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### Title

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## Hepatic steatosis is negatively associated with bone mineral density in children

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### Abstract

**Objective:** To evaluate the relationship between hepatic steatosis and bone mineral density (BMD) in children. Additionally, to assess 25-hydroxyvitamin D levels in the relationship between hepatic steatosis and BMD.

**Study design:** A community-based sample of 235 children was assessed for hepatic steatosis, BMD, and serum 25-hydroxyvitamin D. Hepatic steatosis was measured by liver magnetic resonance imaging proton density fat fraction (MRI-PDFF). BMD was measured by whole-body dual-energy X-ray absorptiometry (DXA).

**Results:** The mean age of the study population was 12.5 years (SD 2.5 years). Liver MRI-PDFF ranged from 1.1% to 40.1% with a mean of 9.3% (SD 8.5%). Across this broad spectrum of

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hepatic fat content, there was a significant negative relationship between liver MRI-PDFF and BMD Z-score ( $R=-0.421$ ,  $p<0.001$ ). Across the states of sufficiency, insufficiency and deficiency, there was a significant negative association between 25-hydroxyvitamin D and liver MRI-PDFF ( $p<0.05$ ); however, there was no significant association between vitamin D status and BMD Z-score ( $p=0.94$ ). Finally, children with clinically low BMD Z-scores were found to have higher ALT ( $p<0.05$ ) and GGT ( $p<0.05$ ) levels compared with children with normal BMD Z-scores.

**Conclusions:** Across the full range of liver MRI-PDFF, there was a strong negative relationship between hepatic steatosis and BMD Z-score. Given the prevalence of NAFLD and the critical importance of childhood bone mineralization in protecting against osteoporosis, clinicians should prioritize supporting bone development in children with NAFLD.

### Keywords

pediatric; NAFLD; osteoporosis; osteopenia; vitamin D; MRI-PDFF

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In the United States, 5 to 10% of children have Nonalcoholic fatty liver disease (NAFLD), making management and the consideration of comorbidities a pertinent concern for modern pediatric practice [1]. Although many of the comorbidities of NAFLD in children are well understood [2–4], there remains a paucity of knowledge relating to bone health in this population. Although obesity is often associated with increased bone mineral density (BMD) in children [5], our prior work suggested that children with obesity and severe NAFLD may have lower BMD than weight-matched children [6]. Subsequently, studies based upon liver ultrasound and alanine aminotransferase to classify children with obesity as having NAFLD have yielded conflicting results regarding BMD [7,8].

Bone mineralization during childhood is important in protecting against future osteoporosis and minimizing fracture risk. Osteoporosis, which usually manifests later in life but also can occur during childhood [9], greatly increases fracture risk. Additionally, osteoporosis is associated with substantial comorbidities and earlier mortality [10,11], as well as increased health expenses [12–14]. Because peak bone mass is achieved within the first two decades of life [15], childhood is the most important, and likely most effective, time to positively influence bone mineralization, and in doing so, decrease the risk of osteoporosis and its complications across an individual's lifespan.

Because NAFLD may be associated with lower BMD in children, this could represent an opportunity to identify children at greater risk for osteoporosis and fractures. To understand the strength of such an association, it is important to know the relationship between hepatic steatosis across its full biological range with BMD. Furthermore, given that vitamin D is metabolized by the liver and integral to optimal bone health, it has been proposed that the relationship between hepatic steatosis and BMD observed in NAFLD might be driven, in part, by vitamin D deficiency [8]. To address these clinical questions, we used liver Magnetic Resonance Imaging Proton Density Fat Fraction (MRI-PDFF), a quantitative noninvasive marker of hepatic steatosis [16,17], as a continuous measure of liver fat; assessed BMD using dual-energy x-ray absorptiometry (DXA); and measured serum 25-hydroxyvitamin D (25(OH)D) in a community-based sample of 235 children. The aims of our study were to determine the relationship between hepatic steatosis and BMD in

children;) to describe the features of children with clinically low BMD; and to investigate a possible role of vitamin D in the association between liver fat and lower BMD.

## METHODS

A community sample of children between the ages of 8 and 17, without a prior diagnostic workup for NAFLD, were recruited for metabolic health screening from community health centers and primary care practices in the County of San Diego. They were evaluated at the Altman Clinical and Translational Research Institute with the approval of the Institutional Review Board at the University of California, San Diego. At the time of enrollment, parents gave written consent and children provided written assent.

Exclusion criteria included having a history of chronic liver disease, chronic kidney disease, rheumatologic disease, cerebral palsy, or other conditions that may affect bone density; inability to undergo MRI-evaluation; vitamin D supplement-use; and pregnancy. Of 263 children who were screened, 242 were found to be eligible based upon the aforementioned exclusion criteria; of these, 7 were excluded due to incomplete data, resulting in 235 children ultimately being included (Figure 1; available at [www.jpeds.com](http://www.jpeds.com)).

The sample size calculation was based upon a projected correlation between liver MRI-PDF and BMD of 0.25; with an  $\alpha$  of 0.05 and a  $\beta$  of 0.05, a minimum of 202 participants were required.

