Compromised cortical bone compartment in type 2 diabetes mellitus patients with microvascular disease

Vikram V Shanbhogue, Stinus Hansen, Morten Frost, Niklas Rye Jørgensen1, Anne Pernille Hermann, Jan Erik Henriksen and Kim Brixen
Department of Endocrinology, Odense University Hospital, Institute of Clinical Research, University of Southern Denmark, Kloevervaenget 6.1.sal, DK-5000 Odense C, Denmark and 1Departments of Diagnostics and Medicine M, Research Center for Ageing and Osteoporosis, Glostrup Hospital, Copenhagen, Denmark

Abstract

Objective and design: Patients with type 2 diabetes mellitus (T2D) have an increased fracture risk despite a normal or elevated bone mineral density (BMD). The aim of this cross-sectional in vivo study was to assess parameters of peripheral bone microarchitecture, estimated bone strength and bone remodeling in T2D patients with and without diabetic microvascular disease (MVD+ and MVD− respectively) and to compare them with healthy controls.

Methods: Fifty-one T2D patients (MVD+ group: n = 25) were recruited from Funen Diabetic Database and matched for age, sex and height with 51 healthy subjects. High-resolution peripheral quantitative tomography (HR-pQCT) was used to assess bone structure at the non-dominant distal radius and tibia. Estimated bone strength was calculated using finite element analysis. Biochemical markers of bone turnover were measured in all participants.

Results: After adjusting for BMI, MVD+ patients displayed lower cortical volumetric BMD (P = 0.02) and cortical thickness (P = 0.02) and higher cortical porosity at the radius (P = 0.02) and a trend towards higher cortical porosity at the tibia (P = 0.07) compared to controls. HR-pQCT parameters did not differ between MVD− and control subjects. Biochemical markers of bone turnover were significantly lower in MVD+ and MVD− patients compared to controls (all P < 0.01). These were no significant correlations between disease duration, glycemic control (average glycated hemoglobin over the previous 3 years) and HR-pQCT parameters.

Conclusion: Cortical bone deficits are not a characteristic of all T2D patients but of a subgroup characterized by the presence of microvascular complications. Whether this influences fracture rates in these patients needs further investigation.

Introduction

The recognition of an association between glycemic control and reduced risk of the classical complications of diabetes mellitus (1, 2) has led to increased interest in the effects of diabetes on the skeleton. While the bulk of evidence in type 2 diabetes mellitus (T2D) points to a normal or modestly elevated bone mineral density (BMD), even after accounting for a larger body size (3, 4), the fracture risk is paradoxically increased up to 1.7 times in comparison with age- and sex-matched controls (3, 5, 6). This seems counterintuitive as the higher BMD and body weight typically seen in these patients should protect the skeleton. However, increased BMD does not necessarily translate into stronger bones because the bone-breaking strength, and thus the risk of fracture, depends not only on bone mass but also on bone quality and bone structure (7).

The advent of high-resolution peripheral quantitative computed tomography (HR-pQCT) allows assessment of trabecular and cortical microarchitecture at the distal radius and tibia simultaneously with assessment of the volumetric BMD (vBMD) and bone geometry. Using this
technique, Burghardt et al. (8) were the first to demonstrate increased cortical porosity at the distal radius and increased trabecular vBMD due to thicker trabeculae at the tibia in a limited cohort of 19 post-menopausal, diabetic women of mixed ethnicity compared with age-matched healthy subjects. Although subsequent studies failed to consistently reproduce these findings (9, 10), the increase in cortical porosity has been reported in distinct subsets of diabetics. For example, Yu et al. (11) noted increased cortical porosity at the distal radius in post-menopausal diabetic women of African-American ethnicity, and Patsch et al. (12) reported similar findings at the distal radius and distal tibia in a subset of post-menopausal women with T2D and prevalent fragility fractures in comparison to T2D women with no previous fractures. The discrepancies in the results may be due to the limited number of patients (n < 30) and heterogeneity in patient characteristics, particularly duration of diabetes, level of glycemic control and the presence of diabetic microvascular complications. As most histomorphometric and biochemical studies indicate that T2D is associated with low bone turnover (13, 14, 15), it is surprising that an increase in cortical porosity was the most consistent abnormality reported in the HR-pQCT studies (8, 11, 12). It is not known if this is the result of an additional insult such as the manifestation of microvascular disease (MVD), which has been linked to an increase in fracture risk in patients with T2D (16).

The primary aim of the current study was to assess bone compartment-specific structure, bone biomechanics and biochemical markers of bone turnover in patients with T2D with and without MVD in comparison to age-, height- and sex-matched control subjects. We hypothesized that patients with MVD have higher bone turnover and deficits in cortical microarchitecture that result in compromised bone strength, compared with healthy control subjects and T2D patients without MVD.

