMFx, $p = 0.779$; P1NP: DM vs. DMFx, $p = 0.934$).

Fracture prevalence and fracture age were similar between the nondiabetic Fx group and the DMFx group: in total, 53 fragility fractures were observed across both groups ($n = 38$, Fx: $n = 22$, DMFx: $n = 31$, chi-squared test $p = 0.157$), and fractures of both groups were sustained in similar rates and at similar sites including the ankle (Fx: $n = 9$ vs. DMFx: $n = 7$), vertebra (Fx: $n = 6$ vs. DMFx: $n = 6$), humerus (Fx: $n = 2$ vs. DMFx: $n = 3$), and wrist (Fx: $n = 2$ vs. DMFx: $n = 2$). Patella ($n = 1$), elbow ($n = 1$), and rib ($n = 1$) were additional fracture locations in the DMFx group, which counted more metatarsal fractures than the Fx group (DMFx: $n = 10$ vs. Fx: $n = 3$). Women of the Fx group had sustained their latest fragility fracture on average 3.3 ± 3.7 years ago, while DMFx women had an average fracture age of 3.2 ± 2.7 years (independent *t*-test; $p = 0.845$). In each group, 16 patients had remote fractures that were older than 11 months, two patients had a fracture that was reportedly 5 months old, and one patient with a 6-month-old (Fx) and a 9-month-old (DMFx) fracture, respectively. In all four study groups, no occult acute vertebral fractures were detected on spinal MRI.

Volumetric bone mineral density and bone geometry measurements

Means and standard deviations of the QCT-derived volumetric bone mineral density (vBMD) and geometry measures are outlined in Table 2. DMFx subjects showed significantly lower integral vBMD at the femoral neck (-9% , $p = 0.041$), the trochanter (-7.5% , $p = 0.039$), and the total proximal femur (-7.8% , $p = 0.035$) compared to DM subjects. Moreover, trabecular vBMD was significantly lower in the DMFx compared to the DM group at all femoral subregions (neck: -32.7% , $p = 0.021$; trochanter: -18.7% , $p = 0.009$; total proximal femur: -20.4% , $p = 0.008$). Cortical vBMD of all three regions did not differ significantly between DMFx and DM subjects. When comparing DM subjects and Co women, DM subjects displayed higher mean integral vBMD than controls, but these differences did not always attain statistical significance (neck: $+7.8\%$, $p = 0.031$; trochanter: $+6.6\%$, $p = 0.423$; total proximal femur: $+7.0\%$, $p = 0.157$).

DMFx subjects tended to have larger femoral bone volume ($+10\%$, $p = 0.055$) compared to DM subjects. In addition, DMFx femurs exhibited thinner cortices (overall cvol/ivol -5.8% , $p = 0.046$) relative to DM femurs, comparable in volume and thickness to Co femurs. The minimal cross-sectional area of the femoral neck (Min neck CSA) was similar between DMFx and DM subjects, while both T2D groups had significantly smaller Min neck CSA compared to controls (DM vs. Co: -8.3% , $p = 0.009$; DMFx vs. Co: -6.3% , $p = 0.037$). The femoral bone volume in DM subjects was significantly smaller than that in controls (-11% , $p =$

Table 1 Descriptive characteristics of all study participants ($n=77$) with sclerostin measurements. Data are expressed as mean \pm SD. eGFR is expressed as median [25th–75th percentile]

