Similarities in trabecular hypertrophy with site-specific differences in cortical morphology between men and women with type 2 diabetes mellitus

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Abstract

The goal of our study was to investigate interactions between sex and type 2 diabetes mellitus (T2DM) with regard to morphology of the peripheral skeleton. We recruited 85 subjects (mean age, 57±11.4 years): women with and without T2DM (n = 17; n = 16); and men with and without T2DM (n = 26; n = 26). All patients underwent high-resolution, peripheral, quantitative, computed tomography (HR-pQCT) imaging of the ultradistal radius (UR) and tibia (UT). Local bone geometry, bone mineral density (BMD), and bone microarchitecture were obtained by quantitative analysis of HR-pQCT images. To reduce the amount of data and avoid multi-collinearity, we performed a factor-analysis of HR-pQCT parameters. Based on factor weight, trabecular BMD, trabecular number, cortical thickness, cortical BMD, and total area were chosen for post-hoc analyses. At the radius and tibia, diabetic men and women exhibited trabecular hypertrophy, with a significant positive main effect of T2DM on trabecular number. At the radius, cortical thickness was higher in diabetic subjects (+20.1%, p = 0.003). Interestingly, there was a statistical trend that suggested attenuation of tibial cortical hypertrophy in diabetic men (cortical thickness, $p_{interaction}$ = 0.052). Moreover, we found an expected sexual dichotomy, with higher trabecular BMD, Tb.N, cortical BMD, Ct.Th, and total area in men than in women (p$\leq$ 0.003) at both measurement sites. Our results suggest that skeletal hypertrophy associated with T2DM is present in men and women, but appears attenuated at the tibial cortex in men.

Introduction

Fragility fractures are increasingly recognized as a skeletal secondary complication of type 2 diabetes mellitus (T2DM) [1–4]. Although subjects with T2DM carry a high risk of falls due to impaired eyesight, polyneuropathy, and fatty atrophy of the musculature, these factors have been shown to be insufficient to explain the disproportionately high rate of fractures [5]. Currently, the pathogenesis of diabetic bone disease and associated fragility fractures is not sufficiently understood. Bone mineral density (BMD)—as measured by dual-energy, x-ray absorptiometry (DXA) or quantitative computed tomography (QCT)—is typically high to normal or only mildly reduced in patients with T2DM [6]. Potential explanations for the paradoxical positive association of high BMD and fragility fractures include micro-architectural and matrix-based causes, such as cortical porosity [7, 8], and deposition of advanced glycation end products (AGEs) [9]. On a cellular level, diabetic bone disease is characterized by low bone turnover [10, 11], and there are also numerous suggestions of a significant imbalance of the WNT/SOST/PTH pathway, possibly through osteocyte dys-function [12, 13].

High-resolution, peripheral, quantitative computed tomography (HR-pQCT) has been used by several researchers to study bone geometry, compartment-specific volumetric bone mineral density (vBMD), and bone microarchitecture—including cortical porosity—of the ultradistal extremities in subjects with T2DM [7, 8, 11, 14, 15].

In the past decade, HR-pQCT has been validated with bone biopsies (i.e., the gold standard method for the quantitative assessment of bone microarchitecture), DXA, and QCT of the axial and peripheral skeleton [16–19]. HR-pQCT has provided key insights into the morphology and pathophysiology of diabetic bone disease in elderly subjects. Poor cortical bone quality, particularly high cortical porosity, has been reported by several researchers ([7, 8, 14]. At the same time, trabecular BMD and trabecular microarchitecture, as determined by HR-pQCT, appear to be stable or even relatively high in subjects with T2DM [7, 15]. The above-mentioned microstructural findings have been well documented in postmenopausal diabetic women, but only few studies have investigated bone microarch-itecture in men with T2DM [15]. Recently, Paccou et al. reported unfavorable associations between bone quality and T2DM in men. Specifically, they found cortical bone quality to be pathologically altered in both elderly men and women, with more pronounced findings in men.

In the general, non-diabetic population, sex-specific differences in bone geometry, bone mineral density (BMD), and bone microarchitecture are well recognized and viewed as the causes for the clinical differences in fracture prevalence between men and women [20, 21]. Using HR-pQCT, it has been confirmed that young men have larger bones with higher tra-becular bone volume and higher trabecular thickness than young women [22]. With aging, trabecular bone volume decreases proportionately in both men and women, but trabecular microarchitecture remains better preserved in men. Cortical thickness appears to be com-parable in younger and middle-aged men and women, but over time, especially at older ages, thickness decreases are larger in women [22]. Interestingly, the cross-sectional area of long bones increases with normal aging by periosteal apposition in both sexes [20, 23, 24]. Of importance, metabolic bone diseases can alter this physiologic pattern (e.g., as seen in male idiopathic osteoporosis) [25]. Considering the importance of age- and sex-specific skeletal differences for the modulation of fracture risk in the general population, and accumulating evidence for impaired bone quality in women and men with T2DM, we designed a study to investigate the interactions between sex and T2DM in the peripheral adult skeleton.

