Relaciones en forma de U-invertida entre la densidad mineral ósea y el grado de esteatosis hepática cuantificada por VCTE en adolescentes: evidencia de NHANES

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Resumen

Introducción

Es posible que haya imprecisiones sobre la esteatosis hepática en investigaciones anteriores que evalúan la relación entre el metabolismo óseo y la esteatosis hepática. El objetivo de la investigación actual era observar las asociaciones entre la densidad mineral ósea (DMO) y la esteatosis y fibrosis hepática que se detectan mediante elastografía de transición a vibración controlada (VCTE, por sus siglas en inglés) en adolescentes de los Estados Unidos.

Métodos

Se utilizaron modelos de regresión lineal múltiple ponderada y ajuste de curvas suavizadas para investigar la asociación entre la DMO y el grado de esteatosis y fibrosis hepática en adolescentes.

Resultados

En 829 adolescentes de entre 12 y 19 años de edad se encontró una asociación negativa entre la DMO y el parámetro de atenuación controlada (CAP, por sus siglas en inglés) [-32,46 (-58,98; -9,05)] y una asociación positiva significativa entre la DMO lumbar y la medición de rigidez hepática (LSM, por sus siglas en inglés) [1,35 (0,19; 2,51)]. Se encontraron relaciones en forma de U-invertida entre la DMO total, la DMO lumbar, la DMO pélvica y el CAP con puntos de inflexión de 221,22 dB/m, 219,88 dB/m, y 216,02 dB/m, respectivamente.

Conclusiones

En adolescentes, una mayor DMO se asocia significativamente a niveles más bajos de esteatosis hepática y niveles más elevados de rigidez hepática.

RESEARCH ARTICLE

Inverted U-shaped relationships between bone mineral density and VCTE-quantified degree of hepatic steatosis in adolescents: Evidence from the NHANES

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Abstract

Introduction

There may be inaccuracies in hepatic steatosis in past research assessing the relationship between bone metabolism and liver steatosis. The goal of the current research was to look at the associations between bone mineral density (BMD) and the hepatic steatosis and fibrosis as detected by vibration-controlled transient elastography (VCTE) in teenagers in the United States.

Methods

Weighted multiple linear regression models and smoothed curve fitting were used to investigate the association between BMD and the degree of hepatic steatosis and fibrosis in adolescents.

Results

In 829 adolescents aged 12–19 years we found a negative association between total BMD and CAP (controlled attenuation parameter) [-32.46 (-58.98, -9.05)] and a significant positive association between lumbar BMD and LSM (liver stiffness measurement) [1.35 (0.19, 2.51)]. The inverted U-shaped relationships were founded between total BMD, lumbar BMD, pelvis BMD, and CAP with inflection points of 221.22 dB/m, 219.88 dB/m, and 216.02 dB/m, respectively.

Conclusions

In adolescents, higher BMD is significantly associated with lower levels of hepatic steatosis and higher levels of liver stiffness.

1. Introduction

According to global research data, NAFLD has become one of the most common chronic liver diseases worldwide [1, 2], the prevalence of NAFLD has reached 25%, 24% and 23% in Asia, North America and Europe, respectively [3]. The prevalence of NAFLD is higher in people with metabolic syndromes such as obesity, diabetes and hyperlipidemia [4]. The continuation of liver steatosis in infancy into adulthood may be a cause of significant hepatic and metabolic illness, making early risk factor diagnosis and screening crucial [5].

Bone mineralization during adolescence is critical for preventing future osteoporosis and reducing fracture risk because during childhood or adolescence, the development and growth of bone mass is faster, so measures need to be taken to prevent osteoporosis as well [6, 7]. Although osteoporosis usually manifests itself in old age, a small percentage of people also develop it in childhood and adolescence [8]. Therefore, early prevention of osteoporosis in children and adolescents is of great significance to the growing public health challenge [9, 10]. Although research on the relationship between NAFLD and bone mineral density (BMD) in teenagers have been conducted [11, 12], these studies have almost always relied on index calculations to determine hepatic steatosis, an assessment method that is considered biased [13–15]. There is still a lack of strong evidence linking the bone metabolism with degree of hepatic steatosis and fibrosis in teenagers.

We therefore conducted a population-based study based on adolescents in the National Health and Nutrition Examination Survey (NHANES) to investigate a more accurate relationship between BMD with liver steatosis and fibrosis among US adolescents.

2. Methods

Data source

NHANES is a large nationwide sample-based survey with a core component of investigating the nutrition and health status of adults and minors [16, 17]. The study procedure was approved by the National Center for Health Statistics (NCHS) Research Ethics Review Committee. The guardians of all minor individuals obtained written approval. Fig 1 illustrates the inclusion exclusions: 5585 participants without available BMD data, 619 participants with missing vibration control transient elastography (VCTE) data, 32 adolescents with viral hepatitis, and 2189 adults were excluded, resulting in the inclusion of 829 adolescent participants.

VCTE

VCTE controlled attenuation data was used to determine the degree of hepatic steatosis and fibrosis. A skilled operator took at least ten times measurements from each subject, and the machine computed a median controlled attenuation parameter (CAP) [18]. The degree of fibrosis was determined by liver stiffness measurement (LSM) and optimized using the Jorden index [19].