	Co (n=19)	Fx (n=19)	DM (n=20)	DMFx (n=19)	p-values *
Demographics and Anthropometry					
Age (years)	57.7 \pm 4.8	64.7 \pm 5.8 ^a	59.6 \pm 4.0	63.3 \pm 6.1	
Height (cm)	161.8 \pm 5.9	162.4 \pm 8.2	159.7 \pm 7.1	160.2 \pm 6.9	
Weight (kg)	69.4 \pm 13.7	67.2 \pm 10.4	71.5 \pm 13.3	74.1 \pm 14.7	
Body mass index (kg/m ²)	26.4 \pm 4.4	25.5 \pm 3.4	27.8 \pm 3.7	28.9 \pm 5.6	
Racial composition					
Caucasian (%)	63.2%	84.2%	35.0%	42.1 %	$p=0.688^b$
Asian (%)	21.0 %	10.5%	35%	21.0%	
African American (%)	5.3%	0.0%	20.0%	31.6%	
Hispanic (%)	10.5%	5.3%	5.0%	0.0%	
Pacific Islander/Native Hawaiian (%)	0.0%	0.0%	5.0%	5.3%	
Diabetic status					
Duration of type 2 diabetes (years)	n.a.	n.a.	8.4 \pm 4.6	13.6 \pm 8.9 ^b	
HbA _{1c} (%)	5.8 \pm 0.3	5.8 \pm 0.4	7.9 \pm 1.5 ^c	8.0 \pm 2.7	
Fasting glucose (mg/dl)	91.1 \pm 11.9	91.7 \pm 11.8	159.6 \pm 70.4 ^c	151.0 \pm 70.0	
Current insulin use (%)	n.a.	n.a.	20%	44.4%	$p=0.204^b$
Current metformin use (%)	n.a.	n.a.	19 (95%)	14 (73.7%)	$p=0.161^b$
Current sulfonylurea use (%)	n.a.	n.a.	35%	36.8 %	$p=0.905^b$
Fragility Fracture Status					
Fragility fracture prevalence (n)	0	22	0	31	$p=0.157^d$
Fragility fracture age (years)	n.a.	3.3 \pm 3.7	n.a.	3.2 \pm 2.7	$p=0.870^d$
Time since last fragility fracture	n.a.	\geq 5 months	n.a.	\geq 5 months	
MR Spine - occult acute vertebral fx (%)	0%	0%	0%	0%	
Metabolism					
Calcium (mg/dl)	9.6 \pm 0.3	9.5 \pm 0.4	9.5 \pm 0.4	9.6 \pm 0.4	
PTH (pg/ml)	37.8 \pm 14.2	32.3 \pm 23.9	38.4 \pm 15.8	41.8 \pm 26.1	
Total 25-OH vitamins D (ng/ml)	28.0 \pm 11.5	42.4 \pm 11.6 ^a	27.4 \pm 11.5	32.4 \pm 12.9	
P1NP (ng/ml)	62.8 \pm 19.0	59.6 \pm 35.6	41.3 \pm 15.4 ^c	51.0 \pm 30.6 ^e	
CTX I (ng/ml)	0.489 \pm 0.25	0.426 \pm 0.28	0.315 \pm 0.17 ^c	0.267 \pm 0.12 ^e	
eGFR (estimated glomerular filtration rate (ml/min/1.73 m ²))	86.4 [77.2 - 97.2]	82.4 [69.0 - 86.0]	98.1 [76.8 - 117.0]	86.4 [68.9 - 99.9]	

Co refers to nondiabetic postmenopausal women without history of fragility fractures; Fx refers to nondiabetic postmenopausal women with history of fragility fractures; DM refers to type 2 diabetic postmenopausal women without history of fracture (low-fracture risk group) and DMFx refers to type 2 diabetic postmenopausal women with a positive history of fragility fractures after the onset of T2D (high-fracture risk group)

n.a. not applicable

^a $p < 0.05$ Co versus Fx; ^b $p < 0.05$ DM versus DMFx; ^c $p < 0.05$ Co versus DM; ^d $p < 0.05$ DMFx vs. Fx; ^e $p < 0.05$ Co vs. DMFx

* p values listed in the right column were calculated using either Pearson's χ^2 test or independent t -test as appropriate.

0.038). DM subjects also tended to have thicker cortices than Co subjects in all femoral subregions, but these trends were marginally nonsignificant (neck cvol/ivol: +6.3 %, $p=0.058$; trochanteric cvol/ivol: +5.6 %, $p=0.062$; total femur cvol/ivol: +5.6 %, $p=0.063$).