Patients and methods

Subjects

Thirty-three women and 52 men were recruited into one of four groups: women with type 2 diabetes mellitus (WT2DM; n = 17); women without type 2 diabetes mellitus (WCo; n = 16); men with type 2 diabetes mellitus (MT2DM; n = 26); and men without type 2 diabetes mellitus (MCo; n = 26). Diabetic subjects were recruited from the Endocrine Outpatient Unit of the Department of Internal Medicine III of the Medical University of Vienna, Austria. Healthy women (WCo) were recruited by the VINFORCE study group/Department of Internal Medicine II, St. Vincent Hospital Vienna, Austria. Healthy (i.e., non-diabetic) men (MCo) were recruited as part of the STRAMBO study, an epidemiologic cohort study conducted by the Université de Lyon, France [26]. The study was approved by the ethics committees of the Medical University of Vienna, the St. Vincent Hospital Vienna, and the Université de Lyon. All participants gave written, informed consent.

Inclusion criteria for all subjects were age 40–75 years and written, informed consent. Diabetic men and women had to be treated with standard antidiabetics and have HbA$_1$C values ranging from 6–10%. Women had to be postmenopausal.

Current or previous use of rosiglitazone, steroids, antiepileptic drugs, vitamin K antagonists, bisphosphonates, fluorides, PTH, strontium ranelate, raloxifen, denosumab, and calcitonin were defined as exclusion criteria. Severe hepatic and/or renal failure, active malignancy, or a history of malignancy, and pregnancy excluded subjects from study participation.

HR-pQCT imaging

All subjects underwent HR-pQCT imaging of the non-dominant ultradistal radius and the left tibia (XtremeCT; Scanco Medical AG, Brüttisellen, Switzerland). Diabetic subjects and female non-diabetic controls were scanned at the Medical University of Vienna, Austria. Male non-diabetic controls underwent imaging in Lyon, France. To highlight cross-calibration validity and exclude a multi-center bias, an additional seven non-diabetic men were scanned and analyzed in Vienna. The two HR-pQCT scanners (first-generation devices) used in this study were cross-calibrated, as published by Burghardt et al. [27]. The identical standard *in vivo* protocol [22, 28] was used in both Vienna and Lyon, and was defined by the following settings: 60kVP; 900 µA; and 100 ms integration time. In case of local fracture history, the contralateral extremity was scanned. After the acquisition of a local scout view, a reference line was placed on the joint surface of the radius and tibia. From the reference line, fixed offsets were used to define the scan region (radius offset: 9.5mm; tibia offset: 22.5mm). The final scan volume covered a length of 9.02mm, corresponding to 110 slices. The nominal resolution of HR-pQCT images was isotropic (82 x 82 x 82 µm). The effective dose was < 4 µSv, the scan time was < 3 minutes per scan.

Image analysis

For quality control, visual semiquantitative motion grading was performed prior to quantitative image analysis. According to the criteria established by Pialat et al., only scans reaching image-quality grades 1–3 were used for quantitative analyses [29]. HR-pQCT images were segmented semi-automatically and analyzed with the standard protocol provided by the manufacturer of the device. Semiautomatic contours were reviewed for accuracy, and manual adjustment was limited to clear contour deviations from the anatomical periosteal boundaries. Volumetric BMD and morphometric parameters were obtained for trabecular and cortical bone [30]. Trabecular bone volume fraction (BV/TV) was derived from trabecular BMD using

an assumed density of 100% for compact mineralized bone (1200 mg HA/cm^3) and background marrow (0 mg HA/cm^3). Trabecular number and the standard deviation of inter-trabecular distances were calculated by distance transformation [31]. Trabecular thickness and trabecular separation were derived from trabecular BMD and trabecular number [32]. Cortical thickness was obtained by annular approximation [30, 33].

