The impact of Non-alcoholic Fatty Liver disease on Bone Mineral Density of Lumbar Spine in Type 2 Diabetes Mellitus

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Research Article

Keywords: Nonalcoholic fatty liver, Diabetes, Lumbar, Bone mineral density

Posted Date: May 13th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1628977/v1

Article type : Original Article

Running title: Association between NAFLD and BMD

The impact of Non-alcoholic Fatty Liver disease on Bone Mineral Density of Lumbar Spine in Type 2 Diabetes Mellitus

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Abstract

Objective: To evaluate the association between Nonalcoholic fatty liver disease (NAFLD) and lumbar spine bone mineral density (BMD) in type 2 diabetes mellitus (T2DM).

Methods: The lumbar BMD of 1088 subjects was measured using dual-energy X-ray absorptiometry (DXA). Liver fat content was quantified via ultrasound. According to clinical diagnosis, subjects were divided into T2DM and non diabetes groups. The groups were further divided into NAFLD and non-

NAFLD groups. Student's t-test assessed the differences in BMD between the NAFLD and non-NAFLD groups. Multivariable linear regression analysis adjusted for confounders was performed to evaluate the association between lumbar BMD and NAFLD.

Results: The lumbar BMD in the T2DM group and the non diabetes group was higher in the NAFLD group than in the non-NAFLD group (P<0.001). Multivariate regression analysis in the T2DM group showed that after adjusting for confounders, the association between lumbar spine BMD and NAFLD remained (P=0.027). In the non diabetes group, after adjusting for confounders, the association between NAFLD and lumbar spine BMD disappeared.

Conclusions: The lumbar BMD of NAFLD patients is higher than that of non-NAFLD. After adjusting for confounding factors, lumbar BMD was associated with NAFLD in T2DM patients but not in non diabetes patients.

Keywords: Nonalcoholic fatty liver; Diabetes; Lumbar; Bone mineral density.

INTRODUCTION

Nonalcoholic fatty liver (NAFLD) is a disease in which excessive fat deposits in liver cells in the absence of excessive drinking or other causes of liver damage and is related to hepatic lipotoxicity(1). Lipotoxicity is often secondary to the accumulation of toxic metabolites derived from triglycerides (TGs), which leads to inflammatory diseases and the activation of insulin resistance(2). The liver and bones are both active endocrine organs that have various metabolic functions(3). Insulin resistance and obesity are the key pathogenic factors for NAFLD and type 2 diabetes mellitus (T2DM)(4). Therefore, these two diseases

usually coexist. Studies have indicated that 75% of T2DM patients have NAFLD. (5, 6). Currently, clear evidence suggests that the bone mineral density (BMD) of T2DM patients is higher than that of non diabetes people, especially in the spine and hips. However, T2DM is associated with an increased risk of fractures(7, 8). In addition, some studies have suggested that there is a latent association between NAFLD and BMD. In addition to osteoporosis, which is commonly thought of as an age-dependent disease, other latent factors are associated with liver and bone tissue(9).

Although some previous studies have separately examined the effects of NAFLD and diabetes on BMD, there is little work discussing the impact of NAFLD coexisting with T2DM on BMD. Moreover, most of the previous studies have been concentrated in specific groups, such as postmenopausal women and obese adolescents. Therefore, in this study, we examined the association between BMD and NAFLD in T2DM patients.

Subjects and methods

Subjects

In this study, 1300 subjects who underwent dual-energy X-ray absorptiometry (DXA) and abdominal ultrasonography between January 2016 and March 2020 were included.

Inclusion criteria：1)All participants were ≥18 years old, 2) NAFLD patients were diagnosed with an ultrasound examination； 3) BMD was measured by dual energy X-ray absorptiometry.

Exclusion criteria: 1) autoimmune, viral or drug-induced hepatitis disease; 2) excessive alcohol consumption (over 20 g per day); 3) patients with diabetes other than T2DM; 4) other diseases that may affect BMD except fatty liver and diabetes (hyperthyroidism, hyperparathyroidism, malignant tumors, etc.); 5) long-term use of drugs that affect BMD (such as glucocorticoids, steroids).