BMD

BMD in adolescents is measured by dual-energy X-ray (DEXA) scans, and in this study, we selected three sites for investigation (total BMD, lumbar BMD, and pelvic BMD).

Covariates

Age, gender, race, ratio of family income to poverty, Vitamin D, high-density lipoprotein cholesterol, physical activities, BMI (body mass index), alkaline phosphatase, diabetes status,
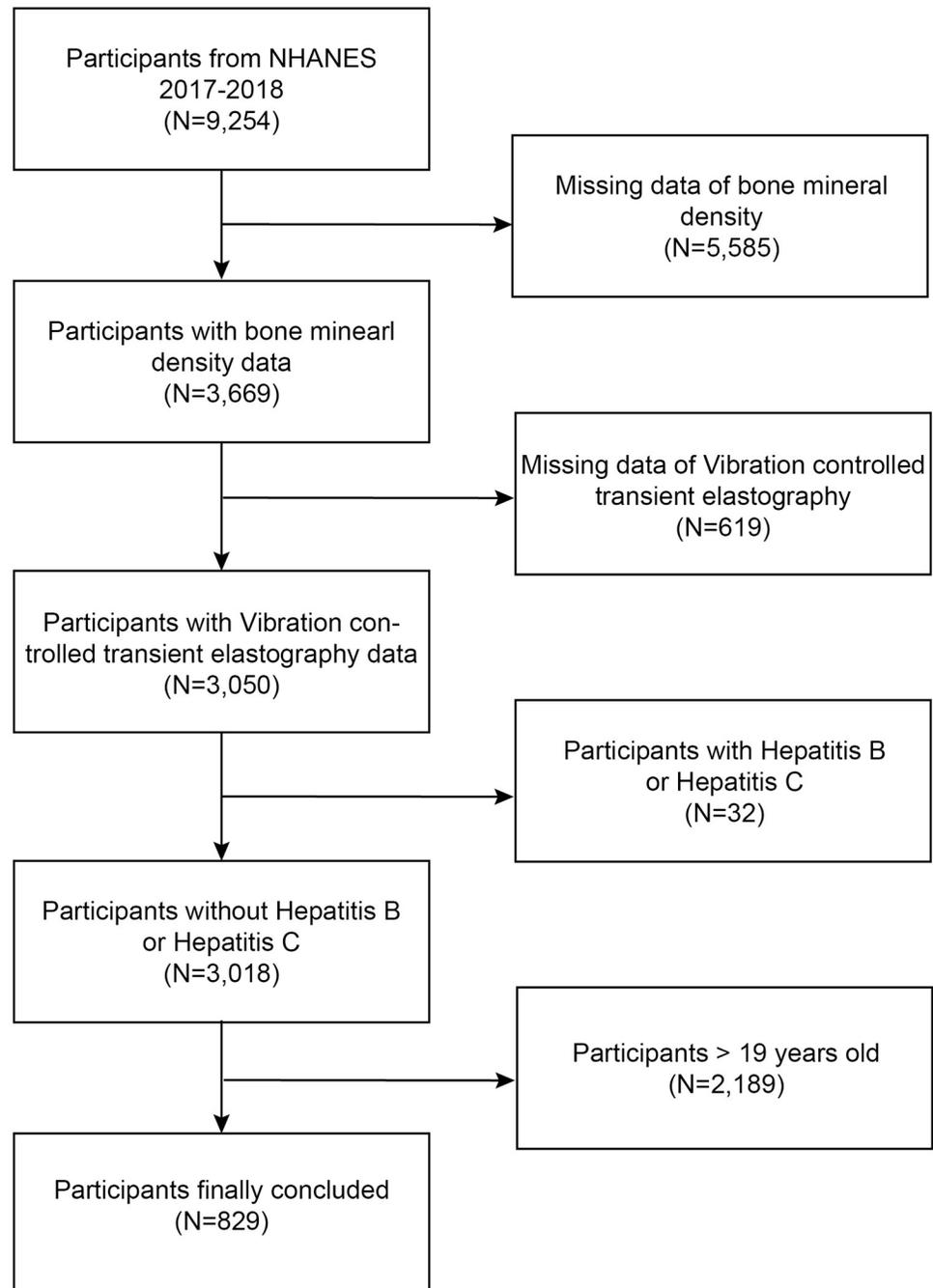
Fig 1. Flow chart of participants selection. NHANES, National Health and Nutrition Examination Survey.

aspartate aminotransferase, high-sensitivity C-reactive protein, triglycerides, low-density lipo-protein cholesterol and total fat percent were all covariates in this study.

Statistical analysis

R (version 4.2) and Empowerstats (version 5.0) were used for all analyses. The demographic parameters of the individuals by CAP quartile were assessed using the One-Way ANOVA test.

The relationships between BMD and CAP and LSM were investigated using multivariate linear regression models. Smoothed curve fit (penalized spline approach) and two-segment linear models were used to explore the nonlinear association between BMD and CAP and LSM [20, 21]. To evaluate changes in the aforesaid relationships across gender, race, and age, subgroup analysis and interaction tests were utilized. Statistical significance was defined as a two-tailed P value of 0.05.

