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Characteristics of bone metabolism in the male patients with diabetic neuropathy

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Abstract

Background: This study aimed to evaluate the characteristics of bone metabolism and fracture risk in the type 2 diabetes mellitus (T2DM) patients with distal symmetric polyneuropathy (DSPN).

Methods: A total of 198 T2DM individuals were recruited from January 2017 to December 2020. Patients with DSPN were evaluated by strict clinical and sensory thresholds. Biochemical parameters and bone mineral density (BMD) were measured. The BMD, bone turnover markers, and probability of fracture were compared between two groups, and the factors related to BMD and probability of hip fracture in 10 years were further explored.

Results: Compared with type 2 diabetes mellitus without distal symmetric polyneuropathy (T2DN–) patients, type 2 diabetes mellitus with distal symmetric polyneuropathy (T2DN+) patients had lower level of cross-linked C-telopeptide (CTX) (0.32 ± 0.19 vs 0.38 ± 0.21 ng/mL, $p = 0.038$) and higher level of bone-specific alkaline phosphatase (BALP) (15.28 ± 5.56 vs 12.58 ± 4.41 μg/mL, $p = 0.003$). T2DN+ patients had higher BMD of lumbar L1-L4 (1.05 ± 0.19 vs 0.95 ± 0.37, $p = 0.027$) and higher probability of hip fracture (0.98 ± 0.88 vs 0.68 ± 0.63, $p = 0.009$) as compared to T2DN– individuals. Univariate correlation analysis showed that BALP level (coefficient (coef) = –0.054, $p = 0.038$), CTX level (coef = –2.28, $p = 0.001$), and hip fracture risk (coef = –1.02, $p < 0.001$) were negatively related to the BMD of L1-L4. As for the risk of hip fracture evaluated by WHO Fracture Risk Assessment Tool (FRAX), age (coef = 0.035, $p < 0.001$), use of insulin (coef = 0.31, $p = 0.015$), and levels of BALP (coef = 0.031, $p = 0.017$) and CTX (coef = 0.7, $p = 0.047$) were positively related to the risk of hip fracture. Multivariate regression analysis showed that CTX level (coef = –1.41, $p = 0.043$) was still negatively related to BMD at the lumbar spine.

Conclusion: This study indicates that T2DM patients with DSPN have special bone metabolism represented by higher BALP level and lower CTX level which may increase BMD at the lumbar spine.

Keywords: Bone mineral density; Diabetic neuropathy; Polyneuropathy; Sensory thresholds; Type 2 diabetes mellitus

1. INTRODUCTION

Diabetes mellitus, characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both, has been one of the most common metabolic diseases worldwide,[1] in which type 2 diabetes mellitus (T2DM) is the predominant subtype, and accounts for up to 90%-95%.[2] T2DM is recognized as one of risk factors for fractures[3] and individuals with T2DM have 69% increased fracture risk than those without diabetes.[4] However, patients with T2DM usually have normal or even higher bone mineral density (BMD)[5] which may be related to their unique bone metabolism.[6]

The mechanisms for the increased fracture risk and special bone metabolism of T2DM patients are not fully established, but it is likely to be multifactorial, in which diabetic neuropathy is one of the most important factors. Diabetic neuropathy is a common complication of diabetes, and can be divided into several subtypes, including distal symmetric polyneuropathy (DSPN), autonomic neuropathies, atypical neuropathies, etc.[7] A retrospective cohort study involving 2 798 309 older male veterans showed that as much as 21% of the increased fracture risk in T2DM patients can be explained by diabetic neuropathy, which is the highest contributing factor for the increased fracture risk of T2DM patients during all examined diabetic complications.[8] The increased fracture risk in the DSPN patients is attributed to different mechanisms. First, diabetic neuropathy is usually related to prolonged uncontrolled glucose level which may influence bone strength by inducing the accumulation of advanced glycation end products (AGEs) in bone and increasing the production of nonenzymatic cross-links within collagen fibers.[9] Second, DSPN is related to at least two-fold increase in fall risk by impairing the ability of balancing.[10] Third, the nervous system has been showed to regulate bone metabolism directly, including central[11] and peripheral nervous systems.[12] Sensory nerve is one of the most important parts of peripheral nerve system, and bones have abundant sensory nerves that frequently innervate trabecular bone, periosteum, and fracture callus.[13] Patients with sensory nerve dysfunction or loss have increased fracture risk and significantly reduced bone regeneration after