After excluding subjects who did not meet the criteria and had incomplete data, 1088 subjects were included. The subjects were divided into the T2DM group and the non diabetes group. The diagnosis of T2DM was based on the recommendations of the current guidelines of the American Diabetes Association(10). T2DM patients were divided into a T2DM with NAFLD group (181 patients) and a T2DM without NAFLD group (353). The non diabetes group was divided into the NAFLD group (144) and the non-NAFLD group (410).

Methods

Dual-energy X-ray absorptiometry to measure lumbar spine BMD

According to the World Health Organization (WHO) diagnostic criteria, the T-score, Z-score and BMD value at the lumbar spine (L1–L4) were measured using DXA (GE Lunar Health Care, DPX-L, USA).

NAFLD diagnosis via abdominal ultrasound

The sonographer used a 3-5 MHz probe to examine and evaluate the liver. The NAFLD diagnostic criteria based on ultrasound are the presence of signs of liver steatosis, such as bright liver echo patterns, increased echo beam attenuation, and loss of structural details in the liver(11).

Collection of laboratory and baseline data

The height, weight, and smoking and alcohol consumption history of the participants were collected. Body mass index (BMI) is the weight (kg) divided by the standing height squared (m^2). (12). Laboratory data included total serum cholesterol (TC), TGs, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), creatinine, uric acid (UA), alanine transaminase (ALT), alanine aminotransferase (AST), glycosylated hemoglobin A1c (HbA1c), calcium, and fasting and postprandial blood sugar.

Statistical analysis

Statistical analyses were performed with IBM SPSS (version 22.0, IBM SPSS Inc., Armonk, New York, US). Continuous standard variables are expressed as the mean±standard deviation. Categorical variables are expressed in numbers (percentages) and were compared using the χ^2 test. Student's t-test was used to evaluate the difference between the NAFLD and non-NAFLD groups. Linear regression analysis was used to evaluate the correlation between NAFLD and lumbar spine BMD. First, we used the average lumbar spine BMD as the dependent variable and selected variables based on the clinical background as independent variables for univariate regression analysis. The confounding factors with $p<0.1$ in the univariate analysis were included in the multivariate analysis. To avoid multicollinearity, the variance inflation factor was evaluated before adjustment.

Results

The baseline characteristics and laboratory data of the T2DM group and non diabetes group are shown in Table 1. There was no significant difference in age, sex, or smoking or alcohol consumption history between the two subgroups (P>0.05). In the T2DM group, the average weight, BMI, TGs, ALT, AST, UA, and calcium in the NAFLD subgroup were higher than those in the non-NAFLD subgroup (P<0.05). The HDL-C and creatinine levels of the non-NAFLD subgroup were higher than those of the NAFLD subgroup (P<0.05). In the non diabetes group, the BMI, TGs, LDL-C, and ALT in the NAFLD subgroup were higher than those in the non-NAFLD subgroup (P<0.05). The non-NAFLD subgroup's HDL-C was higher than that of the NAFLD subgroup (P<0.05).

Figure 1 describes lumbar spine BMD according to NAFLD presence. Lumbar spine BMD in the T2DM group (-0.91±1.68 vs -1.3±1.63 g/cm²) and the non diabetes group (-1.2±1.76 vs -2.12±1.74 g/cm²), NAFLD group were significantly higher than non-NAFLD group (P< 0.001).

The relationship between lumbar spine BMD and NAFLD in different groups is shown in Table 2 and Table 3. In the T2DM group, univariate analysis revealed an association of lumbar spine BMD with NAFLD (p<0.05). After adjusting for confounding factors (BMI, sex, age, TGs, HDL-C, serum calcium, UA, creatinine, ALT, glycosylated hemoglobin), NAFLD and lumbar spine BMD were still associated (P<0.05). In the non diabetes group, univariate analysis revealed an association of lumbar spine BMD with NAFLD

(P<0.05). After adjusting for confounding factors (TGs, HDL-C, sex, age, ALT, blood calcium, UA, creatinine, calcium, glycosylated hemoglobin), there was no correlation between lumbar spine BMD and NAFLD (p>0.05).

Discussion

This study assessed the correlation between NAFLD and lumbar spine BMD in T2DM patients and non diabetes patients. The main findings are as follows: First, the lumbar spine BMD in the NAFLD group was higher than that in the non-NAFLD group regardless of whether the patients had T2DM. Second, after adjusting for confounding factors, NAFLD was still associated with lumbar spine BMD in the T2DM group, while NAFLD was not associated with lumbar spine BMD in the non diabetes group.