3. Results

Baseline characteristics

At the time of assessment, the mean (SD) age of the 829 adolescents was 15.51 (2.26) years, with 52.747% of male participants. The weighted mean total BMD, CAP, and LSM were 1.04 (0.12) g/cm^2, 221.76 (55.94) dB/m, and 5.01 (2.28) kPa, respectively. The characteristics of the study population according to the quartiles of the CAP are shown in Table 1. Adolescents in the higher quartiles of CAP were more likely to be Mexican Americans, to have a higher BMI and waist circumference, with higher pelvis BMD and LSM, and lower vitamin D levels.

Association between BMD with hepatic steatosis and fibrosis

Table 2 presents the results of multivariate linear regression models between BMD with CAP and LSM. When not adjusted for any covariates, there are positive correlations between the three sites of BMD and CAP. However, in the partially adjusted model and the fully adjusted model, these associations shifted to negative correlations, particularly a negative association between total BMD and CAP, with each 32.46 dB/m decrease in CAP followed by a 1 g/cm^2 increase in total BMD [-32.46 (-58.98, -9.05)]. The association between BMD and liver stiffness was positive in all models, with a positive association between lumbar BMD and LSM. The linear correlation between lumbar BMD and LSM remained significant after adjusting for all covariates, with each 0.84 kPa increase in LSM followed by a 1 g/cm^2 increase in lumbar BMD [1.35 (0.19, 2.51)].

In subgroup analyses stratified by gender, race, and age, the associations between BMD and LSM remained positive in all subgroups (Table 3). The associations between BMD and CAP were negative in all subgroups except race. In non-Hispanic blacks, the negative correlation changed into a positive, moreover, the results of the interaction test showed that race modified the association between BMD and CAP among adolescents (P for interaction < 0.05).

In addition, Fig 2 illustrates the nonlinear relationship between BMD with CAP and LSM. The results indicated there are inverted U-shaped relationships between BMD and CAP at all three sites, and we subsequently used threshold effects analysis and two-segment linear regression models to further investigate the effects of inflection points and two segments in the inverted U-shaped relationship. The results showed that the inflection points of CAP for total BMD, lumbar BMD, and pelvis BMD were 221.22 dB/m, 219.88 dB/m, and 216.02 dB/m, respectively (Table 4).

4. Discussion

In this study, we assessed the associations between BMD with the degree of hepatic steatosis and fibrosis in U.S. adolescents and found that lower CAP and higher LSM were significantly associated with higher BMD. In addition, we identified an inverted U-shaped correlation between BMD and CAP and the point of infection, which may further explain the potentially complex association between the degree of hepatic steatosis and fibrosis and bone metabolism

Table 1. Basic characteristics of participants by CAP quartile.

Characteristics	Q1 (N = 205)	Q2 (N = 205)	Q3 (N = 211)	Q4 (N = 208)	*P*-value
Age (years)	15.44 ± 2.27	15.44 ± 2.34	15.68 ± 2.20	15.47 ± 2.25	0.656
Sex, n (%)					0.741
Male	103 (50.24%)	109 (53.17%)	108 (51.18%)	115 (55.29%)	
Female	102 (49.76%)	96 (46.83%)	103 (48.82%)	93 (44.71%)	
Race/ethnicity, n (%)					0.003
Non-Hispanic White	74 (36.10%)	82 (40.00%)	56 (26.54%)	49 (23.56%)	
Non-Hispanic Black	35 (17.07%)	40 (19.51%)	40 (18.96%)	36 (17.31%)	
Mexican American	35 (17.07%)	31 (15.12%)	41 (19.43%)	57 (27.40%)	
Other race/multiracial	61 (29.76%)	52 (25.37%)	74 (35.07%)	66 (31.73%)	
Diabetes, n (%)					0.429
Yes	1 (0.49%)	1 (0.49%)	4 (1.90%)	1 (0.48%)	
No	202 (98.54%)	203 (99.02%)	207 (98.10%)	205 (98.56%)	
Borderline	2 (0.98%)	1 (0.49%)	0 (0.00%)	2 (0.96%)	
Days of physical activities, n (%)					0.699
0–3	97 (47.32%)	81 (39.51%)	96 (45.50%)	92 (44.23%)	
4–7	108 (52.68%)	124 (40.49%)	115 (54.50%)	116 (55.77%)	
BMI (kg/m^2)	21.03 ± 4.09	22.20 ± 3.71	23.97 ± 5.39	29.85 ± 6.89	<0.001
PIR	2.18 ± 1.51	2.13 ± 1.59	2.19 ± 1.57	1.97 ± 1.44	0.506
Triglycerides (mg./dL)	61.55 ± 32.63	68.31 ± 36.79	73.96 ± 58.81	89.38 ± 50.64	<0.001
HDL-C (mg/dL)	55.18 ± 10.99	55.09 ± 12.49	51.52 ± 10.78	47.94 ± 11.30	<0.001
LDL-C (mg/dL)	85.14 ± 27.05	85.20 ± 25.16	90.10 ± 22.70	94.62 ± 23.84	0.011
Waist circumference (cm)	74.48 ± 10.26	77.62 ± 9.51	81.28 ± 12.56	96.97 ± 16.73	<0.001
Vitamin D (nmol/L)	62.00 ± 24.22	60.47 ± 21.10	56.07 ± 21.46	51.23 ± 17.34	<0.001
Hs-CRP (mg/L)	2.08 ± 7.07	1.39 ± 3.60	1.92 ± 6.06	2.46 ± 3.56	<0.001
Total fat percent, (%)	26.63 ± 7.79	27.31 ± 8.37	29.81 ± 9.53	36.22 ± 7.81	<0.001
ALP (IU/L)	146.01 ± 103.44	151.48 ± 97.73	140.65 ± 96.03	149.10 ± 99.72	0.564
AST (U/L)	19.58 ± 5.96	20.14 ± 10.37	20.55 ± 14.32	21.79 ± 8.48	0.029
Blood urea nitrogen (mg/dL)	12.24 ± 3.41	11.93 ± 3.40	12.38 ± 3.96	11.51 ± 3.05	0.245
Pelvis BMD (g/cm^2)	1.15 ± 0.18	1.17 ± 0.19	1.19 ± 0.18	1.25 ± 0.16	<0.001
Lumbar BMD (g/cm^2)	0.97 ± 0.16	0.98 ± 0.16	0.98 ± 0.15	0.97 ± 0.14	0.667
Total BMD (g/cm^2)	1.03 ± 0.12	1.04 ± 0.12	1.05 ± 0.12	1.05 ± 0.11	0.167
LSM (kPa)	4.61 ± 1.53	4.61 ± 1.21	4.73 ± 1.23	6.10 ± 3.73	<0.001

