



# Vascular Complications in Individuals with Type 2 Diabetes Mellitus Additionally Increase the Risk of Femoral Neck Fractures Due to Deteriorated Trabecular Microarchitecture

Aleksandar Cirovic<sup>1</sup> · Marko Vujacic<sup>2</sup> · Bojan Petrovic<sup>2</sup> · Ana Cirovic<sup>1</sup> · Vladimir Zivkovic<sup>3</sup> · Slobodan Nikolic<sup>3</sup> · Danijela Djonic<sup>1</sup> · Zoran Bascarevic<sup>2</sup> · Marija Djuric<sup>1</sup> · Petar Milovanovic<sup>1</sup> 

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## Abstract

Individuals with diabetes mellitus type 2 (T2DM) have an increased risk of hip fracture, especially if vascular complications are present. However, microstructural origins of increased bone fragility in T2DM are still controversial. DXA measurement of the contralateral hip and three-dimensional microCT analyses of femoral neck trabecular microarchitecture were performed in 32 individuals (26 women and 6 men,  $78 \pm 7$  years). The specimens were divided to two groups: T2DM individuals with hip fracture (DMFx,  $n = 18$ ) and healthy controls (CTL,  $n = 14$ ). DFMx group consisted of individuals with vascular complications (DMFx\_VD,  $n = 8$ ) and those without vascular complications (DMFx\_NVD,  $n = 10$ ). T-score was significantly lower in DFMx\_VD and DFMx\_NVD than in controls ( $p < 0.001$ ). BV/TV, Tb.N, Tb.Sp, SMI, and FD varied among DFMx\_NVD, DFMx\_VD, and CTL groups ( $p = 0.023$ ,  $p = 0.004$ ,  $p = 0.008$ ,  $p = 0.001$ ,  $p = 0.007$ , respectively). Specifically, BV/TV of DFMx\_VD was significantly lower than that of DFMx\_NVD group ( $p = 0.020$ ); DFMx\_NVD group had higher Tb.N and lower Tb.Sp compared with DFMx\_VD ( $p = 0.006$ ,  $p = 0.012$ , respectively) and CTL ( $p = 0.026$ ,  $p = 0.035$ , respectively). DFMx group and healthy controls showed similar BV/TV, Tb.Th, Tb.N, Tb.Sp, Conn.D, DA, and FD ( $p = 0.771$ ,  $p = 0.503$ ,  $p = 0.285$ ,  $p = 0.266$ ,  $p = 0.208$ ,  $p = 0.235$ ,  $p = 0.688$ , respectively), while SMI was significantly higher in controls ( $p = 0.005$ ). Two distinct phenotypes of bone fragility were identified in T2DM patients: patients with vascular complications showed impaired trabecular microarchitecture, whereas bone fragility in the group without vascular complications was independent on trabecular microarchitecture pattern. Such heterogeneity among T2DM patients may explain contradicting literature data and may set a basis for further studies to evaluate fracture risk related to T2DM.

**Keywords** T2DM · Fracture · Femoral neck · Microarchitecture · Trabeculae

## Introduction

Diabetes mellitus (DM) is one of the fastest expanding, public health problems of the twenty-first century. According to World Health Organization (WHO), DM occurs in

every twelfth non-pediatric individual, and it is estimated that in 2045, there will be 700 million individuals with DM worldwide [1]. Poorly controlled glucose levels accelerate the development of diabetic complications, such as diabetic neuropathy, diabetic retinopathy, etc. Moreover, patients with DM complications are at an increased risk of falls and higher risk of bone fracture [2–4]. Despite frequently having normal or even a higher bone mineral density (BMD) [5–8], patients with type 2 DM (T2DM) are at an increased risk of sustaining hip fracture [2, 5, 6, 9–12]. Almost a third of patients with hip fracture die within the first year after fracture [13], while mortality after hip fracture in patients with coexisting DM is approximately two times higher [11, 14, 15].