**Subjects and methods**

This cross-sectional study was conducted at Odense University Hospital (OUH), Denmark, between August 2013 and February 2015 after ethical approval from the Regional Scientific Committee of Southern Denmark (S-20130038) and obtaining verbal and written informed consent from all participants. Patients with T2D were recruited from the Funen Diabetic Database (FDDB), a clinical database from the Island of Funen consisting of 1470 patients with T2D who were treated at OUH. Of note, most uncomplicated diabetes cases are managed by the general practitioner (GP), and referral to OUH and subsequent registration in FDDB is dependent on the patient’s and GP’s personal preferences, any diagnostic uncertainty or inability to manage such cases in the periphery. FDDB thus represents only a fraction of the T2D cases on Funen and is probably not representative of the background T2D population.

Patient recruitment is outlined in Fig. 1. Briefly, every fifth patient (n = 294) registered in the FDDB was assessed for eligibility to participate in the study. The inclusion criteria were a diagnosis of T2D (in accordance with WHO criteria (17)) based on the available clinical data and biochemical parameters (absence of glutamic acid decarboxylase antibodies and high C-peptide levels > 700 pmol/l), and the use of and response to anti-diabetic medications where applicable. Exclusion criteria were thyroid or parathyroid disease, renal or hepatic dysfunction (defined as estimated glomerular filtration rate < 60 ml/min calculated according to the Modification of Diet in Renal Disease (MDRD) formula and serum aspartate aminotransferase (AST) > 3 times the upper limit of normal respectively), diabetic macrovascular disease (cardiovascular, cerebrovascular and peripheral vascular disease), use of glucocorticoids or anticonvulsants in the preceding year, current treatment with thiazolidinediones or anti-coagulants, current estradiol replacement therapy, current or past treatment for osteoporosis and pregnancy.

Fifty-one patients who satisfied the inclusion/exclusion criteria were recruited to the study and matched for age, sex and height with 51 healthy controls who were recruited from a previous population-based study (18) aiming to establish HR-pQCT reference data (and are...
currently in a follow-up study examining age-related changes in bone structure). None of the controls had diabetes mellitus as evidenced from normal fasting blood glucose measurement and self-reported absence of anti-diabetic medications. None of the participants had a recent history of immobilization for a period of more than 1 month.

Assessment of MVD

The presence of MVD was assessed through patient medical records and clinical examinations after recruitment into the study. Patients were classified as having i) diabetic retinopathy (yes/no) in at least one eye, as assessed by an ophthalmologist and documented by retinal photography (at three consecutive visits), ii) diabetic nephropathy (yes/no) based on the presence of urinary albumin creatinine ratio > 30 mg/g in a random voided-urine sample (where two of three tests were positive and after exclusion of urinary tract infection, uncontrolled hypertension and heart failure) and iii) diabetic peripheral neuropathy (yes/no) based on clinical examinations (at three consecutive visits) including monofilament testing (reduced sensation defined as inability to feel three of four touches at the distal halluces for 10 g monofilament), vibration perception test (detected using a 128 Hz tuning fork) and/or examination of foot reflexes. Patients were classified into the MVD+ group based on the presence of retinopathy, nephropathy or neuropathy. In total, 25 patients had evidence of MVD, including retinopathy in 19, neuropathy in 12 and nephropathy in 12. Six patients had both retinopathy and nephropathy, five patients had retinopathy and neuropathy, three patients had nephropathy and neuropathy, and two patients had evidence of all three complications.

The study was performed according to the guidelines of the Declaration of Helsinki.

Study protocol

Data on medical and fracture history, medications, calcium intake (the amount of milk and dairy products consumed daily), smoking and alcohol were ascertained through patient interview and self-administered questionnaire, supplemented by medical records if necessary. Fractures of the fingers, toes and face were disregarded in the analysis of fractures. Body weight and height were measured to the nearest 0.1 kg on a Seca model 708 scale (Seca, Hamburg, Germany) and the nearest 0.1 cm on a wall-mounted Harpenden stadiometer (Holtain Ltd, Crymich, UK) respectively, with the patient in casual indoor clothing and without shoes.

Areal BMD by dual energy X-ray absorptiometry

Dual energy X-ray absorptiometry (DXA; Hologic Discovery, Waltham, MA, USA) was used to measure areal BMD (aBMD) at the total hip and lumbar spine (L1–L4). The coefficient of variation is 1.5% at both the spine and hip in our unit.