Serum sclerostin levels

Results of serum sclerostin levels per group are plotted in Fig. 1a. Sclerostin concentrations were highest in the DMFx group. DMFx subjects had +31.4 % higher

serum sclerostin levels than non-fractured DM subjects ($p=0.013$). Moreover, DMFx women showed +25.2 % higher serum sclerostin levels than Fx subjects ($p=0.033$) and +22.4 % higher sclerostin concentrations than nondiabetic Co subjects ($p=0.028$).

Discussion

In this study, we aimed to investigate volumetric bone density, bone geometry features, as well as serum

Table 2 QCT-derived, volumetric bone mineral density (vBMD) and bone geometry parameters of the left proximal femur in nondiabetic postmenopausal women with (Fx) and without (Co) history of fragility fractures and in fracture-free type 2 diabetic (T2D) postmenopausal women (DM; assigned low-fracture risk T2D group) and in T2D

postmenopausal women with a positive history of fragility fractures after the onset of T2D (DMFx, assigned high-fracture risk T2D group). Data are expressed as unadjusted means \pm SD. Min neck CSA=minimal cross-sectional area of the femoral neck

	Co (n=20)	Fx (n=18)	DM (n=20)	DMFx (n=17)
Volumetric Bone Mineral Density (vBMD)				
Femoral Neck				
Integral Neck vBMD (g/cm ³)	0.282 \pm 0.023	0.277 \pm 0.023	0.306 \pm 0.026 ^c	0.281 \pm 0.036 ^b
Trabecular Neck vBMD (g/cm ³)	0.104 \pm 0.042	0.071 \pm 0.028	0.119 \pm 0.042	0.080 \pm 0.041 ^b
Cortical Neck vBMD (g/cm ³)	0.548 \pm 0.030	0.567 \pm 0.032	0.570 \pm 0.040	0.572 \pm 0.037
Trochanteric Region				
Integral Trochanteric vBMD (g/cm ³)	0.268 \pm 0.028	0.253 \pm 0.029	0.287 \pm 0.021	0.267 \pm 0.027 ^b
Trabecular Trochanteric vBMD (g/cm ³)	0.128 \pm 0.034	0.112 \pm 0.022	0.139 \pm 0.019	0.113 \pm 0.023 ^b
Cortical Trochanteric vBMD (g/cm ³)	0.528 \pm 0.022	0.526 \pm 0.034	0.549 \pm 0.035	0.546 \pm 0.033
Total Proximal Femoral Region				
Integral Femoral vBMD (g/cm ³)	0.270 \pm 0.027	0.257 \pm 0.027	0.289 \pm 0.020	0.268 \pm 0.027 ^b
Trabecular Femoral vBMD (g/cm ³)	0.125 \pm 0.034	0.108 \pm 0.021	0.137 \pm 0.021	0.109 \pm 0.024 ^b
Cortical Femoral vBMD (g/cm ³)	0.522 \pm 0.020	0.525 \pm 0.032	0.542 \pm 0.032	0.543 \pm 0.032
Bone geometry				
Proximal Femoral Bone Volume (cm ³)	90.40 \pm 18.25	88.74 \pm 14.05	80.01 \pm 17.29 ^c	88.48 \pm 18.89
Min Neck CSA (cm ²)	11.05 \pm 1.73	10.45 \pm 1.06	10.20 \pm 1.34 ^c	10.40 \pm 1.36 ^d
Cortical Femoral Thickness (FEMcvol/ivol) *	0.341 \pm 0.028	0.329 \pm 0.023	0.360 \pm 0.023 ^e	0.339 \pm 0.025 ^b
Cortical Neck Thickness (NECKcvol/ivol) *	0.384 \pm 0.032	0.384 \pm 0.029	0.408 \pm 0.042	0.382 \pm 0.042
Cortical Trochanteric Thickness (TROCHcvol/ivol) *	0.322 \pm 0.029	0.309 \pm 0.023	0.340 \pm 0.024 ^e	0.323 \pm 0.024

^a p <0.05 Co versus Fx; ^b p <0.05 DM versus DMFx; ^c p <0.05 Co versus DM; ^d p <0.05 Co versus DMFx; ^e p <0.05 Fx versus DM

*cvol/ivol=cortical volume to integral volume as a measure of cortical thickness

sclerostin levels in two specific T2D subsets with different fracture risk profiles. We included a high-risk group (DMFx), as indicated by a positive history of one or more fragility fractures after the onset of T2D, and a low-risk group (DM=T2D) which was reportedly fracture-free and compared their results to nondiabetic controls with and without fragility fractures.