Studies of bone microarchitecture were conducted to try to unravel the reasons for higher bone fragility in spite of

✉ Petar Milovanovic  
drpmilovanovic@gmail.com;  
petar.milovanovic@med.bg.ac.rs

<sup>1</sup> Laboratory of Bone Biology and Bioanthropology, Institute of Anatomy, Faculty of Medicine, University of Belgrade, Dr Subotica 4/2, Belgrade, Serbia

<sup>2</sup> Institute for Orthopedic Surgery “Banjica”, Mihaila Avramovića 28, Belgrade, Serbia

<sup>3</sup> Institute of Forensic Medicine, Faculty of Medicine, University of Belgrade, Deligradska 31a, Belgrade, Serbia

BMD out of osteoporotic range. According to studies which used high-resolution peripheral quantitative computed tomography (HR-pQCT), cortical and trabecular microarchitecture of distal tibia and radius is at least equally good or even slightly improved in T2DM individuals with no vascular complications or history of hip fracture compared with healthy controls [16–18]. In contrast, some studies suggested deteriorated microarchitecture of distal radius and tibia in T2DM individuals with microvascular complications such as diabetic retinopathy, neuropathy, or nephropathy [18] or who had previously sustained hip fracture [16] compared with controls.

Although hip fractures are more common in T2DM patients [8, 12], data on femoral microarchitecture in those patients are still limited. Wölfel et al. recently identified a subgroup of T2DM individuals with increased subtrochanteric cortical porosity and a subgroup with normal cortical porosity [19]. Osima et al. [20] also examined subtrochanteric region of the femoral diaphysis using low-resolution computed tomography (CT) and showed lower cortical porosity in postmenopausal women with T2DM compared with those without DM. In contrast, Karim et al. did not find any significant differences in cortical or trabecular bone microarchitecture of the femoral neck and head between T2DM and non-diabetic patients undergoing total hip replacement surgery for osteoarthritis [21]. Hunt et al. [22] reported that in men undergoing total hip arthroplasty for osteoarthritis, a lower trabecular separation and a trend to higher trabecular number were observed at the femoral neck of T2DM patients compared with non-DM controls. However, those studies of femoral microarchitecture did not include T2DM patients who sustained a hip fracture, and most of them analyzed specimens with osteoarthritis. Considering an overall trend of improved microarchitecture of the femoral neck in individuals with hip osteoarthritis [23] and likely reduced hip fracture risk [24, 25], assessment of patients with severe osteoarthritis has a limited value for understanding the real fracture risk. Therefore, it is important to assess femoral bone microarchitecture in DM patients who sustained a hip fracture. Moreover, considering that it was suggested that DM patients with cardiovascular complications have a greater fracture risk [2, 3], it should

be analyzed whether the presence of vascular complications influences bone microarchitecture in T2DM patients. Considering lack of microarchitectural assessments of the femoral neck in T2DM patients with hip fracture as well as contradicting results obtained in previous HR-pQCT and microCT studies, the aims of our study were to examine whether there is microarchitectural basis for bone fragility in patients with T2DM and to determine whether trabecular microarchitecture in T2DM patients is affected by the presence of vascular complications. Therefore, here, we compared trabecular microarchitecture of the femoral neck between T2DM patients who sustained a hip fracture and had confirmed vascular complications, T2DM patients with hip fracture and no positive history of vascular complications, and age-matched healthy controls; moreover, we pooled all individuals with T2DM to compare trabecular microarchitecture of the femoral neck between T2DM subjects with fracture and healthy controls.

## Material and Methods

### Groups of Individuals

For this study, we obtained the femoral neck specimens of 32 individuals (age  $78 \pm 7$  years; 26 women and 6 men). The specimens were divided to two groups: T2DM individuals with hip fracture (DMFx,  $n = 18$ ) and healthy controls (CTL,  $n = 14$ ). In DMTx group, we identified two subgroups: individuals with vascular complications (DMFx\_VD,  $n = 8$ ) and individuals without vascular complications (DMFx\_NVD,  $n = 10$ ).

