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ORIGINAL RESEARCH

Association of Plasma Sex-Related Hormones Levels with Bone Mineral Densities and Risk of Osteoporosis and Osteopenia in Men and Menopausal Women with Type 2 Diabetes Mellitus

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Objective: This study aimed to examine associations between plasma sex-related hormones with bone mineral density (BMD) and risks of osteoporosis or osteopenia in men and postmenopausal women patients with type 2 diabetes mellitus (T2DM).

Methods: Baseline information on an ongoing cohort of 149 men and 102 postmenopausal women with T2DM in Xiamen, China were analyzed. Plasma estradiol (E2), total testosterone (T), follicle-stimulating hormone (FSH), luteinizing hormone (LH) and prolactin (PRL) were measured. BMD of lumbar spine (L2-4), femoral neck (FN) and total hip (TH) were determined by dual-energy X-ray absorptiometry (DXA). Osteoporosis or osteopenia was defined as the minimum T-scores of BMD of these three different sites of −1.0 or below.

Results: T2DM patients with osteoporosis/osteopenia (66.4% in men and 79.4% in postmenopausal women), compared to those without, showed significantly decreased level of E2 (75.3±28.9 vs. 107.8±25.9pmol/L and 18.4 (18.4–29.5) vs. 22.8 (18.4–40.5) pmol/L for men and postmenopausal women, respectively, both p-values <0.05), but not other sex-related hormones (including T, FSH, LH, or PRL). For all T2DM patients together and men separately, multivariable linear regression and logistic regression analyses showed that higher E2 levels were significantly associated with higher BMD T-scores in L2-4, FN, TH and minimum of these three different sites, lower 10-year probability of major osteoporotic fractures (MOF) and hip fractures (HFs) estimated by Fracture Risk Assessment Tool score, as well as decreased risk of osteoporosis/osteopenia. As for postmenopausal women T2DM patients, E2 level was positively associated with BMD T-scores in L2-4 and minimum of three different sites but was not independently associated with risk of osteoporosis/osteopenia.

Conclusion: Higher plasma E2 was significantly associated with increased BMD and lower risk of osteoporosis or osteopenia in T2DM patients, especially for men. Screening of BMD and estradiol levels as well as evaluating risks of osteoporosis/osteopenia are important for T2DM patients.

Keywords: estradiol, bone mineral density, osteoporosis, osteopenia, diabetes, BMD

Introduction

Type 2 diabetes mellitus (T2DM) and osteoporosis are both common chronic non-communicable diseases with increasing prevalence and public health burden worldwide during recent decades.[1] Osteoporosis is characterized by reduced bone mass and damaged bone tissue microstructure and induce increased bone fragility and risk of fracture.[2,3] Patients with T2DM are at elevated risk for osteoporosis and its related fractures, such as in hips, spines and even any sites, compared

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with healthy individuals,[4,5] although some studies found that bone mineral density (BMD) is relatively higher in patients with T2DM than those age-matched individuals without diabetes.[6,7]

Some of the previous studies have found that sex hormones affect bone health, but the results were indistinct. Different kinds of sex hormones, including androgen, estrogens, and gonadotropins (luteinizing hormone [LH], follicle-stimulating hormone [FSH], etc.), have been shown different effects on BMD and fracture risk.[8,9] Sex hormones changes, for example, testosterone and estradiol levels decline with aging, play important roles in osteoporosis development for aging populations. Previous studies have reported associations between testosterone, free testosterone (FT) and estrogen (E2) with BMD and risk of fractures with indistinct results,[10–12] and seldom of them focused on postmenopausal women. Therefore, the relationship of sex-related hormones with osteoporosis and fracture risk remains unclear, especially in terms of gender-specific association between them.[13–16] Furthermore, previous studies showed the levels of sex hormones in T2DM patients were different as compared to healthy individuals.[17,18] Therefore, future study is warranted to clarify the gender-specific associations of sex-related hormones with BMD and risk of osteoporosis or osteopenia, especially for postmenopausal women with T2DM.

In the present study, we firstly aimed to explore the independent associations of plasma sex-related hormones levels, such as E2, total testosterone (T), FSH, LH and prolactin (PRL), with BMD and risk of osteoporosis/osteopenia in all patients with T2DM. Secondly, we aimed to test the sex-specific associations of sex-related hormones levels with BMD and risks of osteoporosis/osteopenia for men and postmenopausal women patients with T2DM separately.

Methods

Ethics Statement

The study was approved by the Human Research Ethics Committee of the Zhongshan Hospital (Xiamen), Fudan University (No. B2019-015). All participants provided written informed consent.

Study Population

Patients' selection and measurements have been described previously.[19] From January 2018 to April 2020, all the Chinese patients with T2DM, with a total number of 490, who had been hospitalized in the Department of Endocrinology, Zhongshan Hospital (Xiamen), Fudan University (Xiamen, China) has been recruited into the present ongoing cohort. 239 patients without BMD and 19 premenopausal women without complete data on clinical measurements were excluded. Finally, 149 men and 102 postmenopausal women were included for the present study.

Patients were diagnosed as diabetes based on American Diabetes Association (ADA) 2018 criteria. Negative results on glutamic acid decarboxylase antibody (GAD-Ab) tests were used to exclude type 1 diabetes mellitus. Inclusion criteria included: (1) T2DM; (2) age $\geq$ 18 years; (3) postmenopausal women or men; and (4) underwent sex steroid and gonadotropin hormone testing and dual-energy X-ray absorptiometry (DXA) measurement. Exclusion criteria included: other type of diabetes (type 1 diabetes mellitus, secondary diabetes), severe liver and renal dysfunction, have received or are currently receiving E2 and progesterone drugs, glucocorticoids, calcium tablets and anti-osteoporosis drugs, the presence of metal implants affects the detection of BMD, other secondary osteoporosis such as multiple myeloma, rheumatic immune disease, hyperthyroidism, perimenopausal status, menopause by surgical intervention or at an unnatural age, unwilling to participate in research.

