

# Association Between Nonalcoholic Fatty Liver Disease and Reduced Bone

## Mineral Density in Children: A Meta-Analysis

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## ABSTRACT

Recent cross-sectional studies have examined the association between nonalcoholic fatty liver disease (NAFLD) and bone mineral density (BMD) in children or adolescents, but these have produced conflicting results. We performed a systematic review and meta-analysis of these published studies to quantify the magnitude of the association, if any, between NAFLD and BMD. We searched publication databases from January 2000 to September 2018, using predefined keywords to identify relevant observational studies conducted in children or adolescents in which NAFLD was diagnosed either by imaging or by histology, and BMD Z score was measured by dual energy X-ray absorptiometry. Data from selected studies were extracted, and meta-analysis was performed using random-effects modelling. A total of eight observational cross-sectional or case-control studies enrolling 632 children and adolescents (mean age: 12.8 years), 357 of whom had NAFLD, were included in the final analysis. Meta-analysis showed significant differences in whole-body or lumbar BMD Z scores between children/adolescents with and without NAFLD (n=6 studies; pooled weighted mean difference [WMD]: -0.48, 95%CI -0.74 to -0.21;  $I^2=55.5\%$ ), as well as between those with biopsy-confirmed nonalcoholic steatohepatitis (NASH) and those with no-NASH (n=4 studies; pooled WMD: -0.27, 95%CI -0.40 to -0.13;  $I^2=0\%$ ). The aforementioned WMDs in BMD Z scores were independent of common clinical risk factors, such as age, sex, race/ethnicity and body mass index. Sensitivity analyses did not modify these findings. Funnel plot and Egger test did not reveal significant publication bias. **Conclusion:** This meta-analysis shows that the presence and severity of NAFLD is significantly associated with reduced whole-body BMD Z scores in children and adolescents. However, the observational design of the studies included does not allow for proving causality.

## **ABBREVIATION LIST**

BMD, bone mineral density

BMI, body mass index

WMD, weighted mean difference

CI, confidence interval

DEXA, dual energy X-ray absorptiometry

NOS, Newcastle-Ottawa Quality Assessment Scale

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## INTRODUCTION

Pediatric non-alcoholic fatty liver disease (NAFLD) has become a major public health problem due to the striking increase in its prevalence and the clinical implications for future development of liver dysfunction, type 2 diabetes mellitus and other cardiometabolic complications (1-3). Indeed, NAFLD is the most common cause of chronic liver disease in children and adolescents of Western countries. Its steep rise in prevalence is closely associated with the epidemic increase in obesity in the pediatric population worldwide (1-3).

Over the past decade, convincing evidence has emerged that NAFLD is a multisystem disease that affects several extra-hepatic organ systems, and interacts with the regulation of multiple metabolic, endocrine and proinflammatory pathways (4,5). On this background of evidence, it has been demonstrated that NAFLD is strongly associated with an increased risk of developing both cardiovascular disease and other important extra-hepatic diseases (such as type 2 diabetes, chronic kidney disease and colorectal cancer) (6-9).

Osteoporosis is defined as a systemic skeletal disease characterized by low bone mineral density (BMD) and micro-architectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fractures (10). Osteoporosis is a public health problem worldwide due to the high cumulative rates of bone fractures with advancing age (10,11). Recently, the existence of a possible association between NAFLD and osteoporosis has created considerable scientific interest (12). A comprehensive systematic review and meta-analysis of twelve observational cross-sectional or case-control studies (involving a total of nearly 30,000 adult individuals of predominantly Asian ethnicity) recently showed that imaging-defined or biopsy-proven NAFLD was significantly associated with a self-reported history of osteoporotic fractures (principally in Chinese men), but not with low BMD, in middle-aged and elderly individuals (13).

To date, a number of cross-sectional studies have also explored the association between NAFLD and BMD Z scores (as measured by dual energy X-ray absorptiometry [DEXA], which is the reference method for measuring BMD in clinical practice) in children and adolescents (14-21). However, there have been conflicting findings from these studies so far. Some studies have

reported a significant association between the presence of, or severity of NAFLD and low BMD Z scores (14,15,17,19,20,21), whereas others failed to find any significant association between NAFLD and BMD (16,18). Thus, it is currently uncertain whether NAFLD is associated with low BMD in children and adolescents. Given the growing clinical and quality-of-life burden associated with NAFLD in pediatric population, we consider that it is clinically important to better understand if this condition adversely affects BMD in childhood.