Mean ± SD for continuous variables: the P value was calculated by the weighted linear regression model.

(%) for categorical variables: the P value was calculated by the weighted chi-square test.

Abbreviation: Q, quartile; PIR, Ratio of family income to poverty; BMI, body mass index; HDL-C, High-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CAP, Controlled Attenuation Parameter; LSM, Liver stiffness measurement; BMD, bone mineral density; Hs-CPR, high-sensitivity C-reactive protein; ALP, alkaline phosphatase; AST, aspartate aminotransferase.

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in adolescents. To our knowledge, this is the first study that investigated BMD in adolescents in relation to the hepatic steatosis and liver stiffness quantified by VCTE.

Past epidemiological studies investigating BMD and NAFLD in adolescents have been few and inconsistent. A study from Turkey included 82 adolescents with obesity and 30 control participants and showed that NAFLD has a detrimental effect on bone health in adolescents and is associated with increased insulin resistance [22]. However, in this study, as most epidemiological studies investigating NAFLD have used indices or ultrasound to diagnose the severity of hepatic steatosis, emerging evidence suggests that the results of indices assessment

Table 2. The associations between bone mineral density with hepatic steatosis and fibrosis.

Bone mineral density	Model 1 [β (95% CI)]	Model 2 [β (95% CI)]	Model 3 [β (95% CI)]
CAP (dB/m)			
Total BMD (g/cm^2)	48.19 (20.24, 75.61)	-34.01 (-62.30, -8.11)	-32.46 (-58.98, -9.05)
Lumbar BMD (g/cm^2)	15.56 (-3.59, 36.76)	-30.54 (-58.69, -13.25)	-24.16 (-45.98, 1.36)
Pelvis BMD (g/cm^2)	58.15 (31.97, 85.25)	-12.77 (-32.09, 8.83)	-6.30 (-25.71, 8.65)
LSM (kPa)			
Total BMD (g/cm^2)	2.48 (1.25, 3.74)	1.27 (-0.09, 2.43)	1.27 (-0.37, 2.76)
Lumbar BMD (g/cm^2)	1.77 (0.79, 2.75)	1.05 (0.03, 1.95)	1.35 (0.19, 2.51)
Pelvis BMD (g/cm^2)	1.96 (1.12, 2.72)	0.74 (-0.05, 1.57)	0.85 (-0.19, 1.79)

Model 1: no covariates were adjusted. Model 2: age, gender, race, and BMI were adjusted. Model 3: age, gender, race, BMI, ALP, AST, PIR, Vitamin D, hs-CRP, total fat percent, physical activities, diabetes status, triglycerides, HDL-C and LDL-C were adjusted. Abbreviation: PIR, Ratio of family income to poverty; BMI, body mass index; HDL-C, High-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CAP, Controlled Attenuation Parameter; LSM, Liver stiffness measurement; BMD, bone mineral density; Hs-CPR, high-sensitivity C-reactive protein; ALP, alkaline phosphatase; AST, aspartate aminotransferase.

underestimate the prevalence of NAFLD [23, 24]. A META analysis that included 8 epidemiological studies of 632 children and adolescents who underwent VCTE or biopsy investigated the relationship between NAFLD and decreased BMD in children, and the results of a random effects model suggested that the presence and severity of NAFLD were significantly associated with reduced whole-body BMD Z scores in children and adolescents [25]. In a recent cross-sectional study, also based on U.S. adolescents, investigating the association between BMD and NAFLD diagnosed by liver enzyme calculations, the authors concluded that adolescents with NAFLD had higher BMD and found gender and racial differences in this association [11]. Our findings also suggest significant differences between non-Hispanic blacks and participants of other races in the association between BMD and hepatic steatosis. In fact, gender and ethnic differences are common in epidemiological studies on BMD [26].

