RESEARCH

Increased risk of non-alcoholic fatty liver disease fibrosis is closely associated with osteoporosis in women but not in men with type 2 diabetes

Zhiyan Yu*, **Yueyue Wu***, **Rui Zhang**, **Yue Li**, **Shufei Zang** and **Jun Liu**

Department of Endocrinology, Shanghai Fifth People's Hospital, Fudan University, Shanghai, China

Correspondence should be addressed to S Zang or J Liu: sophiazsf@fudan.edu.cn or liujun@5thhospital.com

*(Z Yu and Y Wu contributed equally to this work)

Abstract

Background: This study aimed to investigate the association of non-alcoholic fatty liver disease (NAFLD) and liver fibrosis with osteoporosis in postmenopausal women and men over 50 years of age with type 2 diabetes (T2DM).

Methods: In this study, 1243 patients with T2DM (T2DM with coexistent NAFLD, $n = 760$; T2DM with no NAFLD, $n = 483$) were analysed. Non-invasive markers, NAFLD fibrosis score (NFS) and fibrosis index based on four factors (FIB-4), were applied to evaluate NAFLD fibrosis risk.

Results: There was no significant difference in bone mineral density (BMD) between the NAFLD group and the non-NAFLD group or between males and females after adjusting for age, BMI and gender. In postmenopausal women, there was an increased risk of osteoporosis (odds ratio (OR): 4.41, 95% CI: 1.04–18.70, $P = 0.039$) in the FIB-4 high risk group compared to the low risk group. Similarly, in women with high risk NFS, there was an increased risk of osteoporosis (OR: 5.98, 95% CI: 1.40–25.60, $P = 0.043$) compared to the low risk group. Among men over 50 years old, there was no significant difference in bone mineral density between the NAFLD group and the non-NAFLD group and no significant difference between bone mineral density and incidence of osteopenia or osteoporosis among those with different NAFLD fibrosis risk.

Conclusion: There was a significant association of high risk for NAFLD liver fibrosis with osteoporosis in postmenopausal diabetic women but not men. In clinical practice, gender-specific evaluation of osteoporosis is needed in patients with T2DM and coexistent NAFLD.

Key Words

- NAFLD
- osteoporosis
- fibrosis
- T2DM

Endocrine Connections (2022) **11**, **e220174**

Introduction

Osteoporosis is a systemic bone disease characterized by decrease in bone mass and damage to the microstructure of bone tissue, resulting in increased bone fragility and susceptibility to fracture (1). Pain due to osteoporosis can reduce the quality of life, while spinal deformity and fractures can limit a patient's level of activity and ability to self-care and increase the incidence of lung infection and bedsores. These potential sequelae place a heavy economic burden on families and society. Numerous risk factors for osteoporosis include obesity, advanced age, menopause, diabetes, calcium and vitamin D deficiency, low body weight, inappropriate secretion of parathyroid

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-22-0174

hormone, hypercalciuria, low insulin-like growth factor-1 and hypoalbuminemia.

Non-alcoholic fatty liver disease (NAFLD) affecting multiple extrahepatic organs is considered a multi-system disease (2). Recent studies have shown that NAFLD is a high risk factor for osteoporosis (3). Several studies have reported an association of NAFLD with bone mineral density (BMD). A lower BMD has been reported in patients with NAFLD compared to those without (4, 5), as well as a negative association of NAFLD with right-hip BMD (5). Previous studies have shown that NAFLD is closely associated with decreased BMD in adults and children (6, 7), as well as a history of osteoporotic fractures (8). Nonetheless, a meta-analysis failed to show significant differences in BMD measurements at the femoral or lumbar spine level in patients with or without NAFLD (9). It is clear that an association of NAFLD with osteoporosis is controversial.

Liver fibrosis is a major predictor for the development of future liver-related events in patients with NAFLD (10), and an increasing number of studies have shown that liver fibrosis is the main marker of poor disease outcome. Nonetheless between different regions and genders, there is no consensus on the association of NAFLD fibrosis with osteoporosis. Studies from Korea reported that hepatic fibrosis was significantly associated with osteoporosis in both men and women, although a more recent study with large sample size reported no association of liver steatosis or fibrosis with osteopenia or osteoporosis in a US population older than 50 years. NAFLD often coexists with T2DM that is also considered a risk factor for osteoporosis (11). Although small studies reported an association of fibrosis with postmenopausal state (12), no studies have examined sex-specific differences in the effect of NAFLD on osteopenia or osteoporosis in patients with T2DM.

Our study with large sample size investigated the association between NAFLD, hepatic fibrosis and osteoporosis in patients with T2DM. First, analyses were done according to gender and menopausal status, and confounder factors (BMI, age, etc.) were corrected for a detailed stratified assessment. Then, reliable, non-invasive assessment indicators for liver fibrosis (NAFLD fibrosis score (NFS) and fibrosis index based on 4 factors (FIB-4)) were applied and were also modified according to age. The following innovative conclusion was drawn: risk of osteoporosis was significantly increased in women at high risk of liver fibrosis compared to those at low risk but not in men with type 2 diabetes.

Patients and methods

Patients

In this study, we retrospectively analysed 2288 diabetic patients who were admitted between January 2018 and December 2019 to the Endocrinology Department of Shanghai Fifth People's Hospital. Diabetes was confirmed if the patient fulfilled the 2017 ADA diagnostic criteria for diabetes (13). Women over 50 years of age who had ceased to menstruate or had undergone surgical removal of both ovaries in the 12 months prior to admission were considered to be in the menopause.

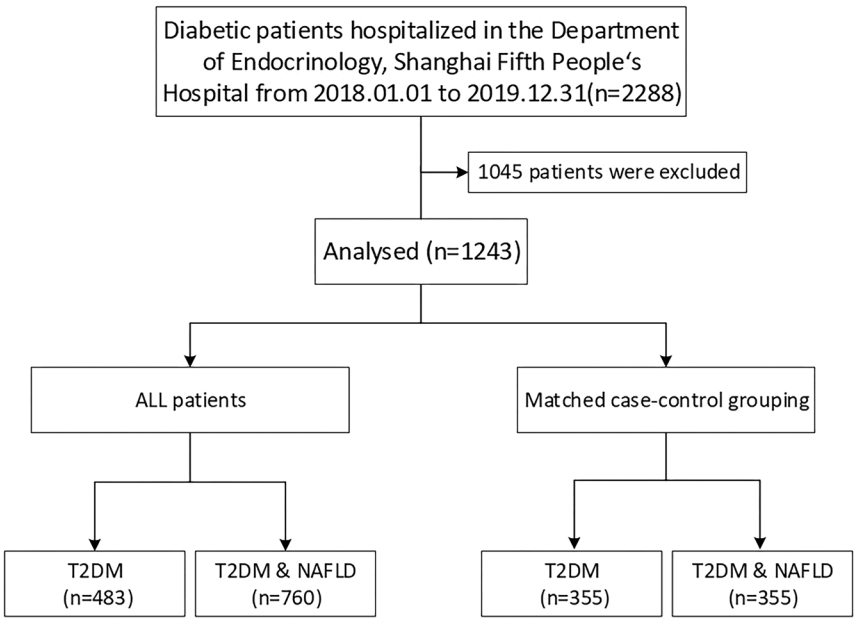

Figure 1
The flowchart of this study.