We therefore carried out a systematic review and meta-analysis of observational studies examining the association between imaging-defined or biopsy-proven NAFLD and BMD in children and adolescents. The major aim of this systematic review and meta-analysis was to precisely gauge the nature and magnitude of this association in the pediatric population. We also examined whether there was a significant association between the histological severity of NAFLD and BMD. Clarification of the magnitude of potential adverse effect(s) of NAFLD on bone health in childhood may have implications for preventing osteoporosis and bone fractures in adulthood.

## **MATERIALS AND METHODS**

### *Registration of review protocol*

The protocol for this systematic review and meta-analysis was registered in advance with PROSPERO (International Prospective Register of Systematic Reviews, no. CRD42018111249).

### *Data sources and searches*

We conducted a systematic literature search from January 1, 2000 to September 30, 2018 in PubMed, Scopus and Web of Science for identifying observational studies examining the association between NAFLD and bone mineral density (BMD). The search free text terms were “nonalcoholic fatty liver disease” (OR “fatty liver” OR “NAFLD” OR “nonalcoholic steatohepatitis”) AND “bone mineral density” OR “osteoporosis” IN “children” or “adolescents”. We also searched for MeSH (Medical Subject Headings) terms. Searches were restricted to human studies. No language restrictions were applied. Additionally, we reviewed references from relevant original papers and

review articles for identifying further eligible studies not covered by the original database searches. We performed a systematic review in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Because the included studies were observational in design, we followed the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines for the meta-analysis of these studies (22).

### *Study selection*

Original studies were included if they met the following inclusion criteria: 1) observational cross-sectional or case-control studies that explored the association between NAFLD and BMD measurements; 2) all studies that reported data of BMD Z score (as measured by DEXA) that was expressed (or that could be calculated) as mean $\pm$ SD in individuals with and without NAFLD; and 3) all studies in which the diagnosis of NAFLD was based on either liver biopsy or imaging techniques, in the absence of significant alcohol consumption and other competing causes of chronic liver disease. Study participants included in the meta-analysis were children and adolescents (aged <18 years) of either sex without any restriction in terms of age, race or ethnicity. No prospective studies were available in the literature for the analysis.

Exclusion criteria were as follows: 1) reviews, practice guidelines, commentaries, editorials, congress abstracts, case reports and theses; 2) studies where the diagnosis of NAFLD was based exclusively on serum liver enzyme levels or other surrogate markers of NAFLD; 3) studies which did not exclude individuals with significant alcohol consumption and other known causes of chronic liver disease or those who took drugs affecting bone metabolism; 4) studies which did not specifically report any BMD Z score; and 5) studies conducted in adult individuals (aged  $\geq$ 18 years).

Two investigators (AM and GT) independently examined all titles and abstracts, and obtained full texts of potentially relevant papers. Papers were read by both investigators, and whether they met inclusion criteria were then assessed. Discrepancies were resolved by consensus, referring back to the original article, in consultation with a third author.

### *Data extraction and quality assessment*

For the eligible studies, we extracted information on study design, study size, publication year, study country, main participants characteristics (such as age, sex, and body mass index [BMI]), methods used for diagnosing and staging NAFLD, outcome of interest (i.e., mean $\pm$ SD of BMD Z score), and list of covariates adjusted in multivariable regression analyses. In the case of multiple publications, the most up-to-date or comprehensive information was included.

Two investigators (AM and GT) assessed the risk of bias independently. Any discrepancies were addressed by a re-evaluation of original articles by a third author. Quality assessment was performed according to the Newcastle-Ottawa Quality Assessment Scale (NOS), which is a validated scale for non-randomized studies in meta-analyses (23). A NOS scale adapted for the cross-sectional studies was used (24). The NOS scale uses a star system to assess the quality of a study in three domains: selection, comparability, and outcome/exposure. The NOS assigns a maximum of five stars for selection (for cross-sectional studies), two stars for comparability, and three stars for outcome/exposure. Studies achieving a score of at least eight stars were classified as being at low risk of bias (i.e., thus reflecting the highest quality).

#### *Data synthesis and analysis*

The primary outcome measure was BMD Z score at various skeletal sites (expressed as mean $\pm$ SD) between patients with and without NAFLD. Z scores are used for diagnosing osteoporosis in children or adolescents (i.e., subjects not yet attaining peak bone mass) as they reflect SD scores from the mean in comparison to BMD of healthy children of the same age and sex (13). When the eligible studies reported only mean BMD values but not also SD, we calculated this latter value from standard errors or confidence intervals or range values that were related to the differences between means in two groups by using validated formulas (23).