HR-pQCT

Bone geometry, vBMD, microarchitecture and estimated bone strength were assessed using a HR-pQCT system (Xtreme CT, Scanco Medical, AG, Brüttisellen, Switzerland). The manufacturer’s default protocols for in vivo imaging, image acquisition and validation of the method have been described previously (19, 20, 21, 22). Briefly, after immobilizing the non-dominant distal radius or distal tibia (or the opposite limb in the presence of a previous fracture) in a carbon fiber cast, a single anteroposterior scout projection of the scan site was made to place a reference line at the distal radial and tibial joint surfaces and the tomographic acquisition was initiated at 9.5 mm and 22.5 mm from this reference line respectively. Each scan corresponded to a 3D representation of 9.02-mm-thick cross-sections (110 slices in the axial direction) with an isotopic image voxel size of 82 μm. A maximum of three scans per anatomic site were allowed to obtain images of optimum quality. Image quality was graded by one of the authors (V V S) using a five-step scale (23), and only scans graded 1–3 were used in the subsequent image analysis. This led to one tibial and three radial measurements in patients with T2D (none in controls) being disregarded due to poor image quality. The scans were first analyzed using the manufacturer’s standard evaluation protocol where the images were semi-automatically segmented using a chaperoned iterative contouring procedure. The total vBMD (mg hydroxyapatite/cm³) and total area (mm²) were calculated (21, 24) based on this evaluation. To characterize cortical and trabecular geometry, vBMD and microarchitecture in greater detail, an extended analysis was performed where the periosteal and endosteal surfaces of the cortex were extracted using an automated dual threshold segmentation algorithm (25), with manual correction of the endosteal contour as required. The trabecular vBMD (Tb vBMD, mg hydroxyapatite/cm³) was calculated as...
the average mineral density in the trabecular volume of interest. The trabecular bone volume fraction (BV/TV) was derived on the assumption that fully mineralized bone has a mineral density of 1200 mg hydroxyapatite/cm². The trabecular number (Tb.N, mm⁻¹) was directly measured using 3D distance transformation (22) and the trabecular thickness (Tb.Th, mm) and trabecular separation (Tb.Sp, mm) were derived from the Tb BV/TV and Tb.N as previously described (26). The extended analysis also calculated the following parameters: cortical and trabecular areas (mm²), periosteal and endosteal diameters (mm), cortical vBMD (Ct vBMD, mg hydroxyapatite/cm²), cortical thickness (Ct.Th, mm) and cortical porosity (Ct.Po, %), as described in detail elsewhere (25, 27). Finally, the mechanical properties of the radius and tibia cross-sections were estimated using a micro-finite element (FE) analysis solver provided by the manufacturer (Finite Element Analysis Software v1.15, Scanco Medical). Estimated failure load (kN) and total bone stiffness (kN/mm) were calculated as described previously (28, 29).

Biochemistry

Blood samples were drawn between 0800 h and 1000 h in the fasting state and stored at −80 °C until analysis. Bone turnover markers were measured using a fully automated immunoassay system (iSYS, Immunodiagnostic Systems Ltd, Boldon, England). The samples were analyzed in a single run with the same batch of reagents/assays. Serum pro-collagen type I amino-terminal propeptide (P1NP), osteocalcin (OC) and C-telopeptide of type I collagen (CTX-I) were measured using chemiluminescence. The intra- and inter-assay coefficients of variation (CV) for P1NP were 3% and 5–8% respectively (normal range in men and women 27.7–127.6 µg/l). The intra- and inter-assay CV for OC were 3% and 6–9% respectively (normal range in men and women 27.7–127.6 µg/l). The intra- and inter-assay CV for CTX-I were <5% and 7–10% respectively (normal range in men 0.115–0.748 µg/l, premenopausal women 0.112–0.738 µg/l, postmenopausal women 0.142–1.351 µg/l). HbA1c, serum creatinine, AST, TSH, PTH, calcium and 25-OH Vitamin D were measured as part of the clinical routine in T2D patients.

Statistical analysis

SPSS statistical package version 21 (IBM SPSS Statistics, Armonk, NY, USA) was used for data analysis. The distribution of data for each parameter was explored using normal probability plots, and data are expressed as mean±s.d. or median (inter-quartile range) as appropriate. To address our primary objective, we compared T2D patients without MVD (MVD−) with their respective controls (Co.MVD−), and T2D patients with MVD (MVD+) with their respective controls (Co.MVD+). These comparisons were performed using the χ² test for categorical variables and the independent unpaired Student’s t test or the Mann–Whitney U test for normally or non-normally distributed continuous variables respectively. Due to the difference in BMI between patients and controls, we adjusted for BMI in the MVD−/Co.MVD− and MVD+/Co.MVD+ comparisons using linear regression analysis. Non-normally distributed data were logarithmically transformed and model assumptions were checked using histograms and normal probability plots of residuals. T2D group and MVD+/MVD− subgroup specific correlations between the HR-pQCT parameters, biochemical markers of bone turnover and glycemic control (assessed as the average of all the HbA1c values in the preceding 3 years), disease duration were assessed using Spearman’s partial rank-order correlation after accounting for age, gender and BMI. Bonferroni correction was applied to account for the large number of tests in correlation analysis. Statistical tests were twotailed and P values <0.05 were considered statistically significant.