Table 3. Subgroup analysis of the association between total bone mineral density with hepatic steatosis and fibrosis.

Subgroup	CAP [β (95%CI)]	P for interaction	LSM [β (95%CI)]	P for interaction
Sex		0.098		0.361
Male	-21.75 (-51.18, 17.60)		1.20 (-0.41, 2.65)	
Female	-55.21 (-107.27, -2.92)		1.14 (-2.11, 4.38)	
Age		0.613		0.332
12–15	-24.11 (-65.33, 21.72)		2.09 (0.13, 4.01)	
16–19	-41.07 (-88.61, 2.60)		0.83 (-1.63, 3.51)	
Race/ethnicity		0.021		0.860
Non-Hispanic White	-3.71 (-59.22, 51.69)		1.69 (-1.71, 4.15)	
Non-Hispanic Black	30.65 (-49.93, 111.24)		1.31 (-2.68, 4.97)	
Mexican American	-140.28 (-222.80, -57.77)		0.76 (-3.13, 4.87)	
Other race/multiracial	-51.92 (-110.19, 5.20)		1.58 (-1.45, 5.11)	

Age, gender, race, BMI, ALP, AST, PIR, Vitamin D, hs-CRP, total fat percent, physical activities, diabetes status, triglycerides, HDL-C and LDL-C were adjusted. Abbreviation: PIR, Ratio of family income to poverty; BMI, body mass index; HDL-C, High-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CAP, Controlled Attenuation Parameter; LSM, Liver stiffness measurement; BMD, bone mineral density; Hs-CPR, high-sensitivity C-reactive protein; ALP, alkaline phosphatase; AST, aspartate aminotransferase.
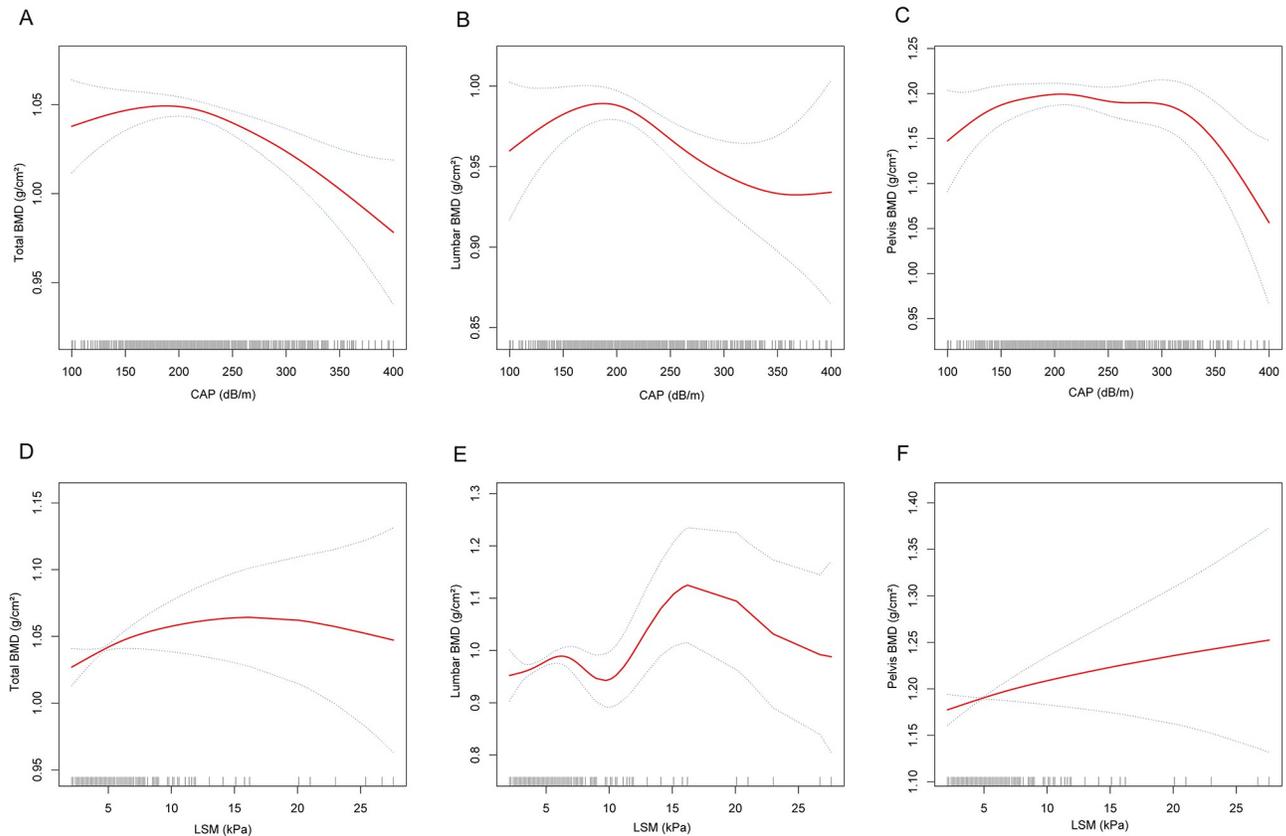
Fig 2. The nonlinear associations between BMD with degree of hepatic steatosis and fibrosis. The solid red line represents the smooth curve fit between variables. Blue bands represent the 95% of confidence interval from the fit. A. total BMD and CAP; B. lumbar BMD and CAP; C. pelvis BMD and CAP; D. total BMD and LSM; E. lumbar BMD and LSM; F. pelvis BMD and LSM.