Measurements

Body weight, height, BMI, waist circumference (WC), hip circumference (HC), waist–hip ratio (WHR) and arterial blood pressure (BP) were measured as previously described.[19]

Venous blood samples were collected in the morning after a 12-h overnight fasting. Fasting plasma glucose (FPG), lipid profiles and bone turnover markers were tested in the clinical laboratory of Zhongshan Hospital (Xiamen), Fudan University (Xiamen, China). Serum creatinine (CRE), uric acid (UA), triglyceride (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-c), serum calcium and phosphorus were determined by using the same methods as previously described.[19] Low-density lipoprotein cholesterol (LDL-c) was calculated using the Friedewald formula.[20]

FPG concentration was measured by the hexokinase method. Serum fasting insulin and C-peptide were measured by electrochemiluminescence immunoassay (Roche Elecsys Insulin Test, Roche Diagnostics). Homeostasis model assessment (HOMA-IR) were used for estimating insulin resistance. 25-hydroxyvita-min D3 [25(OH)D] and bone turnover markers, such as intact parathyroid hormone (PTH), osteocalcin (OCN) and procollagen type 1 N-terminal propeptide (P1NP) were measured using the automatic electrochemiluminescence analyzer (Cobas e602, Roche). T, LH, E2, PRL and FSH were measured by chemiluminescent immunoassay analysis (Cobas e602, Roche Diagnostics). Assay sensitivities were 0.025ng/mL for testosterone, 0.100 mIU/mL for LH, and 0.100 mIU/mL for FSH. The intra- and inter-assay coefficients of variation were <8% and 10% for T, <3% and 2.9% for LH, <2.9% and 2.7% for FSH, respectively. Glycated hemoglobin (HbA1c) was determined by using high performance liquid chromatography (VARIANT II TURBO, Bio-Rad).

BMD Measurement and Definition of Osteoporosis or Osteopenia

BMD was measured using DXA (QDR4500A, Hologic Inc., Waltham MA, USA) as previously described.[19] Three different sites, including total lumbar, femur neck (FN), and TH, were checked for each patient. Osteoporosis or osteopenia was defined by the minimum T-score of BMD at three different sites of −1.0 or below. And the 10-year probability of major osteoporotic fractures (MOFs) and hip fractures (HFs) were estimated by using Fracture Risk Assessment Tool (FRAX) score.[21]

Statistical Analyses

Skewness and kurtosis normality test for continuous variables were conducted. Data were presented as the mean ± standard deviation for continuous variables which followed approximation of normal distributions, median (interquartile range [IQR]) for non-normally distributed continuous variables or number and percentage for categorical variables. Differences between subjects categorized as osteopenia/osteoporosis (vs. normal) were analyzed using independent samples *t*-test for normally distributed continuous variables, nonparametric test for non-normally distributed continuous variables and chi-square test for categorical variables. One-way ANOVA was used to compare differences of sex-related hormones levels across tertiles of T-score of BMD. Multivariable linear regression was conducted to analyze associations of E2 with T-score of BMD (L2-4, FN, TH, minimum), FRAX MOFs and HFs for men, postmenopausal women and all, separately. Multivariable logistic regression was used to calculate adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of E2 with osteoporosis/osteopenia (vs. normal) in men, postmenopausal women and all. Multivariable regression analyses were adjusted for potential confounders, including age, ever smoking and drinking habits, BMI, diabetes duration, FBG, HbA1c, diabetes medical treatment, TC, TGs, HDL-C, LDL-C, CRE, and UA. All p-values were two-sided and p-value <0.05 was considered statistically significant. All analyses were performed using SPSS 21.0 software (IBM Corporation).

Results

Demographic and Clinical Characteristics Across Osteoporosis/Osteopenia

Among the total of 251 T2DM patients, the mean (±SD) of ages and duration of diabetes were 57.8 (±11.8) and 7.9 (±6.8) years, respectively; 149 were men and 102 were postmenopausal women with the mean duration of menopause of 14.0 (±3.7) years. Among them, 99 (66.4%) men and 81 (79.4%) postmenopausal women were identified as osteoporosis/osteopenia. Table 1 and Table 2 showed differences of baseline characteristics across gender and osteoporosis/osteopenia (vs. normal BMD) for men and postmenopausal women separately. Generally, patients with osteoporosis/osteopenia, compared to those with normal BMD, showed significantly lower levels of T-score on L2-4, FN, TH and minimum of three different sites, body weight, height and BMI, as well as higher scores of FRAX MOFs and HFs. However, there was no significant difference on other clinical characteristics, including BP, biochemical measurements, OCN, P1NP, β-crosslaps and 1,25 (OH) 2D3. Estradiol (E2) but no other sex-related hormones, such as LH, FSH, progesterone, T and PRL, was significantly decreased in those with osteoporosis/osteopenia for both men and postmenopausal women T2DM patients.