### Clinical and Laboratory Evaluation

Each child's age, sex, ethnicity and self-identified race was recorded. Height and weight were measured to the nearest tenth of a centimeter and kilogram, respectively. These measurements were made with participants standing, wearing light clothing and no shoes. Body mass index (BMI) was calculated as the weight in kilograms divided by height, measured in meters squared. Fasting serum samples were collected to measure alanine aminotransferase (ALT), aspartate transaminase (AST), gamma-glutamyl transferase (GGT), glucose, insulin and 25(OH)D. Homeostatic model assessment of insulin resistance

(HOMA-IR) was calculated using the formula 
$$\frac{\text{Glucose} \left( \frac{\text{mg}}{\text{dL}} \right) \times \text{Insulin} \left( \frac{\text{mU}}{\text{mL}} \right)}{405}$$
.

25(OH)D status was determined using clinical guidelines set by the United States Endocrine Society, with >30 ng/mL considered as sufficient, <30 ng/mL and >20 ng/mL considered as insufficient, and <20 ng/mL considered deficient [18].

### MRI Evaluation

Liver MRI-PDF was measured using confounder-corrected chemical-shift-encoded MRI at 3 Tesla (3T), which has been shown to correlate well with histology-based steatosis grade [16,17]. Briefly, a multi-echo gradient-recalled echo sequence was acquired with low flip angle (10°) and a 150 millisecond repetition time to avoid T1 bias. Six gradient-recalled echoes were acquired at successive nominal out-of-phase and in-phase echo times to permit simultaneous estimation of fractional fat signal and T2\* decay [19–22]. MRI-PDF maps were reconstructed from the magnitude images by applying a custom algorithm pixel by

pixel that corrected for exponential T2\* signal decay and incorporated a multi-peak spectral fat model to correct for the multifrequency interference effects of fat proton signals [23]. For each participant, a circular region of interest of 1-centimeter radius was placed in all evaluable Couinaud liver segments on the MRI-PDFF maps. MRI-PDFF measurements from each of the evaluable regions of interest were averaged to calculate the composite PDFF value for that participant.

### **Bone Densitometry**

All children underwent whole-body and lumbar spine (L1-L4) DXA scans using a fan-beam densitometer (Hologic Discovery W, Bedford, MA). Measures of BMD and percent body fat were determined using data obtained from these whole-body scans using Apex software (Version 4.0.1). All scans were performed by a single technologist. In vitro precision was calculated using a Hologic spine phantom. The phantom precision, expressed as a percentage coefficient of variation (%CV) was 0.418. Z-scores for whole-body BMD, defined as total body minus head BMD, were calculated based on race and sex-specific lambda-mu-sigma (LMS) curves based on over 1500 children [24]. Low BMD Z-scores were defined as being less than or equal to  $-2.0$ , consistent with the guidelines set by the International Society for Clinical Densitometry [25]. In order to create a clear distinction between children with clinically low BMD Z-scores and those with “normal” BMD Z-scores for analysis, “normal” was defined as having a BMD Z-score of  $-1.0$ .

### **Classification of NAFLD and Exclusion of Other Disease**

Participants were further classified as having or not having NAFLD based upon the amount of liver fat and the exclusion of other causes of chronic liver disease. Elevated liver fat was set as liver MRI-PDFF  $\geq 5\%$ . children were evaluated for other causes of chronic liver disease and other pertinent medical problems, based on methods that have been previously described [26,27]. Subjects were tested for viral hepatitis, and completed questionnaires to evaluate alcohol and medication use, as well as past medical history.

### **Statistical Analyses**

Normality of the data was assessed with descriptive statistics and graphing. Liver MRI-PDFF, serum insulin and HOMA-IR were not normally distributed and thus were logarithmically transformed for analysis. Correlation testing between continuous variables was performed using the Pearson test. Group comparisons between dichotomous variables were made using the Student T-tests, whereas comparisons across the three different 25(OH)D status groups were made using Kruskal-Wallis testing. Comparisons between categorical variables were completed by Chi-square and Fisher Exact testing.

The associations between whole-body BMD and BMD Z-score with MRI-PDFF were analyzed with and without adjustments for sex, age, ethnicity, height and weight. Serum 25(OH)D, percent body fat, serum insulin, and HOMA-IR were also analyzed in relation to whole-body BMD and BMD Z-score.

Z-scores for height and BMI were calculated using Epi Info 7 (CDC, Atlanta, Georgia). All other analyses were conducted using SPSS v25 (IBM Corp, Armonk, New York). Two-sided testing differences of  $p < 0.05$  were considered statistically significant.

## RESULTS

### Study Population

We studied 235 children, ranging from 8 to 17 years of age, with a mean age of 12.5 years (SD of 2.5 years). Males comprised 65.5% of our study sample. The majority of children were of Hispanic ethnicity (82.6%). Mean ALT was 37 U/L (SD of 38 U/L). The mean liver MRI-PDFF was 9.3% (SD of 8.5%). BMD Z-scores had a mean of 0.3 (SD of 1.1), with 12 (5.1%) of children classified as having clinically low bone density for age (Z-score  $< -2.0$ ). A detailed description of our entire study sample can be found in Table 1.

### Liver Fat and Bone Mineral Density

Liver MRI-PDFF was negatively correlated with BMD Z-score (Pearson  $R = -0.421$ ,  $p < 0.001$ ; Figure 2). In a linear regression model, this relationship remained significant after controlling for age, sex, ethnicity, weight and height ( $R^2 = 0.205$ ,  $p < 0.001$ ; Table 2). In this model, every 5.4 percentage-point increase in liver MRI-PDFF corresponded to a 1-point decrease in BMD Z-score.