For example, in the association between lipid metabolism and BMD, Xie et al. found a significant nonlinear association between HDL-C and BMD in men and whites, but not in blacks [27], and a negative linear association between LDL-C and lumbar BMD only in whites and Mexican Americans, but not in blacks [28]. However, after reviewing the past literature, we found that the potential mechanism regarding this situation is currently unexplained. In addition, the association of BMD with CAP and LSM differed across age subgroups, which

Table 4. Threshold effect analysis of CAP on BMD by two-piecewise linear regression model.

CAP (dB/m)	Fitting by the standard linear model	Fitting by the two-piecewise linear model			
		Inflection point (K)	< K-segment effect	> K-segment effect	Log likelihood ratio
Total BMD (g/cm²)	-37.87 (-70.93, -4.81)	221.22	68.12 (-32.71, 168.95)	-61.88 (-101.30, -22.46)	0.029
Lumbar BMD (g/cm²)	-23.46 (-47.38, 0.45)	219.88	342.40 (118.91, 565.90)	-37.25 (-62.46, -12.04)	0.001
Pelvis BMD (g/cm²)	-5.16 (-26.30, 15.98)	216.02	45.09 (-11.99, 102.18)	-19.34 (-45.21, 6.53)	0.062

Models were adjusted for age, gender, race, BMI, ALP, AST, PIR, Vitamin D, hs-CRP, total fat percent, physical activities, diabetes status, triglycerides, HDL-C and LDL-C were adjusted. Abbreviation: PIR, Ratio of family income to poverty; BMI, body mass index; HDL-C, High-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CAP, Controlled Attenuation Parameter; LSM, Liver stiffness measurement; BMD, bone mineral density; Hs-CPR, high-sensitivity C-reactive protein; ALP, alkaline phosphatase; AST, aspartate aminotransferase.

likely stems from dynamic changes in bone and liver metabolism during adolescent growth and development [29].

According to the current study, there are several hypotheses for a significant association between BMD and the degree of hepatic steatosis and fibrosis. First, some investigators have suggested that insulin resistance is the main mediator of this relationship and that insulin resistance affects bone metabolism by influencing the increase or decrease of multiple inflammatory metabolic factors and bone metabolites, which leads to an acceleration of bone density loss [30, 31]. Pacifico et al. demonstrated in a case-control study that CRP and other inflammatory mediators may accelerate bone mass loss in adolescents with NAFLD [32]. In addition, past studies on TNF-α have demonstrated its ability to inhibit osteoblast differentiation and enhance osteoclast activity and osteoblast apoptosis [33–35]. More importantly, the relationship between BMD and the degree of hepatic steatosis and fibrosis is largely mediated by obesity and body fat content, and adolescents with obesity may help prevent bone loss by increasing mechanical load [36, 37]. This was also verified in our multivariate linear model, where there was even a positive association between BMD and CAP when unadjusted for BMI.

There are various limitations to our research. First, due to the limitations of the cross-sectional investigation, we were unable to show a causal relationship between BMD and the degree of hepatic steatosis and fibrosis. Furthermore, because we excluded teenage individuals with viral hepatitis, our findings in this cohort should be regarded with care. Despite these flaws, our research offers some advantages. In this study, we used VCTE findings to more accurately quantify the degree of hepatic steatosis and fibrosis in teenagers than index estimates. More importantly, we discovered a substantial inverted U-shaped link between BMD and the degree of hepatic steatosis in adolescents, as well as an inflection point that may assist clinicians and public health officials avoid osteoporosis in adolescents.

5. Conclusion

According to our findings, BMD is negatively connected to the degree of hepatic steatosis and positively related to LSM in teenagers.

Acknowledgments

Author Contributions

Conceptualization: Shengmao He, Yun Zhang, Wenfu Tan, Bingliang Yin.

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Writing – original draft: Shengmao He, Yun Zhang.

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References

1. Mann JP, Valenti L, Scorletti E, Byrne CD, Nobili V: Nonalcoholic Fatty Liver Disease in Children. *Semin Liver Dis* 2018, 38(1):1–13. https://doi.org/10.1055/s-0038-1627456 PMID: 29471561

2. Brunt EM, Wong VW, Nobili V, Day CP, Sookoian S, Maher JJ, Bugianesi E, Sirlin CB, Neuschwander-Tetri BA, Rinella ME: Nonalcoholic fatty liver disease. *Nat Rev Dis Primers* 2015, 1:15080. https://doi.org/10.1038/nrdp.2015.80 PMID: 27188459