In addition, HR-pQCT images were reviewed for the presence of vascular calcifications, which were defined as linear or tubular hyperdensity zones of circular, semi-circular, or crescent-like shape, which corresponded to the anatomical territory of the anterior tibial artery, the posterior tibial artery, the radial artery, the ulnar artery, the interosseous branches, or smaller intramuscular or subcutaneous arterioles [34]. Skin calcifications or other non-vascular soft tissue calcifications were not included.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics version 22. Metric data were described using means +/- standard deviation (SD) if normally distributed or, in case of highly skewed data, medians [min; max]. Categorical data were presented using absolute numbers and percentages. As there was a large number of highly correlated measures obtained for the tibia and radius, principal axis factor analyses (FA) was used to reduce the number of necessary statistical tests and to minimize an error of the first type. Only parameters with the highest loading within a factor were used for subsequent analyses.

In order to test the moderation effect of sex on the effect of diabetes, two-way analyses of variance were used.

Unpaired student t-tests were used to determine differences in age and laboratory data. To compare the percentage of male and female patients with and without calcifications, a Fisher's exact test was applied. Due to the limited sample size, we refrained from using multiplicity corrections to avoid decreasing power. In order to rule out a multi-center bias, a small subset of non-diabetic male participants from Lyon was compared with non-diabetic men from Vienna who were not part of the original study. A p-value equal to or below 5% was considered to indicate significant results.

Results

Subject characteristics

Demographics and clinical characteristics are given in Table 1. There were no significant differences in age. Laboratory data were available only from subjects with T2DM and not from non-diabetic controls. Comparing diabetic men and women, there were no significant differences in fasting blood glucose (p = 0.804), HbA1c-levels (p = 0.411), serum insulin (p = 0.730), PTH (p = 0.126), and 25-OH-vitamin D (p = 0.074). Serum creatinine was higher in diabetic men, but remained within normal limits (1.0 mg/dl; p = 0.013). As determined from visual assessment of HR-pQCT scans by a board-certified radiologist (JMP), there were no significant differences between lower leg vascular calcification frequencies in diabetic men (50% with calcifications) and women (42.4% with calcifications, p = 0.607). Likewise, at the upper extremity, there were no significant differences between vascular calcification frequencies in diabetic men (19.2% with calcifications) and women (9.1% with calcifications, p = 0.284).

HR-pQCT

For all four subject groups (i.e., WT2DM, WCo; MT2DM, MCo), means and standard deviations of HR-pQCT parameters are given in Table 2. Moreover, Table 2 provides relative

Table 1. Demographics and clinical characteristics.

	WT2DM (n = 17)	WCo (n = 16)	MT2DM (n = 26)	MCo (n = 26)
Age (years)	58 ± 14.29	55 ± 8.10	57 ± 11.11	57 ± 11.19
Height (m)	1.62 ± 0.07	1.69 ± 0.06	1.73 ± 0.06	1.71 ± 0.09
BMI (kg/m^2)	33.47 ± 4.95	24.19 ± 3.50	29.65 ± 4.35	26.01 ± 2.92
HbA1c (%)	7.91 ± 1.52	n/a	7.54 ± 1.15	n/a
Blood glucose	148.21 ± 45.05	90 ± 6.93 (n = 3)	144.68 ± 38.80	n/a
Insulin	9.85 ± 5.25	n/a	10.82 ± 8.83	n/a
PTH	50.90 ± 27.05	n/a	40.05 ± 15.09	n/a
25-OH-Vit D	33.34 ± 15.96	n/a	47.38 ± 23.79	n/a
Calcitriol	29.31 ± 12.34	n/a	41.79 ± 13.06	n/a
Folic acid	18.91 ± 8.47	n/a	20.50 ± 7.11	n/a
Vitamin B12	282.31 ± 193.92	n/a	299.35 ± 182.87	n/a
Calcium	2.40 ± 0.10	n/a	2.46 ± 0.05	n/a
Creatinine	0.83 ± 0.15	n/a	0.99 ± 0.21	n/a
Uric acid	5.53 ± 0.94	n/a	5.60 ± 1.28	n/a
Medical history n (%)				
Never used tobacco	8 (53.3%)	n/a	8 (33.3%)	n/a
Hypertension	14 (93.3%)	n/a	16 (66.6%)	n/a
Duration of T2DM (in years)	8.21 ± 5.82	n/a	7.71 ± 7.12	n/a
Current medication n (%)				
Metformin	11 (73.3%)	n/a	19 (79.17%)	n/a
Sulfonylureas	10 (66.6%)	n/a	9 (37.5%)	n/a
Insulin-sensitizer	0	n/a	0	n/a
Insulin	1 (6.6%)	n/a	1 (4.17%)	n/a
DPP-4	4 (26.6%)	n/a	6 (25%)	n/a
Pioglitazone	7 (46.6%)	n/a	14 (58.3%)	n/a

differences in HR-pQCT parameters between subjects with and without T2DM (with separate analyses for men and women). Fig 1 (radius) and Fig 2 (tibia) illustrate bone morphology in men and women with and without T2DM.