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injury.[14] The peripheral nerve fibers can regulate bone metabolism directly[15] or by their neurotransmitters.[16] One study showed that a small molecule, which can increase prostaglandin E2 (PGE2) level in the bone locally, could significantly boost bone formation, however this effect was absent in EP4 (PGE2 receptor in sensory nerves) knockout mice.[15] Another study showed that mice lacking Sema3A in neurons ($Sema3_{asynapsin}$–/–) had lower bone mass, accompanied by decreased number of sensory innervations on trabecular bone.[17] These results indicate that sensory nerves on bone may control bone homeostasis and promote bone regeneration. Sensory nerves also contribute to the chondrogenic differentiation during limb growth in embryonic development by regulating calcitonin gene-related peptides (CGRPs) and substance P (SP).[16] The peripheral nervous system may also regulate bone metabolism through neuroregulation of skeletal vascular supply.[18] In human bones, 95% of nerves are associated with blood vessels[19] and angiogenesis is a key component of both bone modeling and remodeling.[20] Sensory neuropeptides CGRP and substance P are highly potent vasodilators,[18] can also promote angiogenesis[21] and thus regulate bone metabolism indirectly.

Available studies have shown that about half of T2DM patients may develop DSPN during the course of T2DM,[22] and thus it is important to recognize the influence of DSPN on bone metabolism in T2DM population. In this study, the characteristics of bone metabolism and fracture risk were evaluated in T2DM patients with DSPN and their related risk factors were explored.

2. METHODS

2.1. Participants

This is an observational cross-sectional study. This study was approved by the Ethics Committee of Shanghai Tongji Hospital, School of Medicine, Tongji University (No. KYSB-2018-136). All participants signed the informed consent.

BMD usually peaks at the age of 35 to 40 years, and thus the enrolled patients older than 40 years were recruited into present study. A total of 198 T2DM individuals were recruited into this study from January 2017 to December 2020 in Shanghai Tongji Hospital. There were 109 individuals with DSPN and 89 individuals without DSPN. The inclusion criteria were as follows: (1) patients were older than 40 years; (2) patients were males; (3) patients had a history of T2DM. Exclusion criteria were as follows: (1) there was a history of fractures in prior 6 months (fractures can influence bone turnover markers [BTMs]); (2) patients had secondary osteoporosis (such as hyperthyroidism, parathyroidism, Cushing syndrome, hypogonadism, growth hormone deficiency); (3) patients had malignant tumors; (4) patients had autoimmune diseases; (5) there was liver dysfunction (alanine aminotransferase >80 U/L or aspartate aminotransferase >70 U/L or glutamyl transferase >90 U/L) or renal dysfunction (estimated glomerular filtration rate <90 mL/min/1.73 m^2); (6) patients were treated with drugs that can affect bone metabolism such as thiazolidinediones, sodium-glucose cotransporter, glucocorticoid, thyroid hormones, estrogen, and antiosteoporosis drugs.

2.2. Neuropathy assessment

Patients with DSPN are evaluated by strict clinical and sensory thresholds. The Toronto Clinical Neuropathy Score (TCNS) and the foot vibration perception threshold (VPT) were employed to assess DSPN.[23] The sensory symptoms and clinical signs of DSPN were combined in scores.

TCNS, involving symptoms, reflexes, and sensory, is a continuous variable ranging from a minimum of 0 (no neuropathy) to a maximum of 19 points. The TCNS is shown in Table 1. Six points are derived from symptoms, eight from lower limb reflexes, and five from sensory examination distally at the toes. All participants were evaluated by one examiner, and classified according to their scores as no neuropathy (0-5), mild neuropathy (6-8), moderate neuropathy (9-12), and severe neuropathy (>12).

Table 1

The TCNS

Symptom scores	Reflex scores	Sensory test scores
Foot	Knee reflexes	Pinprick
Pain	Ankle reflexes	Temperature
Numbness		Light touch
Tingling		Vibration
Weakness		Position
Ataxia		
Upper-limb symptoms		

Sensory testing was performed on the first toe. Symptom scores: present = 1; absent = 0. Reflex scores: absent = 2; reduced = 1, normal = 0. Sensory test score: abnormal = 1. normal = 0. Total scores range from normal = 0 to maximum of 19.
TCNS = Toronto Clinical Neuropathy Score.