A linear regression model was also developed for whole-body BMD based upon liver MRI-PDFF controlled for age, sex, Hispanic ethnicity, height and weight ( $R^2 = 0.675$ ,  $P < .001$ ; Table II). The relationship between liver MRI-PDFF and whole-body BMD was even stronger than the relationship between liver MRI-PDFF and BMD Z-score. In the model for whole-body BMD, every 4 percentage-point increase in liver MRI-PDFF corresponded to a 10  $\text{g}/\text{cm}^2$  decrease in whole-body BMD.

Neither serum 25(OH) D nor percent body fat were associated with either liver whole-body BMD ( $p = 0.48$  and  $0.13$ , respectively) or BMD Z-score ( $p = 0.76$  and  $0.24$ , respectively), and after adjustment for these factors in multivariate models, there were no differences in the results. Additionally, although serum insulin and HOMA-IR were weakly correlated with BMD Z-score, neither were associated with whole-body BMD. Furthermore, adjustment for serum insulin and HOMA-IR within the multivariate model for BMD Z-score did not alter the relationship between BMD Z-score and liver MRI-PDFF. Thus, serum 25(OH) D, percent body fat, serum insulin and HOMA-IR values were excluded from the final multivariate model.

### NAFLD and Bone Mineral Density

Within our study cohort there were 127 children with NAFLD and 108 children without NAFLD (Table 1), with mean MRI-PDFF levels of 14.7% and 2.9%, respectively. These groups did not differ in age or sex. Children with NAFLD had significantly higher body weight (75.1 kg vs 68.7 kg,  $p < 0.05$ ) and BMI (30.0  $\text{kg}/\text{m}^2$  vs 27.0  $\text{kg}/\text{m}^2$ ,  $p < 0.001$ ). Although BMI was significantly higher in children with NAFLD, BMI Z-scores were not statistically different between children with and without NAFLD (2.0 vs 1.9,  $p > 0.1$ ).

Measures of bone mineralization, including BMD and BMD Z-score were all significantly lower in children with NAFLD compared with children without NAFLD (0.964 vs 1.005 g/cm<sup>2</sup> for BMD,  $p<0.001$ ;  $-0.1$  vs  $0.7$  for BMD Z-score,  $p<0.001$ ).

Out of the 235 children in our study sample, 12 had clinically low BMD Z-scores of  $-2.0$ , with an average Z-score of  $-2.5$  (SD of  $0.3$ ; Table 3 [available at [www.jpeds.com](http://www.jpeds.com)]). All 12 of these children had NAFLD and were compared with 104 children who had NAFLD and a BMD Z-score greater than or equal to  $-1.0$  (Figure 4, A; available at [www.jpeds.com](http://www.jpeds.com)). Children with NAFLD and low BMD Z-score had significantly higher ALT than children with NAFLD and normal BMD z-score ( $78$  vs  $50$  U/L,  $p<0.05$ ; Figure 4, B [available at [www.jpeds.com](http://www.jpeds.com)]). Similarly, GGT was significantly greater in children with low BMD Z-score ( $43.9$  vs  $28.9$  U/L,  $p<0.01$ ; Figure 4, C [available at [www.jpeds.com](http://www.jpeds.com)]).

### 25(OH)D, Hepatic Steatosis and Bone Mineral Density

The mean 25(OH)D level in our study sample was  $24.8$  ng/mL. The distribution of vitamin D status was  $21.7\%$  ( $51/235$ ) sufficient,  $45.5\%$  ( $107/235$ ) insufficient, and  $32.8\%$  ( $77/235$ ) deficient. MRI-PDFF was significantly different across these three groups, with average liver MRI-PDFF values of  $7\%$ ,  $5.7\%$  and  $4.1\%$  in children deficient, insufficient and sufficient in vitamin D, respectively ( $p<0.05$ , Figure 3, A). Notably, there was no difference between BMD Z-score or whole-body BMD across vitamin D status groups ( $p=0.94$ , Figure 3, B). Similarly, the correlation between whole-body BMD and 25(OH)D serum levels was not significant ( $R=0.075$ ,  $p=0.26$ ). Interestingly, 25(OH)D was inversely correlated with BMI ( $R=-0.183$ ,  $p<0.001$ ) but not with MRI-PDFF ( $R=-0.014$ ,  $p=0.114$ ).

## DISCUSSION

We studied a large community-based sample of children and found a strong negative relationship between liver fat content and bone density. Serum vitamin D status was associated with liver MRI-PDFF, but was not associated with BMD. All children found to have clinically low bone density were classified as having NAFLD. Among children with NAFLD, those that had clinically low bone density also had higher ALT and GGT than those with normal bone density.

We demonstrated a strong inverse relationship between liver fat and bone density across the full range of liver fat and across a broad range of age. Our results expand upon the findings of a Spanish study conducted by Labayen et al, which measured BMD and MRI-determined hepatic fat fraction in pre-adolescent children [28]. This study utilized baseline data from the Prevention of Diabetes in Kids (PREDIKID) clinical trial [29] and reported a negative association by between MRI-determined liver fat fraction and BMD in 115 children (age 8–12 years) with overweight or obesity, and mild to moderate hepatic steatosis. In our study, we included a larger sample from a broader age range (8–17 years) with a more complete range of hepatic steatosis (1 to 40%). These results suggest that liver fat has a strong biological relationship with bone mineral density in childhood.