Data reduction by factor-analysis yielded four factor groups ('first level factors') identical for the radius and tibia parameters. Detailed results for factor-analysis are given in Table 3. Based on the highest factor weight within factor groups (Table 3), four representative HR-pQCT parameters were chosen from each group for selective post-hoc testing. Trabecular BMD (chosen from the factor-group that contained trabecular density, trabecular bone volume fraction, and trabecular thickness), trabecular number (chosen from the factor-group that contained trabecular number, trabecular separation, and trabecular heterogeneity), cortical thickness (chosen from the factor-group that contained cortical area, total density, cortical density, and cortical thickness), and total area (chosen from the factor-group that contained total area, trabecular area, and cortical perimeter). Due to independent information provided by cortical BMD, additional post-hoc testing was performed for cortical BMD as a fifth parameter. The post-hoc choice of HR-pQCT parameters was identical for the radius and tibia (Table 3).

At the ultradistal radius, trabecular BMD (+25.8%, p<0.001), trabecular number (+7.4%, p = 0.003), cortical thickness (+21.9%, p = 0.002), and total area (+36.2%, p<0.001) were significantly higher in men than in women. There were no significant sex-specific differences in cortical BMD. Trabecular number (+14.7%, p<0.001) and cortical thickness (+20.1%,

Table 2. HR-pQCT parameters of the ultradistal radius and the ultradistal tibia in men and women with and without type 2 diabetes mellitus.

	WT2DM (n = 17)	WCo (n = 16)	Mean Relative Difference (%)	MT2DM (n = 26)	MCo (n = 26)	Mean Relative Difference (%)
Ultradistal Radius						
Total area (mm²)	266±73	247±48	+7.4%	346±50	380±76	-9%
Cortical area (mm²)	53.1±12.4	41.2±8.3	+29%	76.3±20.5	67.2±14.9	+13.6%
Trabecular area (mm²)	205±70	200±49	+2.6%	263±56	304±73	-13.3%
Total BMD (mgHA/mm³)	331±49	291±75	+14%	362±94	326±63	+10.9%
Cortical BMD (mgHA/mm³)	865±99	847±77	+2.2%	887±56	848±54	+4.6%
Trabecular BMD (mgHA/mm³)	158±30	143±36	+10.3%	193±53	187±36	+3.2%
Cortical thickness (mm)	0.79±0.21	0.64±0.17	+24%	0.96±0.30	0.80±0.19	+19.9%
Bone volume fraction (BV/TV, %)	0.13±0.02	0.12±0.03	+10.3%	0.16±0.04	0.16±0.03	+3.2%
Trabecular number (1/mm)	2.02±0.29	1.75±0.18	+15.6%	2.18±0.23	1.91±0.22	+14.4%
Trabecular thickness (mm)	0.07±0.01	0.07±0.01	-4.9%	0.07±0.02	0.08±0.01	-9.8%
Trabecular separation (mm)	0.44±0.08	0.51±0.08	-13.4%	0.39±0.05	0.45±0.07	-13.5%
Trabecular heterogeneity (mm)	0.19±0.05	0.22±0.05	-16.7%	0.17±0.015	0.19±0.04	-11.1%
Ultradistal Tibia						
Total area (mm²)	674±90	673±120	+0.2%	861±120	825±135	+4.4%
Cortical area (mm²)	109±23	83±15	+30.1%	148±35	147±32	+0.2%
Trabecular area (mm²)	555±103	593±106	-6.4%	714±126	672±131	+6.2%
Total BMD (mgHA/mm³)	290±42	246±51	+18%	312±64	316±55	-1.4%
Cortical BMD (mgHA/mm³)	835±72	807±62	+3.5%	872±55	881±55	-1.0%
Trabecular BMD (mgHA/mm³)	168±29	155±27	+8.9%	189±42	183±37	+2.7%
Cortical thickness (mm)	1.08±0.26	0.86±0.20	+25.9%	1.28±0.34	1.31±0.27	-2.1%
Bone volume fraction (BV/TV, %)	0.14±0.02	0.13±0.02	+8.5%	0.16±0.03	0.15±0.03	+2.8%
Trabecular number (1/mm)	2.03±0.35	1.63±0.23	+24.6%	2.26±0.29	1.81±0.26	+25%
Trabecular thickness (mm)	0.07±0.01	0.08±0.01	-12.1%	0.07±0.01	0.08±0.01	-18%
Trabecular separation (mm)	0.44±0.08	0.54±0.09	-19.4%	0.38±0.06	0.48±0.09	-20.8%
Trabecular heterogeneity (mm)	0.19±0.05	0.25±0.06	-22.3%	0.16±0.04	0.22±0.07	-28.3%