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-22-0174

In total, 1045 patients were excluded for the following reasons: premenopausal female ($n = 239$); male age below 50 years ($n = 156$); presence of viral liver disease or autoimmune hepatitis ($n = 171$); presence of thyroid, parathyroid, adrenal or gonadal disorder ($n = 48$); absence of liver ultrasound ($n = 182$); absence of bone density test results ($n = 153$); previous gastrointestinal resection ($n = 7$); history of drug or alcohol abuse ($n = 16$); previous hip replacement ($n = 6$), malignant tumour ($n = 34$) and long-term bed rest ($n = 33$).

A total of 1243 diabetic patients were studied; 483 without fatty liver and 760 with fatty liver (Fig. 1).

Clinical and laboratory data

During the hospital admission, height (cm), weight (kg) and body mass index (BMI), calculated as weight (kg)/height^2 (m^2), were recorded for all patients. Blood pressure was recorded after the patient had rested for 10 min, and the blood was drawn for the measurement of HbA1c, fasting blood glucose (FBG), fasting insulin (FINS), fasting C-peptide (FCP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (GGT), platelet count, creatinine, albumin, globulin, serum calcium, PTH, P1NP, β-CTX, and OC. Estimated glomerular filtration rate (eGFR) was calculated according to the Chronic Kidney Disease Epidemiology Collaborative Group (CKD-epi) equation.

BMD assessment

Dual-energy X-ray absorptiometry (DXA) examination is considered the gold standard to assess bone mineral density. It is easy to use and has a fast detection speed. In our study, DXA examination was performed by doctors with radiological qualifications in the Department of Nuclear Medicine. Test results were based on the lower T score: T score >−1 was considered normal. Osteopenia was defined as a T score between −1.0 and −2.5 and osteoporosis as a T score ≤−2.5.

NAFLD diagnosis

Abdominal ultrasonography was performed, and fatty liver was characterized when echogenicity of the liver significantly increased relative to that of the kidneys. The ultrasound beam was attenuated with the diaphragm indistinct or when the echogenic walls of the portal veins were less visible (14). NAFLD was defined as ultrasonographically proven fatty changes in the absence of competing aetiologies of fatty liver disease, such as: alcoholism, viral or autoimmune chronic liver disease, steatogenic drug history and thyroid disorder. Alcoholism was defined as an intake that exceeded 210 g/week for men and 140 g/week for women.

FIB-4 index and NFS index

FIB-4 index = age (years) × AST (U/L) / (PLT ($\times 10^9$/L) × $\sqrt{\text{ALT (U/L)}}$).

NFS index = −1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m^2) + 1.13 × impaired fasting glucose/diabetes (yes = 1, no = 0) + 0.99 × (AST/ALT) − 0.013 × PLT ($\times 10^9$/L) − 0.66 × albumin (g/dL). Different thresholds were applied for patients aged ≥65 years in the stratification of liver fibrosis (15).

Statistical analysis

Data are presented as mean ± S.D. when continuous data distribution was normally distributed or close to normal. Skewed data are presented as median and quartiles. Frequency and percentage are used in categorical data. All analyses were performed using SPSS 23.0 software. The *t* test was used for normally distributed data, two independent sample rank sum test for skewed data, and chi-square test or Fisher's exact chi-square test for categorical data. This was a 1:1-matched case–control study and age, gender and BMI match tolerances were 0, 0, and 2 respectively. We determined the relationship between FIB4 and NFS and BMD by logistic regression analysis: first, we correlated each factor with BMD. Then, potential clinical confounders with $P < 0.05$ in univariate analysis and FIB4 and NFS were included in multivariate logistic analysis.

Results

Characteristics of subjects in the cross-sectional study

A total of 1243 subjects with T2DM were included in this study of whom 654 were male (266 non-NAFLD, 388 NAFLD) and 589 were female (217 non-NAFLD and 372 NAFLD). The clinical and laboratory characteristics are shown in Table 1. Compared with non-NAFLD patients, those with T2DM and NAFLD had significantly higher BMI, TC, TG, LDL, FINS, FBG, FCP, ALT, AST, and GGT but lower age and HDL (all $P < 0.001$). Patients with T2DM and

Table 1 Study population characteristics.

	Total participants (n = 1243)			**Men** (n = 654)			**Women** (n = 589)		
	T2DM	T2DM and NAFLD	*P*-value	T2DM	T2DM and NAFLD	*P*-value	T2DM	T2DM and NAFLD	*P*-value
N	483	760		266	388		217	372	
BMI (kg/m^2)	23.89 ± 3.50	26.34 ± 3.51	<0.001	23.89 ± 3.15	26.58 ± 3.23	<0.001	23.88 ± 3.89	26.10 ± 3.76	<0.001
Age (year)	68.93 ± 9.52	65.52 ± 8.76	<0.001	67.63 ± 9.44	64.79 ± 8.34	<0.001	70.52 ± 9.39	66.28 ± 9.12	<0.001
Duration of diabetes (year)	10.00 (6.00, 20.00)	10.00 (6.00, 18.00)	0.155	10.00 (5.00, 15.00)	10.00 (4.00, 16.00)	0.847	13.00 (8.00, 20.00)	10.00 (7.00, 18.00)	0.054
ALT (U/L)	15.70 (11.00, 22.00)	21.00 (14.00, 29.80)	<0.001	16.00 (11.00, 22.00)	21.70 (16.00, 33.25)	<0.001	15.00 (11.00, 21.00)	18.75 (13.00, 29.00)	<0.001
AST (U/L)	16.80 (13.00, 22.00)	18.00 (14.30, 25.28)	<0.001	16.80 (13.00, 22.55)	18.00 (14.73, 24.75)	0.008	16.50 (13.00, 21.00)	17.80 (14.00, 26.75)	0.002
ALB (g/L)	40.51 ± 4.80	42.78 ± 4.66	<0.001	40.40 ± 5.04	42.90 ± 4.61	<0.001	40.66 ± 4.46	42.65 ± 4.71	<0.001
γ- GT (U/L)	20.00 (14.00, 33.00)	26.00 (19.00, 39.00)	<0.001	21.00 (14.00, 35.00)	28.00 (21.00, 42.00)	<0.001	19.00 (14.00, 30.25)	24.00 (18.00, 35.00)	<0.001
Calcium (mmol/L)	2.22 ± 0.12	2.27 ± 0.12	<0.001	2.21 ± 0.13	2.25 ± 0.11	<0.001	2.23 ± 0.12	2.28 ± 0.12	<0.001
25(OH)D (nmol/L)	43.02 ± 20.67	43.74 ± 18.15	0.594	44.97 ± 22.88	47.03 ± 17.58	0.286	40.76 ± 17.58	40.84 ± 18.17	0.963
PTH (pmol/L)	4.10 (3.00, 5.20)	4.20 (3.10, 5.45)	0.327	3.90 (2.88, 5.10)	4.10 (3.10, 5.10)	0.291	4.30 (3.20, 5.30)	4.40 (3.00, 5.73)	0.799
OC (ng/mL)	12.89 (9.75, 17.66)	11.90 (8.92, 16.12)	0.133	11.07 (8.40, 15.90)	10.61 (8.30, 13.87)	0.140	14.53 (10.97, 18.79)	14.35 (10.88, 18.11)	0.493
P1NP (ng/mL)	40.09 (27.86, 55.54)	35.56 (26.60, 46.87)	0.016	35.15 (26.37, 53.87)	32.16 (23.93, 41.65)	0.052	44.83 (28.88, 59.64)	38.07 (28.69, 50.31)	0.045
β-CTX (pg/mL)	388.55 (242.65, 588.70)	351.40 (226.20, 492.65)	0.017	319.30 (213.95, 495.30)	316.95 (212.33, 457.73)	0.331	466.40 (271.80, 666.00)	377.20 (246.25,554.40)	0.010
Lumbar BMD (T-score)	−0.19 ± 1.93	0.15 ± 1.80	0.011	0.82 ± 1.86	1.00 ± 1.59	0.286	−1.27 ± 1.35	−0.59 ± 1.63	<0.001
Lumbar BMD (Z-score)	1.30 ± 1.55	1.12 ± 1.49	0.094	1.50 ± 1.78	1.44 ± 1.53	0.720	1.09 ± 1.24	0.84 ± 1.39	0.068
Left-hip BMD (T-score)	−1.13 ± 1.26	−0.72 ± 1.15	<0.001	−0.67 ± 1.17	−0.38 ± 1.13	0.012	−1.62 ± 1.16	−1.02 ± 1.09	<0.001
Left-hip BMD (z-score)	0.27 ± 1.02	0.29 ± 1.01	0.850	0.31 ± 1.12	0.46 ± 1.06	0.176	0.23 ± 0.91	0.13 ± 0.95	0.312
BMD			<0.001			0.090			<0.001
Normal BMD	122 (39.87%)	270 (53.78%)		93 (58.49%)	162 (69.23%)		29 (19.73%)	108 (40.30%)	
Osteopenia	128 (41.83%)	181 (36.06%)		60 (37.74%)	66 (28.21%)		68 (46.26%)	115 (42.91%)	
Osteoporosis	56 (18.30%)	51 (10.16%)		6 (3.77%)	6 (2.56%)		50 (34.01%)	45 (16.79%)	