Given the high prevalence of NAFLD, understanding the mechanisms driving the observed negative relationship between NAFLD and BMD could be used to improve long-term bone

health outcomes at a population level. Although the precise biology underlying low BMD in NAFLD is not well understood, this phenomenon may be partially driven by the increased bone marrow adiposity that occurs in the setting of NAFLD. We previously demonstrated a positive relationship between liver MRI-PDFF and bone marrow adiposity in children [30]. Studies in adults and children have additionally found an inverse relationship between bone marrow adiposity and BMD [31–33], which may be explained by the fact that osteoblasts and adipocytes are both of mesenchymal stem cell origin [34].

The demonstrated relationship between liver MRI-PDFF and BMD expands upon previously published studies on the relationship between NAFLD and BMD in children [6–8,28]. Within our study, 1 in 20 children had clinically low BMD Z-scores, all of whom had NAFLD. In addition, these children had substantially elevated levels of ALT and GGT, suggesting children with low BMD may have higher severity liver injury than peers with NAFLD and normal BMD. Further supporting this finding, studies have also shown that children with nonalcoholic steatohepatitis (NASH) have significantly lower BMD than children with NAFLD [6,35]. Overall, the results of our study support that low BMD is a common comorbidity of NAFLD in children; however, only 13% of pediatric gastroenterologists conduct bone health screening for their patients with NAFLD [36]. Given the risk for low BMD in children with NAFLD, improving bone health screening and support for this growing patient population has potential to greatly reduce the risk for fractures and osteoporosis. Research studies should evaluate bone health outcomes in patients with NAFLD and assess the effect of prevention and treatment strategies. Additionally, studies should investigate the biological mechanisms linking BMD and hepatic steatosis, including potential genetic factors.

To reduce risk, lifestyle modification should be recommended for children with NAFLD and concomitant low BMD. Although current NASPGHAN guidelines broadly suggest daily exercise for children with NAFLD, recommendations for children with NAFLD and low BMD should place specific emphasis on weight-bearing exercise and increasing lean mass, both of which have been shown to improve bone strength [37,38]. Recommending increased intake of fortified dairy products, fatty fish, and dark leafy greens may also support adequate levels of calcium and vitamin D [39]. Additional recommendations for children at risk for osteoporosis includes the supplementation of calcium and vitamin D, as well as bisphosphonate therapy on a case-by-case basis [40].

Our results confirmed that Vitamin D insufficiency and deficiency are common in children with NAFLD [41], but showed no association between vitamin D status and BMD, which is consistent with previous findings [42]. Thus, vitamin D is unlikely to be a major factor in the pathophysiology underlying lower BMD in children with NAFLD. Moreover, vitamin D supplementation was shown to be an ineffective intervention for NAFLD in multiple randomized control trials in adults [43,44]. Despite this, it should be noted that calcium and vitamin D supplementation is recommended for children at risk for osteoporosis [40]. The decision to prescribe vitamin D supplementation should not be based upon a diagnosis of NAFLD in the absence of clinical vitamin D deficiency, or the presence of risk factors for osteoporosis.



One of the primary strengths of this study was the use of a highly accurate measure for hepatic steatosis, liver MRI-PDFF [16]. Our study was made generalizable with the use of a large community-based sample. One potential modifier not assessed in our study was weight-bearing exercise, which is known to positively impact BMD. Data on Tanner staging and menarche were not collected, and thus our analysis was not adjusted for these factors. In addition, our study population was predominantly Hispanic. This population is at greater risk for NAFLD, thus how well these data will replicate in other groups should be investigated. Finally, the cross-sectional design of our study provides information on correlation, but not causation.

Our study shows that high liver fat content is a risk factor for low BMD. Whether improvement in liver fat is associated with improvement in bone mineralization is an important question that will need to be addressed in interventional studies. In the meantime, we speculate that early screening and proactively supporting bone development in children with NAFLD could help decrease the lifelong risk for fractures and osteoporosis.

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## LIST OF ABBREVIATIONS

<b>ALT</b>	alanine aminotransferase
<b>AST</b>	aspartate transaminase
<b>BMD</b>	bone mineral density
<b>BMI</b>	body mass index
<b>DXA</b>	dual-energy X-ray absorptiometry
<b>GGT</b>	gamma-glutamyl transferase
<b>HOMA-IR</b>	homeostatic model assessment of insulin resistance
<b>LMS</b>	lambda-mu-sigma
<b>MRI</b>	magnetic resonance imaging
<b>MSC</b>	mesenchymal stem cell

<b>NAFL</b>	nonalcoholic fatty liver
<b>NAFLD</b>	nonalcoholic fatty liver disease
<b>NASH</b>	nonalcoholic steatohepatitis
<b>PDFF</b>	proton density fat fraction
<b>PPAR-<math>\gamma</math></b>	peroxisome proliferator-activated receptor $\gamma$
<b>SD</b>	standard deviation