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p = 0.003) were significantly higher in subjects with T2DM than in non-diabetic subjects. We found a trend toward higher cortical BMD in subjects with T2DM (+3.6%, p = 0.076). Regarding total area, there was a trend toward a significant interaction between sex and T2DM (p = 0.074). For visualization of data (including interactions) and a complete list of p-values, please see Fig 3.
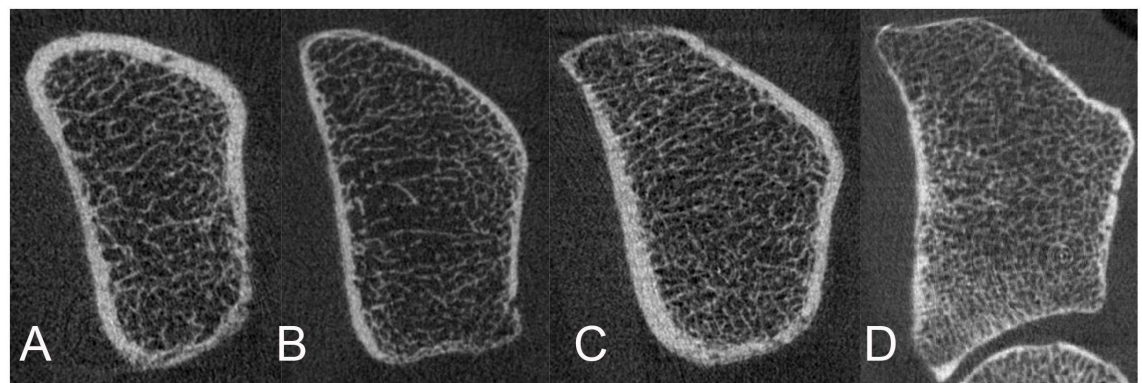

Fig 1. HR-pQCT of the ultradistal radius: Representative images. A) Woman with T2DM. B) Woman without T2DM. C) Man with T2DM. D) Man without T2DM.
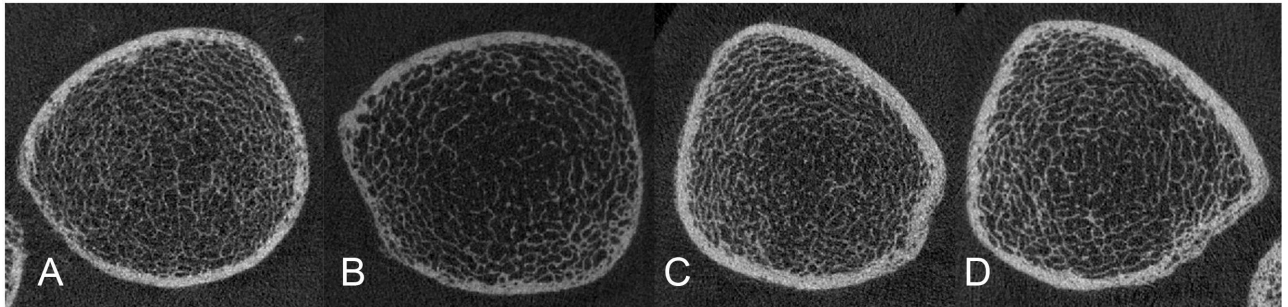
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Fig 2. HR-pQCT of the ultradistal tibia: Representative images. A) Woman with T2DM. B) Woman without T2DM. C) Man with T2DM. D) Man without T2DM.

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At the ultradistal tibia, trabecular BMD (+13.1%, p = 0.003), trabecular number (+10.4%, p = 0.002), cortical thickness (+34.1%, p<0.001), cortical BMD (+6.8%; p<0.001), and total area (+25.0%, p<0.001) were significantly higher in men than in women. T2DM had a significant association with trabecular number (+24.1%, p<0.001), but no significant associations with trabecular BMD, cortical thickness, cortical BMD, or total area. We found a trend toward

Table 3. Factor-analysis of HR-pQCT parameters of the ultradistal radius and the ultradistal tibia. Numbers in columns are factor loadings per parameter (in rows).