Results

Clinical characteristics and DXA-based aBMD measurements are presented in Table 1. The matching for age, sex and height ensured that these parameters were virtually identical for MVD− vs Co.MVD− and MVD+ vs Co.MVD+ groups. However, patients in the MVD− and MVD+ groups had a significantly higher body weight and thus a higher BMI than Co.MVD− and Co.MVD+ groups. While ten of 17 women in the MVD− group and nine of 17 women in the Co.MVD− group were postmenopausal (mean age at menopause: 47.9±4.7 and 47.2±4.7 years respectively), all women in the MVD+ and Co.MVD+ groups were post-menopausal (mean age at menopause: 49.5±5.6 and 51.0±4.0 years respectively). Two and four women in the MVD− and MVD+ groups and one and two women in the Co.MVD− and Co.MVD+ groups respectively, had received estradiol replacement therapy for <2 years. Details of anti-diabetic medications are provided in Supplementary Table 1 (Data not shown Available on request).

As expected, use of antihypertensive medications was more prevalent among T2D patients than controls (50% vs 33%, P=0.04 in the MVD− and Co.MVD− groups; 80% vs 16%, P<0.01 in the MVD+ and Co.MVD+ groups
respectively). Fourteen diabetic patients (three MVD− and 11 MVD+ patients) and seven control subjects (two Co.MVD− and five Co.MVD+ subjects) were on thiazide diuretics alone or in combination with other commonly prescribed antihypertensives (Supplementary Table 1 (Data not shown)). Thirty-nine T2D patients and five control subjects were on statin therapy for dyslipidemia. There were no significant differences between the groups in the prevalence of smoking, alcohol use, daily calcium intake or previous fragility fractures. Fracture sites for the T2D group included wrist (n=2 each in the MVD− and MVD+ groups), shoulder (n=1 each in the MVD− and MVD+ groups) and forearm (n=1 in MVD+ group). In the control group, fracture sites included ankle (n=1), shoulder (n=1), elbow and wrist (n=1), wrist (n=3), and forearm (n=1).

DXA measurements of spine and hip

The MVD− group had higher aBMD at the spine than the Co.MVD− group (+6%, P=0.024). The MVD+ group had a higher aBMD at both the spine (+13%, P<0.01) and hip (+11%, P=0.03) in comparison with the Co.MVD+ group. After correcting for BMI, only the aBMD at the spine remained significantly different between the MVD+ and Co.MVD+ groups (P<0.01).

HR-pQCT measurements of the distal radius and tibia

HR-pQCT parameters are shown in Table 2 for the radius and in Table 3 for the tibia. Comparing MVD− patients with Co.MVD− subjects, there were no significant differences in geometric, densitometric, structural or estimated bone strength parameters at the radius or tibia.

At the radius, however, MVD+ patients had lower Ct vBMD (-3%, P=0.04) and a trend towards higher Ct.Po (+32%, P=0.08) compared to Co.MVD+ subjects. The Ct.Th was 9% lower in MVD+ patients but this was not statistically significant (P=0.13). None of the trabecular parameters were significantly different between the groups, although Tb.N was 12% higher in MVD+ patients in comparison with Co.MVD+ subjects (P=0.07). In regression analysis adjusting for BMI, the group differences were more pronounced for Ct vBMD (P=0.02), Ct.Th (P=0.02) and Ct.Po (P=0.02), while the differences in Tb.N were generally muted (P=0.19), although the relative effects exhibited similar trends to the uncorrected differences.

### Table 1 Clinical characteristics, DXA findings and biochemical markers of bone turnover in patients with type 2 diabetes and controls. Data are expressed as mean±s.d., median (inter-quartile range) or patient numbers (N) as appropriate. Significant P values are shown in bold. Inter-group differences were assessed using the unpaired Student’s t test, the Mann–Whitney U test or the χ² tests as appropriate.