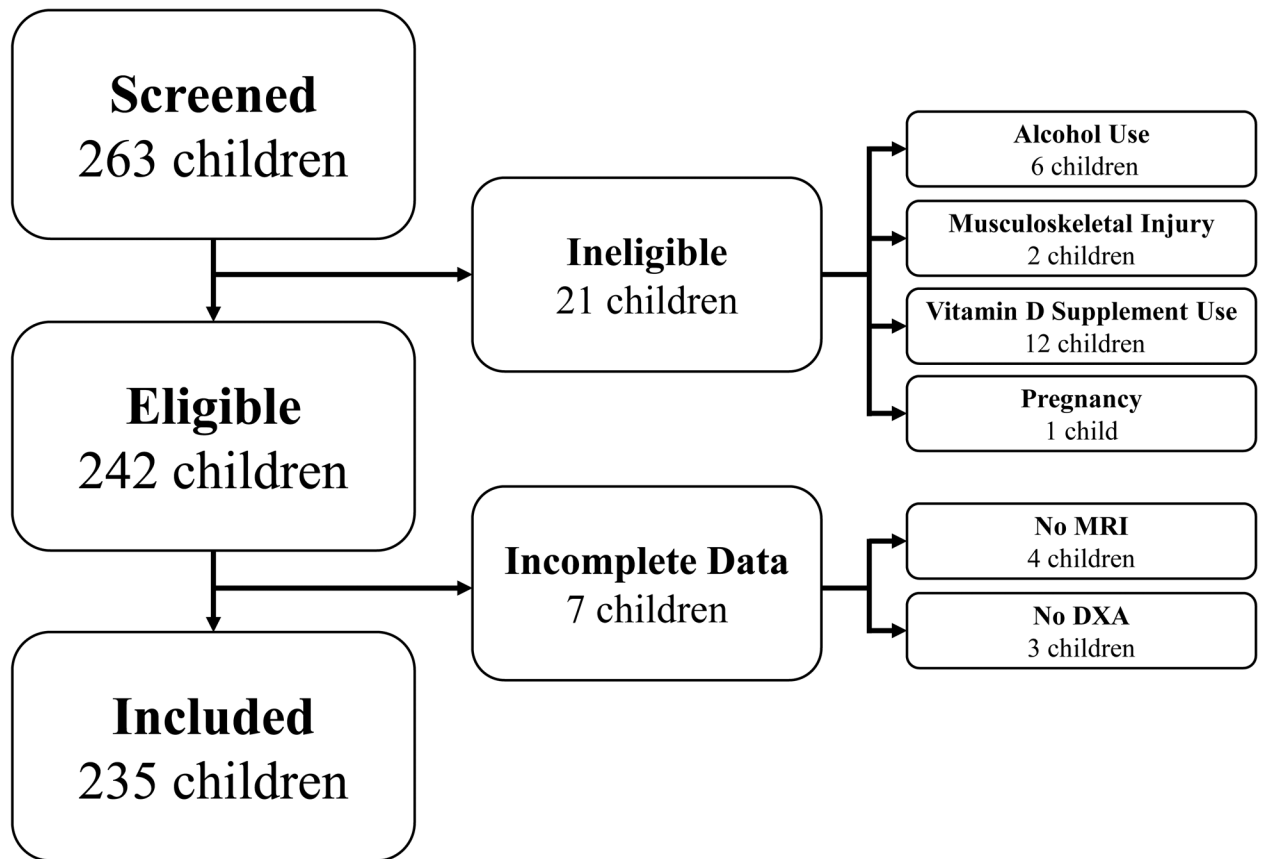
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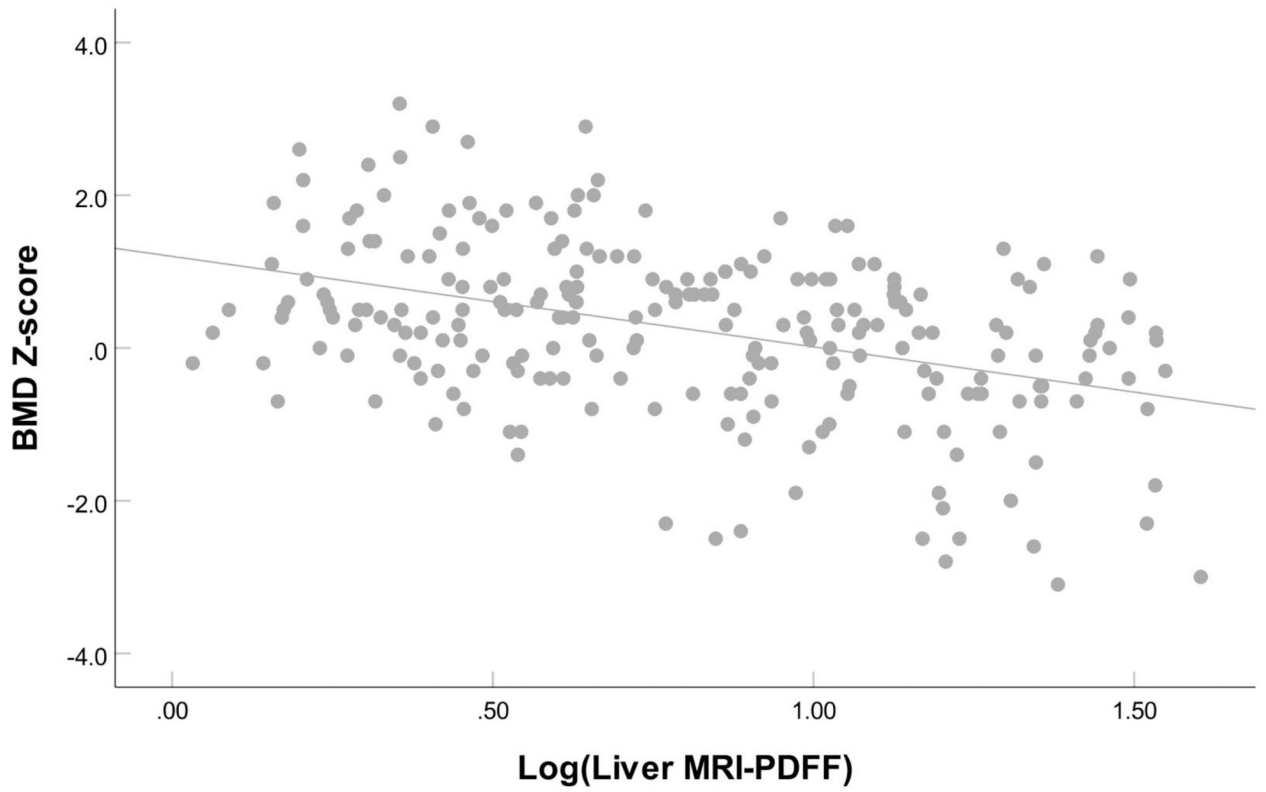