VPT is an important method of quantitative sensory examination. It is simple, easy, noninvasive, and reproducible and plays an important role in the early diagnosis and assessment of diabetic peripheral neuropathy.[24] The TCNS has been validated against VPT. It is performed on both feet using the validated digital VPT device named sensiometer A200 (ChinaCache, Beijing, China) to confirm the presence of DSPN. Scoring was performed twice according to the manufacturer's instructions, and the average was calculated. Patients were classified as normal group (VPT ≤15 V on both feet), mild abnormality (VPT 16-24v on both feet,) or severe abnormality (VPT ≥25V on either foot).

Thus, participants with neuropathy (type 2 diabetes mellitus with distal symmetric polyneuropathy (T2DN+); n = 109) were defined by a combination of TCNS score ≥6 and VPT ≥16, while participants with TCNS score ≤5 and VPT ≤15 on both feet were considered neuropathy negative (type 2 diabetes mellitus without distal symmetric polyneuropathy (T2DN–); n = 89).

2.3. Demographics and clinical characteristics

The demographics and clinical characteristics (including age, weight, height, body mass index [BMI], status of current smoking, current drinking, and others) were collected from all the participants. BMI (kg/m^2) was calculated by dividing weight (kg) by the square of height (m^2). The smoking status means currently smoking. The status of drinking was defined as drinking more than or equal to three or more units of alcohol daily (one unit of alcohol means 8 g of alcohol).

2.4. Biochemical parameter assessment

The peripheral venous blood was collected from each subject after 8-hour fasting. The following parameters were detected in all the participants: liver and kidney function, lipid profile (total cholesterol [TCH], triglyceride [TG], low-density lipoprotein [LDL] and high-density lipoprotein [HDL]), glycosylated hemoglobin (hemoglobin A1c, HbA1c), BTM, alkaline phosphatase (ALP), bone ALP (BALP), procollagen type I intact N-terminal (P1NP), osteocalcin (OC), tartrate-resistant acid phosphatase-5b (TRACP-5b), c-terminal cross-linking telopeptide of type I collagen (CTX), calcium (Ca), corrected Ca and phosphorus (P), total protein, albumin, hemoglobin (Hb), fasting blood glucose (FBG), fasting insulin (FIS), C-peptide, Home-IR, and atherosclerosis index. The levels of thyroid hormone, thyroid stimulating hormone, parathyroid hormone, sex hormone (estrogen and progesterone), vitamin D, growth hormone, and insulin-like growth factor-1 were also detected in all the participants.

Liver function, renal function, lipid profiles, and electrolytes were detected by an automatic chemistry analyzer using serum. HbA1c was detected by high-performance liquid chromatography using whole blood. Serum P1NP, OC, and CTX were measured by electrochemiluminescence assay (Roche Diagnostics). Serum BALP and TRACP-5b were measured by enzyme immunoassay (IDS Ltd coefficient of variation of intra- and inter-assay <10%). ALP was measured by ALP assay kit. Hb was detected with the blood cell analyzer. Total protein and albumin were detected by biochemical analyzer. Insulin and C-peptide are detected by chemiluminescence particle immunoassay. Corrected Ca (mmol/L) was calculated according to the following formula: Ca (mmol/L) + 0.02 × [40-albumin (g/L)]. Atherosclerosis index was calculated with the following formula: [TC (mmol/L)-HDL (mmol/L)/HDL (mmol/L)]. Home-IR was calculated according to the following formula: [FBG (mmol/L) × FINS (μU/mL)].

2.5. BMD by dual-energy x-ray absorptiometry

The BMD at lumbar spine L1-L4, femur neck, and total hip was detected by dual-energy x-ray absorptiometry (DEXA, HOLOGIC Discovery; coefficient of variation <1%) in all the participants.

2.6. WHO FRAX and FRAX-adjustment scores

The probability of hip fracture in 10 years and any important fracture probability in 10 years (clinical spine, forearm, hip, or shoulder fracture) by World Health Organization (WHO) Fracture Risk Assessment Tool (FRAX) were calculated in all the participants. The FRAX algorithm includes femoral neck BMD T-score, age, sex, BMI, previous history of fracture, parental history of hip fracture, current smoking, recent use of corticosteroids, presence of rheumatoid arthritis (RA), and at least three alcoholic beverages per day. T2DM is a risk factor for osteoporotic fracture, but not a direct input variable to FRAX. There are several proposals to improve the performance of FRAX for those with T2DM. Three methods provided in the studies were employed to calculate the FRAX-adjustment,[25] including (1) the RA input to FRAX; (2) reducing the femoral neck T-score input to FRAX by 0.5 SD; (3) increasing the age input to FRAX by 10 years. The fracture risk assessment scale was used for fracture risk assessment in each participant.