<table>
<thead>
<tr>
<th></th>
<th>MVD− (n=26)</th>
<th>Co.MVD− (n=26)</th>
<th>P</th>
<th>MVD+ (n=25)</th>
<th>Co.MVD+ (n=25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>51.4 ± 11.1</td>
<td>51.3 ± 11.3</td>
<td>0.97</td>
<td>65.3 ± 7.1</td>
<td>65.1 ± 6.7</td>
<td>0.91</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>86.7 ± 21.5</td>
<td>73.7 ± 13.1</td>
<td>0.01</td>
<td>92.9 ± 17.3</td>
<td>81.7 ± 14.0</td>
<td>0.01</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.9 ± 7.2</td>
<td>168.9 ± 6.9</td>
<td>0.60</td>
<td>171.0 ± 8.2</td>
<td>171.5 ± 8.3</td>
<td>0.94</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.6 ± 6.6</td>
<td>25.9 ± 4.6</td>
<td>&lt;0.01</td>
<td>31.7 ± 5.0</td>
<td>27.9 ± 4.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Sex N (female/male)</td>
<td>17/9</td>
<td>17/9</td>
<td>–</td>
<td>13/12</td>
<td>13/12</td>
<td>–</td>
</tr>
<tr>
<td>Post-menopausal status N (%)</td>
<td>10 (59)</td>
<td>9 (53)</td>
<td>–</td>
<td>13 (100)</td>
<td>13 (100)</td>
<td>–</td>
</tr>
<tr>
<td>Hypertension N (%)</td>
<td>13 (50)</td>
<td>7 (27)</td>
<td>0.04</td>
<td>20 (80)</td>
<td>4 (16)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Fractures N (%)</td>
<td>3 (12)</td>
<td>4 (16)</td>
<td>0.43</td>
<td>4 (16)</td>
<td>3 (12)</td>
<td>0.50</td>
</tr>
<tr>
<td>Alcohol consumptiona N (%)</td>
<td>1 (4)</td>
<td>3 (12)</td>
<td>0.31</td>
<td>4 (16)</td>
<td>8 (32)</td>
<td>0.16</td>
</tr>
<tr>
<td>Daily calcium intake (mg)</td>
<td>500 (400–800)</td>
<td>550 (375–725)</td>
<td>0.91</td>
<td>600 (400–1000)</td>
<td>800 (400–850)</td>
<td>0.84</td>
</tr>
<tr>
<td>Diabetic status</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
<td>49.4 ± 8.5</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>42.3 ± 5.6</td>
<td>NA</td>
<td>–</td>
<td>17.2 ± 7.1</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>Duration of disease (years)</td>
<td>10.0 ± 5.6</td>
<td>NA</td>
<td>–</td>
<td>63.5 ± 10.4</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>59.3 ± 14.4</td>
<td>NA</td>
<td>–</td>
<td>65.1 ± 10.4</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>DXA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spine BMD (g/cm²)</td>
<td>1.01 ± 0.10</td>
<td>0.95 ± 0.10</td>
<td>0.02</td>
<td>1.06 ± 0.15</td>
<td>0.94 ± 0.10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Spine T-score</td>
<td>−0.4 ± 0.9</td>
<td>−1.0 ± 0.9</td>
<td>0.02</td>
<td>0.1 ± 1.3</td>
<td>−1.2 ± 0.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total Hip BMD (g/cm²)</td>
<td>0.98 ± 0.14</td>
<td>0.94 ± 0.10</td>
<td>0.22</td>
<td>1.00 ± 0.17</td>
<td>0.90 ± 0.12</td>
<td>0.03</td>
</tr>
<tr>
<td>Total hip T-score</td>
<td>0.1 ± 1.0</td>
<td>−0.3 ± 0.7</td>
<td>0.20</td>
<td>0.1 ± 1.2</td>
<td>−0.6 ± 0.8</td>
<td>0.02</td>
</tr>
</tbody>
</table>

MVD−, no microvascular disease; Co.MVD−, control subjects matched for age, height and gender to MVD− patients; MVD+, presence of microvascular disease; Co.MVD+, presence of microvascular disease in the presence of smoking, alcohol use, daily calcium intake or previous fragility fractures. Fracture sites for the T2D group included wrist (n=2 each in the MVD− and MVD+ groups), shoulder (n=1 each in the MVD− and MVD+ groups) and forearm (n=1 in MVD+ group). In the control group, fracture sites included ankle (n=1), shoulder (n=1), elbow and wrist (n=1), wrist (n=3), and forearm (n=1).

*aConsumption of ≥7 units of alcohol/week.

*bSignificant (P<0.05) after adjusting for BMI.
European Journal of Endocrinology

At the tibia, cortical and trabecular vBMDs were similar between MVD+ and Co.MVD+ groups. However, MVD+ patients tended towards higher Ct.Po (+36%, P=0.06) and Tb.Th (+14%, P=0.06), and lower Tb.N (−9%, P=0.05) than Co.MVD+ subjects. These trends were less obvious for Ct.Po (P=0.07), while differences in Tb.Th and Tb.N were not significant after correction for BMI (P=0.12 and P=0.33 respectively). Estimates of bone strength were similar between the groups.

Biochemical markers of bone turnover

Biochemical markers of bone formation and resorption were lower in MVD− group in comparison to Co.MVD− group (all P<0.01) and in the MVD+ group in comparison with the Co.MVD+ group (all P<0.01) (Table 4).

Association between duration of disease, glycemic control, markers of bone turnover and HR-pQCT parameters

Spearman’s partial correlation indicated an inverse relationship between radial failure load and glycemic control, tibial Tb.Th and glycemic control and tibial failure load and glycemic control in T2D group, and tibial Tb.Th and glycemic control in MVD+ group when controlling for age, gender and BMI. However, none of these correlations were significant after adjusting for multiple comparisons (data not shown).

Discussion

Mounting evidence has suggested that patients with diabetes have increased fracture risks despite a normal to elevated aBMD (3, 30, 31), prompting a number of high-resolution 3D imaging studies investigating the hypothesis that diabetes-associated alterations in bone quality increase skeletal fragility independent of aBMD (8, 9, 10, 11, 12). Although some of these studies indicated significant deficits in radial cortical microarchitecture due to an increase in cortical porosity (8, 11, 12), none of these studies explored the association between diabetic MVD and bone structure. The present study is the first HR-pQCT study assessing parameters of bone quality such as geometry and microarchitecture as well as estimated biomechanics of the appendicular skeleton in type 2 diabetic patients with and without clinical evidence of diabetic MVD. We found that patients with MVD had lower cortical vBMD and cortical thickness and higher cortical porosity.