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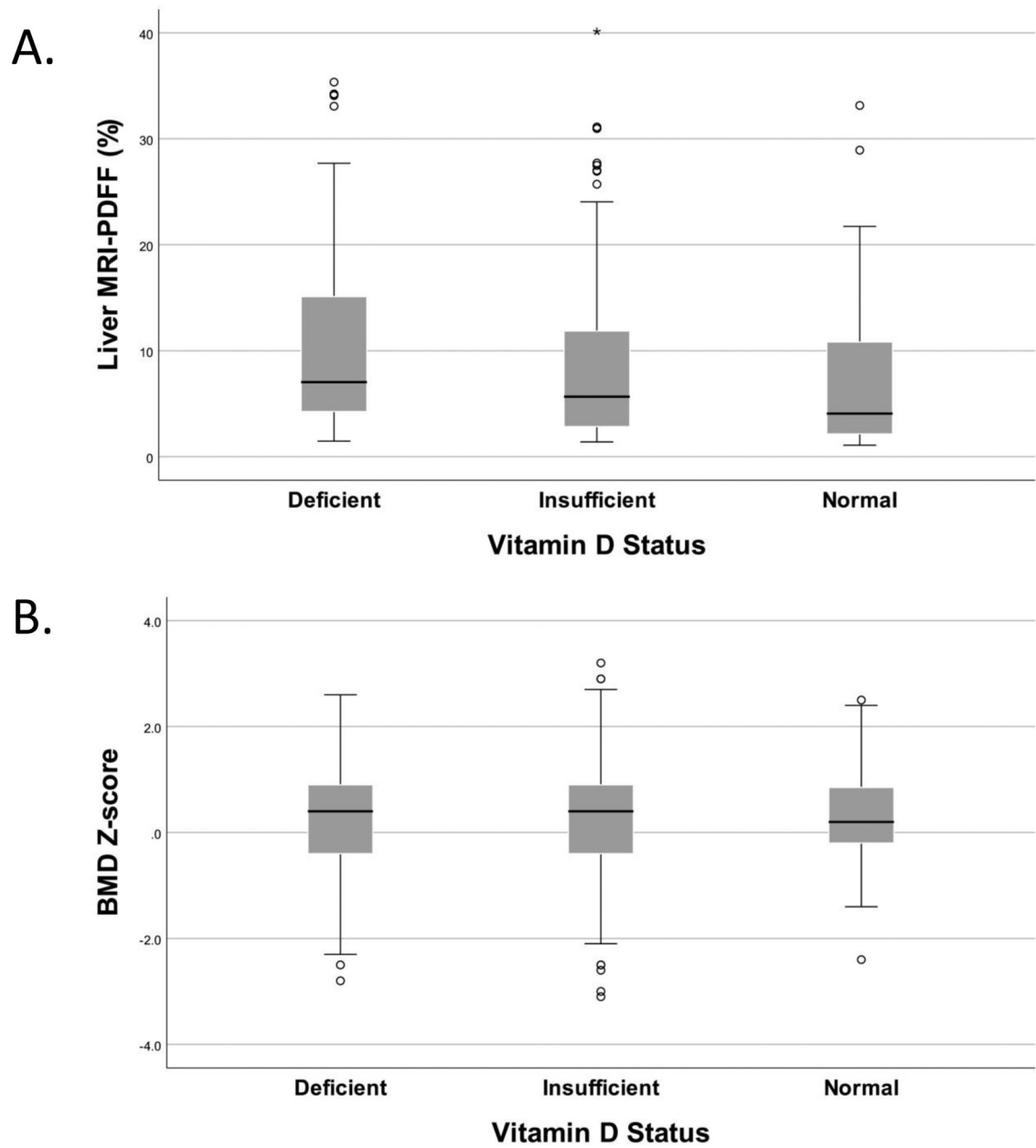
**Online Figure 1: Study Flow Diagram**