One of our main findings was that T2D postmenopausal women with (DMFx) and without (DM) fractures displayed discordant proximal femoral volumetric BMD (vBMD) measurements. In general, DMFx subjects exhibited significantly lower integral vBMD than DM subjects at all femoral sites. Interestingly, this lower vBMD in DMFx subjects was mainly attributable to a reduction in trabecular vBMD. So far, there has been only one study examining the bone structural basis in a T2D diabetic postmenopausal cohort via quantitative computed

tomography of the femur [11]. In this study, Melton et al. assessed the three-dimensional BMD in 28 postmenopausal T2D women, but did not consider fracture status. Consistent with our results for the fracture-free diabetic group (DM), he found a higher femoral neck BMD in T2D women compared to nondiabetic controls. He also observed a slightly higher trabecular BMD in T2D relative to nondiabetic controls [11]. Extending on his findings, our study design included an additional T2D group susceptible to fragility fractures (DMFx) which exhibited significantly lower volumetric BMD at the femur and in particular, lower trabecular BMD than the low-risk DM group. Consistent with our findings at the central skeleton, we previously observed trends for lower trabecular BMD and trabecular number in the DMFx group at the distal tibia and radius measured by HR-pQCT [8]. In summary, our data suggest a well-preserved or even hypertrophic

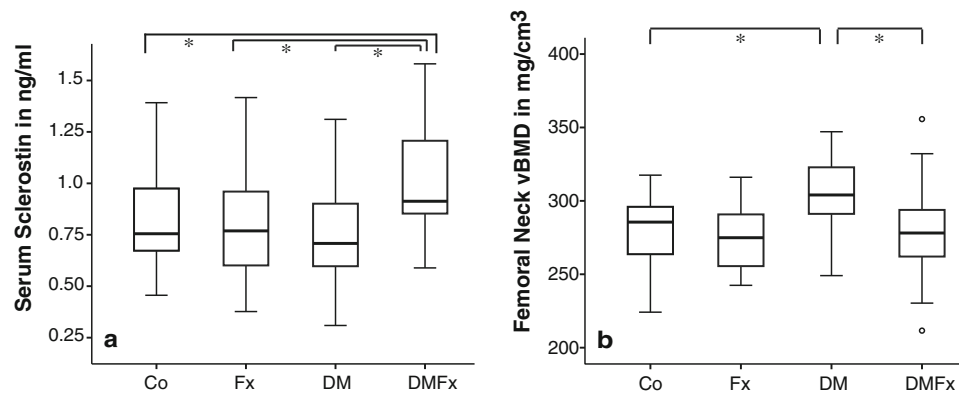


Fig. 1 Serum sclerostin levels in ng/mL (**a**) and integral volumetric bone mineral density (vBMD) of the femoral neck in mg/cm^3 (**b**) by group. *Co*, nondiabetic postmenopausal women without history of fracture; *Fx*, nondiabetic postmenopausal women with history of fragility fractures; *DM*, type 2 diabetic (T2D) postmenopausal women without history of

skeletal fragility fractures (low-fracture risk T2D group); *DMFx*, T2D postmenopausal women with a positive history of fragility fractures after the onset of T2D (low-fracture risk T2D group). The asterisks represent significant intergroup differences ($p < 0.05$)

trabecular volumetric bone mineral density in the low-risk DM group and is indicative of a diminished trabecular bone quality in the high-risk DMFx group. In addition, our data show for the first time that femoral trabecular vBMD by QCT is an important skeletal feature that can distinguish between low- and high-fracture risk T2D postmenopausal women.