Table 2 Bone geometry, vBMD, microarchitectural characteristics and FE-based estimated bone strength as assessed using HR-pQCT at the radius. Data are expressed as mean±s.d. or median (inter-quartile range). Significant values are shown in bold. Inter-group differences were assessed using the unpaired Student’s t test or the Mann–Whitney U test as appropriate.

<table>
<thead>
<tr>
<th></th>
<th>MVD− (n=25)*</th>
<th>Co.MVD− (n=26)</th>
<th>P</th>
<th>MVD+ (n=23)*</th>
<th>Co.MVD+ (n=25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Geometry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total bone area (mm²)</td>
<td>274.2 (244.2–313.4)</td>
<td>253.9 (222.7–317.0)</td>
<td>0.38</td>
<td>337.5 (257.3–387.3)</td>
<td>286.8 (237.5–364.7)</td>
<td>0.16</td>
</tr>
<tr>
<td>Ct. area (mm²)</td>
<td>67.4 ±13.2</td>
<td>67.7 ±13.7</td>
<td>0.95</td>
<td>70.0 ±15.8</td>
<td>69.9 ±15.4</td>
<td>0.99</td>
</tr>
<tr>
<td>Tb. Area (mm²)</td>
<td>204.4 (179.5–231.6)</td>
<td>181.1 (166.5–251.6)</td>
<td>0.29</td>
<td>284.0 (203.5–319.5)</td>
<td>225.7 (180.6–298.0)</td>
<td>0.23</td>
</tr>
<tr>
<td>Periosteal perimeter (mm)</td>
<td>71.8 (68.4–79.0)</td>
<td>68.1 (63.5–79.1)</td>
<td>0.16</td>
<td>82.6 (70.5–94.9)</td>
<td>74.8 (66.8–83.9)</td>
<td>0.12</td>
</tr>
<tr>
<td>Endosteal perimeter (mm)</td>
<td>65.0 (61.3–70.3)</td>
<td>60.6 (56.5–72.0)</td>
<td>0.16</td>
<td>75.2 (63.4–87.4)</td>
<td>68.4 (58.8–78.3)</td>
<td>0.19</td>
</tr>
<tr>
<td><strong>Volumetric density</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total vBMD (mg/cm³)</td>
<td>357.8 ±53.7</td>
<td>369.3 ±66.9</td>
<td>0.50</td>
<td>319.3 ±73.5</td>
<td>330.8 ±77.9</td>
<td>0.60</td>
</tr>
<tr>
<td>Ct. vBMD (mg/cm³)</td>
<td>982.9 ±42.1</td>
<td>1004.4 ±42.0</td>
<td>0.08</td>
<td>935.3 ±55.3</td>
<td>966.0 ±47.0</td>
<td>0.04b</td>
</tr>
<tr>
<td>Tb. vBMD (mg/cm³)</td>
<td>186.8 ±41.5</td>
<td>181.1 ±40.1</td>
<td>0.62</td>
<td>184.3 ±49.1</td>
<td>165.7 ±51.7</td>
<td>0.21</td>
</tr>
<tr>
<td><strong>Microarchitecture</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tb.N (1/mm)</td>
<td>2.00 ±0.23</td>
<td>2.02 ±0.27</td>
<td>0.80</td>
<td>2.09 ±0.44</td>
<td>1.88 ±0.33</td>
<td>0.07</td>
</tr>
<tr>
<td>Tb.Th (mm)</td>
<td>0.077 ±0.012</td>
<td>0.074 ±0.013</td>
<td>0.40</td>
<td>0.074 ±0.014</td>
<td>0.072 ±0.014</td>
<td>0.80</td>
</tr>
<tr>
<td>Tb.Sp (mm)</td>
<td>0.428 ±0.062</td>
<td>0.429 ±0.075</td>
<td>0.95</td>
<td>0.434 ±0.163</td>
<td>0.475 ±0.107</td>
<td>0.12</td>
</tr>
<tr>
<td>Ct.Th (mm)</td>
<td>1.07 ±0.26</td>
<td>1.05 ±0.17</td>
<td>0.71</td>
<td>0.91 ±0.20</td>
<td>1.00 ±0.20</td>
<td>0.13b</td>
</tr>
<tr>
<td>Ct.Po (%)</td>
<td>2.16 (1.59–2.95)</td>
<td>1.94 (1.54–2.87)</td>
<td>0.72</td>
<td>4.05 (2.40–5.08)</td>
<td>3.07 (1.83–4.50)</td>
<td>0.08b</td>
</tr>
<tr>
<td><strong>Estimated bone strength</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone stiffness (kN/mm)</td>
<td>91.0 ±24.5</td>
<td>86.2 ±21.3</td>
<td>0.46</td>
<td>90.5 ±29.1</td>
<td>88.1 ±24.6</td>
<td>0.76</td>
</tr>
<tr>
<td>Failure load (kN)</td>
<td>4.6 ±1.2</td>
<td>4.3 ±1.0</td>
<td>0.44</td>
<td>4.6 ±1.5</td>
<td>4.4 ±1.2</td>
<td>0.40</td>
</tr>
</tbody>
</table>

MVD−, no microvascular disease; Co.MVD−, control subjects matched for age, height and gender to MVD− patients; MVD+, presence of microvascular disease; Co.MVD+, control subjects matched for age, height and gender to MVD+ patients.