Studies have been conducted on the correlation between NAFLD and BMD, but the results are still controversial. A retrospective study found that NAFLD harms male femoral neck BMD but positively affects lumbar spine BMD in postmenopausal women(13). Another study that used liver biopsy as a diagnostic method for NAFLD found that the BMD of the lumbar spine in the NAFLD group was higher than that in the control group(14). Nevertheless, there was no significant difference in femoral neck BMD between the two groups. These results show that NAFLD does not reduce lumbar spine BMD or that NAFLD increases lumbar spine BMD, which is partly consistent with our findings. We speculate that one reason for this difference in BMD between the lumbar spine and the femoral neck may be related to different

body fat distributions. Subcutaneous and visceral fat have different metabolic characteristics(15). They may have different effects on BMD in different areas, and this correlation may vary with age and sex. Previous studies have shown that fat in the upper body can prevent bone loss and is associated with higher BMD in the lumbar spine(16). The effect of NAFLD on increased lumbar spine BMD may be related to the structural characteristics of the lumbar spine. NAFLD may prevent bone loss by increasing mechanical load and enhancing cortical bone formation. Some studies have also observed that serum fetuin-A is elevated in NAFLD patients(17-19). Nevertheless, research on fetuin-A is currently limited to in vitro experiments.

We noticed that several cross-sectional studies have reported that NAFLD is associated with a decrease in BMD. However, the subjects in these studies were mostly children and adolescents, and some were postmenopausal women(20-22). These findings indicate that the relationship between NAFLD and BMD is different among different people. At present, the mechanism underlying the low BMD in adolescents and postmenopausal women with NAFLD is not completely clear. In addition to being related to age, this association may also be related to low calcium, low growth hormone (GH) and low insulin growth factor (IGF-1)(23-25), yet these mechanisms have not yet been fully clarified.

Although there have been some studies on the relationship between NAFLD and BMD, few studies have examined the relationship between NAFLD and BMD in patients with T2DM. Our research revealed that the BMD of NAFLD patients was higher than that of non-NAFLD people regardless of diabetes status. After adjusting for confounding factors, lumbar BMD in T2DM patients with NAFLD was still higher

than that in non-NAFLD patients. NAFLD and T2DM are both metabolic diseases and have similar disease mechanisms. Most of the current studies support that the BMD of T2DM subjects is identical or higher than that of people without T2DM, which may be related to the higher obesity rate of T2DM patients(26). Obesity may lead to an increase in mechanical load and strain, thus increasing BMD(27). Several previous studies on the relationship of T2DM with liver fibrosis and bone found low bone turnover(28). The investigators did not mention the relationship with BMD, and these study sample sizes were insufficient. Most of the subjects were postmenopausal women. Our study found that lumbar spine BMD increased in patients with T2DM and may be associated with pancreatic and intestinal hormone secretion in T2DM patients. T2DM can affect many hormones that act on bone through endocrine pathways, affecting bone metabolism and increasing bone formation(15).

There are several limitations to this study. First, in this study, we did not use liver biopsy to evaluate NAFLD. Liver biopsy is the gold standard for NAFLD diagnosis, but it is invasive and difficult to implement widely. Therefore, we chose abdominal ultrasound, which has been widely used in the clinic as a diagnostic method. Although it is not the gold standard, we believe that this method is well-tested and reliable. Second, although the current research considers many other factors that were not evaluated in previous research, it is still impossible to completely exclude all confounding factors, such as bone turnover biomarkers, vitamin D, and steroids. However, we believe that these factors will not significantly impact the results of this study. Third, our study found differences in NAFLD's effect on BMD according to diabetes status, but this was only a cross-sectional study. More prospective and mechanism-related studies are needed to evaluate the

relationships among T2DM, NAFLD and BMD.

Conclusions

This study shows differences in the relationship between NAFLD and lumbar spine BMD according to diabetes status. In addition, increasing BMI only within a specific range has a positive effect on lumbar spine BMD. As T2DM and NAFLD coexist commonly, the impact of NAFLD on bone needs to be evaluated in different clinical backgrounds. When clinical intervention is required, it is necessary to consider the different effects of different metabolic factors on patients.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of Sichuan Provincial center for disease control and prevention.