Table 1 Demographic, Clinical Characteristics and Sex-Related Hormones of Subjects Stratified by Osteoporosis/Osteopenia (vs. Normal BMD) in Men T2DM Patients

	All	Normal BMD	Osteoporosis/Osteopenia	P value
		T-Score ≥-1	T-Score <-1	
Demographics and clinical characteristics				
N (%)	149 (100.0)	50 (33.6)	99 (66.4)	
Age (years)	57.3±10.0	53.7±10.9	57.3±9.8	0.513
Ever smoking (n, %)	87 (58.4)	30 (60.0)	57 (57.6)	0.882
Ever drinking (n, %)	62 (41.6)	20 (40.0)	42 (42.4)	0.778
Height (cm)	168.0±4.1	171.1±5.5	167.9±41	0.033*
Weight (kg)	67.2±8.9	74.1±11.43	67.1±8.7	0.004*
BMI (kg/m^2)	23.8±3.1	25.3±3.8	23.8±3.0	0.016*
Waist (cm)	89.2±6.58	91.5±9.5	89.0±6.5	0.024*
Hip (cm)	93.6±5.6	95.3±7.3	93.6±5.5	0.008*
Waist–hip ratio	0.95±0.05	0.96±0.05	0.95±0.05	0.518
Systolic blood pressure (mmHg)	129.2±16.3	130.1±17.0	128.7±16.0	0.624
Diastolic blood pressure (mmHg)	83.2±9.7	82.7±10.7	83.4±9.2	0.676
Diabetes duration (years)	5.0 (1.0–10.0)	4.0 (0.6–8.0)	5.0 (1.0–10.0)	0.232
Fasting plasma glucose (mmol/L)	7.84±2.37	8.53±2.73	7.78±2.33	0.760
HbA1c (%)	9.22±2.32	8.96±2.16	9.27±2.28	0.786
HOMA-IR ($*10^{-6}mol*IU*L^{-2}$)	2.5 (1.5–3.7)	2.1 (1.2–3.8)	2.6 (1.5–3.5)	0.929
Triglyceride (mmol/L)	1.24 (0.91–1.69)	1.35 (1.04–1.86)	1.30 (0.92–1.67)	0.577
Total cholesterol (mmol/L)	4.24 (3.51–5.31)	4.24 (3.90–4.66)	4.26 (3.55–5.25)	0.866
HDL-cholesterol (mmol/L)	1.14±0.38	1.03±0.23	1.13±0.37	0.862
LDL-cholesterol (mmol/L)	2.40 (1.88–3.16)	2.38 (2.13–3.09)	2.41 (1.91–3.16)	0.270
Blood uric acid (μmol/L)	354.6±76.6	359.2±79.9	355.5±75.1	0.992
Creatinine (umol/L)	73.5 (69.0–84.8)	79.0 (72.0–92.5)	72.0 (69.0–83.5)	0.272
C-reactive protein (mg/L)	0.90 (0.30–1.70)	1.35 (0.73–2.25)	0.80 (0.30–1.70)	0.513
Procalcitonin (pg/mL)	2.0 (2.0–2.0)	2.0 (0.0–3.1)	2.0 (2.0–2.0)	0.966
Serum calcium(mmol/L)	2.31 (2.26–2.35)	2.34 (2.26–2.40)	2.31 (2.26–2.35)	0.740
Serum phosphorus(mmol/L)	1.19±0.17	1.14±0.14	1.19±0.17	0.119
Parathyroid hormone (pg/mL)	37.5 (32.9–45.4)	36.3 (27.6–41.7)	38.1 (33.3–45.3)	0.321
Osteocalcin (ng/mL)	12.5±3.2	12.3±3.5	12.6±3.1	0.197
P1NP (ng/mL)	41.1 (30.0–47.0)	36.2 (29.3–51.1)	44.0 (30.1–47.4)	0.415
β-Crosslaps (ng/mL)	0.46±0.22	0.40±0.25	0.46±0.21	0.515
1,25 (OH) 2D3 (nmol/L)	61.79±22.02	61.62±34.60	62.76±22.01	0.495
Diabetes treatment (n (%))				
Biguanides	69 (46.3)	19 (38.0)	50 (50.5)	0.148
Glycosidase inhibitor	26 (17.5)	6 (12.0)	20 (20.2)	0.213
Sulfonylureas	40 (26.9)	13 (26.0)	27 (27.3)	0.869
TZD	8 (5.4)	7 (14.0)	1 (1.0)	0.008
Glinides	13 (8.7)	5 (10.0)	8 (8.1)	0.695
GLP-1 agonists	1 (0.7)	1 (2.0)	0 (0.0)	0.158
DPP-4 inhibitors	22 (14.8)	7 (14.0)	15 (15.2)	0.852
SGLT-2 inhibitors	1 (0.7)	0 (0.0)	1 (1.0)	0.476
Oral hypoglycemic medications use	99 (66.4)	32 (64.0)	67 (67.7)	0.654
Insulin use	46 (30.9)	15 (30.0)	31 (31.3)	0.870
Sex-related hormones				
Estradiol (E2, pmol/L)	76.6±28.8	107.8±25.9	75.3±28.9	<0.001*
Luteinizing hormone (LH, mIU/mL)	7.15 (5.35–8.55)	8.60 (6.80–12.85)	6.90 (5.15–8.50)	0.060
FSH (mIU/mL)	7.85 (6.40–11.30)	8.40 (5.80–12.55)	8.00 (6.40–11.50)	0.800
Progesterone (nmol/L)	0.40 (0.20–0.60)	0.40 (0.16–0.60)	0.40 (0.20–0.60)	0.491
Total testosterone (nmol/L)	14.07±5.50	16.19±5.07	13.89±5.45	0.314
Prolactin (PRL, mIU/L)	254.7 (207.4–385.8)	323.9 (253.9–433.6)	265.7 (208.9–378.9)	0.486

(*Continued*)

Table 1 (Continued).

	All	Normal BMD	Osteoporosis/Osteopenia	P value
		T-Score ≥-1	T-Score <-1	
BMD T-score				
Lumbar 2–4	−2.35±0.83	−0.15±0.66	−2.32±0.83	<0.001*
Femoral neck	−2.30±0.47	−0.67±0.31	−2.30±0.46	<0.001*
Total hip	−1.70±0.51	−0.26±0.31	−1.74±0.50	<0.001*
Minimum T-score	−1.42±0.85	−0.50±0.38	−1.88±0.62	<0.001*
FRAX MOF	1.72±0.66	1.50±0.35	2.55±1.29	<0.001*
FRAX HF	0.37±0.42	0.20±0.17	0.96±1.04	<0.001*

Note: * p<0.05.