The DMTx group encompassed patients with T2DM who were undergoing total hip arthroplasty due to hip fracture at a tertiary level, orthopedic university hospital (Institute for Orthopedic Surgery “Banjica”, Belgrade). This was a consecutive group of patients treated during 2019, until the Institute was turned to a COVID-19 hospital as a governmental measure in securing enough beds for COVID-19 patients with moderately severe disease. The inclusion criteria were as follows: (i) T2DM treated with oral antidiabetic medications (documented in clinical records and medical

**Fig. 1** Representative 3D reconstructions of femoral neck trabecular bone in T2DM individuals with hip fracture and with vascular complications (DMFx\_VD), T2DM individuals with hip fracture and without vascular complications (DMFx\_NVD), and healthy controls (CTL)



history); (ii) unilateral fracture of the femoral neck caused by fall at the same level; (iii) clinical decision to treat the fracture surgically using hip arthroplasty; (iv) patient's consent to surgery; (v) patient's informed consent to be included in the study. The exclusion criteria encompassed the presence of other diseases or treatments with significant effects on bone metabolism or structure, as follows: (i) rheumatoid arthritis; (ii) inborn skeletal anomalies; (iii) chronic liver disease; (iv) any type of malignancy; (v) endocrine diseases such as acromegaly, hypo- or hyperparathyroidism; (vi) treatment with bisphosphonates, glucocorticoids, estrogen, anticonvulsants, or antipsychotics; and (vii) hip osteoarthritis. All included patients had intracapsular fracture of the femoral neck, whereas patients with trochanteric fracture were not included in the study. Before arthroplasty, DXA of the spine and contralateral hip was conducted, and HbA1c level was measured. Data about patients' health status, including vascular complications and medications used, were collected from anamnesis, clinical records, and medical history. All the individuals with vascular complications had mainly macrovascular complications such as peripheral artery disease, stroke, and ischemic heart disease including myocardial infarction, or carotid artery disease (Supplement 1). We calculated the FRAX score for each patient who

underwent total hip arthroplasty. Characteristics of patients with T2DM and non-diabetic controls are summarized in Table 1. T2DM was relatively well controlled at the time before fracture, considering that HbA1c values were on average  $6.07\% \pm 1.42\%$  [range 4.3–8.4%]. The patients signed informed consent, and the procedures were approved by the institutional review board of the Institute for Orthopedic Surgery "Banjica."

To ensure having the same and consistent bony part available for the microCT analysis, control group (CTL) included individuals who were admitted to the Institute of Forensic Medicine in Belgrade for autopsy. Their main causes of death were natural or sudden deaths, such as cardiac arrest, respiratory failure, car accidents with preserved hip region, and violent death. Inclusion criteria were as follows: (i) no T1DM and T2DM and (ii) no history of hip fracture. Exclusion criteria were based on medical history and autopsy reports, and they encompassed the following: (i) rheumatoid arthritis; (ii) inborn skeletal anomalies; (iii) chronic kidney disease; (iv) chronic liver disease; (v) any type of malignancy in the moment of death; (vi) acromegaly, and hypo- or hyperparathyroidism; (vii) known treatment with bisphosphonates, glucocorticoids, estrogen, anticonvulsants, or antipsychotics; (viii) hip osteoarthritis; and (ix) manifest

**Table 1** Characteristics of T2DM individuals with fracture and no known vascular complications (DMFx\_NVD), T2DM individuals with fracture and known vascular complications (DMFx\_VD), and non-diabetic controls (CTL)