Flow chart demonstrates the recruitment of study participants and reasons for exclusion.



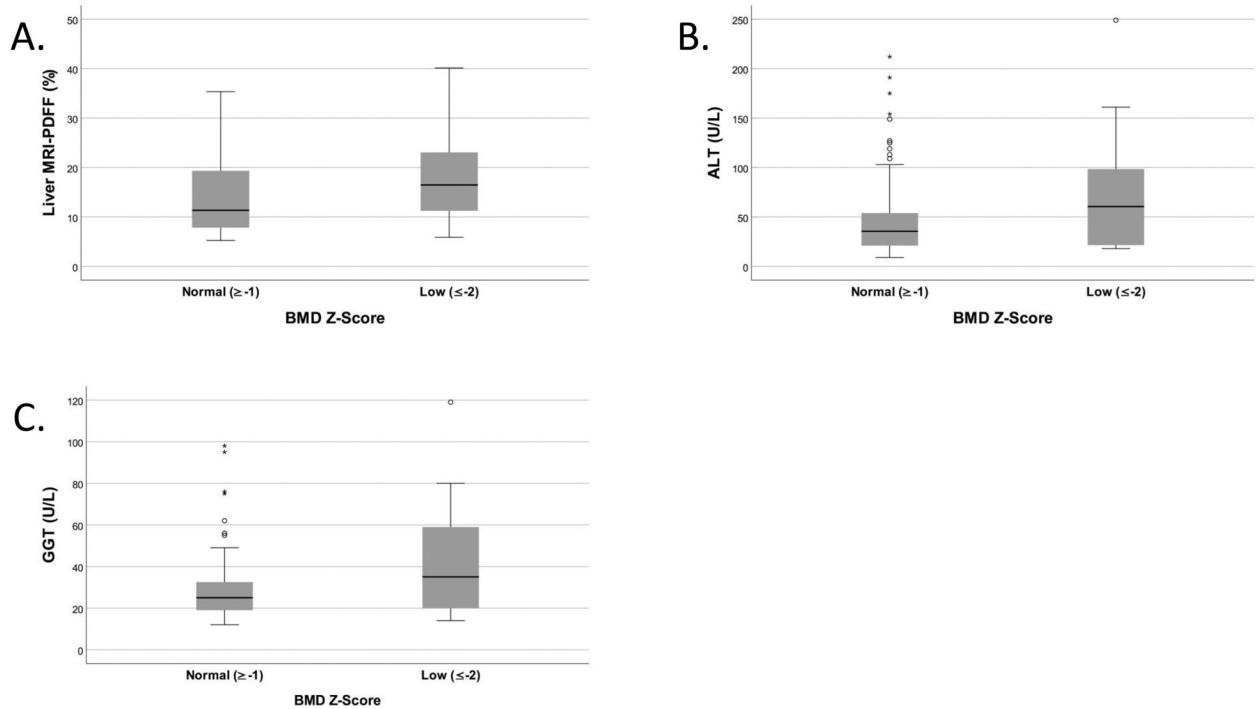
**Figure 2: Relationship between MRI-PDFF and BMD Z-score**

Scatter plot shows the distribution BMD z-score by log transformed liver MRI-PDFF and that these two measures were significantly negatively correlated ( $R = -0.414$ ;  $p < 0.001$ ).



**Figure 3: Distribution of Liver MRI-PDFF and BMD by Vitamin D Status**

Box and whisker plots show (A) Liver MRI-PDFF and (B) BMD z-score across groups by vitamin D status; categorized as sufficient ( $\geq 30$  ng/mL), insufficient (between 20 and 30 ng/mL) or deficient ( $< 20$  ng/mL). Liver MRI-PDFF was significantly different across groups by vitamin D status ( $p=0.023$ ). BMD was not significantly different across groups by vitamin D status ( $p = 0.923$ ).



**Figure 4: Distribution of Liver MRI-PDFF, ALT and GGT in children with NAFLD by BMD Status**

Among children with NAFLD, box and whisker plots show the distribution of (A) Liver MRI-PDFF, (B) ALT and (C) GGT in those with normal BMD z-score versus those with low BMD z-score. Normal BMD z-score was defined as  $\geq -1.0$  and low BMD was defined as  $\leq -2.0$ . Children with low BMD Z-scores had higher amount of liver fat than children with normal BMD Z-scores (18.7 vs 14.1%,  $p = 0.078$ ). Children with low BMD Z-scores had significantly higher serum ALT levels compared to children with normal BMD Z-scores (78.1 vs 48.4 U/L,  $p < 0.05$ ). Children with low BMD Z-scores had significantly higher serum GGT levels than children with normal BMD Z-scores (43.9 vs 28.9 U/L,  $p < 0.05$ ).