γ-GT, gamma-glutamyl transferase; A/G, albumin to globulin ratio; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMD, bone mineral density; DBP, diastolic blood pressure; FBG, fast blood glucose; Fins, fast insulin; GLB, globulin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NAFLD, non-alcoholic fatty liver disease; PTH, parathyroid hormone; SBP, systolic blood pressure; TC, total cholesterol; TG, Triglyceride.

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-22-0174

Table 2 Comparison of parameters between non-NAFLD and NAFLD patients with T2DM by matching confounding factors.

	Total participants (n = 710)			**Men** (n = 396)			**Women** (n = 314)		
	T2DM	T2DM and NAFLD	*P*-value	T2DM	T2DM and NAFLD	*P*-value	T2DM	T2DM and NAFLD	*P*-value
N	355	355		198	198		157	157	
BMI (kg/m^2)	24.69 ± 2.75	24.80 ± 2.67	0.580	24.83 ± 2.74	24.95 ± 2.64	0.654	24.53 ± 2.77	24.62 ± 2.70	0.786
Age (year)	67.55 ± 8.56	67.33 ± 8.55	0.810	66.55 ± 8.34	66.29 ± 8.26	0.764	68.71 ± 8.70	68.54 ± 8.75	0.865
Duration of diabetes (year)	10.00 (6.00, 20.00)	10.00 (5.00, 18.00)	0.433	10.00 (5.00, 18.00)	10.00 (3.00, 18.00)	0.458	12.00 (7.00, 20.00)	10.00 (7.00, 18.00)	0.487
ALT (U/L)	16.00 (11.38, 23.40)	18.50 (13.00, 27.25)	0.002	17.35 (12.00, 23.15)	19.90 (14.83, 28.00)	0.008	15.15 (11.00, 21.43)	16.00 (11.95, 24.45)	0.089
AST (U/L)	17.00 (13.03, 22.15)	17.00 (14.00, 23.70)	0.241	17.00 (13.50, 22.70)	17.60 (14.30, 23.70)	0.428	16.80 (12.90, 21.00)	16.20 (13.05, 24.00)	0.325
ALB (g/L)	41.08 ± 4.76	42.45 ± 4.28	<0.001	41.08 ± 5.05	42.55 ± 4.20	0.005	41.08 ± 4.40	42.34 ± 4.38	0.018
γ-GT (U/L)	21.00 (15.00, 33.50)	24.00 (18.00, 37.00)	0.002	22.00 (15.00, 36.75)	26.00 (20.00, 40.75)	0.006	19.00 (14.00, 31.00)	22.00 (16.00, 31.50)	0.096
Calcium (mmol/L)	2.23 ± 0.13	2.26 ± 0.10	0.011	2.22 ± 0.13	2.24 ± 0.10	0.088	2.24 ± 0.13	2.27 ± 0.11	0.055
25(OH)D (nmol/L)	44.87 ± 19.71	43.83 ± 19.78	0.565	48.42 ± 2.54	47.91 ± 18.04	0.848	40.95 ± 15.15	39.59 ± 20.69	0.564
PTH (pmol/L)	4.20 (3.00, 5.30)	4.20 (3.13, 5.60)	0.610	4.00 (2.90, 4.98)	4.20 (3.20, 5.20)	0.399	4.40 (3.40, 5.40)	4.40 (3.15, 6.00)	0.934
OC (ng/mL)	11.70 (9.28, 17.01)	11.90 (8.98, 16.12)	0.633	10.81 (8.12, 15.44)	9.68 (8.17, 14.85)	0.695	14.22 (10.45, 18.53)	13.95 (10.92, 17.36)	0.904
P1NP (ng/mL)	40.16 (28.17, 55.39)	32.85 (25.69, 43.79)	0.003	35.81 (28.18, 53.87)	31.13 (23.93, 41.31)	0.037	44.83 (27.94, 59.11)	34.38 (27.61, 45.03)	0.021
β-CTX (pg/mL)	356.20 (240.53, 563.83)	334.40 (231.35, 478.15)	0.200	308.30 (218.38, 484.33)	330.65 (214.50, 462.05)	0.790	416.05 (246.08, 647.40)	341.10 (245.60, 525.80)	0.153
Lumbar BMD (T-score)	0.03 ± 1.89	-0.17 ± 1.75	0.258	1.04 ± 1.84	0.67 ± 1.6	0.111	−1.03 ± 1.23	−0.99 ± 1.48	0.816
Lumbar BMD (Z-score)	1.36 ± 1.59	1.08 ± 1.47	0.056	1.64 ± 1.8	1.21 ± 1.56	0.063	1.06 ± 1.29	0.94 ± 1.37	0.505
Left-hip BMD (T-score)	−0.98 ± 1.21	−0.90 ± 1.11	0.477	−0.57 ± 1.15	−0.64 ± 1.08	0.657	−1.41 ± 1.12	−1.16 ± 1.09	0.093
Left-hip BMD (z-score)	0.31 ± 1.01	0.29 ± 1.01	0.803	0.38 ± 1.09	0.27 ± 1.02	0.433	0.24 ± 0.92	0.30 ± 1.01	0.608
BMD			0.733			0.945			0.160
Normal BMD	96 (44.24%)	107 (47.98%)		71 (63.39%)	68 (61.26%)		25 (26.04%)	39 (34.82%)	
Osteopenia	90 (41.47%)	86 (38.57%)		37 (33.04%)	39 (35.14%)		53 (55.21%)	47 (41.96%)	
Osteoporosis	31 (14.29%)	30 (13.45%)		4 (3.57%)	4 (3.60%)		18 (18.75%)	26 (23.21%)	

γ-GT, gamma-glutamyl transferase; A/G, albumin to globulin ratio; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMD, bone mineral density; DBP, diastolic blood pressure; FBG, fast blood glucose; Fins, fast insulin; GLB, globulin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NAFLD, non-alcoholic fatty liver disease; PTH, parathyroid hormone; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-22-0174

NAFLD had much higher lumbar anteroposterior T score (0.15 ± 1.80 vs −0.19 ± 1.93, $P=0.011$), higher left hip anteroposterior T score (−0.72 ± 1.15 vs −1.13 ± 1.26, $P<0.001$), lower P1NP (35.56 (26.60, 46.87) vs 40.09 (27.86, 55.54), $P=0.016$) and lower β-CTX (351.40 (226.20, 492.65) vs 388.55 (242.65, 588.70), $P=0.017$) than those without NAFLD. The incidence of osteoporosis and osteopenia in patients with T2DM and NAFLD was significantly lower than in those without NAFLD (10.16% vs 18.30%), $P<0.001$).