Characteristics	DMFx_NVD (N=10)	DMFx_VD (N=8)	CTL (N=14)	<i>p</i>	Post-hoc analyses
<b>Demographic</b>					
Age (years) <sup>‡</sup>	75.1 ± 6.7	80.87 ± 5.1	77.93 ± 6.8	0.179	NA
Sex (women) <sup>#</sup>	9 (90%)	6 (75%)	11 (78.6%)	0.729	NA
Duration of T2DM (years)*	6.4 ± 7.3	13.8 ± 10.5	NA	0.158	NA
Duration of menopause (years)*	24.2 ± 6.1	29.2 ± 1.3	NA	0.08	NA
<b>Anthropometry</b>					
Body mass index (kg/m <sup>2</sup> ) <sup>‡</sup>	26.3 ± 3.5	24.7 ± 2	25.5 ± 5.7	0.814	NA
<b>Biochemical</b>					
Hemoglobin A1c (HbA1c, %)*	6 ± 1.4	6.2 ± 1.6	NA	0.801	NA
Creatinine*	93.5 ± 19.5	107.6 ± 47.7	NA	0.437	NA
<b>Diabetes medications</b>					
Metformin <sup>#</sup>	9/10 (90%)	5/8 (62.5%)	NA	0.274	NA
Sulfonylureas <sup>#</sup>	4/10 (40%)	4/8 (50%)	NA	1	NA
FRAX score <sup>‡</sup>	2.5 ± 1.7	2.5 ± 1.1	3.9 ± 3.2	0.536	NA
T-score femoral neck <sup>‡</sup>	−2.1 ± 0.7	−2.2 ± 0.6	1.6 ± 0.5	<0.001	<0.001 (DMFx_NVD vs CTL) <0.001 (DMFx_VD vs CTL)
T-score lumbar spine*	−1.33 ± 1.1	−1.4 ± 0.6	NA	0.886	NA

\**T* test for independent samples

<sup>‡</sup>One-way ANOVA

<sup>#</sup>Fisher's exact test

vascular complications. For each individual in the control group, postmortem DXA measurement was performed and FRAX score was calculated. All the procedures were approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade.

### Collection and Preparation of Bone Specimens

Immediately after removal at surgery or autopsy, the femoral neck samples were stored in 4% formaldehyde solution at 4 °C. From each of the 32 samples, an approximately 10 mm × 10 mm trabecular bone cube was cut directly inferior to the epiphyseal line of the femoral head/neck interface in the direction of the femoral head fovea, as suggested in a previous study [26], by using a water-cooled low-speed diamond saw. The excised trabecular region was chosen due to following reasons: (i) it represents the continuation of the inferomedial femoral neck compartment at the base of the femoral head and (ii) the examined bone cube was not affected by the fracture line, so the samples could be harvested and analyzed in a consistent manner.

### Microcomputed Tomography

Each specimen was placed on a sample holder in a consistent orientation and scanned using Skyscan 1172 microcomputed tomography system (Bruker microCT, Skyscan, Belgium) with the following scanning conditions and parameters: 80 kV, 124 μA, and 1200 ms exposure time. A combined aluminum and copper filter was used. 2 K camera binning was used, leading to 10 μm isotropic resolution. Rotation step of 0.40° and triple frame averaging were chosen. We reconstructed the obtained projection images using NRecon software (Bruker microCT, Belgium) on InstaRecon platform (InstaRecon, USA) with Gaussian smoothing of 3, thermal drift correction, misalignment compensation, ring artifact, and beam hardening corrections as needed. A global gray-level threshold of 95/255 was selected to distinguish between the mineralized and non-mineralized tissue. We manually marked the region of interest (ROI) for each slice to obtain the volume of interest (VOI). We analyzed at least 1000 slices per sample, meaning that the sample thickness of at least 1 cm per subject was evaluated. After importing all 32 VOIs to CT.An software (ver. 1.16.4.1: Skyscan, Belgium), we analyzed the following trabecular bone parameters: trabecular bone volume fraction (BV/TV, %), trabecular number (Tb.N, 1/mm), trabecular thickness (Tb.Th, mm), trabecular separation (Tb.Sp, mm), connectivity density (1/mm<sup>3</sup>), structure model index (SMI, dimensionless), fractal dimension (FD, dimensionless), and degree of anisotropy (DA, dimensionless).