**Table 1:**

## Study sample characteristics

	All N = 235	Normal N = 108	NAFLD N = 127	p-value
Age, years (SD)	12.5 (2.5)	12.6 (2.7)	12.3 (2.4)	0.305
Sex				0.447
Male (%)	154 (65.5)	68 (62.9)	86 (67.7)	
Female (%)	81 (34.5)	40 (37.1)	41 (32.3)	
Race, n (%)				<b>0.018</b>
American Indian	6 (2.5)	2 (1.9)	4 (3.1)	
Asian	13 (5.5)	1 (0.9)	12 (9.4)	
Black	14 (6.0)	9 (8.3)	5 (3.9)	
White	63 (26.8)	33 (30.6)	30 (23.6)	
Other	139 (59.1)	63 (58.3)	76 (59.8)	
Ethnicity, n (%)				<b>0.004</b>
Hispanic	194 (82.6)	81 (75.0)	113 (89.0)	
Non-Hispanic	41 (17.4)	27 (25.0)	14 (11.0)	
Weight, kg (SD)	72.2 (20.1)	68.7 (19.6)	75.1 (20.2)	<b>0.015</b>
Height, cm (SD)	157.6 (12.4)	158.5 (13.2)	156.9 (11.7)	0.304
Height Z-score (SD)	0.81 (1.13)	0.80 (1.05)	0.82 (1.20)	0.927
Body Mass Index, kg/m <sup>2</sup> (SD)	28.6 (5.6)	27.0 (5.3)	30.0 (5.4)	< <b>0.001</b>
BMI Z-Score (SD)	1.9 (0.6)	1.7 (0.7)	2.1 (0.4)	< <b>0.001</b>
Percent Body Fat, % (SD)	38.4 (8.2)	37.8 (8.8)	39.0 (7.7)	0.239
ALT, U/L (SD)	37 (38)	18 (8)	52 (46)	< <b>0.001</b>
AST, U/L (SD)	31 (18)	30 (16)	33 (20)	0.235
GGT, U/L (SD)	25 (16)	18 (6)	31 (19)	< <b>0.001</b>
Serum Vitamin D, ng/mL (SD)	24.8 (7.5)	25.6 (6.6)	24.1 (8.3)	0.136
Blood Glucose, mg/dL (SD)	86.5 (11.4)	85.4 (7.4)	87.3 (13.6)	0.196
Insulin, mU/dL (SD)	27.8 (24.3)	20.1 (15.3)	34.4 (28.4)	< <b>0.001</b>
HOMA-IR (SD)	5.97 (5.27)	4.26 (3.37)	7.44 (6.05)	< <b>0.001</b>
Liver MRI-PDFF, % (SD)	9.3 (8.5)	2.9 (1.0)	14.7 (8.3)	< <b>0.001</b>
Bone Mineral Density of Lumbar-Spine, g/cm <sup>2</sup> (SD)	0.909 (0.195)	0.942 (0.207)	0.882 (0.181)	<b>0.019</b>
Whole-body BMD, g/cm <sup>2</sup> (SD)	0.964 (0.145)	1.005 (0.153)	0.928 (0.128)	< <b>0.001</b>
Bone Mineral Density, Z-score (SD)	0.3 (1.1)	0.7 (1.0)	-0.1 (1.1)	< <b>0.001</b>

Data are presented as means (SD) or percentages. NAFLD cases were determined based on a liver MRI-PDFF cut-off of greater than or equal to 5%.

P-values less than 0.05 are presented in bold. Significance was determined by two-sided Student's t-test for numerical data, and by Chi-square or Fisher Exact test for categorical data.

**Table 2:**

Adjusted linear regression models predicting whole-body BMD and BMD Z-score

	BMD Z-score		Whole-body BMD (g/cm <sup>2</sup> )	
	Adjusted R <sup>2</sup> =0.205, p < 0.001		Adjusted R <sup>2</sup> =0.675, p < 0.001	
	Beta	P-value	Beta	P-value
<b>MRI-PDFF, %</b>	-0.053	<0.001	-0.004	<0.001
<b>Age, years</b>	-0.136	0.001	0.031	<0.001
<b>Sex, male</b>	0.394	0.009	0.018	0.156
<b>Ethnicity, Hispanic</b>	-0.038	0.828	-0.010	0.502
<b>Weight, kg</b>	0.004	0.390	0.000	0.783
<b>Height, cm</b>	0.025	0.017	0.003	<0.001

Linear regression models using liver MRI-PDFF to predict BMD Z-score and whole-body BMD. Both models were adjusted for age, sex, height, weight and Hispanic ethnicity. P-values less than 0.05 are presented in bold.

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**Table 3:**

Children with NAFLD and low BMD Z-scores

	Normal BMD N = 104	Low BMD N = 12	P-value
Age, years (SD)	12.2 (2.4)	11.8 (2.6)	0.557
Sex			0.124
Male, %	63.5	83.3	
Female, %	36.5	16.7	
Weight, kg (SD)	74.4 (19.8)	68.4 (16.8)	0.319
Height, cm (SD)	156.7 (11.5)	151.2 (11.5)	0.118
Height Z-score (SD)	0.9 (1.2)	0.6 (0.7)	0.422
Body Mass Index, kg/m <sup>2</sup> (SD)	29.8 (5.2)	29.4 (3.8)	0.813
BMI Z-Score (SD)	2.0 (0.6)	2.0 (0.4)	0.779
Percent Body Fat, % (SD)	38.6 (8.1)	39.4 (5.9)	0.747
Serum 25-OH Vitamin D, ng/mL (SD)	24.3 (8.7)	23.6 (4.9)	0.799
Blood Glucose, mmol/L (SD)	86.6 (14.9)	87.9 (4.5)	0.767
Insulin, mg/dL (SD)	28.2 (17.8)	29.1 (24.0)	0.874
ALT, U/L (SD)	48.4 (40.0)	78.1 (69.6)	<b>0.028</b>
AST, U/L (SD)	33.6 (19.3)	30.8 (28.9)	0.653
GGT, U/L (SD)	28.9 (15.5)	43.9 (31.6)	<b>0.006</b>
Liver MRI-PDF, % (SD)	14.1 (8.1)	18.7 (10.3)	0.078
Bone Mineral Density of Lumbar-Spine, g/cm <sup>2</sup> (SD)	0.881 (0.182)	0.817 (0.186)	0.254
BMD Z-Score (SD)	0.3 (0.7)	-2.5 (0.3)	<b>&lt;0.001</b>
Whole-body BMD, g/cm <sup>2</sup> (SD)	0.951 (0.118)	0.746 (0.095)	<b>&lt;0.001</b>

Data are presented as means (SD) or percentages. BMD Z-scores less than or equal to -2.0 were classified as low, and those greater than or equal to -1.0 were classified as normal.

P-values less than 0.05 are presented in bold. Significance was determined by two-sided student's t-test.