3. Shaunak M, Byrne CD, Davis N, Afolabi P, Faust SN, Davies JH: Non-alcoholic fatty liver disease and childhood obesity. *Arch Dis Child* 2021, 106(1):3–8. https://doi.org/10.1136/archdischild-2019-318063 PMID: 32409495

4. Eslam M, Alkhouri N, Vajro P, Baumann U, Weiss R, Socha P, et al: Defining paediatric metabolic (dysfunction)-associated fatty liver disease: an international expert consensus statement. *Lancet Gastroenterol Hepatol* 2021, 6(10):864–873. https://doi.org/10.1016/S2468-1253(21)00183-7 PMID: 34364544

5. Weihe P, Weihrauch-Blüher S: Metabolic Syndrome in Children and Adolescents: Diagnostic Criteria, Therapeutic Options and Perspectives. *Curr Obes Rep* 2019, 8(4):472–479. https://doi.org/10.1007/s13679-019-00357-x PMID: 31691175

6. Chun LF, Yu EL, Sawh MC, Bross C, Nichols J, Polgreen L, et al: Hepatic Steatosis is Negatively Associated with Bone Mineral Density in Children. *J Pediatr* 2021, 233:105–111.e103. https://doi.org/10.1016/j.jpeds.2021.01.064 PMID: 33545191

7. Baxter-Jones AD, Faulkner RA, Forwood MR, Mirwald RL, Bailey DA: Bone mineral accrual from 8 to 30 years of age: an estimation of peak bone mass. *J Bone Miner Res* 2011, 26(8):1729–1739. https://doi.org/10.1002/jbmr.412 PMID: 21520276

8. Ward LM, Konji VN, Ma J: The management of osteoporosis in children. *Osteoporos Int* 2016, 27(7):2147–2179. https://doi.org/10.1007/s00198-016-3515-9 PMID: 27125514

9. Cauley JA: Public health impact of osteoporosis. *J Gerontol A Biol Sci Med Sci* 2013, 68(10):1243–1251. https://doi.org/10.1093/gerona/glt093 PMID: 23902935

10. Burge R, Dawson-Hughes B, Solomon DH, Wong JB, King A, Tosteson A: Incidence and economic burden of osteoporosis-related fractures in the United States, 2005–2025. *J Bone Miner Res* 2007, 22(3):465–475. https://doi.org/10.1359/jbmr.061113 PMID: 17144789

11. Xie R, Zhang Y, Yan T, Huang X, Xie S, Liu C, et al: Relationship between nonalcoholic fatty liver disease and bone mineral density in adolescents. *Medicine (Baltimore)* 2022, 101(41):e31164. https://doi.org/10.1097/MD.0000000000031164 PMID: 36253982

12. Chang EJ, Yi DY, Yang HR: Vitamin D Status and Bone Mineral Density in Obese Children with Nonalcoholic Fatty Liver Disease. *J Korean Med Sci* 2015, 30(12):1821–1827. https://doi.org/10.3346/jkms.2015.30.12.1821 PMID: 26713058

13. Xie R, Zhang Y: Is assessing the degree of hepatic steatosis and fibrosis based on index calculations the best choice for epidemiological studies? *Environ Pollut* 2023, 317:120783. https://doi.org/10.1016/j.envpol.2022.120783 PMID: 36460186

14. Xie R, Zhang Y: Index-based calculation or Transient Elastography to assess the degree of hepatic steatosis and fibrosis. *J Nutr* 2023, 153(3):909. https://doi.org/10.1016/j.tjnut.2022.10.015 PMID: 36774231

15. Xie R, Zhang Y: Associations between dietary flavonoid intake with hepatic steatosis and fibrosis quantified by VCTE: Evidence from NHANES and FNDDS. *Nutr Metab Cardiovasc Dis* 2023. https://doi.org/10.1016/j.numecd.2023.03.005 PMID: 36964061

16. Zhang Y, Xie R, Ou J: A U-shaped association between serum albumin with total triiodothyronine in adults. *J Clin Lab Anal* 2022, 36(6):e24473. https://doi.org/10.1002/jcla.24473 PMID: 35524426

17. Xie R, Zhang Y: Association between 19 dietary fatty acids intake and rheumatoid arthritis: Results of a nationwide survey. *Prostaglandins Leukot Essent Fatty Acids* 2023, 188:102530. https://doi.org/10.1016/j.plefa.2022.102530 PMID: 36586398

18. Vilar-Gomez E, Nephew LD, Vuppalanchi R, Gawrieh S, Mladenovic A, Pike F, et al: High-quality diet, physical activity, and college education are associated with low risk of NAFLD among the US population. *Hepatology* 2022, 75(6):1491–1506. https://doi.org/10.1002/hep.32207 PMID: 34668597

19. Ciardullo S, Perseghin G: Statin use is associated with lower prevalence of advanced liver fibrosis in patients with type 2 diabetes. *Metabolism* 2021, 121:154752. https://doi.org/10.1016/j.metabol.2021.154752 PMID: 33716004

20. Xie R, Ning Z, Xiao M, Li L, Liu M, Zhang Y: Dietary inflammatory potential and biological aging among US adults: a population-based study. *Aging Clinical and Experimental Research* 2023. https://doi.org/10.1007/s40520-023-02410-1 PMID: 37186209

21. Xie R, Liu X, Wu H, Liu M, Zhang Y: Associations between Systemic Immune-Inflammation Index and Abdominal Aortic Calcification: the NHANES 2013–2014. *Nutrition*, *Metabolism and Cardiovascular Diseases* 2023.