*Three radial measurements were disregarded due to poor image quality.

bSignificant (P<0.05) after adjusting for BMI.
at the radius and a tendency towards higher cortical porosity at the tibia in comparison with age- and sex-matched control subjects after correcting for the differences in body mass. Bearing in mind the cross-sectional design of our study that limits the ability to assess causality, our observations of i) compromised cortical bone compartment in MVD+ patients compared to Co.MVD+ subjects and ii) similar cortical bone parameters in MVD− vs Co.MVD− subjects, indicate that cortical deficits are not a characteristic of all T2D but of a subgroup characterized by the presence of microvascular complications. Furthermore, this could also suggest that the development of microvascular complications may be a critical point of progression in the occurrence of T2D-related cortical deficits. Comparing the results from the current study with population-based HR-pQCT reference data from our previous study (18), we found that MVD+ patients exhibited cortical porosity values that were not even reached by control subjects in the eighth decade, indicating an accelerated deterioration of cortical bone in T2D patients with MVD. On the other hand, MVD+ patients had a longer duration of disease and were more likely to be on treatment with insulin than MVD− patients (64% vs 31%, Supplementary Table 1). Therefore, the association between MVD and skeletal deficits may be a reflection of the fact that their disease has lasted longer and is more severe with the presence of MVD serving as a proxy for more general disease severity. However, we did not find any correlation between vBMD, bone structure or estimated bone strength.

Table 4  Biochemical markers of bone turnover in patients and controls. Data are expressed as median (inter-quartile range). Significant values are shown in bold. Inter-group differences were assessed using the Mann–Whitney U test. Normal range for PINP: 27.7–127.6 µg/l. Normal range for OC: 10.4–45.6 µg/l. Normal range for CTX-I, men: 0.115–0.748 µg/l, premenopausal women: 0.112–0.738 µg/l, postmenopausal women 0.142–1.351 µg/l.

<table>
<thead>
<tr>
<th>PINP (µg/l)</th>
<th>MVD− (n=26)</th>
<th>Co.MVD− (n=26)</th>
<th>P</th>
<th>MVD+ (n=25)</th>
<th>Co.MVD+ (n=25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>36.7 (29.1–42.6)</td>
<td>51.2 (35.2–65.0)</td>
<td>&lt; 0.01</td>
<td>32.7 (25.8–40.6)</td>
<td>47.4 (36.7–67.2)</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>12.7 (8.9–17.4)</td>
<td>24.9 (17.4–34.4)</td>
<td>&lt; 0.01</td>
<td>14.5 (8.8–17.1)</td>
<td>21.3 (17.2–30.6)</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>0.21 (0.14–0.38)</td>
<td>0.49 (0.29–0.74)</td>
<td>&lt; 0.01</td>
<td>0.13 (0.06–0.29)</td>
<td>0.43 (0.29–0.87)</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
</tbody>
</table>

PINP, amino-terminal propeptide of type I procollagen; OC, Osteocalcin; CTX-I, cross-linked C-telopeptide 1.
parameters and duration of disease or degree of glycemic control in MVD−/MVD+ subgroups. This is consistent with most previous studies and a meta-analysis investigating the association between glycemic control, duration of disease and aBMD (3). While this could indicate that the level of metabolic control does not directly affect bone architecture over longer time periods, the average HbA1c in our study cohort (mean 61.4 ± 12.7 mmol/mol) indicated fair metabolic control and thus it does not discount the fact that short-term reversible changes may be linked to glycemic control in poorly controlled diabetic patients (32).

In contrast to the cortical bone deficits, most of the trabecular bone parameters were either equivalent or showed a non-significant tendency towards superior trabecular microarchitecture in MVD+ patients compared to MVD− patients and control subjects. While previous HR-pQCT studies have described a generally advantageous peripheral trabecular bone microarchitecture in T2D (8, 10), the primary emphasis and comparisons in these studies were between T2D patients and healthy controls, with lack of information about MVD in the T2D cohorts. In their computed tomography study, Melton et al. (33) found a higher trabecular vBMD at the lumbar spine and femoral neck in patients with T2D compared to non-diabetics, although approximately 40% of the diabetic cohort in that study had evidence of chronic complications of diabetes. One potential explanation for the preserved trabecular bone mass in patients with MVD in our study could be the inclusion of cortical remnants in the endosteal ‘transition zone’ as trabeculae, thus leading to an overestimation of trabecular vBMD (34). Despite significant intracortical erosion, the endocortical boundaries were still visually discernable in these patients, thus making it possible to manually adjust the automated endosteal contour to ensure an appropriate assignment of these features to the cortical and trabecular compartments.