Abbreviations: BMD, bone mineral density; BMI, body mass index; DPP-4, dipeptidyl peptidase 4; FRAX, fracture risk algorithm; FSH, follicle-stimulating hormone; GLP-1, glucagon-like peptide 1; HDL, high-density lipoprotein; HF, hip fractures; HOMA-IR, homeostasis model assessment - insulin resistance; LDL, low-density lipoprotein cholesterol; MOF, major osteoporotic fractures; P1NP, N-terminal peptide of procollagen type 1; SGLT-2, Sodium glucose co-transporter 2; T2DM, type 2 diabetes mellitus; TZD, trazodone.

Table 2 Demographic, Clinical Characteristics and Sex-Related Hormones of Subjects Stratified by Osteoporosis / Osteopenia (vs. Normal BMD) in Postmenopausal Women T2DM Patients

	All	Normal BMD	Osteoporosis/Osteopenia	P value
		T-Score ≥-1	T-Score <-1	
Demographics and clinical characteristics				
N (%)	102 (100.0)	21 (20.6)	81 (79.4)	
Age (years)	63.5±7.9	61.4±8.8	66.4±7.9	0.155
Ever smoking (n (%))	3 (2.9)	1 (4.8)	2 (2.5)	0.834
Ever drinking (n (%))	2 (2.0)	1 (4.8)	1 (1.2)	0.301
Height (cm)	158 (153–161)	160 (158–163)	155 (151–160.25)	0.013*
Weight (kg)	60.6±10.4	67.0±11.3	54.2±10.0	0.001*
BMI (kg/m^2)	24.9±5.1	27.1±7.1	22.4±3.5	0.025*
Waist (cm)	85.5±9.5	86.5±10.2	81.2±9.5	0.628
Hip (cm)	92.8±7.4	95.9±7.4	89.7±7.9	0.055
Waist–hip ratio	0.92±0.07	0.90±0.07	0.91±0.07	0.189
Systolic blood pressure (mmHg)	134.2±17.4	130.8±16.7	135.1±17.5	0.307
Diastolic blood pressure (mmHg)	80.4±10.3	79.9±11.1	80.5±10.2	0.831
Diabetes duration (years)	9.0 (4.0–14.8)	9.0 (1.0–11.0)	10.0 (3.8–19.5)	0.308
Fasting plasma glucose (mmol/L)	7.60 (6.00–8.80)	8.30 (6.88–9.40)	6.10 (5.30–8.20)	0.051
HbA1c (%)	9.05±2.18	9.50±2.51	8.80±2.09	0.296
HOMA-IR ($*10^{-6}mol*IU*L^{-2}$)	2.9 (1.7–4.6)	3.3 (1.7–4.5)	1.9 (1.3–3.5)	0.557
Triglyceride (mmol/L)	1.61 (1.21–2.15)	1.46 (1.21–1.81)	1.41 (0.94–1.82)	0.489
Total cholesterol (mmol/L)	4.76±2.02	4.51±1.26	4.94±3.66	0.541
HDL-cholesterol (mmol/L)	1.18 (0.99–1.35)	1.24 (1.03–1.30)	1.97 (1.61–2.68)	0.849
LDL-cholesterol (mmol/L)	2.56±1.10	2.57±1.07	2.15±0.86	0.931
Blood uric acid (μmol/L)	335.80±100.45	333.76±91.31	316.45±103.08	0.918
Creatinine (umol/L)	59.0 (50.3–70.8)	53.0 (50.0–63.0)	63.5 (56.5–73.3)	0.125
C-reactive protein (mg/L)	1.10 (0.50–2.80)	1.20 (0.50–2.80)	0.80 (0.50–1.65)	1.000
Procalcitonin (pg/mL)	2.0 (0.5–2.0)	1.8 (0.5–2.0)	1.5 (0.5–2.0)	0.356
Serum calcium(mmol/L)	2.33 (2.25–2.42)	2.33 (2.27–2.38)	2.32 (2.22–2.46)	0.529
Serum phosphorus(mmol/L)	1.27 (1.16–1.41)	1.30 (1.22–1.51)	1.21 (1.11–1.37)	0.540
Parathyroid hormone (pg/mL)	34.9±12.2	36.5±10.9	39.5±11.4	0.497
Osteocalcin (ng/mL)	16.0 (12.9–19.1)	15.1 (11.7–18.9)	16.3 (14.6–24.8)	0.596
P1NP (ng/mL)	45.1 (34.9–63.1)	49.9 (34.6–70.9)	57.2 (38.8–69.1)	0.299
β-Crosslaps (ng/mL)	0.39 (0.29–0.57)	0.39 (0.29–0.61)	0.41 (0.34–0.58)	0.957
1,25 (OH) 2D3 (nmol/L)	59.53±18.58	54.80±22.83	58.47±17.42	0.194

(*Continued*)

Table 2 (Continued).