22. Pirgon O, Bilgin H, Tolu I, Odabas D: Correlation of insulin sensitivity with bone mineral status in obese adolescents with nonalcoholic fatty liver disease. *Clin Endocrinol (Oxf)* 2011, 75(2):189–195. https://doi.org/10.1111/j.1365-2265.2011.04038.x PMID: 21521307

23. Kim D, Cholankeril G, Loomba R, Ahmed A: Prevalence of Fatty Liver Disease and Fibrosis Detected by Transient Elastography in Adults in the United States, 2017–2018. *Clin Gastroenterol Hepatol* 2021, 19(7):1499–1501.e1492. https://doi.org/10.1016/j.cgh.2020.08.017 PMID: 32801011

24. Xie R, Xiao M, Li L, Ma N, Liu M, Huang X, et al: Association between SII and hepatic steatosis and liver fibrosis: A population-based study. *Front Immunol* 2022, 13:925690. https://doi.org/10.3389/fimmu.2022.925690 PMID: 36189280

25. Mantovani A, Gatti D, Zoppini G, Lippi G, Bonora E, Byrne CD, et al: Association Between Nonalcoholic Fatty Liver Disease and Reduced Bone Mineral Density in Children: A Meta-Analysis. *Hepatology* 2019, 70(3):812–823. https://doi.org/10.1002/hep.30538 PMID: 30706504

26. Xie R, Liu Y, Wang J, Zhang C, Xiao M, Liu M, et al: Race and Gender Differences in the Associations Between Cadmium Exposure and Bone Mineral Density in US Adults. *Biol Trace Elem Res* 2022. https://doi.org/10.1007/s12011-022-03521-y PMID: 36508128

27. Xie R, Huang X, Liu Q, Liu M: Positive association between high-density lipoprotein cholesterol and bone mineral density in U.S. adults: the NHANES 2011–2018. *J Orthop Surg Res* 2022, 17(1):92. https://doi.org/10.1186/s13018-022-02986-w PMID: 35168655

28. Xie R, Huang X, Zhang Y, Liu Q, Liu M: High Low-Density Lipoprotein Cholesterol Levels are Associated with Osteoporosis Among Adults 20–59 Years of Age. *Int J Gen Med* 2022, 15:2261–2270. https://doi.org/10.2147/IJGM.S353531 PMID: 35250302

29. Ramírez-Vélez R, García-Hermoso A, Correa-Rodríguez M, Izquierdo M: Defining values for controlled attenuation parameter and liver stiffness in youth without liver disease. *Pediatr Res* 2022, 91(4):912–920. https://doi.org/10.1038/s41390-021-01441-6 PMID: 33846557

30. Yilmaz Y: Review article: non-alcoholic fatty liver disease and osteoporosis—clinical and molecular crosstalk. *Aliment Pharmacol Ther* 2012, 36(4):345–352. https://doi.org/10.1111/j.1365-2036.2012.05196.x PMID: 22730920

31. Guañabens N, Parés A: Osteoporosis in chronic liver disease. *Liver Int* 2018, 38(5):776–785. https://doi.org/10.1111/liv.13730 PMID: 29479832

32. Pacifico L, Bezzi M, Lombardo CV, Romaggioli S, Ferraro F, Bascetta S, et al: Adipokines and C-reactive protein in relation to bone mineralization in pediatric nonalcoholic fatty liver disease. *World J Gastroenterol* 2013, 19(25):4007–4014. https://doi.org/10.3748/wjg.v19.i25.4007 PMID: 23840146

33. Kudo O, Fujikawa Y, Itonaga I, Sabokbar A, Torisu T, Athanasou NA: Proinflammatory cytokine (TNFalpha/IL-1alpha) induction of human osteoclast formation. *J Pathol* 2002, 198(2):220–227. https://doi.org/10.1002/path.1190 PMID: 12237882

34. Gilbert L, He X, Farmer P, Rubin J, Drissi H, van Wijnen AJ, et al: Expression of the osteoblast differentiation factor RUNX2 (Cbfa1/AML3/Pebp2alpha A) is inhibited by tumor necrosis factor-alpha. *J Biol Chem* 2002, 277(4):2695–2701. https://doi.org/10.1074/jbc.M106339200 PMID: 11723115

35. Bush H, Golabi P, Younossi ZM: Pediatric non-alcoholic fatty liver disease. *Children* 2017, 4(6):48. https://doi.org/10.3390/children4060048 PMID: 28598410

36. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al: Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 2018, 15(1):11–20. https://doi.org/10.1038/nrgastro.2017.109 PMID: 28930295

37. Ouyang Y, Quan Y, Guo C, Xie S, Liu C, Huang X, et al: Saturation Effect of Body Mass Index on Bone Mineral Density in Adolescents of Different Ages: A Population-Based Study. *Front Endocrinol (Lausanne)* 2022, 13:922903. https://doi.org/10.3389/fendo.2022.922903 PMID: 35865310