Consent for publication

All the authors were consent for publication

Availability of data and materials

The supporting data can be acquired via correspondence author.

Competing interests

The authors declare no competing interests.

Funding

Not applicable

Authors' contributions

JD designed the study. JD analyzed the data and wrote the manuscript. CQH participated in the study design, data analyze, editing and review of the manuscript. XPZ supervised the overall study and contributed to study design, editing and review of the manuscript. YM, HMLwere responsible for collecting, sorting and statistical data. XPZ is the guarantor of this work and, as such, had full access to all the data in the study and takes

responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript.

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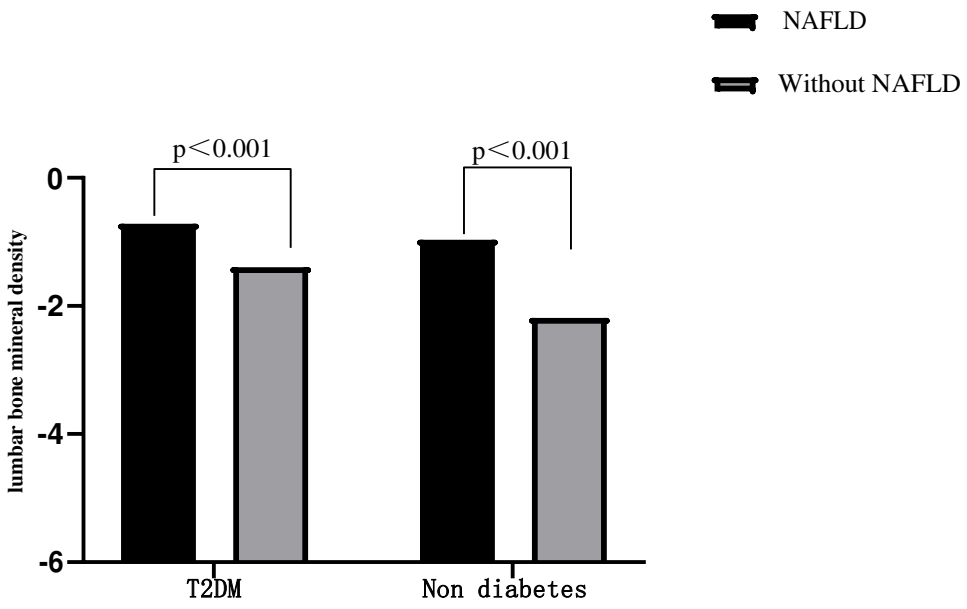
Figure legends

Figure 1 Mean values of lumbar BMD (L1-L4) in different diabetes status classification by NAFLD.



Note: NAFLD, non-alcoholic fatty liver disease; T2DM, type 2 diabetes mellitus.

P values calculated by t-test.

Table legends

Table 1 General characteristics of the study population with and without NAFLD after their classification according to T2DM.

	T2DM（n=534）			Non diabetes （n=554）		
	With NAFLD	Without NAFLD	p-value	With NAFLD	Without NAFLD	p-value
Demographics						
Age (years)	65.71±10.13	67.91±11.19	0.069	69.9±10.7	70.5±12.4	0.785
Female	113（62.6））	202（57.2）	0.293	100（68.7）	243（59.5）	0.237
Height (cm)	159.3±9.3	158.3±8.6	0.433	155.5±8.1	155.9±9.9	0.808
Body weight (kg)	67.9±10.5	59.4±9.4	0.001*	67.6±12.5	54.6±10.1	0.001*
BMI (kg/cm2)	26.6±3.7	23.7±3.3	0.001*	28.2±3.7	22.2±2.9	0.001*
Smoking history	51（28.1）	86（24.3）	0.392	21（14.6）	94（22.9）	0.204
Drinking history	38（20.7）	76（21.7）	0.765	24（16.7）	76（18.5）	0.762
Laboratory data						
HbA1c (%)	8.8±2.1	8.9±2.4	0.677	5.9±1.1	5.61±0.5	0.174
T-chol (mmol/L)	4.7±1.9	4.6±1.4	0.373	4.7±1.4	4.4±0.9	0.208
Triglyceride (mmol/L)	2.8±2.6	1.9±1.4	0.001*	2.0±1.0	1.33±0.9	0.001*
HDL-C (mmol/L)	1.2±0.4	1.3±0.3	0.001*	1.36±0.4	1.43±0.4	0.01*
LDL-C (mmol/L)	2.7±0.9	2.6±0.9	0.189	2.79±1.14	2.4±0.79	0.03*