Further comparisons between males and females revealed that BMI, age, FINS, FCP, FBG, HOMA-IR, blood lipids, ALT, AST and eGFR showed the same uniform differences between T2DM patients with and without NAFLD to those seen in the overall patient population. Among female T2DM patients, those with NAFLD had higher lumbar anteroposterior T score, higher left hip anteroposterior T score, lower P1NP, low β-CTX and decreased osteoporosis, whereas for male T2DM patients, no bone metabolites showed any difference (Table 1 and Supplementary Table 1, see section on supplementary materials given at the end of this article).

Comparison of parameters between non-NAFLD and NAFLD patients with T2DM by matching confounding factors

A 1:1 case–control analysis was performed to avoid the potential bias of covariates that were not evenly distributed between non-NAFLD and NAFLD patients (Table 2 and Supplementary Table 2). After matching for age, gender and BMI, there was no significant difference in BMD between the NAFLD group and the non-NAFLD group in terms of T score or Z value and no significant difference in incidence of osteopenia or osteoporosis. Further gender-specific analyses revealed no significant difference in the distribution of bone mineral density between non-NAFLD and NAFLD patients with T2DM, for males or females.

Comparison of parameters between different NAFLD fibrosis risk stages

We also investigated the association of NAFLD severity and osteoporosis in T2DM patients with co-existing NAFLD. When stratified according to FIB-4 (Table 3 and Supplementary Table 3), in all patients with intermediate or high risk, as well as the overall population, males and females had lower albumin. In the overall population, compared to those with low fibrosis risk, patients with intermediate- and high-risk FIB-4 showed much lower left hip T score (−0.88 ± 1.22 and −0.89 ± 1.14 vs −0.54 ± 1.11, $P=0.008$) and much higher incidence of osteoporosis (13.47% and 12.35% vs 5.22%). In females, those with intermediate- or high-risk FIB-4 had much lower lumbar spine T score, left hip T score and P1NP, resulting in a much higher occurrence of osteoporosis (23.35% and 25.00% vs 7.27%). In males, although 25(OH)D and serum calcium were lower in high fibrosis risk patients, there was no significant difference in BMD and incidence of osteopenia or osteoporosis among the three groups of FIB-4 risk: low, intermediate or high.

When stratified according to NFS (Table 4 and Supplementary Table 4), few patients were considered ruled-in low fibrosis risk (14.34% vs 45.39%, respectively) compared to FIB-4. In the total patient population, compared with low-risk and intermediate-risk patients, those with high risk had higher BMI and lower calcium, 25(OH)D and left hip T score (−1.02 ± 1.08 vs −0.51 ± 1.12 and −0.66 ± 1.17, $P=0.016$), resulting in a much higher incidence of osteoporosis (16.27% vs 7.34% and 9.48%). In the female population, compared with low-risk patients, those with high risk had much higher BMI and PTH, lower 25(OH)D, P1NP, lumber T score (−1.07 ± 1.48 vs −0.15 ± 1.20, $P=0.005$) and left hip T score (−1.44 ± 0.92 vs −0.52 ± 0.91, $P<0.001$) and higher incidence of osteoporosis (26.37% vs 6.45% and 18.26%). In the male population, compared with those at low risk, patients at high risk showed lower calcium and 25(OH)D. There was no significant difference in BMD or incidence of osteopenia or osteoporosis among the three groups of NFS: low, intermediate or high risk.

Increased NAFLD fibrosis risk was closely associated with BMD

Correlation analyses were performed to investigate the association between lumber T score, left hip T score and clinical parameters. In the overall and female population, both lumbar and left hip T score were negatively correlated with age, FIB4 ($P<0.001$ and $P=0.025$) and NFS ($P=0.027$ and $P\leq0.001$) and positively correlated with BMI. In addition, left hip T score was positively correlated with ALB and negatively correlated with PTH. In males, lumber T score and left hip T score were negatively correlated with age and positively correlated with BMI, but there was no significant correlation with other factors (Table 5).

Increased NAFLD fibrosis risk was an independent risk factor for osteoporosis in women

Logistic regression analyses were performed on NAFLD fibrosis risk for osteoporosis in patients with NAFLD and

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-22-0174

Table 3 Comparison of parameters between different NAFLD fibrosis risk stages stratified according to FIB-4.