### Statistical Analysis

The Kolmogorov–Smirnov test was used to verify that all measured parameters complied with the normal distribution. One-way ANOVA was performed to check for overall differences in age, BMI, femoral neck and lumbar spine T-scores, FRAX scores, and microarchitectural bone parameters between DMF<sub>x</sub>\_VD, DMF<sub>x</sub>\_NVD, and CTL groups; when overall ANOVA showed *p* value < 0.05, pairwise comparisons (post-hoc tests) under Bonferroni correction for multiple testing were conducted. *T* tests for independent samples were used to compare microarchitectural bone parameters between DMF<sub>x</sub> and CTL, as well as quantitative parameters between the two DMF<sub>x</sub> subgroups (disease duration, menopause duration, hemoglobin A1c level, creatinine level). Fisher's exact probability test was used to evaluate the difference in sex distribution and medications between the groups. All analyses were performed two-tailed in SPSS software ver. 15 at the significance level of 0.05.

### Results

We compared femoral trabecular microarchitecture among individuals with T2DM who experienced hip fracture and had confirmed vascular complications (DMF<sub>x</sub>\_VD, *n* = 8), individuals with T2DM who sustained hip fracture and had no vascular complications (DMF<sub>x</sub>\_NVD, *n* = 10), and healthy controls (CTL, *n* = 14).

DMF<sub>x</sub>\_NVD, DMF<sub>x</sub>\_VD, and CTL groups did not differ in age (*p* = 0.179), sex (*p* = 0.729), and BMI (*p* = 0.814). FRAX score for hip fracture was similar between the groups (*p* = 0.536); nevertheless, we found a significantly reduced femoral neck T-score in DMF<sub>x</sub>\_VD and DMF<sub>x</sub>\_NVD groups compared with CTL group (*p* < 0.001). DMF<sub>x</sub>\_VD and DMF<sub>x</sub>\_NVD groups did not differ in HbA1c (*p* = 0.801), duration of disease (*p* = 0.158), or creatinine level (*p* = 0.437). Moreover, T-score of the femoral neck and lumbar spine did not vary significantly between the DMF<sub>x</sub> subgroups (*p* = 0.906, *p* = 0.886, respectively).

### Microarchitectural Analyses of DMF<sub>x</sub> and CTL Groups

Comparison between DMF<sub>x</sub> and CTL groups revealed no significant differences in BV/TV, Tb.Th, Tb.N, Tb.Sp, Conn.D, DA, and FD (*p* = 0.771, *p* = 0.503, *p* = 0.285, *p* = 0.266, *p* = 0.208, *p* = 0.235, *p* = 0.688, respectively), whereas SMI was slightly but significantly higher in controls (*p* = 0.005) (Table 2).

**Table 2** Trabecular microarchitectural parameters of T2DM individuals with fracture (DMFx) and non-diabetic control group (CTL)

Parameter	Groups	n	Mean	SD	p value
BV/TV [%]	DMFx	18	25.52	3.45	<i>p</i> =0.771
	CTL	14	25.04	5.79	
Tb.Th [mm]	DMFx	18	0.20	0.02	<i>p</i> =0.503
	CTL	14	0.21	0.03	
Tb.N [1/mm]	DMFx	18	1.27	0.15	<i>p</i> =0.285
	CTL	14	1.20	0.19	
Tb.Sp [mm]	DMFx	18	0.66	0.07	<i>p</i> =0.266
	CTL	14	0.70	0.10	
Conn.D [1/mm <sup>3</sup> ]	DMFx	18	12.57	4.39	<i>p</i> =0.208
	CTL	14	14.47	3.80	
DA	DMFx	18	1.96	0.18	<i>p</i> =0.235
	CTL	14	2.05	0.24	
FD	DMFx	18	2.55	0.05	<i>p</i> =0.688
	CTL	14	2.54	0.06	
SMI	DMFx	18	0.85	0.42	<i>p</i> =0.005*
	CTL	14	1.35	0.51	