	All	Normal BMD	Osteoporosis/Osteopenia	P value
		T-Score ≥-1	T-Score <-1	
Diabetes treatment (n (%))				
Biguanides	58 (56.9)	11 (52.4)	47 (58.0)	0.642
Glycosidase inhibitor	32 (31.4)	6 (28.6)	26 (32.1)	0.756
Sulfonylureas	41 (40.2)	9 (42.9)	32 (39.5)	0.780
TZD	8 (7.8)	2 (9.5)	6 (7.4)	0.748
Glinides	10 (9.8)	2 (9.5)	8 (9.9)	0.961
GLP-1 agonists	0 (0.0)	0 (0.0)	0 (0.0)	NA
DPP-4 inhibitors	28 (27.5)	5 (23.8)	23 (28.4)	0.675
SGLT-2 inhibitors	0 (0.0)	0 (0.0)	0 (0.0)	NA
Oral hypoglycemic medications use	86 (84.3)	16 (79.2)	70 (86.4)	0.251
Insulin use	34 (33.3)	8 (38.1)	26 (32.1)	0.603
Sex-related hormones				
Estradiol (E2, pmol/L)	18.4 (18.4–31.6)	22.8 (18.4–40.5)	18.4 (18.4–29.5)	0.027*
Luteinizing hormone (LH, mIU/mL)	30.99±14.76	31.43±19.13	32.23±13.97	0.879
FSH (mIU/mL)	53.8 (42.2–67.2)	46.0 (39.1–56.2)	61.6 (45.6–75.0)	0.228
Progesterone (nmol/L)	0.30 (0.16–0.50)	0.20(0.16–0.40)	0.30 (0.16–0.50)	0.582
Total testosterone (nmol/L)	0.40 (0.20–0.70)	0.60 (0.30–0.80)	0.30 (0.09–0.48)	0.123
Prolactin (PRL, mIU/L)	328.6 (249.3–406.5)	332.1 (271.9–406.6)	321.2 (242.7–371.5)	0.640
BMD T-score				
Lumbar 2–4	−1.56±1.46	−0.06±0.78	−3.25±0.92	<0.001*
Femoral neck	−1.61±1.09	−0.12±0.59	−2.87±0.74	<0.001*
Total hip	−1.22±1.04	0.15±0.58	−2.46±0.62	<0.001*
Minimum T-score	−1.67±1.03	−0.49±0.42	−2.14±0.80	<0.001*
FRAX MOF	4.03±2.27	2.27±0.54	4.50±2.34	<0.001*
FRAX HF	1.23±1.50	0.19±0.19	1.50±1.59	<0.001*

Note: *p<0.05.

Abbreviations: BMD, bone mineral density; BMI, body mass index; DPP-4, dipeptidyl peptidase 4; FRAX, fracture risk algorithm; FSH, follicle-stimulating hormone; GLP-1, glucagon-like peptide 1; HDL, high-density lipoprotein; HF, hip fractures; HOMA-IR, homeostasis model assessment - insulin resistance; LDL, low-density lipoprotein cholesterol; MOF, major osteoporotic fractures; P1NP, N-terminal peptide of procollagen type 1; SGLT-2, Sodium glucose co-transporter 2; T2DM, type 2 diabetes mellitus; TZD, trazodone.

Difference of Sex-Related Hormones Across Tertiles of BMD T-Score

Table 3 showed differences of sex-related hormones across tertiles of BMD T-score on L2-4, FN, TH and minimum of them. For men, estradiol (E2) levels were significantly elevated with increasing tertiles of BMD T-score in L2-4, FN, TH and minimum of them; and T was significantly higher with increasing tertiles of BMD score only in L2-4; LH, FSH, progesterone or PRL were not significantly associated with BMD T-score in any site. For postmenopausal women T2DM patients, differences of all the sex-related hormones, including E2, were not statistically significant across tertiles of BMD T-score in any site.

Associations of E2 with BMD T-Score and FRAX Scores

Associations of E2 with BMD T-scores in L2-4, FN, TH and minimum of them as well as scores of FRAX MOFs and HFs in men, postmenopausal women and all together were tested using multivariable linear regression analyses; and regression coefficients (beta) with standard errors (SEs) were shown in Table 4. For men and all patients together, E2 levels were significantly and positively associated with BMD T-scores in L2-4, FN, TH and minimum of them but negatively associated with scores of FRAX MOFs and HFs even after adjustment for potential confounding variables. For postmenopausal women, E2 was positively associated with BMD T-scores in L2-4 and minimum of three different sites but was not significantly associated with BMD T-scores in FN, TH or FRAX scores with adjustment in the multivariable linear regression models.

Table 3 Difference of Sex-Related Hormones Across Tertiles of BMD T-Score in L2-4, FN, and TH in Men and Postmenopausal Women T2DM Patients