	Total participants (n = 760)				**Men** (n = 388)				**Women** (n = 372)			
	FIB4-LR	FIB4-IR	FIB4-HR	*P*-value	FIB4-LR	FIB4-IR	FIB4-HR	*P*-value	FIB4-LR	FIB4-IR	FIB4-HR	*P*-value
n	345	334	81		180	167	41		165	167	40	
BMI (kg/m^2)	26.63 ± 3.46	26.10 ± 3.52	26.29 ± 3.46	0.195	26.88 ± 3.29	26.44 ± 3.11	26.05 ± 2.71	0.309	26.35 ± 3.63	25.74 ± 3.90	26.49 ± 3.98	0.343
Duration of diabetes (year)	10.00 (5.75, 15.00)	10.00 (6.00, 20.00)	10.00 (7.00, 20.00)	0.421	8.50 (3.25, 12.75)	10.00 (5.00, 18.50)	17.00 (3.00, 20.00)	0.399	10.00 (8.00, 15.00)	10.00 (6.25, 20.00)[a]	10.00 (9.25, 20.00)[a]	0.894
ALB (g/L)	43.55 ± 4.79	42.51 ± 4.49[a]	40.96 ± 4.23[a,b]	<0.001	43.50 ± 4.68	43.02 ± 4.42	40.28 ± 4.37[a,b]	0.001	43.62 ± 4.92	41.98 ± 4.52[a]	41.73 ± 3.99[a]	0.006
γ- GT (U/L)	25.00 (19.00, 36.00)	26.00 (19.00, 38.00)	29.00 (21.00, 50.25)	0.225	26.00 (21.00, 45.00)	27.50 (20.00, 41.75)	29.50 (22.00, 39.00)	0.702	23.00 (18.00, 34.00)	24.00 (19.00, 34.75)	29.00 (19.50, 59.75)	0.200
Ca (mmol/L)	2.27 ± 0.12	2.26 ± 0.11	2.24 ± 0.14	0.060	2.27 ± 0.10	2.25 ± 0.10	2.20 ± 0.13[a,b]	0.002	2.28 ± 0.14	2.27 ± 0.11	2.28 ± 0.13	0.825
25(OH)D (nmol/L)	43.74 ± 16.44	45.09 ± 20.06	38.34 ± 15.56	0.104	45.59 ± 14.05	43.46 ± 15.48	39.09 ± 18.45[a,b]	0.021	41.92 ± 18.37	40.00 ± 18.34	37.86 ± 13.78	0.528
PTH (pmol/L)	4.30 (3.20, 5.50)	4.10 (3.10, 5.40)	4.10 (2.90, 6.20)	0.611	4.30 (3.20, 5.25)	4.05 (3.20, 5.10)	3.50 (2.70, 6.10)	0.688	4.50 (3.10, 5.55)	4.30 (2.90, 5.80)	5.65 (3.45, 6.50)	0.366
OC (ng/mL)	9.95 (12.34, 16.12)	11.59 (8.21, 15.50)	10.69 (8.45, 16.57)	0.210	10.97 (9.16, 14.13)	9.15 (7.97, 12.23)	9.31 (8.30, 13.68)	0.124	14.60 (11.00, 18.95)	14.75 (11.41, 18.85)	13.93 (10.65, 16.77)	0.807
P1NP (ng/mL)	41.00 (30.12, 78.30)	35.58 (25.29, 47.52)	32.38 (25.59, 45.32)	0.078	35.97 (26.63, 58.51)	31.41 (22.36, 41.65)	30.24 (23.78, 36.93)	0.078	43.29 (33.55, 56.09)	36.90 (27.54, 42.30)	34.00 (26.30, 37.14)[a]	0.042
β-CTX (pg/mL)	370.40 (240.70, 524.55)	346.90 (214.95, 475.60)	329.15 (236.75, 535.38)	0.516	329.10 (226.20, 458.70)	299.95 (189.45, 438.00)	302.70 (250.95, 492.03)	0.226	378.80 (271.73, 574.35)	397.85 (247.95, 579.75)	388.30 (190.48, 551.73)	0.859
Lumbar BMD (T-score)	0.29 ± 1.68	0.11 ± 1.92	−0.56 ± 1.54	0.065	0.96 ± 1.57	1.08 ± 1.68	0.78 ± 0.76	0.654	−0.35 ± 1.53	−0.79 ± 1.67[a]	−1.39 ± 1.31[a]	0.005
Lumbar BMD (Z-score)	0.98 ± 1.43	1.28 ± 1.53	0.94 ± 1.28	0.070	1.30 ± 1.49	1.56 ± 1.57	1.58 ± 1.12	0.384	0.67 ± 1.30	1.02 ± 1.45	0.54 ± 1.24	0.096
Left-hip BMD (T-score)	−0.54 ± 1.11	−0.88 ± 1.22[a]	−0.89 ± 1.14[a]	0.008	−0.32 ± 1.18	−0.37 ± 1.15	−0.36 ± 1.01	0.937	−0.74 ± 1.00	−1.36 ± 1.08[a]	−1.22 ± 1.12[a]	<0.001
Left-hip BMD (z-score)	0.23 ± 1.02	0.36 ± 1.05	0.33 ± 1.00	0.380	0.36 ± 1.08	0.59 ± 1.11	0.71 ± 0.81	0.245	0.11 ± 0.95	0.15 ± 0.95	0.09 ± 1.06	0.818
BMD				0.002				0.870				<0.001
Normal BMD	206 (59.71%)	167 (50.00%)	39 (48.15%)		126 (70.00%)	114 (68.26%)	28 (68.29%)		80 (48.48%)	53 (31.74%)	11 (27.50%)	
Osteopenia	121 (35.07%)	122 (36.53%)	32 (39.51%)		48 (26.67%)	47 (28.14%)	13 (31.71%)		73 (44.24%)	75 (44.91%)	19 (47.50%)	
Osteoporosis	18 (5.22%)	45 (13.47%)	10 (12.35%)		6 (3.33%)	6 (3.59%)	0 (0.00%)		12 (7.27%)	39 (23.35%)	10 (25.00%)	

The parameters are presented as means ± s.d. or medians (interquartile ranges). *P* values were calculated from three tests for categorical variables, Student's *t* tests for continuous variables and samples non-parametric test.

[a]$P < 0.05$ vs FIB4-LR; [b]$P < 0.05$ vs FIB4-IR.

γ-GT, gamma-glutamyl transferase; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMD, bone mineral density; DBP, diastolic blood pressure; FBG, fast blood glucose; FIB4-HR, FIB4 high risk; FIB4-IR, FIB4 intermediate risk; FIB4-LR, FIB4 low risk; Fins, fast insulin; GLB, globulin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PTH, parathyroid hormone; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-22-0174

Table 4 Comparison of parameters between different NAFLD fibrosis risk stages stratified according to NFS.

	Total participants (n = 760)				**Men** (n = 388)				**Women** (n = 372)			
	NFS-LR	NFS-IR	NFS-HR	*P*-value	NFS-LR	NFS-IR	NFS-HR	*P*-value	NFS-LR	NFS-IR	NFS-HR	*P*-value
n	109	485	166		47	266	75		62	219	91	
BMI (kg/m^2)	25.42 ± 3.00	26.30 ± 3.59	27.08 ± 3.33[a,b]	0.002	25.84 ± 2.77	26.56 ± 3.17	27.39 ± 3.36[a]	0.048	25.08 ± 3.16	25.99 ± 4.03	26.82 ± 3.31[a]	0.040
Duration of diabetes (year)	11.00 (4.25, 15.00)	10.00 (6.00, 15.00)	10.00 (5.50, 20.00)	0.366	7.50 (3.50, 15.75)	10.00 (5.00, 14.00)	10.00 (4.00, 20.00)	0.772	11.50 (6.50, 15.00)	10.00 (7.00, 15.25)	12.50 (9.50, 20.00)	0.308
ALB (g/L)	45.87 ± 4.58	43.39 ± 4.05[a]	39.99 ± 4.21[a,b]	<0.001	45.84 ± 4.79	43.64 ± 4.04[a]	39.56 ± 4.22[a,b]	<0.001	45.90 ± 4.45	43.09 ± 4.06[a]	40.34 ± 4.19[a,b]	<0.001
γ- GT (U/L)	26.00 (18.00, 40.00)	26.00 (20.00, 40.50)	24.00 (18.00, 35.00)	0.297	34.00 (22.75, 58.50)	26.00 (20.00, 42.25)	26.00 (21.00, 40.00)	0.182	21.00 (17.00, 34.00)	25.00 (19.00, 38.00)	22.50 (16.25, 32.75)	0.138
Calcium (mmol/L)	2.29 ± 0.14	2.27 ± 0.11	2.24 ± 0.10[a,b]	0.001	2.29 ± 0.10	2.26 ± 0.10	2.21 ± 0.10[a,b]	<0.001	2.29 ± 0.17	2.29 ± 0.12	2.26 ± 0.10	0.162
25(OH)D (nmol/L)	45.35 ± 21.57	43.14 ± 17.03	38.80 ± 17.61[a,b]	0.012	43.51 ± 15.26	42.18 ± 17.34	41.59 ± 18.60[b]	0.027	46.65 ± 25.21	41.01 ± 15.72	36.59 ± 16.64[a]	0.031
PTH (pmol/L)	4.20 (3.15, 5.25)	4.20 (3.10, 5.25)	4.40 (3.20, 6.10)	0.283	4.20 (3.25, 5.20)	4.10 (3.20, 5.10)	3.85 (2.73, 5.05)	0.534	4.20 (3.10, 5.28)	4.40 (3.00, 5.50)	5.50 (3.60, 7.15)[a,b]	0.010
OC (ng/mL)	12.28 (9.69, 18.35)	11.52 (8.78, 15.43)	13.25 (8.82, 20.18)	0.210	10.98 (9.16, 14.13)	10.05 (8.12, 12.36)	9.53 (8.06, 17.38)	0.415	12.82 (11.01, 19.12)	14.60 (10.97, 17.75)	15.68 (11.52, 20.71)	0.634
P1NP (ng/mL)	37.59 (30.12, 67.27)	35.37 (25.74, 45.08)	34.58 (25.29, 47.52)	0.056	30.24 (23.78, 36.93)	32.88 (23.94, 41.13)	33.97 (26.63, 63.77)	0.101	43.29 (33.55, 67.09)	37.90 (27.54, 52.30)	33.00 (30.30, 47.14)[a]	0.045
β-CTX (pg/mL)	305.40 (223.35, 471.90)	366.00 (231.35, 484.25)	359.55 (221.63, 530.38)	0.736	300.10 (185.20, 415.60)	327.50 (217.23, 454.88)	266.05 (198.40, 475.75)	0.750	322.65 (249.93, 606.70)	388.90 (269.40, 559.90)	401.80 (235.63, 546.80)	0.820
Lumbar BMD (T-score)	0.26 ± 1.48	0.20 ± 1.87	−0.25 ± 1.72	0.177	0.86 ± 1.65	1.07 ± 1.66	0.93 ± 1.30	0.852	−0.15 ± 1.20	−0.66 ± 1.65[a]	−1.07 ± 1.48[a]	0.005
Lumbar BMD (Z-score)	0.89 ± 1.35	1.14 ± 1.52	1.14 ± 1.32	0.383	1.13 ± 1.58	1.49 ± 1.61	1.48 ± 1.23	0.503	0.72 ± 1.16	0.79 ± 1.34	0.92 ± 1.35	0.611
Left-hip BMD (T-score)	−0.51 ± 1.12	−0.66 ± 1.17	−1.02 ± 1.08[a,b]	0.016	−0.50 ± 1.38	−0.28 ± 1.14	−0.42 ± 1.01	0.493	−0.52 ± 0.91	−1.03 ± 1.08[a]	−1.44 ± 0.92[a,b]	<0.001
Left-hip BMD (z-score)	0.18 ± 1.00	0.33 ± 1.08	0.30 ± 0.89	0.557	0.09 ± 1.24	0.54 ± 1.11	0.57 ± 0.86	0.115	0.24 ± 0.80	0.12 ± 1.01	0.12 ± 0.88	0.759
BMD				0.002				0.084				0.002
Normal BMD	63 (57.80%)	275 (56.70%)	67 (40.36%)		31 (65.96%)	190 (71.43%)	45 (60.00%)		32 (51.61%)	85 (38.81%)	22 (24.18%)	
Osteopenia	38 (34.86%)	164 (33.81%)	72 (43.37%)		12 (25.53%)	70 (26.32%)	27 (36.00%)		26 (41.94%)	94 (42.92%)	45 (49.45%)	
Osteoporosis	8 (7.34%)	46 (9.48%)	27 (16.27%)		4 (8.51%)	6 (2.26%)	3 (4.00%)		4 (6.45%)	40 (18.26%)	24 (26.37%)	