2.7. Statistical analysis

Quantitative data are represented as mean ± SD. Categorical variables are described as number and percentages. The quantitative data with normal distribution were compared with independent sample *t* test, and those without normal distribution with Wilcoxon rank sum test. The categorical variables were compared with chi-square test. Univariate correlation analysis was employed to analyze whether each variable was associated with the BMD of lumbar L1-L4 and the probability of hip fracture in 10 years. Variables with possible associations ($p < 0.1$ according to the univariate correlation analysis) were included in multivariate regression analysis to adjust for potential confounding factors. The results of multivariate regression analysis are expressed in the form of forest map. A value of two-tailed $p < 0.05$ was considered statistically significant. Statistical analysis was conducted with Statistical Package for the Social Science version 20.0 (SPSS; IBM, Armonk, NY).

3. RESULTS

In our study, 80% of patients with diabetic neuropathy experienced pain, numbness, and weakness in daily life. Most of these symptoms were found in the feet, only a small number of participants had upper-limb symptoms, and 75% had decreased temperature and pain sensation. Almost all patients had decreased knee and ankle reflexes, but <10% of patients had ataxia. The mean TCNs scores were 6 to 8 in the participants, which is suggestive of mild neuropathy.

Comparing to the T2DN– patients, T2DN+ patients had longer course of T2DM, and longer duration of drinking. Moreover, T2DN+ patients had higher HbA1c level (9.67 ± 2.16 vs 8.66 ± 2.14, $p = 0.0053$), higher FBG (9.17 ± 3.69 vs 7.85 ± 3.16, $p = 0.0242$) and lower serum P level (1.16 ± 0.16 vs 1.22 ± 0.18, $p = 0.0233$) as compared to T2DN– patients (Table 2). Meanwhile, blood lipid profile, liver function, renal function, islet function (serum insulin and C-peptide), and insulin resistance were comparable between two groups.

In addition, T2DN+ patients had lower CTX level (0.32 ± 0.19 vs 0.38 ± 0.21 ng/mL, $p = 0.0378$) and higher BALP level (15.28 ± 5.56 vs 12.28 ± 4.41 μg/mL, $p = 0.0025$), but the

Table 2

Baseline characteristic of T2DN– and T2DN+ patients

Factors	T2DN– (n = 89)	T2DN+ (n = 109)	*p*
Characteristics			
Age, y	62.81 ± 7.79	64.86 ± 6.85	0.0841
BMI, kg/m^2	24.59 ± 2.79	24.47 ± 2.67	0.7892
Duration, y	8.70 ± 6.10	11.38 ± 7.52	0.0180
Hypertension, %	43 (48.61%)	67 (61.73%)	0.1031
Current smoking, %	45 (50.56%)	40 (37.04%)	0.1061
Current drinking, %	13 (14.81%)	31 (29.17%)	0.0312
Previous fracture, %	0	2 (2.47%)	0.1796
Use of insulin, %	49 (55.56%)	75 (69.14%)	0.0829
Height shorting, %	6 (6.94%)	13 (12.35%)	0.2621
Blood biochemical indicators			
Hb, g/mL	137.29 ± 13.89	139.69 ± 15.93	0.3264
TCH, mmol/L	4.27 ± 1.10	4.40 ± 1.25	0.5156
TG, mmol/L	1.83 ± 1.53	1.85 ± 1.57	0.9257
LDL, mmol/L	2.81 ± 0.88	2.92 ± 0.88	0.4211
HDL, mmol/L	1.01 ± 0.22	1.04 ± 0.48	0.7338
AI	3.33 ± 1.19	3.47 ± 1.01	0.4334
FFA, mmol/L	0.42 ± 0.24	0.50 ± 0.23	0.0852
AST, mmol/L	22.71 ± 19.58	18.56 ± 7.25	0.0810
ALT, mmol/L	25.49 ± 30.71	21.66 ± 13.87	0.3194
GGT, mmol/L	46.34 ± 99.91	34.88 ± 27.97	0.3364
HCY, μmol/L	12.89 ± 3.68	13.39 ± 6.27	0.5997
CREA, μmol/L	82.72 ± 17.09	83.61 ± 12.10	0.7120
ACR, mg/g	4.87 ± 17.39	11.19 ± 29.04	0.2837
Ca, mmol/L	2.24 ± 0.093	2.27 ± 0.11	0.0543
Correct Ca, mmol/L	2.84 ± 0.26	2.81 ± 0.25	0.4623
P, mmol/L	1.22 ± 0.18	1.16 ± 0.16	0.0233
HbA1c, %	8.66 ± 2.14	9.67 ± 2.16	0.0053
Albumin, g/L	39.08 ± 3.75	39.47 ± 5.78	0.6307
Total protein, g/L	65.89 ± 5.14	65.88 ± 6.49	0.9908
FBG, mmol/L	7.85 ± 3.16	9.17 ± 3.69	0.0242
FINS, μU/mL	11.96 ± 13.21	14.08 ± 11.59	0.3256
C-peptide, ng/mL	1.48 ± 1.09	1.31 ± 0.89	0.3053
Home-IR	113.45 (191.96)	123.94 (95.32)	0.6871