Apart from the observed differences in volumetric bone mineral density, our low-risk (DM) and high-risk (DMFx) diabetics also differed in their femoral bone geometry. DMFx subjects had significantly thinner femoral cortices and by trend, larger femoral bone volume compared to DM subjects. At the same time, DM subjects tended to have thicker cortices and significantly smaller bones than nondiabetic controls. In keeping with our results for the low-risk (DM) group, previous studies have also reported thicker femoral cortices [11] and smaller, but denser bones in T2D individuals when compared to nondiabetic controls [30]. These findings were mainly attributed to a reduced bone turnover in T2D [31]. However, these studies did not take into consideration the fracture status. Here, we report that thicker cortices are not a feature of T2D per se, but are a characteristic of the low-risk DM group. The higher BMD and thicker cortices might protect the DM group against fractures and put it at low fracture risk. In contrast, our high-risk DMFx group exhibited significantly thinner cortices and tended to have a larger femoral bone volume than low-risk DM patients. Patterns of cortical thinning have been described in nondiabetic populations in the context of functional adaptation as it occurs in age-related bone loss [32]. In this study, DMFx subjects were slightly, but insignificantly older than DM subjects. However, the results presented here have been adjusted for age, indicating that the observed geometric differences represent an age-independent effect. Although T2D bone

disease has been generally linked to low bone turnover, the geometric features observed in our high-risk DMFx group give reason to speculate that in these patients, bone turnover might be imbalanced towards impaired periosteal apposition and continued net endocortical resorption. However, additional analysis of serum bone turnover markers showed low, yet similar levels of bone resorption (CTX I) and bone formation (P1NP) in both diabetic groups (DM and DMFx). This finding is contrary to our above proposed theory of imbalanced bone turnover in the DMFx. At first glance, this finding also contradicts the findings of Ardawi et al. who reported increased CTX I serum levels in T2D women with vertebral fractures compared to non-fractured T2D individuals [14]. However, Ardawi et al. did not assess vertebral fracture age. Thus, his findings of elevated CTX levels in T2D with vertebral fractures may have reflected increased bone resorption in the course of fracture healing.

At this point, it remains unclear how our findings of low bone turnover markers in the DMFx group, and especially of low bone resorption (CTX I), can be explained in synopsis with the observed differences in femoral vBMD and bone geometry. The failure to detect significant differences in CTX I levels between DM and DMFx groups may be due to the relatively small sample size ($n=19$, respectively 20 per group). In addition, osteoclast-mediated bone resorption is a multistep process which involves the dissolution of the inorganic phase by acidification of the extracellular microenvironment followed by proteolytic cleavage of the organic collagen type I matrix by proteases such as cathepsin K and matrix metalloproteinases (MMP-9 and MMP-13) [33]. In other organs, such as the kidney, T2D has been linked to a decreased proteolytic activity of matrix metalloproteinases [34]. The effect of T2D on bone matrix metalloproteinases has yet to be determined. Furthermore, it is to date unclear if

the CTX I ELISA assay that we used is designed to also detect glycated CTX I products. High blood glucose levels cause glycation of circulation proteins including peptides such as CTX I. Our assay may have therefore missed out on quantifying the full amount of CTX I available in the serum of DMFx patients. According to Vashishth et al., osteoclasts are not able to degrade collagen type 1 that has been glycated over time as a result of glucose-induced nonenzymatic glycation (advanced glycation end product (AGE) collagen or AGE-distorted collagen) [35]. Since AGEs accumulate with longer duration of T2D, and DMFx subjects had a significantly longer history of T2D than DM subjects, it is possible that the CTX I assay in DMFx patients may have captured only the amount of non-distorted degradable collagen, irrespective of the true amount of collagen type 1 deposited in the bone extracellular matrix.

In summary, the differences in femoral geometry and thickness that we detected between DM and DMFx subjects in this study cannot at the moment be explained by the groups' bone turnover profiles. There may be other unique yet unidentified mechanisms acting such as vascular network expansion that might drive the higher bone fragility in the DMFx group.