The parameters are presented as means ± s.d. or medians (interquartile ranges). *P* values were calculated from three tests for categorical variables, Student's *t* tests for continuous variables and samples non-parametric test.

[a]$P < 0.05$ vs NFS-LR; [b]$P < 0.05$ vs NFS-IR.

γ-GT, gamma-glutamyl transferase; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMD, bone mineral density; DBP, diastolic blood pressure; FBG, fast blood glucose; Fins, fast insulin; GLB, globulin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NFS-HR, NFS high risk; NFS-IR, NFS intermediate risk; NFS-LR, NFS low risk; PTH, parathyroid hormone; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-22-0174

co-existent T2DM. FIB-4 high risk was an independent risk factor for osteoporosis in women (OR = 4.41; 95% CI, 1.04–18.70; in the high-risk vs the low-risk group, $P = 0.039$) after correction for duration of diabetes, PTH, 25(OH)D and BMI (Table 6). Similarly, NFS high risk also was an independent risk factor for osteoporosis in women (OR = 5.98; 95% CI, 1.40–25.60 in the high-risk group vs the low-risk group, $P = 0.043$) after correction for duration of diabetes, PTH and 25(OH)D (Table 6).

Discussion

Our study is the first large study to investigate the association of NAFLD and hepatic fibrosis with osteoporosis in male and female T2DM patients. Our data analysis of the overall population showed that the prevalence of osteoporosis was significantly lower in the NAFLD group, and fatty liver appeared to be a protective factor for osteoporosis. Nonetheless, after matching for sex, age and BMI, there was no correlation between NAFLD and osteoporosis. This may be because patients with NAFLD had a higher BMI, and an appropriate increase in BMI is a protective factor for BMD (16). This may also explain the inconsistent results of previous studies.

Another important finding was that liver fibrosis, stratified by FIB-4 and NFS, was closely associated with osteoporosis in females and the overall population, but no correlation was evident in males. It is speculated that the overall population correlation may be attributable to women, who are at increased risk of developing osteoporosis due to the diminished protective effect of oestrogen after menopause. Nonetheless, the gender differences between hepatic fibrosis and osteoporosis were not clear. Gonadal hormones including testosterone (T), oestrogens (E), follicle-stimulating hormone (FSH) and serum sex-hormone binding globulin (SHBG) interact to determine bone mass accrual and BMD maintenance (17). During ageing, men have a greater periosteal apposition and similar endocortical resorption to women (18). We speculate that the difference in hormones contributes to the differences in hepatic fibrosis and osteoporosis.

At present, the link between liver fibrosis and osteoporosis has not been fully clarified. Previous studies showed that bone cortical thickness in patients with obvious fibrosis is significantly thinner and may be related to enhanced intracortical bone resorption (19). NASH can lead to an increase in the release of inflammatory factors such as interleukin-6 and tumour necrosis factor ɑ that can promote a decrease in bone density. For example,

Table 5 Factors associated with bone mineral density in subjects with NAFLD.

	Total participants				Men				Women			
	Lumbar BMD (T-score)		Left-hip BMD (T-score)		Lumbar BMD (T-score)		Left-hip BMD (T-score)		Lumbar BMD (T-score)		Left-hip BMD (T-score)	
	R	*P*-value	R	*P*-value	R	*P*-value	R	*P*-value	R	*P*-value	R	*P*-value
Age (year)	−0.143	0.001	−0.325	<0.001	−0.029	0.049	−0.180	0.006	−0.264	<0.001	−0.446	<0.001
BMI (kg/m^2)	0.293	<0.001	0.265	<0.001	0.257	<0.001	0.287	<0.001	0.326	<0.001	0.238	<0.001
Duration of diabetes (year)	−0.100	0.122	−0.243	<0.001	−0.082	0.400	−0.170	0.080	−0.015	0.861	−0.254	0.003
FBG (mmol/L)	0.016	0.756	0.073	0.165	0.057	0.454	0.164	0.131	−0.096	0.186	−0.049	0.505
FINS (pmol/L)	−0.033	0.604	−0.006	0.930	−0.061	0.509	−0.067	0.469	−0.068	0.450	0.052	0.561
HOMA-IR	−0.025	0.705	0.092	0.163	0.175	0.065	−0.196	0.056	−0.175	0.056	0.026	0.774
HbA1C (%)	−0.002	0.971	−0.079	0.101	−0.114	0.101	−0.144	0.058	−0.022	0.740	−0.093	0.163
TC (mmol/L)	−0.054	0.272	−0.019	0.694	−0.075	0.293	−0.044	0.533	0.068	0.312	0.074	0.271
TG (mmol/L)	0.036	0.466	0.058	0.231	0.032	0.651	0.101	0.153	0.084	0.213	0.054	0.421
HDL-C (mmol/L)	0.036	0.189	0.032	0.156	0.055	0.096	0.133	0.060	−0.038	0.110	0.008	0.908
LDL-C (mmol/L)	−0.009	0.860	−0.011	0.815	−0.055	0.437	−0.079	0.264	0.076	0.260	0.077	0.255
ALB (g/L)	0.011	0.991	0.170	<0.001	0.001	0.875	0.134	0.054	0.024	0.721	0.202	0.002
PTH (pmol/L)	−0.052	0.260	−0.167	<0.001	0.042	0.535	−0.050	0.454	−0.040	0.528	−0.218	<0.001
25(OH)D (nmol/L)	0.125	0.006	0.147	0.001	0.073	0.283	0.105	0.120	0.045	0.473	0.108	0.083
Calcium (mmol/L)	−0.013	0.774	0.039	0.402	0.009	0.899	0.011	0.869	0.023	0.722	0.096	0.129
FIB4	−0.167	<0.001	−0.107	0.025	0.001	0.994	0.020	0.768	−0.229	<0.001	−0.152	0.022
NFS	−0.109	0.027	−0.190	<0.001	0.064	0.376	−0.039	0.590	−0.242	<0.001	−0.316	<0.001