Normal distribution values are shown as means ± SD or number (percentage). Values with non-normal distribution are expressed as median (quartile).

ACR = urinary microalbumin creatinine ratio; AI = atherosclerosis index; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI, body mass index; Ca = calcium; Corrected Ca = corrected calcium; CREA = creatinine; Duration = diabetes course; FBG = fasting blood glucose; FFA = free fatty acids; FIS = fasting insulin; GGT = γ- glutamyl transferase; Hb = hemoglobin; HbA1c = glycosylated haemoglobin; HCY = homocysteine; HDL = high density lipoprotein; Home-IR = insulin resistance index; LDL = low density lipoprotein; P = phosphorus; TCH = total cholesterol; TG = triglyceride; T2DN+ = type 2 diabetes mellitus patients without distal symmetric polyneuropathy; T2DN- = type 2 diabetes mellitus patients with distal symmetric polyneuropathy.

levels of TRACP-5b, total ALP, P1NP, and OC were comparable between two groups (Table 3). T2DN+ patients had higher BMD of lumbar L1-L4 (1.05 ± 0.19 vs 0.95 ± 0.37, $p = 0.0273$) but there was no significant difference in the femur neck and total hip between T2DN– individuals and T2DN+ individuals (Table 3). When the fracture risk was evaluated by FRAX, the probability of hip fracture was higher in T2DN+ patients (0.98 ± 0.88 vs 0.68 ± 0.63, $p = 0.0092$) than in T2DN– patients (Table 3). However, there was no significant difference in the FRAX-adjustment between two groups (Table 3).

Univariate correlation analysis showed higher BMD of lumbar L1-L4 and increased hip fracture risk in T2DN+ individuals. The BALP level (coef =–0.054, $p = 0.038$), CTX level (coef = –2.28, $p = 0.001$), and hip fracture risk (coef = –1.02, $p < 0.001$) were negatively related to the BMD of L1-L4 (Table 4). As for the risk of hip fracture evaluated by FRAX, age (coef = 0.035, $p < 0.001$), use of insulin (coef = 0.31, $p = 0.015$), and levels of BALP (coef = 0.031, $p = 0.017$) and CTX (coef = 0.7, $p = 0.047$) were positively related to the risk of hip fracture (Table 4), but the BMDs of L1-L4 (coef = –0.27, $p < 0.0001$), femoral neck (coef = –0.71, $p < 0.0001$), and total hip (coef = –0.62, $p < 0.0001$) were negatively related to the risk of hip fracture (Table 4).

The variables that were related to the BMD of L1-L4 and hip fracture risk in the univariate correlation analysis were included into multivariate regression model. As shown in Fig. 1, neuropathy in diabetics was positively (coef = 0.57, $p < 0.021$) and the level of CTX was negatively (coef = –1.41, $p = 0.043$) related to the BMD of lumbar spine (Fig. 1A). The age (coef = 0.323, $p < 0.001$) and level of BALP (coef = 0.027, $p = 0.025$) were positively related to the risk of hip fracture, and BMD of L1-L4 was still negatively related to the risk of hip fracture individually (coef = –0.28, $p < 0.001$) (Fig. 1B).