In addition to the discrepancies seen in bone mineral density and geometry, we also observed differences in serum sclerostin concentrations between low- and high-risk T2D women. Serum sclerostin levels were significantly higher in the DMFx group. This remained true, when comparing DMFx women to nondiabetic controls with (Fx) and without (Co) fractures. To date, two studies have investigated the association of serum sclerostin and T2D postmenopausal women in the setting of fracture and found higher sclerostin levels in T2D women with vertebral fractures compared to controls [13, 14]. However, unlike our study, these studies were limited to vertebral fractures [13, 14] and lacked a second nondiabetic control group with fragility fractures (Fx). In addition, they did not evaluate fracture age or screen for occult vertebral fractures. As serum sclerostin levels have been found to be elevated during the course of fracture healing, these previous diabetic sclerostin reports run the risk of being confounded by recent bone fracture [15]. In our study, fractures in both fracture groups (DMFx and Fx) were generally remote (mean fracture age 3.2 to 3.3 years) and had a similar prevalence. Moreover, fractures in both groups were sustained at similar skeletal regions, supporting the homogeneous fracture profile of the DMFx and Fx groups. As we did not detect a parallel increase of serum sclerostin levels in our Fx and DMFx groups, this minimizes the possibility that our diabetic serum sclerostin measurements have been confounded by fracture repair effects. Unlike all previous studies, our high-risk DMFx group consisted of women with fractures from

various skeletal sites, including the vertebra, humerus, wrist, and ankle. This is consistent with the most prevalent sites of fragility fractures reported for T2D postmenopausal women in large epidemiologic studies [3–5]. By doing so, we sampled a diabetic patient subset with generally increased bone fragility as it can be typically found in routine clinical practice and did not restrict our enrollment to patients of one fracture type. Confirming and extending on the existing literature, our results are the first to establish a significant association between elevated serum sclerostin levels and a higher fragility fracture status in T2D postmenopausal women irrespective of skeletal fracture site and not confounded by fracture age. Our results suggest that elevated serum sclerostin levels might be a helpful tool in differentiating low- and high-risk T2D patients.

Taken together, we found differences in volumetric femoral bone density, bone geometry, and serum sclerostin levels between T2 diabetics with and without prevalent fragility fractures. Integral vBMD and cortical thickness, while decreased in DMFx subjects, were higher in DM than in controls. These results seem to suggest that DM subjects without fractures may be protected against bone fractures through beneficial bone quality features. By contrast, DMFx subjects seem to be a specific T2D subset at higher risk for fractures despite a volumetric bone mineral density and geometry comparable to that of nondiabetic fracture-free controls.

At this moment, it remains unclear what factors make the DMFx group so different from the DM group. From a clinical perspective, DM and DMFx patients exhibited highly similar characteristics (Table 1). Kidney function, bone homeostatic hormones (PTH, total serum 25-hydroxyvitamin D, calcium), and glycemic control (as expressed by HbA_{1c} levels and fasting glucose) were comparable between DM and DMFx subjects. Moreover, the racial composition was similar between the two groups. Both groups reported similar use of antidiabetic medications such as insulin and metformin. The main clinical difference was a longer duration of diabetes in the DMFx compared to DM subjects (13.6 years vs. 8.4 years, respectively). In combination with poorly controlled HbA_{1c}, these findings make it reasonable to speculate that DMFx subjects were exposed to the detrimental effects of hyperglycemia for a longer time than DM subjects. The effect of chronic hyperglycemia at the structural and cellular bone level is an ongoing subject of research [31, 36]. Advanced glycosylation end products (AGEs) might aggregate over time in the bone matrix or in the osteocytes and alter osteocyte function [37]. Given the tight connectivity between the vascular and the osseocanalicular systems [38], glucose may also accumulate in the canalicular liquid and alter bone cell function [39, 40]. In this context, it seems remarkable that osteocyte mechanosensation was found reduced in an *in vitro* high-