*T* test for independent samples

\**p* < 0.05

**Microarchitectural Analyses of DMFx\_VD, DMFx\_NVD, and CTL Groups**

We found that BV/TV, Tb.N, Tb.Sp, SMI, and FD (*p*=0.023, *p*=0.004, *p*=0.008, *p*=0.001, *p*=0.007, respectively) varied between the examined groups (Table 3, Fig. 1). Tb.Th, Conn.D, and DA were similar between the groups (*p*=0.586, *p*=0.106, *p*=0.286, respectively). Furthermore, post-hoc analyses showed significantly lower BV/TV in DMFx\_VD group compared with DMFx\_NVD group (*p*=0.020), whereas no significant differences in BV/TV were found between CTL and any of the T2DM groups (DMFx\_VD, *p*=0.450; DMFx\_NVD, *p*=0.260). We found significantly higher Tb.N in DMFx\_NVD compared with CTL (*p*=0.030) and DMFx\_VD (*p*=0.006) groups. DMFx\_NVD showed the lowest Tb.Sp (vs. CTL: *p*=0.035; vs. DMFx\_VD, *p*=0.012). Although SMI was significantly lower in DMFx\_NVD compared with CTL (*p*<0.001) and DMFx\_VD (*p*=0.046) groups, it reflected predominance of trabecular plates over rods in all groups. FD was lower in DMFx\_NVD compared with DMFx\_VD group (*p*=0.006) (Table 3).

**Table 3** Trabecular microarchitectural parameters of T2DM individuals with fracture and no known vascular complications (DMFx\_NVD), T2DM individuals with fracture and known vascular complications (DMFx\_VD), and non-diabetic controls (CTL)

Parameter	Groups	n	Mean	SD	p value	Post-hoc analyses (Bonferroni)
BV/TV [%]	DMFx_NVD	10	28.07	1.90	<i>p</i> =0.023*	<i>p</i> =0.020 (DMFx_NVD vs DMFx_VD)
	DMFx_VD	8	22.34	1.85		
	CTL	14	25.04	5.79		
Tb.Th [mm]	DMFx_NVD	10	0.20	0.02	<i>p</i> =0.586	NA
	DMFx_VD	8	0.20	0.01		
	CTL	14	0.21	0.03		
Tb.N [1/mm]	DMFx_NVD	10	1.37	0.08	<i>p</i> =0.004*	<i>p</i> =0.006 (DMFx_NVD vs DMFx_VD); <i>p</i> =0.030 (DMFx_NVD vs CTL)
	DMFx_VD	8	1.14	0.10		
	CTL	14	1.20	0.19		
Tb.Sp [mm]	DMFx_NVD	10	0.61	0.05	<i>p</i> =0.008*	<i>p</i> =0.012 (DMFx_NVD vs DMFx_VD); <i>p</i> =0.035 (DMFx_NVD vs CTL)
	DMFx_VD	8	0.73	0.04		
	CTL	14	0.70	0.10		
Conn.D [1/mm <sup>3</sup> ]	DMFx_NVD	10	14.05	5.14	<i>p</i> =0.106	NA
	DMFx_VD	8	10.71	2.43		
	CTL	14	14.47	3.80		
DA	DMFx_NVD	10	1.91	0.19	<i>p</i> =0.286	NA
	DMFx_VD	8	2.01	0.14		
	CTL	14	2.05	0.24		
FD	DMFx_NVD	10	2.58	0.03	<i>p</i> =0.007*	<i>p</i> =0.006 (DMFx_NVD vs DMFx_VD)
	DMFx_VD	8	2.51	0.02		
	CTL	14	2.54	0.06		
SMI	DMFx_NVD	10	0.62	0.34	<i>p</i> =0.001*	<i>p</i> =0.046 (DMFx_NVD vs DMFx_VD); <i>p</i> <0.001 (DMFx_NVD vs CTL)
	DMFx_VD	8	1.14	0.34		
	CTL	14	1.35	0.51		

One-way ANOVA

\**p* < 0.05

## Discussion

Our analysis showed the poorest femoral trabecular microarchitecture in T2DM patients with vascular complications. In particular, these patients clearly showed worse microarchitectural parameters than T2DM patients without vascular complications, although these subgroups of T2DM individuals did not differ in any of the relevant demographic factors (sex, age, BMI), disease-related factors (duration of the disease, HbA1c level, creatinine level), and type of therapy. Hence, clear variability in the femoral trabecular microarchitecture that is related to the presence or absence of vascular complications indicates that patients with T2DM should not be considered a single and uniform group in studies of the fracture risk.