	Men (n = 149)				Postmenopausal Women (n = 102)			
	Tertile 1	Tertile 2	Tertile 3	P value	Tertile 1	Tertile 2	Tertile 3	P value
Lumbar 2–4 (L2-4)								
T-score of BMD	≤0.937	0.945–1.039	≥1.044		≤0.812	0.813–0.938	≥0.947	
Estradiol (E2, pmol/L)	81.9±35.7	99.8±37.1	121.6±29.4	<0.001*	18.4 (18.4–22.3)	25.4 (18.4–40.5)	18.4 (18.4–31.3)	0.472
Luteinizing hormone (LH, mIU/mL)	6.8 (5.3–8.7)	7.6 (6.4–10.1)	8.5 (6.9–12.5)	0.424	30.4±15.1	29.3±13.1	33.6±16.3	0.689
FSH (mIU/mL)	8.2 (6.4–12.5)	8.3 (5.8–11.5)	9.4 (6.6–13.8)	0.428	57.1 (44.5–70.9)	50.9 (38.6–60.3)	52.3 (43.7–66.9)	0.918
Progesterone (nmol/L)	0.4 (0.2–0.6)	0.3 (0.2–0.5)	0.4 (0.2–0.6)	0.106	0.4 (0.2–0.6)	0.3 (0.2–0.6)	0.3 (0.2–0.5)	0.356
Total testosterone (nmol/L)	13.74±5.84	14.25±5.46	16.15±4.64	0.023*	0.4 (0.2–0.6)	0.4 (0.1–0.7)	0.4 (0.3–0.7)	0.487
Prolactin (PRL, mIU/L)	264.9 (211.6–382.8)	268.6 (177.8–462.6)	289.6 (206.3–402.8)	0.059	328.8 (251.8–444.9)	329.8 (248.7–411.6)	313.6 (242.9–361.1)	0.469
Femoral neck								
T-score of BMD	≤0.722	0.728–0.824	≥0.826		≤0.618	0.622–0.709	≥0.712	
Estradiol (E2, pmol/L)	90.7±37.7	96.6±37.7	116.070±32.874	0.002*	18.4 (18.4–32.8)	18.4 (18.4–23.9)	22.8 (18.4–40.5)	0.873
Luteinizing hormone (LH, mIU/mL)	7.6 (6.3–9.0)	7.7 (6.1–10.4)	7.8 (6.4–11.1)	0.709	29.1±14.1	32.1±17.1	30.9±13.6	0.720
FSH (mIU/mL)	9.1 (6.9–13.2)	8.6 (6.3–11.3)	7.7 (4.6–12.3)	0.420	59.8 (47.9–76.3)	57.1 (39.5–69.9)	47.6 (39.1–55.9)	0.509
Progesterone (nmol/L)	0.4 (0.2–0.6)	0.4 (0.2–0.5)	0.4 (0.3–0.6)	0.247	0.3 (0.2–0.4)	0.3 (0.2–0.5)	0.3 (0.2–0.6)	0.811
Total testosterone (nmol/L)	15.4±6.1	13.5±4.8	15.4±5.2	0.121	0.3 (0.1–0.7)	0.4 (0.1–0.7)	0.5 (0.3–0.8)	0.691
Prolactin (PRL, mIU/L)	278.0 (223.3–432.2)	263.9 (175.7–418.0)	288.4 (239.5–382.8)	0.417	302.7 (208.7–375.1)	350.8 (290.2–414.3)	328.3 (251.0–406.0)	0.515
Total Hip								
T-score of BMD	≤0.864	0.865–0.984	≥0.985		≤0.736	0.739–0.855	≥0.857	
Estradiol (E2, pmol/L)	89.3±39.4	95.5±32.0	118.27±34.7	<0.001*	18.4 (18.4–32.8)	18.4 (18.4–20.8)	24.7 (18.4–40.5)	0.964
Luteinizing hormone (LH, mIU/mL)	7.7 (6.2–10.5)	7.0 (5.5–9.5)	8.2 (6.8–9.8)	0.157	30.6±15.6	30.7±15.5	30.9±13.9	1.000
FSH (mIU/mL)	8.8 (6.7–13.1)	8.3 (6.2–11.3)	8.2 (4.8–11.9)	0.436	58.8 (44.8–75.3)	59.3 (43.5–69.3)	47.6 (39.1–55.9)	0.355
Progesterone (nmol/L)	0.4 (0.2–0.6)	0.4 (0.2–0.6)	0.4 (0.192–0.5)	0.994	0.3 (0.2–0.5)	0.3 (0.2–0.5)	0.2 (0.2–0.4)	0.981
Total testosterone (nmol/L)	14.8±5.6	14.3±5.4	15.1±5.3	0.791	0.4 (0.1–0.7)	0.4 (0.2–0.7)	0.5 (0.3–0.8)	0.678
Prolactin (PRL, mIU/L)	289.4 (220.8–427.5)	286.5 (208.0–463.2)	265.9 (205.4–349.1)	0.696	329.8 (236.1–381.8)	328.3 (248.7–411.6)	328.8 (271.9–406.6)	0.123
Minimum T-score of any site								
T-score of BMD	≤-1.9	−1.8 ~ −1.1	≥-1.0		≤-2.5	−2.4 ~ −1.7	≥-1.6	
Estradiol (E2, pmol/L)	85.0±37.2	100.9±36.8	117.8±32.3	<0.001*	18.4 (18.4–30.6)	18.4 (18.4–30.5)	22.3 (18.4–33.5)	0.142
Luteinizing hormone (LH, mIU/mL)	14.5±5.9	14.4±5.1	15.4±5.2	0.603	0.3 (0.1–0.6)	0.4 (0.2–0.7)	0.6 (0.3–0.8)	0.111
FSH (mIU/mL)	0.4 (0.2–0.6)	0.3 (0.2–0.5)	0.4 (0.2–0.6)	0.348	0.2 (0.2–0.4)	0.4 (0.2–0.6)	0.3 (0.2–0.6)	0.112
Progesterone (nmol/L)	7.0 (5.6–8.9)	7.7 (6.5–9.8)	8.5 (6.7–12.9)	0.079	32.8±13.4	30.7±14.9	29.5±16.4	0.655
Total testosterone (nmol/L)	8.2 (6.4–12.6)	8.9 (6.3–11.6)	8.3 (4.8–12.9)	0.421	60.8 (50.0–73.9)	53.8 (41.8–70.9)	46.0 (38.9–56.7)	0.365
Prolactin (PRL, mIU/L)	253.3 (195.9–343.7)	288.7 (211.9–450.1)	287.4 (214.4–398.9)	0.176	312.5 (236.1–363.2)	349.9 (263.5–453.7)	311.5 (249.3–393.7)	0.349

Note: * p<0.05.