γ-GT, gamma-glutamyl transferase; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMD, bone mineral density; DBP, diastolic blood pressure; FBG, fast blood glucose; Fins, fast insulin; GLB, globulin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR; homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; PTH, parathyroid hormone; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-22-0174

Table 6 Logistic regression analyses of NAFLD fibrosis risk for osteoporosis in women's subjects with NAFLD and co-existing T2DM.

	Model 1		Model 2		Model 3		Model 4		Model 5	
FIB4	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Low-risk		0.001		0.024		0.030		0.073		0.073
Intermediate risk vs Low-risk	2.73 (1.48–5.03)	0.001	2.09 (0.93–4.69)	0.090	2.09 (0.89–4.90)	0.090	1.90 (0.80–4.53)	0.149	2.21 (0.894–5.58)	0.086
High risk vs low risk	4.97 (1.86–13.30)	0.001	5.05 (1.32–19.34)	0.018	4.63 (1.18–18.14)	0.028	4.86 (1.15–19.16)	0.032	4.41 (1.04–18.70)	0.039
	Model 1		**Model 2**		**Model 3**		**Model 4**		**Model 5**	
NFS	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Low risk		0.003		0.023		0.043		0.044		/
Intermediate risk vs low risk	2.47 (0.96–6.36)	0.061	2.52 (0.74–8.55)	0.139	2.34 (0.67–8.16)	0.181	2.15 (0.60–7.64)	0.237		/
High risk vs low risk	5.64 (1.98–16.08)	0.001	4.32 (1.12–16.63)	0.033	5.89 (1.42–24.35)	0.038	5.98 (1.40–25.60)	0.043		/

Model 1 was unadjusted; Model 2 adjusted for duration of diabetes; Model 3 was adjusted for duration of diabetes and PTH; Model 4 was adjusted for 25(OH)D and Model 3; Model 5 was adjusted for BMI and Model 4.

TNF-α can promote osteoblasts and their precursor cells and inhibit the differentiation of osteoblasts. Liver fibrosis, elevated blood copper ions and excessive copper can cause kidney damage, resulting in a large loss of bone calcium and development of osteoporosis (20). It has also been reported that copper can reduce the rate of bone turnover by inhibiting the function of osteoblasts and osteoclasts (21). Various cytokines and pathogenic mediators have been implicated in the pathogenesis of bone loss in chronic liver disease (22, 23). In addition, insulin resistance, hypercoagulation-hypofibrinolysis, overexpression of osteopontin, reduced osteoprotegerin and osteocalcin, decreased leptin, adiponectin and 25-hydroxyvitamin D3 are also involved in the pathogenesis of fatty liver (7).

Accumulating evidence suggests that diabetic bone disease is characterized by low bone turnover and patients with T2DM have a higher long-term risk of fracture despite having similar or slightly higher BMD than age- and sex-adjusted non-diabetic controls (24, 25). In our study, grouping analysis of bone metabolism markers showed that P1NP in the high-risk group of hepatic fibrosis female diabetic patients was significantly higher than that in the low-risk group, while there was no significant difference in OC or β-CTX. We speculate that liver fibrosis in diabetic patients may increase the risk of osteoporosis by affecting bone synthesis. This is slightly different from previous research results but may be related to the sample size and ethnicity of the study population (26).

This study has some limitations. First, it is a cross-sectional study so no clear causal relationship can be confirmed. In addition, abdominal ultrasonography was performed to diagnose fatty liver. Although not the gold standard, an updated meta-analysis has shown that ultrasonography enables reliable and accurate detection of hepatic steatosis (27). In addition, studies have shown that a semi-quantitative ultrasonographic index, ultrasonographic fatty liver indicator (US-FLI) accurately identified histological severity (28) and will be applied in our further study to investigate the association of NAFLD with osteoporosis. Finally, we found that most patients were considered middle or high risk when stratified according to NFS, implying that NFS was inadequate in screening for liver fibrosis in T2DM patients and more accurate tests are needed in further study.

Conclusion

Our study shows that NAFLD liver fibrosis is significantly correlated with osteoporosis in Chinese postmenopausal women with diabetes but not males. Its pathogenic

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-22-0174

mechanism may be related to the decrease in P1NP. Future prospective cohort studies with rigorous control of confounding factors are needed to elucidate the association of NAFLD hepatic fibrosis with osteoporotic fracture risk.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/EC-22-0174.

Declaration of interest

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

Funding

We acknowledge the financial support from Scientific Research Project funded by Shanghai Fifth People's Hospital, Fudan University (NO. 2019WYZD02, 2019WYFY02), Talent Development Plan funded by Shanghai Fifth People's Hospital, Fudan University (No. 2020WYRCZY01), Health Profession Clinical Research Funds of Shanghai Municipal Health Commission (No. 201940295) and Scientific Research Project from Science and Technology Commission of Shanghai Municipality (No. 19ZR1440200).

Ethical statement

The study protocol was approved by the Ethics Committee of Shanghai Fifth People's Hospital. Informed consent for the collection of relevant data was signed by the patient during hospitalization.

Data availability

The datasets generated during and/or analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

References

1 Qaseem A, Forciea MA, McLean RM, Denberg TD, Clinical Guidelines Committee of the American College of Physicians, Barry MJ, Cooke M, Fitterman N, Harris RP, Humphrey LL, *et al.* Treatment of low bone density or osteoporosis to prevent fractures in men and women: a clinical practice guideline update from the American College of Physicians. *Annals of Internal Medicine* 2017 **166** 818–839. (https://doi.org/10.7326/M15-1361)

2 Younossi ZM. Non-alcoholic fatty liver disease – a global public health perspective. *Journal of Hepatology* 2019 **70** 531–544. (https://doi.org/10.1016/j.jhep.2018.10.033)

3 Moon SS, Lee YS & Kim SW. Association of nonalcoholic fatty liver disease with low bone mass in postmenopausal women. *Endocrine* 2012 **42** 423–429. (https://doi.org/10.1007/s12020-012-9639-6)

4 Cui R, Sheng H, Rui XF, Cheng XY, Sheng CJ, Wang JY & Qu S. Low bone mineral density in Chinese adults with nonalcoholic fatty liver disease. *International Journal of Endocrinology* 2013 **2013** 396545. (https://doi.org/10.1155/2013/396545)