Table 3

Bone metabolism-related markers of T2DN– and T2DN+ patients

Factors	T2DN– (n = 89)	T2DN+ (n = 109)	*p*
Bone metabolism-related markers			
Total ALP, U/mL	80.84 ± 33.98	87.81 ± 24.22	0.1598
BALP, μg/mL	12.58 ± 4.41	15.28 ± 5.56	0.0025
P1NP, ng/mL	34.78 ± 12.48	34.77 ± 12.97	0.9985
OC, ng/mL	10.78 ± 3.93	10.64 ± 3.88	0.8337
TRACP-5b, U/L	2.25 ± 0.76	2.51 ± 0.68	0.3026
CTX, ng/mL	0.38 ± 0.21	0.32 ± 0.19	0.0378
DXA			
Lumbar L1-L4, g/cm^2	0.95 ± 0.37	1.05 ± 0.19	0.0273
LS T-Score	–0.89 ± 1.45	–0.38 ± 1.74	0.0398
Femur neck, g/cm^2	0.73 ± 0.11	0.73 ± 0.12	0.7566
FN T-Score	–1.49 ± 0.81	–1.46 ± 0.87	0.7873
Total hip, g/cm^2	0.92 ± 0.12	0.91 ± 0.14	0.4952
TH T-Score	–0.74 ± 0.83	–0.81 ± 0.92	0.6182
FRAX			
Major osteoporotic fracture, %	2.29 ± 0.80	2.51 ± 1.13	0.1345
Hip fracture, %	0.68 ± 0.63	0.98 ± 0.88	0.0092
FRAX-adjustment			
Major osteoporotic fracture, %			
With T-score lowered 0.5 SD	3.48 ± 1.60	3.38 ± 1.44	0.6980
With RA adjustment	3.65 ± 1.54	3.23 ± 1.35	0.3990
With age raised 10 y	3.39 ± 1.45	3.33 ± 1.35	0.5786
Hip fracture, %			
With T-score lowered 0.5 SD	1.62 ± 1.31	1.46 ± 1.10	0.0634
With RA adjustment	2.24 ± 7.79	1.16 ± 1.08	0.2341
With age raised 10 y	1.57 ± 1.29	1.53 ± 1.07	0.0927

Values are shown as means ± SD or number (percentage).

ALP = alkaline phosphatase; BALP = bone alkaline phosphatase; CTX = C-terminal cross-linking telopeptide of type I collagen; DXA = dual-energy x-ray absorptiometry; FRAX = WHO Fracture Risk Assessment Tool; FRAX-adjustment = three adjustments applied to FRAX with diabetes[25]; LS = Lumar L1-L4; OC = osteocalcin; P1NP = procollagen type I intact N-terminal; RA = rheumatoid arthritis; TRACP-5b = tartrate-resistant acid phosphatase-5b; T2DN+ = type 2 diabetes mellitus patients without distal symmetric polyneuropathy; T2DN- = type 2 diabetes mellitus patients with distal symmetric polyneuropathy.

4. DISCUSSION

In the present study, our results showed that T2DM patients with DSPN had longer course of diabetes, longer duration of drinking, higher levels of HbA1c and serum P, higher BALP level, lower CTX level, higher BMD of L1-L4, and higher risk of hip fracture as compared to T2DM patients without DSPN. Moreover, the higher BALP level and the use of insulin were related to the increased hip fracture in the T2DM patients with DSPN.

Older age and longer course of T2DM have been proven to be risk factors for increased hip fracture risk in the T2DM patients.[26,27] Many previous studies have demonstrated that older age may enhance the risk of hip fracture and other types of fracture.[28] Meanwhile, the prolonged course of T2DM increases the risk of hip fracture.[26] A meta-analysis involving 42 clinical trials has revealed that longer course of T2DM is associated with increased risk of hip fracture.[27] Moreover, longer course of T2DM also contributes to the excess mortality after fall-induced hip fracture in patients with diabetes.[29] Longer course of T2DM is also positively related to the loss of muscle mass and function, which therefore increases the probability of falling and related injuries, like fracture.[30]

Several studies have reported that the use of insulin is associated with an increased fracture risk as compared to other glucose-lowering drugs, absence of insulin use, metformin monotherapy, or nondiabetics.[31] The increased fracture risk in the patients with insulin use is related to different factors. First, many hormones secreted by the islet, such as insulin, amylin, and preptin, have been found to have effects on bone cells in vitro and in vivo,[32] and thus the lack of insulin in patients with longer course of T2DM may cause imbalance of bone metabolism. Second, T2DM patients with the use of insulin frequently have multiple complications which can also increase the risk of fractures.[32] Third, the use of insulin may increase hypoglycemia events in T2DM patients which also increases the risk of falls.[33]