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Table 4 Regression Coefficients of Multivariable Linear Regression of Estradiol (E2) for Indices of BMD in Men, Postmenopausal Women and All T2DM Patients

Sites	Unadjusted Regression Coefficient			Adjusted Regression Coefficient		
	Beta	SE	P value	Beta	SE	P value
Men (n = 149)†						
Lumbar 2–4 (L2-4) T-score	0.001	0.000	<0.001*	0.001	0.000	0.001*
Femoral neck T-score	0.001	0.000	<0.001*	0.001	0.000	0.002*
Total hip T-score	0.001	0.000	0.001	0.001	0.000	0.004*
Minimum T-score	0.009	0.002	<0.001*	0.009	0.002	<0.001*
FRAX MOF	−0.007	0.003	0.007*	−0.009	0.003	0.002*
FRAX HF	−0.006	0.002	0.005*	−0.007	0.002	0.001
Postmenopausal Women (n = 102)†						
Lumbar 2–4 (L2-4) T-score	0.001	0.001	0.015*	0.002	0.001	0.006*
Femoral neck T-score	0.001	0.000	0.062	0.000	0.000	0.536
Total hip T-score	0.001	0.000	0.017*	0.001	0.000	0.102
Minimum T-score	0.009	0.004	0.011*	0.009	0.004	0.022*
FRAX MOF	−0.013	0.007	0.090	−0.001	0.006	0.907
FRAX HF	−0.008	0.005	0.117	−0.001	0.004	0.887
All (n = 251)‡						
Lumbar 2–4 (L2-4) T-score	0.001	0.000	<0.001*	0.001	0.000	<0.001*
Femoral neck T-score	0.001	0.000	<0.001*	0.001	0.000	<0.001*
Total hip T-score	0.001	0.000	0.001	0.001	0.000	<0.001*
Minimum T-score	0.009	0.001	<0.001*	0.008	0.001	<0.001*
FRAX MOF	−0.017	0.002	<0.001*	−0.011	0.002	<0.001*
FRAX HF	−0.016	0.002	<0.001*	−0.004	0.002	0.006

Notes: *p<0.05, †Multivariable linear regression was adjusted for age, smoking habit, alcohol consumption.
Abbreviations: BMI, diabetes duration, FBG, HbA1c, diabetes medical treatment, total cholesterol, triglycerides, HDL-C, LDL-C, creatinine, and uric acid. ‡Multivariable linear regression was adjusted for sex, age, smoking habit, alcohol consumption, BMI, diabetes duration, FBG, HbA1c, diabetes medical treatment, total cholesterol, triglycerides, HDL-C, LDL-C, creatinine, and uric acid; BMD, bone mineral density; CI, confidence interval; FRAX, fracture risk algorithm; MOF, major osteoporotic fractures; HF, hip fractures; SE, standard error.

Association of E2 with Risks of Osteoporosis/Osteopenia

Association of E2 with risk of osteoporosis/osteopenia was explored by using multivariable logistic regression analysis. Table 5 showed that the unadjusted and adjusted ORs with 95% CIs of E2 for risks of osteoporosis/osteopenia in men, postmenopausal women and all, separately. Generally, increasing E2 level was significantly associated with decreased risks of osteoporosis/osteopenia, with the unadjusted ORs (95% CIs) of 0.981 (0.971–0.992), 0.983 (0.967–0.999) and 0.986 (0.980–0.992) (all p-values<0.05) for men, postmenopausal women and all, respectively. However, with adjustment for the potential confounding variables, associations of E2 levels with risks of osteoporosis/osteopenia kept statistically significant for men and all T2DM patients but not for postmenopausal women, with the adjusted ORs (95% CIs) of 0.980 (0.968–0.992, p = 0.001), 0.983 (0.972–0.993, p = 0.001) and 0.986 (0.966–1.006, p = 0.176), respectively.

Discussion

In a total sample of 251 patients with T2DM, the current study found that the prevalence rates of osteoporosis/osteopenia were 66.4% in men and 79.4% in postmenopausal women. Patients with osteoporosis/osteopenia, compared to those with normal BMD, showed significantly lower levels of estradiol (E2) but no other sex-related hormones. For all T2DM patients together and men of them, multivariable regression analyses, with adjustment for potential confounding variables, showed that increasing E2 levels were significantly associated with higher BMD T-scores in L2-4, FN, TH

Table 5 Multivariable Logistic Regression of Estradiol (E2) for Risks of Osteoporosis/Osteopenia in Men, Postmenopausal Women and All T2DM. Patients

	OR	95% CI	P value
Men (n = 149)†			
Unadjusted	0.981	0.971–0.992	<0.001*
Adjusted	0.980	0.968–0.992	0.001*
Postmenopausal Women (n = 102)†			
Unadjusted	0.983	0.967–0.999	0.048*
Adjusted	0.986	0.966–1.006	0.176
All (n = 251)‡			
Unadjusted	0.986	0.980–0.992	<0.001*
Adjusted	0.983	0.972–0.993	0.001*

Notes: * p<0.05, †Multivariable logistic regression was adjusted for age, smoking habit, alcohol consumption.

Abbreviations: BMI, diabetes duration, FBG, HbA1c, diabetes medical treatment, total cholesterol, triglycerides, HDL-C, LDL-C, creatinine, and uric acid. ‡Multivariable logistic regression was adjusted for sex, age, smoking habit, alcohol consumption, BMI, diabetes duration, FBG, HbA1c, diabetes medical treatment, total cholesterol, triglycerides, HDL-C, LDL-C, creatinine, and uric acid; CI, confidence interval; OR, odds ratio.

and minimum of them and lower FRAX scores of MOFs and HFs as well as decreased risk of osteoporosis/osteopenia. As for postmenopausal women T2DM patients, E2 level was positively associated with BMD T-scores only in L2-4 and minimum of three different sites but was not independently associated with risk of osteoporosis/osteopenia. No other sex-related hormones, such as LH, FSH, progesterone, T or PRL, was significantly associated with either BMD or risk of osteoporosis/osteopenia for men, postmenopausal women or all T2DM patients.

A lot of existing evidence has shown independent associations between estradiol and BMD in women,[8,9,16] and that decreased estradiol with aging has been a vital factor of osteoporosis in older women.[22,23] Furthermore, hormone therapy has been becoming an optional therapy for postmenopausal women to prevent osteoporosis,[24,25] since E2 deficiency has been found to result in the loss of bone which is the major cause of osteoporosis. However, evidence on the association of estradiol with BMD and risk of osteoporosis/osteopenia in women patients with T2DM was little, especially in postmenopausal women with T2DM. The present study, based on 102 postmenopausal women with T2DM, found that estradiol was significantly and positively associated with BMD T-scores in L2-4 but not in FN or TH. However, we failed to found that increasing estradiol level was independently associated with decreased risk of osteoporosis/osteopenia.