We hypothesized that the presence of MVD is associated with alteration in bone remodeling dynamics resulting in cortical bone deficits. However, in line with most previous studies (13, 14, 15), we found (for MVD+ and MVD− patients alike) significantly lower biochemical markers of bone formation and resorption in comparison with healthy controls. This suggests that alterations in bone turnover are unlikely to be the explanation for the structural differences between the groups. Although it is unclear if circulating bone turnover markers accurately distinguish a specific defect of the cellular activity of one compartment of bone since they reflect the overall activity of the whole skeleton, the differential impact of MVD on cortical and trabecular bone points to other factors such as differences in recruitment of osteoblast (OB) progenitors during remodeling of cortical and trabecular bone. Kristensen et al. (35) have described three distinct routes for recruitment of mature bone-forming OB during trabecular bone remodeling originating from: i) bone surface covering OB-lineage bone lining cells, ii) canopies that represent mesenchymal bone marrow envelopes above bone remodeling sites and iii) capillary pericytes in contact with the bone remodeling sites. In contrast, the only road to remodeling sites buried in the cortex and the crucial source of OB progenitors are the pericytes lining the capillaries (36). As reduction in pericyte numbers and the subsequent pathophysiologic changes underpin diabetic microangiopathy (37), it is possible that the preferential involvement of the cortical compartment in patients with MVD could be related to a greater impact of a theoretical deficiency of pericytes in cortical bone, with the trabecular bone being relatively spared because of the availability of alternative osteoprogenitor sources.

Despite the compromised cortical compartment with increased cortical porosity in patients with MVD, the prevalence of fragility fractures was similar to that of MVD− patients. Although the relative difference in porosity between the MVD− and MVD+ groups was 88% and 83% at the radius and tibia respectively, the absolute difference between the groups was quite small (1.9% and 4.7% at the radius and tibia respectively). At this point in time, it is unclear if these small differences in porosity significantly impact clinical bone strength. Although this study was not designed to identify differences in fracture prevalence, it is possible that the higher porosity may serve as a surrogate marker of disease severity rather than bone fragility in T2D.

This study has several limitations. First, the recruitment of T2D patients using FDBD could introduce a selection bias that limits the possibility to extrapolate the results to T2D patients with less complex management. Nevertheless, FDBD has comprehensive information about the included patients and ensures diagnostic accuracy. Further, since we excluded patients with macrovascular disease to avoid the confounding influence of ischemia due to overt large-vessel disease, the results of our study may provide only conservative estimates of the effects of vascular disease on bone in T2D. Second, the limited sample size and the heterogeneity in the MVD−/MVD+ subgroups and in the representation of microvascular complications in the MVD+ group precluded reliable subgroup analysis and an adequate
assesssment of the separate roles and severity of neuropathy, retinopathy and nephropathy in bone structure. Further studies including a larger number of patients are necessary to address the question if the association between complications and skeletal health are just associations, or if they are causative, independent of the long-term exposure to diabetes. Third, the cross-sectional and observational design of the study makes it impossible to determine the temporal association between the development of MVD and bone deficits, necessitating future prospective studies. Fourth, the HR-pQCT resolution constraints limit detection of intracortical pores ≤ 100 μm (38), thus not accounting for possible differences in microporosity. The significance of these pores with respect to cortical strength and fracture risk is uncertain, however. Finally, the FEA solver was constrained by assumptions of homogenous material properties between the study groups, thus mirroring only the impact of bone geometry and microarchitecture on bone strength and not taking tissue-level differences in mineralization into account.

In summary, our results indicated that T2D patients with no evidence of MVD had normal bone microarchitecture. On the other hand, the presence of MVD may be related to cortical bone deterioration, most noticeably through reduced cortical vBMD and cortical thickness and increased cortical porosity at the radius and a tendency towards increased cortical porosity at the tibia. Further investigation is needed to determine if this relationship between the presence of MVD and cortical bone deficits is causal and impacts fracture rates, and whether diabetic bone disease is an extension of the spectrum of diabetic MVD.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding
This study was supported by grants from the Research Foundation, Odense University Hospital.

Author contribution statement

Acknowledgements
The authors thank Anette Riis Madsen and Steffanie Christensen for their help in coordinating the study, and all the technical staff at the Osteoporosis Clinic, Odense University Hospital, for obtaining consent from the participants and performing the bone scans. We thank Claire Gudex, Department of Endocrinology, Odense University Hospital, for proofreading the manuscript. We also thank Nadia Quardon at the Department of Clinical Biochemistry at Glostrup University Hospital for her help with analysis of biochemical markers of bone turnover.

References
29 MacNeil JA & Boyd SK. Bone strength at the distal radius can be estimated from high-resolution peripheral quantitative computed tomography and the finite element method. Bone 2008 42 1203–1213. (doi:10.1016/j.bone.2008.01.017)

Received 25 August 2015
Revised version received 5 October 2015
Accepted 4 November 2015