BALP, an extracellular enzyme of osteoblasts, is involved in bone formation, is stable in serum, and has been used as a marker of osteoblast maturation and activity. BALP has been found as one of the most accurate markers of bone formation.[34] In the organic matter of bone, 90% of the fraction is type I collagen carboxy-terminal peptide (CTX). The level of CTX reflects the bone resorption activity of osteoclasts, and CTX has been used as a valid marker for metabolic bone diseases.[35] Sensory nerves can sense bone density through the PGE2.[15] PGE2 can bind to EP4 in the sensory nerve and regulate sympathetic nervous activity through the central nervous system to form osteoblastic bone. High sympathetic tone promotes osteoclastic bone resorption by increasing the synthesis of receptor activator of nuclear factor κ-B ligand (rank l) in the osteoblasts. Bone remodeling depends up on a coordinated sequence of bone resorption by osteoclasts, and bone formation by osteoblasts. In our study, T2DM patients with DSPN had special bone metabolism represented by a higher level of BALP and a lower level of CTX. This indicates that T2DM patients with DSPN have decreased bone turnover. The vertebral bone density is elevated, and the risk of fracture is also increased.

Several clinical studies have shown that male patients with diabetic polyneuropathy have higher levels of CTX and P1NP as comparing to patients without neuropathy, which indicates diabetic polyneuropathy may increase bone turnover.[36] However, another study of Mohseni et al[37] failed to the influence

Table 4

Univariate correlation analysis of the potential confounders of BMD and hip fracture

	LS T-score			Hip fracture, %		
Factors	**Coef**	***p***	**95% CI**	**Coef**	***p***	**95% CI**
Age, y	0.016	0.369	(–0.019 to 0.051)	0.035	<0.001	(0.018-0.050)
Duration, y	0.0058	0.759	(–0.031 to 0.043)	0.012	0.178	(–0.0055 to 0.029)
Hypertension						
No						
Yes	0.36	0.170	(–0.16 to 0.88)	–0.22	0.071	(–0.46 to 0.019)
Previous fracture						
No						
Yes	–1.14	0.294	(–3.29 to 1.01)	0.45	0.409	(–0.62 to 1.52)
Current smoking						
No						
Yes	0.10	0.702	(–0.42 to 0.63)	0.074	0.555	(–0.17 to 0.32)
Current drinking						
No						
Yes	0.12	0.710	(–0.52 to 0.75)	–0.082	0.587	(–0.38 to 0.22)
Use of insulin						
No						
Yes	–0.10	0.711	(–0.64 to 0.44)	0.31	0.015	(0.062 to 0.56)
Height shorting						
No						
Yes	–0.55	0.189	(–1.37 to 0.27)	–0.14	0.500	(–0.55 to 0.27)
BALP, μg/mL	–0.054	0.038	(–0.11 to –0.0031)	0.031	0.017	(0.0055-0.058)
CTX, ng/mL	–2.28	0.001	(–3.62 to –0.93)	0.70	0.047	(0.0087-1.39)
AST, mmol/L	–0.0086	0.313	(–0.025 to 0.0082)	0.0042	0.268	(–0.0033 to 0.012)
CREA, μmol/L	0.0012	0.891	(–0.016 to 0.019)	0.0049	0.194	(–0.0025 to 0.012)
P, mmol/L	0.29	0.714	(–1.28 to 1.86)	–0.44	0.183	(–1.10 to 0.21)
HbA1c, %	–0.017	0.779	–0.14 to 0.10	0.044	0.088	(–0.0066 to 0.095)
Hip fracture, %	–1.02	<0.001	(–1.29 to –0.73)	/	/	/
LS T-Score	/	/	/	–0.27	<0.001	(–0.35 to –0.19)
FN T-Score	/	/	/	–0.71	<0.001	(–0.81 to –0.62)
TH T-Score	/	/	/	–0.62	<0.001	(–0.73 to –0.52)

ALP = alkaline phosphatase; AST = aspartate aminotransferase; BALP = bone alkaline phosphatase; BMD = bone mineral density; coef = coefficient; CREA = creatinine; CTX = C-terminal cross-linking telopeptide of type I collagen; Duration = diabetes course; HbA1c = glycosylated haemoglobin; P = phosphorus; / = unanalyzable.