	FACTOR			
	1	2	3	4
	RADIUS			
Total area (mm^2)				.983
Cortical area (mm^2)			.878	
Trabecular area (mm^2)				.966
Total BMD (mgHA/mm^3)	.525		.730	
Cortical BMD (mgHA/mm^3)			.793	
Trabecular BMD (mgHA/mm^3)	.859	.407		
Cortical thickness (mm)			.939	
Bone volume fraction (BV/TV, %)	.856	.413		
Trabecular number (1/mm)		.957		
Trabecular thickness (mm)	.947			
Trabecular separation (mm)		-.914		
Trabecular heterogeneity (mm)		-.862		
	TIBIA			
Total area (mm^2)				.983
Cortical area (mm^2)			.852	
Trabecular area (mm^2)				.969
Total BMD (mgHA/mm^3)	.584		.710	
Cortical BMD (mgHA/mm^3)			.899	
Trabecular BMD (mgHA/mm^3)	.886			
Cortical thickness (mm)			.910	
Bone volume fraction (BV/TV, %)	.885			
Trabecular number (1/mm)		.930		
Trabecular thickness (mm)	.826	-.457		
Trabecular separation (mm)		-.909		
Trabecular heterogeneity (mm)		-.929		

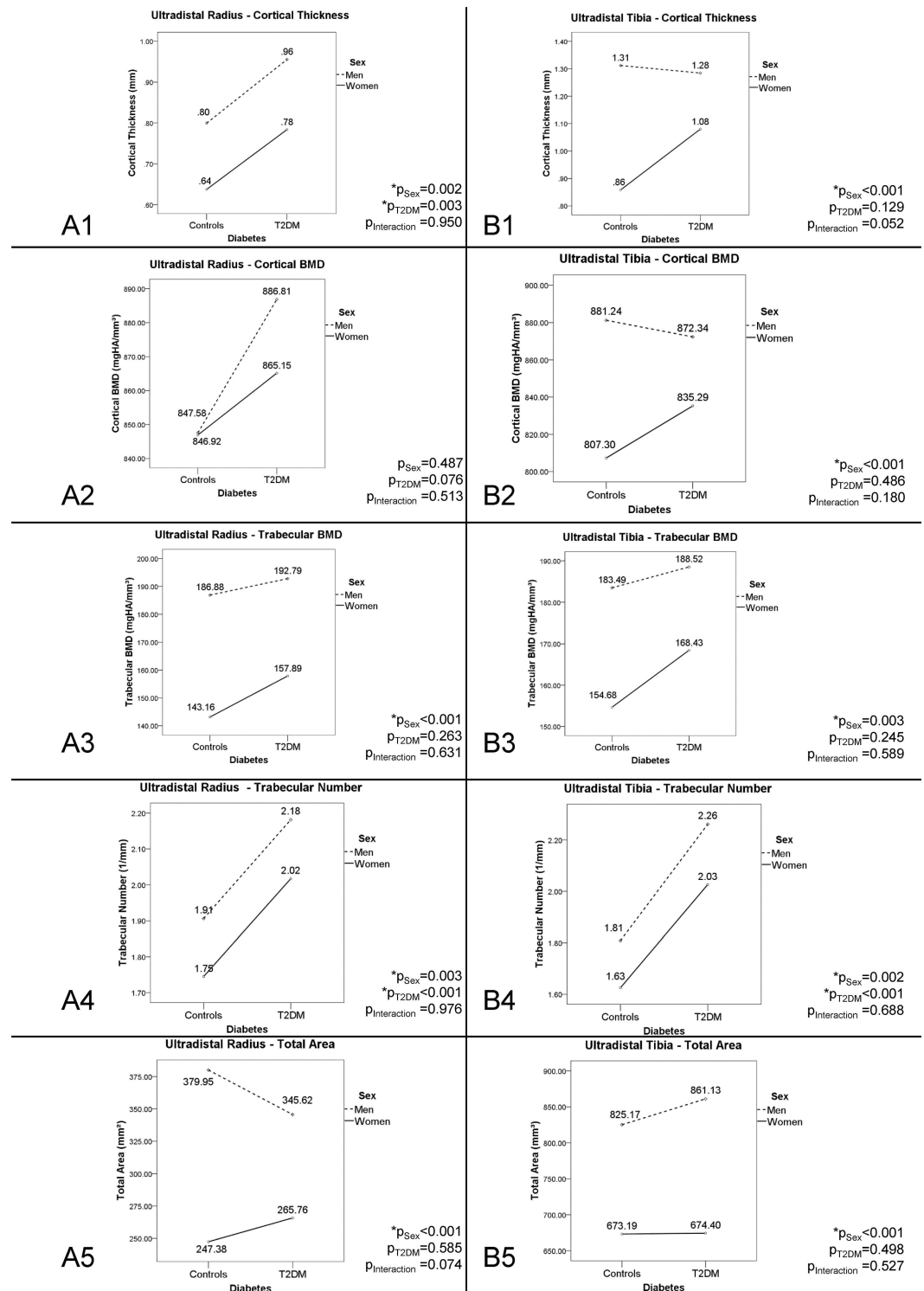https://doi.org/10.1371/journal.pone.0174664.t003