glucose environment [41]. Although our study is cross-sectional by design and therefore not suitable to draw causal inferences, it seems noteworthy that high serum sclerostin levels coincided with low bone mineral density in the DMFx group, while lower sclerostin levels coincided with higher bone mass in the DM group. As high serum sclerostin levels are normally found in mechanically unloaded or bed-ridden patients [42, 43], the high sclerostin findings in the DMFx group could be indicative of an osteocyte “pseudo-unloading” in this patient group that culminates in diminished bone formation. However, further studies are needed to investigate osteocyte function in the setting of diabetes on a cellular level. Another potential mechanism that could help explain the unique findings in the DMFx group, include the concept of diabetes-induced accelerated bone aging [44]. In line with this theory, our DMFx cohort exhibited bone geometry features and serum sclerostin levels that exceeded patterns reached by nondiabetic postmenopausal controls in their eighth decade of life [45]. These findings are complemented by the observations from our previous HR-pQCT study in which the DMFx group exhibited a peripheral cortical bone phenotype that was comparable to those of nondiabetic 80-year-old women [8].

Our study has several limitations. First, this study was cross-sectional by design and included only 80 patients, which limits our ability to consider other potential influencing factors. In addition, our patients emanated from racially diverse backgrounds. However, racial composition was similar among groups, in particular among the DM and DMFx groups (Table 1), and we adjusted for race in our statistical analyses. Another limitation of our study is that we are not able to determine whether alterations in sclerostin or bone mass manifested prior to fracture. One might argue that the observed changes in vBMD and geometry in the DMFx group might be the result of a prior fracture. However, all patients were fully mobile, and by inclusion requirements, patients were not allowed to have experienced immobilization periods of 3 months or longer. Furthermore, 70 % of the reported fractures in the DMFx group were sustained either at the upper extremity or at the contralateral, non-measured lower extremity, and the remainder 30 % ipsilateral fractures were on average 2 years old. These findings decrease the chances that the vBMD results observed in the DMFx group are secondary to fracture.

Recent studies suggest that serum sclerostin measurements depend upon the immunoassay used, therefore introducing additional interstudy-variability and reduced interstudy-comparability [46]. Nevertheless, for our analyses, we deployed a single, widely utilized, well-validated serum sclerostin kit system rendering between-group comparisons and interstudy-comparability more reliable.

Another shortcoming of our study is that we had no biopsies to validate our findings at the tissue level. However, given

that T2D individuals are specifically prone to infections, bone biopsies may not be feasible or ethically appropriate to obtain in the setting of larger-scale studies.

Summary and conclusion

In this study, we investigated volumetric, QCT-derived bone parameters of the proximal femur and serum sclerostin levels in two specific T2D subsets with different fracture risk profiles (low vs. high fracture risk) and compared them to nondiabetic controls with and without fragility fractures. We found that T2D postmenopausal women without fractures (low-risk DM group) and T2D women with a positive history of fragility fractures (high-risk DMFx group) differed in their femoral volumetric bone mineral density, bone geometry, and serum sclerostin concentrations. While the low-risk DM group exhibited more favorable bone properties compared to nondiabetic controls, the high-risk DMFx group did not. Instead, this high-risk DMFx group showed bone parameters similar to nondiabetic controls without fractures. Our data suggest that volumetric bone parameters by QCT and serum sclerostin levels can identify T2D individuals at high risk of fracture and might therefore show promise as clinical tools for fracture risk assessment in type 2 diabetics. Although our study was cross-sectional and observational by design, our coincidental findings of elevated sclerostin levels and low volumetric bone mineral density in the DMFx group raise the hypothesis that these high-risk T2D postmenopausal women may be particularly promising candidates to receive anti-sclerostin antibody treatment. However, future diabetic-specific, randomized clinical trials are needed to test if this hypothesis holds true. In addition, large-scale prospective studies should further examine the evolution of diabetic bone disease and should establish diabetes-specific reference databases for QCT-derived vBMD parameters and for circulating sclerostin levels.

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Conflict of interest None

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