The relation of estradiol and BMD remains unclear in men. Some studies have shown that estradiol is a crucial sex hormone in bone health and bone homeostasis,[26] positively related to BMD and negatively associated with risk of fractures in men.[13–15] But there were still some other studies which have reported that estradiol had no contribution to provide clinically useful improvement in fracture risk discrimination and was limited to predict risk of fracture.[27,28] Woo et al reported that Chinese men with low serum estradiol levels showed elevated bone loss and increased risk of fractures which was similar to findings in Caucasians.[12] In our study of 149 men T2DM patients with mean age of 57.3 years old, we found that increasing estradiol level was significantly associated with higher BMD T-scores in L2-4, FN, TH and minimum of them, and lower FRAX scores of MOFs and HFs, as well as decreased risk of osteoporosis/osteopenia, which was quite similar to findings in all the T2DM patients. The protective effects of estradiol on BMD and risk of osteoporosis/osteopenia have been confirmed for men and all the T2DM patients in the present study. For postmenopausal women with T2DM, we could not rule out the possibly protective effect of estradiol on osteoporosis/osteopenia due to the small sample size. For example, E2 was positively associated with BMD T-scores in L2-4 and minimum of three different sites for postmenopausal

women. Therefore, more studies with larger sample size for postmenopausal women with T2DM are needed, especially in prospective cohort study design in future.

The effect of testosterone on bone health has still been questioned in males. Previous research showed that higher testosterone was associated with higher BMD and decreased fracture risk.[29,30] Mellstrom et al found free testosterone (FT) is an independent predictor of BMD and prevalent fractures in elderly Swedish males.[10] Xia Jin-wei et al founded that the ratio of E2/ testosterone of the type 2 diabetes group was significantly increased, whereas testosterone was obviously decreased compared with healthy people.[18] Testosterone replacement therapy (TRT) in men has been found to increase BMD and decrease osteoporosis/osteopenia,[31,32] but some studies showed TRT has limited effect on increasing BMD,[33] it needs more research to confirm the effect of TRT on bone health in male. As for women, several studies have reported the important role of testosterone in BMD or osteoporosis,[34] while others showed that testosterone was not associated with BMD,[35] and that spin and hip BMD losses during the menopause transition was not related to androgen.[36] In present study, we did not observe T level was associated with BMD and fracture risk either in men or postmenopausal women. Unfortunately, we did not test fT which could reflect the real state of androgen.

As for other sex-related hormones, previous study suggested that FSH and LH levels were associated with BMD changes and osteoporosis occurrence in women.[37] Another study showed that central hypogonadism appears to adversely affect bone health independently of gonadal steroids levels, which may be due to lower LH levels and consequent reduction of vitamin D 25-hydroxylation in the testis.[38] And there is evidence that PRL excess may directly or indirectly induce hypogonadism influence on bone metabolism.[39,40] In present study we did not find significant associations of FSH, LH or PRL with BMD T-score or risk of osteoporosis/osteopenia. Possible mechanisms of osteoporosis in T2DM may be associated with age, obesity, diabetes complication, duration of diabetes, glucose control and some medication such as peroxisome proliferator-activated receptor γ agents.[41,42] However, to date, the potential pathogenesis of osteoporosis caused by T2DM remains unclear and need to be explored further in future.

Our previous publication based on the same cohort found that T2DM patients with osteoporosis showed significantly lower levels of body weight, height and BMI as compared to those without osteoporosis,[19] which were quite similar to the present study. Some studies reported that sex-related hormones might differ in those with obesity, for example, E2 levels were higher in post-menopausal women with increased BMI than their controls.[43] But we believed that obesity might not affect the present findings on the associations of sex-related hormones with BMD and risk of osteoporosis/osteopenia, since we have adjusted for the potential confounding effect of obesity by using the multivariable regression analyses.

Previous studies to explore the relationship between sex-related hormones, BMD and risk of osteoporosis/osteopenia mainly carried out among Caucasians, but our research is one of the few studies in Asian populations with T2DM, especially for postmenopausal women.[44–46] But there were still several limitations in our study. Firstly, the present study was based on the cross-sectional analyses of our ongoing cohort study, therefore we cannot determine the temporal sequence among sex-related hormones, BMD and osteoporosis. Therefore, prospective cohort studies, including changes of sex-related hormones with aging, are key important factors for future studies. Secondly, selection bias was obvious in our study since all patients were sampled from only one hospital and only those hospitalized T2DM patients were included; therefore, we could only generalize the present findings with limited power. Thirdly, the sample size of our T2DM patients, especially for postmenopausal women, was quite small, so we may therefore have not enough power to find the true associations among sex-related hormones, BMD and osteoporosis. Therefore, there is genuine need for future studies with larger sample size, especially for postmenopausal women. Last but not the least, no healthy control subjects were included into the present study, so we could not compare sex-related hormones between them and T2DM patients. Therefore, the present findings could not extrapolate into the general healthy subjects.

Conclusion

The prevalence rate of osteoporosis/osteopenia was quite high in T2DM patients, especially in postmenopausal women. Generally, increasing E2 levels were significantly associated with higher BMD T-scores and lower FRAX scores as well as decreased risks of osteoporosis or osteopenia for men and all the T2DM patients. Therefore, the present findings implied that screening of BMD and E2 levels and evaluating risks of osteoporosis are important for clinical practice from the perspective of fracture prevention for T2DM patients.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the Helsinki Declaration and its later amendments or comparable ethical standards.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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