5 Yang HJ, Shim SG, Ma BO & Kwak JY. Association of nonalcoholic fatty liver disease with bone mineral density and serum osteocalcin levels in Korean men. *European Journal of Gastroenterology and Hepatology* 2016 **28** 338–344. (https://doi.org/10.1097/MEG.0000000000000535)

6 Mantovani A, Gatti D, Zoppini G, Lippi G, Bonora E, Byrne CD, Nobili V & Targher G. Association between nonalcoholic fatty liver disease and reduced bone mineral density in children: a meta-analysis. *Hepatology* 2019 **70** 812–823. (https://doi.org/10.1002/hep.30538)

7 Targher G, Lonardo A & Rossini M. Nonalcoholic fatty liver disease and decreased bone mineral density: is there a link? *Journal of Endocrinological Investigation* 2015 **38** 817–825. (https://doi.org/10.1007/s40618-015-0315-6)

8 Mantovani A, Dauriz M, Gatti D, Viapiana O, Zoppini G, Lippi G, Byrne CD, Bonnet F, Bonora E & Targher G. Systematic review with meta-analysis: non-alcoholic fatty liver disease is associated with a history of osteoporotic fractures but not with low bone mineral density. *Alimentary Pharmacology and Therapeutics* 2019 **49** 375–388. (https://doi.org/10.1111/apt.15087)

9 Upala S, Jaruvongvanich V, Wijarnpreecha K & Sanguankeo A. Nonalcoholic fatty liver disease and osteoporosis: a systematic review and meta-analysis. *Journal of Bone and Mineral Metabolism* 2017 **35** 685–693. (https://doi.org/10.1007/s00774-016-0807-2)

10 Ekstedt M, Hagström H, Nasr P, Fredrikson M, Stål P, Kechagias S & Hultcrantz R. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. *Hepatology* 2015 **61** 1547–1554. (https://doi.org/10.1002/hep.27368)

11 Loosen SH, Roderburg C, Demir M, Qvartskhava N, Keitel V, Kostev K & Luedde T. Non-alcoholic fatty liver disease (NAFLD) is associated with an increased incidence of osteoporosis and bone fractures. *Zeitschrift für Gastroenterologie* 2022 **60** 1221–1227. (https://doi.org/10.1055/a-1482-9236)

12 Zhu X, Yan H, Chang X, Xia M, Zhang L, Wang L, Sun X, Yang X, Gao X & Bian H. Association between non-alcoholic fatty liver disease-associated hepatic fibrosis and bone mineral density in postmenopausal women with type 2 diabetes or impaired glucose regulation. *BMJ Open Diabetes Research and Care* 2020 **8** e000999. (https://doi.org/10.1136/bmjdrc-2019-000999)

13 American Diabetes Association. Standards of medical care in diabetes – 2014. *Diabetes Care* 2014 **37** (Supplement 1) S14–S80. (https://doi.org/10.2337/dc14-S014)

14 Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, Landt CL & Harrison SA. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology* 2011 **140** 124–131. (https://doi.org/10.1053/j.gastro.2010.09.038)

15 McPherson S, Hardy T, Dufour JF, Petta S, Romero-Gomez M, Allison M, Oliveira CP, Francque S, Van Gaal L, Schattenberg JM, *et al.* Age as a confounding factor for the accurate non-invasive diagnosis of advanced NAFLD fibrosis. *American Journal of Gastroenterology* 2017 **112** 740–751. (https://doi.org/10.1038/ajg.2016.453)

16 Leslie WD, Morin SN, Majumdar SR & Lix LM. Effects of obesity and diabetes on rate of bone density loss. *Osteoporosis International* 2018 **29** 61–67. (https://doi.org/10.1007/s00198-017-4223-9)

17 Vescini F, Chiodini I, Falchetti A, Palermo A, Salcuni AS, Bonadonna S, De Geronimo V, Cesareo R, Giovanelli L, Brigo M, *et al.* Management of osteoporosis in men: a narrative review. *International Journal of Molecular Sciences* 2021 **22** 13640. (https://doi.org/10.3390/ijms222413640)

18 Seeman E. Periosteal bone formation – a neglected determinant of bone strength. *New England Journal of Medicine* 2003 **349** 320–323. (https://doi.org/10.1056/NEJMp038101)

19 Culafić Dj, Djonic D, Culafic-Vojinovic V, Ignjatovic S, Soldatovic I, Vasic J, Beck TJ & Djuric MEvidence of degraded BMD and geometry at the proximal femora in male patients with alcoholic liver cirrhosis. *Osteoporosis International* 2015 **26** 253–259. (https://doi.org/10.1007/s00198-014-2849-4)

20 Walshe JM. Copper: not too little, not too much, but just right. Based on the triennial Pewterers Lecture delivered at the National Hospital for Neurology, London. *Journal of the Royal College of Physicians of London* 1995 **29** 280–288.

21 Kozuka H. Interactive exhibition of heavy metal toxicity in bone metabolism. From the viewpoint of deductive toxicology.

Yakugaku Zasshi 1995 **115** 157–169. (https://doi.org/10.1248/yakushi1947.115.3_157)

22 Nakchbandi IA. Osteoporosis and fractures in liver disease: relevance, pathogenesis and therapeutic implications. *World Journal of Gastroenterology* 2014 **20** 9427–9438. (https://doi.org/10.3748/wjg.v20.i28.9427)

23 Gaudio A, Lasco A, Morabito N, Atteritano M, Vergara C, Catalano A, Fries W, Trifiletti A & Frisina N. Hepatic osteodystrophy: does the osteoprotegerin/receptor activator of nuclear factor-kB ligand system play a role? *Journal of Endocrinological Investigation* 2005 **28** 677–682. (https://doi.org/10.1007/BF03347549)

24 Napoli N, Chandran M, Pierroz DD, Abrahamsen B, Schwartz AV, Ferrari SL & IOF Bone and Diabetes Working Group. Mechanisms of diabetes mellitus-induced bone fragility. *Nature Reviews: Endocrinology* 2017 **13** 208–219. (https://doi.org/10.1038/nrendo.2016.153)

25 Hygum K, Starup-Linde J, Harsløf T, Vestergaard P & Langdahl BL. Mechanisms in endocrinology: diabetes mellitus, a state of low bone turnover – a systematic review and meta-analysis. *European Journal of Endocrinology* 2017 **176** R137–R157. (https://doi.org/10.1530/EJE-16-0652)

26 Mantovani A, Sani E, Fassio A, Colecchia A, Viapiana O, Gatti D, Idolazzi L, Rossini M, Salvagno G, Lippi G, *et al.* Association between non-alcoholic fatty liver disease and bone turnover biomarkers in post-menopausal women with type 2 diabetes. *Diabetes and Metabolism* 2019 **45** 347–355. (https://doi.org/10.1016/j.diabet.2018.10.001)

27 Ballestri S, Byrne CD & La TG. Diagnostic accuracy of ultrasonography for the detection of hepatic steatosis: an updated meta-analysis of observational studies. *Metabolism and Target Organ Damage* 2021 **1** 7. (https://doi.org/10.20517/mtod.2021.05)

28 Ballestri S, Nascimbeni F, Baldelli E, Marrazzo A, Romagnoli D, Targher G & Lonardo A. Ultrasonographic fatty liver indicator detects mild steatosis and correlates with metabolic/histological parameters in various liver diseases. *Metabolism: Clinical and Experimental* 2017 **72** 57–65. (https://doi.org/10.1016/j.metabol.2017.04.003)

Received in final form 6 January 2022
Accepted 10 August 2022
Accepted Manuscript published online 21 September 2022