Some of the microarchitectural parameters in T2DM patients who experienced fracture and did not have vascular complications were even better than those in the control group. However, after pooling both DMFx subgroups, there were no differences in trabecular microarchitecture of the femoral neck compared with CTL group. Considering that DMFx\_NVD group experienced hip fracture despite having similar femoral BV/TV and even higher Tb.N compared with the CTL group, it is apparent that the reasons for increased fragility in T2DM patients may also lie beyond trabecular microarchitecture. Of note, by examining cortical microarchitecture of subtrochanteric region, Osima et al. [20] assumed that the reason for increased fragility in T2DM individuals is probably beyond cortical microarchitecture. Since trabecular compartment also significantly contributes to femoral neck strength [27], we examined trabecular bone microarchitecture; clearly, although both DMFx\_VD and DMFx\_NVD groups experienced fracture, they displayed quite different microarchitecture patterns, also highlighting that the risk of fracture does not correlate fully with microarchitecture in all patients, and other determinants of bone fragility should be considered.

Although DXA was able to identify an increased fracture risk in both DMFx subgroups (DMFx\_VD, DMFx\_NVD) compared with controls, we showed that DXA measurement was “blind” for the additional risk observed in individuals with vascular complications. A previous study suggested that diabetic complications such as diabetic nephropathy and vascular diseases may increase the risk of fracture in DM individuals [2]. A large, retrospective study on more than 600,000 men [28] showed a higher prevalence of vascular and cerebrovascular diseases in DM patients who sustained hip fracture than in DM patients without a fracture. Leanza et al. [29] suggested that among T1DM patients, those with vascular diseases more often sustained two or more non-vertebral fractures than those

without cardiovascular diseases. Likewise, Miao et al. [30] reported that T1DM patients hospitalized due to microvascular complications (diabetic retinopathy and neuropathy) or cardiovascular diseases had a much greater relative risk of fracture than individuals without cardiovascular disease or diabetic complications. However, there is limited evidence for the microarchitectural origins of such trends. So far, only an HR-pQCT based study showed that, compared with T2DM patients without microvascular complications (retinopathy, nephropathy, or neuropathy), T2DM patients with complications showed a higher cortical porosity of distal radius [18], but no data are available for the femoral neck, the frequent fracture site. Here, we showed notable differences in femoral trabecular bone microarchitecture between the group of T2DM patients with and without vascular complications. Considering that both groups of T2DM patients sustained a low-energy hip fracture, it is obvious that they had a reduction in femoral bone mechanical competence. In T2DM patients without vascular complications, the fracture occurred despite good trabecular bone microarchitecture reflected in higher Tb.N and similar BV/TV to controls. In contrast, the fracture occurrence in T2DM patients with vascular complications likely stems from deterioration in trabecular bone microarchitecture, probably in addition to other bone changes that occur in patients without complications as well, but further studies are needed to unravel those changes.

Wölfel et al. analyzed subtrochanteric region of T2DM patients; besides deteriorated microarchitecture, they found additional weaknesses, such as impaired mineralization profile and reduced osteon density of endocortical region, mirroring impaired bone quality in T2DM individuals with high cortical porosity. Oren et al. obtained a 32% higher content of pentosidine in tibial plateau of 10 T2DM patients compared with controls [31]. In-vivo studies in T2DM patients confirmed excessive skin accumulation of advanced glycation end products (AGEs) [32], and content of AGEs in the skin positively correlated with quantum of AGEs accumulated in bone [33, 34]. AGEs tend to accumulate more severely in cortical bone [35], and cortical AGEs content is a solid marker of normal bone aging. AGEs could be one of the reasons for bone fragility in DM individuals since accumulation of AGEs is more pronounced in T2DM patients and excessive AGEs tend to deteriorate bone quality directly via interference with osteoblast function [36] and indirectly via non-enzymatic glycosylation of collagen fibers [37].