Fig 3. HR-pQCT of the ultradistal radius and tibia in men and women with and without type 2 diabetes mellitus. Means for cortical thickness (in mm), cortical BMD (in mgHA/mm^3), trabecular BMD (in mgHA/mm^3), trabecular number (in 1/mm), and total area (in mm^2). Dashed line represents mean differences between diabetic and non-diabetic men, and continuous line represents mean differences between diabetic and non-diabetic women. p-values are given for differences between men and women (p_{sex}), differences between subjects with and without T2DM (pT2DM), and interactions between sex and T2DM ($p_{interaction}$).

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Table 4. Comparison of male participants without T2DM from two European HR-pQCT sites.

	Healthy male participants (Austria)	Healthy male participants (France)	p-value
Demographics			
Age (years)	53.54 ± 7.12	54.04 ± 7.61	0.90
Height (m)	1.73 ± 6.43	1.74 ± 10.33	0.98
Weight (kg)	85 ± 22.34	76.86 ± 9.70	0.42
BMI (kg/m^2)	28.23 ± 5.26	25.7 ± 3.90	0.41
HR-pQCT–Ultradistal Radius			
Number of participants (n)	7	7	
Trabecular BMD (mgHA/mm^3)	190.96 ± 37.35	179.47 ± 19.20	0.48
Cortical BMD (mgHA/mm^3)	858.6 ± 73.44	865.37 ± 39.91	0.83
Cortical thickness (mm)	0.88 ± 0.21	0.86 ± 0.15	0.83
Trabecular number (1/mm)	2.18 ± 0.20	1.92 ± 0.21	0.36
HR-pQCT–Ultradistal Tibia			
Number of participants (n)	6	7	
Trabecular BMD (mgHA/mm^3)	186.48 ± 52.15	178.82 ± 33.30	0.69
Cortical BMD (mgHA/mm^3)	836.13 ± 75.55	897.0 ± 29.40	0.08
Cortical thickness (mm)	1.35 ± 0.29	1.33 ± 0.23	0.35
Trabecular number (1/mm)	1.97 ± 0.33	1.76 ± 0.18	0.35

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an interaction between sex and T2DM for cortical thickness (p = 0.052). There were no significant interactions between sex and T2DM with regard to trabecular BMD, trabecular number, cortical BMD, or total area at the tibia. For visualization of data (including interactions) and a complete list of p-values, please see Fig 3.

There were no significant differences in HR-pQCT parameters between an age-matched subset of healthy male participants scanned in Lyon, France, and healthy men from Vienna, Austria (Table 4), reflecting the validity of our multi-center approach.

Discussion

The majority of bone research in subjects with type 2 diabetes mellitus (T2DM) has been conducted in postmenopausal women. A recent publication reported pronounced cortical disease in elderly, male type 2 diabetics, but, overall, there are only limited data about bone microarchitecture in men with T2DM. It thus remains to be determined whether and how bone morphology differs between diabetic men and women. Specifically addressing the issue of potential interactions between sex and T2DM, we recruited men and women with and without T2DM and performed HR-pQCT imaging of the ultradistal extremities.

High BMD without associated fracture risk reduction is recognized as a clinical key feature of diabetic bone disease [35, 36]. In the present study, we found a high trabecular number in men and women with T2DM, thereby supporting the results of other HR-pQCT studies in diabetic subjects [7, 15]. Trabecular hypertrophy has also been reported in pre-diabetic subjects with insulin resistance [37]. Trabecular rarefactions and increases in trabecular heterogeneity appear to be a feature of the later stages of diabetes-related bone disease [8, 38].