ALT (U/l)	32.2±18.4	22.8±14.5	0.001*	31.8±19.3	22.2±13.3	0.003*
AST (U/l)	29.3±15.8	24.7±17.5	0.016*	32.4±23.1	26.5±9.2	0.096
Creatinine (umol/L)	58.6±14.3	63.2±16.4	0.014*	57.3±13.6	63.4±14.8	0.012*
Uric acid（umol/L）	351.1±97.1	316.9±89.8	0.002*	337.9±97.6	319.8±99.2	0.282
calcium（mmol/L）	2.34±0.1	2.29±0.2	0.022*	2.29±0.1	2.25±0.1	0.11

Note: The values are the mean ± SD, Numbers in the brackets are percentages. n, number of patients; T2DM, type 2 diabetes mellitus; BMI, body mass index; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; T-chol, total cholesterol, AST, aspartate aminotransferase; ALT, alanine aminotransferase; HbA1c, glycosylated hemoglobin; *P＜0.05

Table 2 Univariate regression analysis：the effect of the study variables on Lumbar spine BMD.

	T2DM				Non- diabetes			
	β	95% CI		p-value	β	95% CI		p-value
Age (years)	-0.035	-0.05	-0.02	0.001*	-0.04	-0.057	0.023	0.001*
Sex (female)	1.569	1.261	-1.877	0.001*	1.553	1.153	1.954	0.001*
Height (cm)	0.081	0.059	0.103	0.001*	0.078	0.054	0.103	0.001*
Body weight (kg)	0.065	0.046	0.083	0.001*	0.081	0.063	0.098	0.001*
BMI (kg/cm2)	0.069	0.012	0.125	0.017*	0.16	0.097	0.224	0.001*
Smoking history	0.027	-0.361	0.416	0.890	0.001	-0.5	0.502	0.997
Drinking history	-0.026	-0.445	0.394	0.905	0.047	-0.499	0.592	0.866
HbA1c (%)	0.017	-0.059	0.094	0.653	0.26	-0.453	0.972	0.472
T-chol (mmol/L)	-0.011	-0.46	0.124	0.872	-0.051	-0.248	0.147	0.615
Triglyceride (mmol/L)	0.26	-0.02	0.163	0.126	0.186	-0.041	0.413	0.109
HDL-C (mmol/L)	-0.679	-1.167	-0.19	0.007*	-1.238	-1.728	-0.747	0.001*
LDL-C (mmol/L)	-0.022	-0.207	0.163	0.957	0.15	-0.098	0.399	0.235
ALT (U/l)	-0.699	-1.078	-0.32	0.001*	-1.238	-1.897	-0.578	0.001*
AST (U/l)	0.013	0.002	0.023	0.021*	0.021	0.007	0.036	0.004*
Creatinine (umol/L)	0.006	-0.005	0.016	0.305	-0.004	-0.02	0.012	0.621
Uric acid（umol/L）	0.014	0.003	0.025	0.014*	0.009	0.000	0.018	0.056
calcium（mmol/L）	0.014	0.000	0.004	0.044*	0.005	0.002	0.007	0.001*

Note: T2DM, type 2 diabetes mellitus; BMI, body mass index; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; T-chol, total cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HbA1c, glycosylated hemoglobin; *P＜0.05

Table 3 Multivariate linear analysis：the effect of the study variables on Lumbar spine BMD

	β	P-value	R^2
T2DM			
NAFLD	-0.488	0.027	0.401
BMI	0.085	0.004	
Age	-0.03	0.005	
Sex(female)	1.366	0.001	
Non diabetes			
BMI	0.107	0.031	0.498
Sex(female)	2.158	0.001	

Note: For ~~T2DM group, BMI, sex, age, TGs, HDL-C, serum calcium, UA, creatinine,~~ ALT, and glycosylated hemoglobin were adjusted.

For Non diabetes group, TGs, HDL-C, sex, age, ALT, blood calcium, UA, creatinine, calcium, and glycosylated hemoglobin were adjusted.

BMI, body mass index; NAFLD, non-alcoholic fatty liver disease.