In our sample, based on the mean HbA1c value among the included T2DM patients, mid-term glucoregulation was relatively good. However, all the patients sustained the fracture, indicating that bone strength was suboptimal despite having almost desirable levels of HbA1c (<6.5%). Our microstructural phenotyping of T2DM patients with “favorable” HbA1c levels supports the findings from a large

prospective study on the relationship between glycemic control and fracture risk, which showed that persons with diabetes and HbA1c values below 6.5% had similar risk to individuals with HbA1c levels between 7 and 7.9% [38].

This study had several limitations. First, it was limited by investigating only trabecular bone, which was chosen as it could be consistently obtained from surgical specimens of hip fracture cases, whereas cortical bone of the femoral neck is rarely and inconsistently obtained at the surgery. Further studies are needed to elucidate other determinants of bone fragility beyond bone microarchitecture, such as bone matrix characteristics and bone cell phenotypes. Next, our sample size was limited, suggesting that the study may be underpowered to detect all intergroup differences, especially in ANOVA with Bonferroni correction; nevertheless, it is of the same order of magnitude like many microCT studies. With larger sample size, some of the insignificant differences may become significant. Third, the values of HbA1c in our sample may not be representative of the entire population of T2DM patients because the value of HbA1c indicated that the disease was relatively well controlled. However, it should be noted that we included only those patients in whom the medical team estimated that surgical treatment of the fracture is possible, with reasonable level of risk, which may have caused such a distribution of HbA1c values. Nevertheless, our data are valuable as this is the first study documenting microstructure of the femoral neck in individuals with “favorable” HbA1c levels and a fracture. Finally, there may be differences in the level of details and accuracy in diagnosing comorbidities between clinical patients and autopsy individuals. Moreover, complete medical history for cadaver donors is often difficult to obtain, especially the data regarding medications used. Nevertheless, control group was selected at autopsies to ensure consistent harvesting of the same bony region for the analyses and to avoid osteoarthritis as a potential factor influencing femoral neck microarchitecture.

Our study suggested that trabecular bone microarchitecture of the femoral neck in T2DM patients without vascular complications does not explain the fracture risk. Considering that both T2DM groups of patients sustained hip fracture, our data may suggest two distinct mechanisms of bone fragility in T2DM patients, depending on the presence of vascular complications. Namely, despite having experienced a fracture, patients without manifest diabetic complications did not show significant differences in microarchitecture compared with the control group; however, deterioration of trabecular microarchitecture was evident in patients with vascular complications compared with those without. Our data further highlight that consideration of diabetic complications is needed for proper understanding of the mechanisms of bone fragility in T2DM patients, and they may partly explain some of the inconsistencies observed in

previous studies. Physicians should be aware of the higher fracture risk in T2DM individuals with vascular complications, even if HbA1c values are below 6.5%. Here, we observed bone microarchitecture at the common location for fragility fractures, and therefore, our findings may contribute to better understanding of the fracture risk in diabetic patients.

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**Author Contributions** Concept/design of the study: PM, AC, MD, ZB; acquisition of data: AC, MV, BP, VZ, SN; data analysis: AC, PM; data interpretation: AC, PM, AC, DD, MD; drafting of the manuscript: AC, PM. critical revision of the manuscript: MD, DD, AC, ZB, SN, MV, BP, VZ; final approval of the article: all the authors.

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**Data Availability** The data that support the findings of this study are available from the corresponding author, [P.M], upon reasonable request.

## Declarations

**Conflict of interest** Aleksandar Cirovic, Marko Vujacic, Bojan Petrovic, Ana Cirovic, Vladimir Zivkovic, Slobodan Nikolic, Danijela Djonic, Zoran Bascarevic, Marija Djuric, and Petar Milovanovic have no conflicts of interest.

**Ethical Approval** All procedures were approved by the institutional review board of the Institute for Orthopedic Surgery “Banjica” and by the Ethics Committee of the Faculty of Medicine, University of Belgrade.

**Human and Animal Rights** All procedures performed were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of the Institute for Orthopedic Surgery “Banjica” and Ethics Committee of Faculty of Medicine, University of Belgrade.

**Informed Consent** Informed consent was obtained from all individual participants included in the study.

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