With regard to cortical morphology, we found significantly thicker radial cortices in diabetic men and women. Cortical hypertrophy in subjects with T2DM is in keeping with recent QCT data in diabetic subjects without fragility fractures [12]. HR-pQCT studies in elderly, diabetic women without fractures have reported cortical thickening, but this did not reach statistical significance [8, 11]. Supporting this phenotypic concept, cortical hypertrophy has also been found in prediabetic women [37].

Cortical deficits, on the contrary, appear to depend on the clinical characteristics of participants (e.g., with/without prevalent fragility fractures [8], race [14], presence of microvascular disease [39]), and thus, vary in extent from study to study. Interestingly, we found cortical hypertrophy to be partially attenuated in men (Fig 1). Despite recent reports of an unfavorable cortical microarchitecture in elderly, diabetic men [15], this finding was unexpected in our relatively young cohort. The unfavorable association between cortical morphology and male sex is surprising because, in the general (i.e., non-diabetic) population, men are at lower risk for osteoporosis and osteoporotic fractures than same-aged women [20]. From a clinical perspective, it thus remains to be determined whether cortical deficits translate into biomechanical deficits and high fracture risk in diabetic men and women.

At the radius, sex and T2DM tended to interact in terms of cross-sectional bone size. Specifically, we found small bone size in diabetic men, but not in diabetic women. Small cross-sectional bone size has been previously described in subjects with T2DM [12] and insulin resistance [37]. It thus appears to be another morphologic feature of diabetic bone disease. The relevance of small cross-sectional bone size lies in reduced biomechanical stability and higher susceptibility to fractures [40].

Supporting the validity of our dataset, the presence of a strong statistical main effect of sex on the skeleton was in line with the literature. Several HR-pQCT studies have found larger geometry and higher BMD in healthy men than in healthy women, confirming previous studies using different imaging tools, including central quantitative computed tomography (QCT), peripheral QCT, and DXA [41, 42]. In terms of microarchitecture, the male skeleton is known to exhibit greater trabecular number, greater trabecular thickness, and lower intertrabecular separation than the female skeleton [22, 43].

Bearing in mind the large number of HR-pQCT parameters and their multi-collinearity, we approached our dataset by factor-analysis and attempted to reduce the amount of data. From factor-analysis, we obtained four groups of HR-pQCT parameters with strong statistical intra-group connections. From a technical and pathophysiologic perspective, statistical group compositions were plausible. The first factor represented a group of parameters derived from trabecular BMD. The second factor contained microstructural indices of the trabecular compartment that are mathematically dependent on trabecular number. The third factor covered parameters driven by cortical features, including cortical thickness, cortical area, cortical bone mineral density, and total bone mineral density. The fourth factor contained geometric indices. Confirming the validity of this approach, the composition of factor-groups and the subsequent choice of representative post-hoc parameters (based on factor-weight) was identical for independent measurement sites (i.e., the radius and tibia).

With our diabetic participants being relatively young and free of fragility fractures, care must be taken when comparing them to participants from other diabetic cohorts investigated by HR-pQCT. In terms of the 'hypertrophic' bone pattern, the results of our female diabetics ranged between those found in pre-diabetic, hyperinsulinemic women [37] and postmenopausal women with manifest T2DM without fractures [7, 11]. Compared to elderly, male diabetic subjects studied by Paccou et al., our diabetic men exhibited a comparable trabecular phenotype and less pronounced—but manifest—cortical bone deficits [15].

While we consider the relatively young age of our participants and the statistical reduction of HR-pQCT parameters to be strengths of our study, there were several limitations that need to be specifically addressed. The sample size was small. Data were collected with two separate HR-pQCT devices, but they were cross-calibrated by a dedicated multicenter study (previously published) [27]. Identical acquisition protocols and evaluation protocols were used. In addition, we were able to demonstrate that there were no differences in HR-pQCT parameters for healthy (i.e.,non-diabetic) men scanned at either European site (Table 4).

Nevertheless, due to the current convention for scan-site definition (i.e., the use of fixed off-sets from the radiocarpal and tibiotarsal joint), the scan sites were minimally different in men and women. As given by the use of a fixed off-set, scans were acquired more distally in men than in women, with, e.g., cortical thickness slightly underrated. Conversely, bone size was slightly overrated in men when compared to women imaged with identical protocols. We did not include subjects with fragility fractures; thus, it is still unclear whether fragility fractures are linked to similar micro-structural pathologies in men and women.

In conclusion, our results suggest that skeletal hypertrophy is present in men and women with T2DM, but appears attenuated at the cortical sites of the lower extremities in diabetic men. Future investigations are needed to provide explanations for this sex-specific pattern of diabetic bone disease.

Acknowledgments

Author Contributions

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