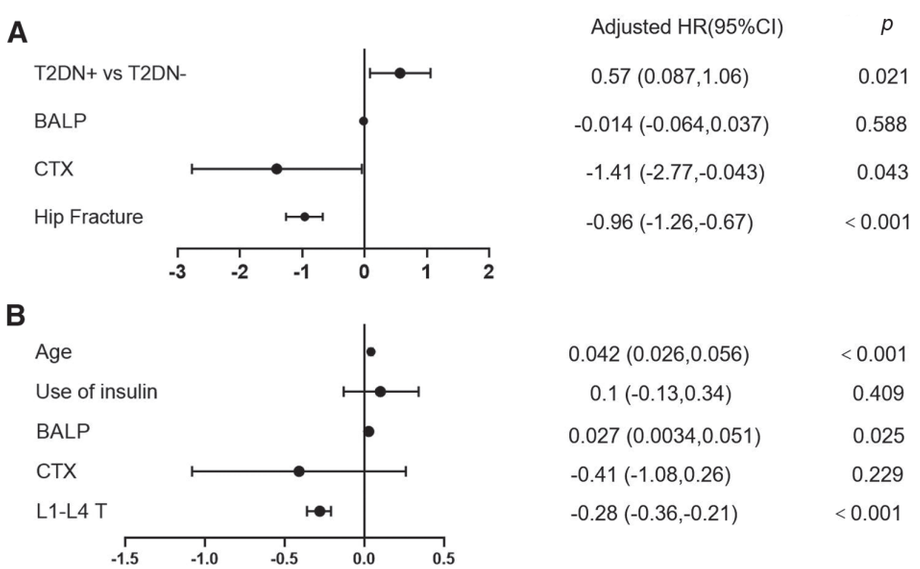

Fig. 1 Multivariate regression analysis of the risk factors of BMD and hip fracture. A, Parameters correlated with BMD of lumbar L1-L4. B, Parameter correlated with hip fracture by FRAX. BALP = bone alkaline phosphatase; BMD = bone mineral density; CTX = C-terminal cross-linking telopeptide of type I collagen; T2DN+ = type 2 diabetes mellitus patients without distal symmetric polyneuropathy; T2DN- = type 2 diabetes mellitus patients with distal symmetric polyneuropathy..

of peripheral neuropathy on bone density or bone turnover in T2DM patients. Some studies have also shown decreased BMD in the patients with DSPN.[37] There is evidence showing that lower level of bone turnover biomarkers (β-CTX, P1NP, and OC) was associated with higher insulin resistance and worse β-cell function in T2DM patients.[38] Animal studies also indicate that mice with inducible sensory denervation have bone loss, combined with significantly decreased number of osteoblasts, amount of osteoid and serum OC level, which confirms that sensory nerve can regulate bone homeostasis through osteoblasts during bone remodeling.[15] However, based on the controversial clinical findings, better measures of peripheral neuropathy and more advanced imaging technologies on bone metabolism are needed to assess the influence of DSPN on the bone metabolism.

Many studies including those of our group have confirmed that the fracture risk is increased in T2DM patients but the BMD can be falsely high in those patients.[6,39,40] One explanation for this is that body or skeletal size may bias the results obtained by DXA, which assesses areal BMD instead of "true" volumetric BMD because of its two-dimensional geometry. Increased insulin and glucose levels due to insulin resistance are other causes.[40–42] Elevated insulin level and accumulation of AGEs alter bone mass, bone microarchitecture (increasing cortical porosity), and osteoblast differentiation. Therefore, T2DM patients have increased bone fragility.[40] In our study, despite T2DN+ patients had higher BMD, the risk of hip fracture in T2DN+ at 10 years (calculated by FRAX) was still higher than in T2DN– patients. It reflects that the risk of fracture in T2DN+ might be higher than predicted.

In conclusion, this study shows that T2DM patients with DSPN have special bone turnover, characterized by higher BALP level and lower CTX level, which may contribute to their increased BMD of lumbar spine, but the increased risk of hip fracture in the T2DM patients with DSPN indicates that some other factors overwhelm the protection of special bone metabolism, including longer course of diabetes and the use of insulin.

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