The effect size of the meta-analysis was expressed as weighted mean difference (WMD) and 95% confidence intervals (CI). The overall estimate of effect size was then calculated using a random-effects model, as this methodology considers any differences between studies even in the absence of statistically significant heterogeneity (23).

Visual inspection of the forest plots was used to investigate the possibility of statistical heterogeneity. The statistical heterogeneity among studies was assessed by the  $I^2$  statistic, which provides an estimate of percentage of variability across studies that is due to heterogeneity rather than chance alone. According to Higgins and Thompson (25), a rough guide to interpretation is as follows:  $I^2$  values of approximately 25% represent low heterogeneity; approximately 50% represent medium heterogeneity; and approximately 75% represent high heterogeneity. The possibility of publication bias was evaluated using the funnel plot and the Egger's regression asymmetry test (26).

To explore the possible sources of (expected) heterogeneity among the eligible studies and to test the robustness of associations, we conducted sensitivity/subgroup analyses and meta-regression analyses. In particular, based on data from the eligible studies, the effect of NAFLD on BMD Z score was assessed by stratifying the studies according to the socio-demographic factors (such as age, sex and BMI), the study country, the various skeletal sites of BMD measurement (i.e., whole body or lumbar spine), the methods used for diagnosing NAFLD (biopsy, magnetic resonance imaging or ultrasonography), and the severity of NAFLD histology (NASH vs. no-NASH). In addition, we tested for possibly excessive influence of individual studies using a meta-analysis influence test that eliminated each of the included studies one at a time. We also performed univariable meta-regression analyses in order to examine the association of WMD in BMD with age, sex and BMI.

P values for chi-square tests are reported in all forest plots. A chi-square test p-value<0.10 was used to determine statistical significance considered for heterogeneity. The proportion of heterogeneity accounted for by between-study variability was also estimated using the  $I^2$  index and adjudicated to be significant if  $I^2$  was >50%. We used STATA® 14.2 (StataCorp, College Station, Tx) for all statistical analyses.

## RESULTS

### *Literature search and study characteristics*

**Figure 1** shows the flow diagram for search and selection processes of the meta-analysis. After excluding duplicates, based on titles and abstracts of 21 citations (in accordance with the aforementioned exclusion criteria of the meta-analysis), we initially identified 10 potentially



relevant studies (14-21,27,28) from PubMed, Scopus or Web of Science prior to September 30, 2018. After examining the full text of these 10 publications, we further excluded two studies, because of unsatisfactory inclusion criteria (27) or unsatisfactory outcome measures (28), as specified in the PRISMA flow diagram.

Overall, eight observational studies were eligible for inclusion in the meta-analysis and were assessed for quality (14-21). **Table 1** shows the main characteristics of these studies. All studies had a cross-sectional or case-control design, whereas no studies had a longitudinal design. Six studies included also a control group of individuals without NAFLD (and were then used for the primary pooled analysis of the meta-analysis) (14-19), whereas two studies were performed only in individuals with histologically confirmed NASH or no-NASH (and these studies were then used only for the subgroup analysis examining the association between BMD Z score and the severity of NAFLD histology) (20,21). The diagnosis of NAFLD was based on ultrasonography (n=3 studies), magnetic resonance imaging (n=1 study), liver biopsy alone (n=3 studies) or combined with magnetic resonance imaging (n=1 study), in the absence of significant alcohol consumption and other known causes of chronic liver disease. In all studies whole body or lumbar BMD Z scores were measured by DEXA in adolescents with and without NAFLD. For the studies included the primary pooled analysis, two studies were carried out in Asia (South Korea and Turkey); two studies were carried out in the United States or Brazil, and two studies were carried out in the Europe (Italy and Spain).

Overall, in the eight cross-sectional or case-control studies included in the meta-analysis there were 632 children and adolescents (mean±SD age: 12.8±2 years), predominantly men (~55%) and overweight or obese (BMI: 29.6±4.1 kg/m<sup>2</sup>); 357 (56.5%) of them had imaging-defined or biopsy-proven NAFLD. Most of these children had a pubertal Tanner stage ≥2.

Of the eight included studies (**supplementary Tables 1-3**), most studies received six (n=2 studies) or seven (n=4 studies) stars on the NOS, whereas two studies received at least eight stars (i.e., thus reflecting an overall medium-high risk of bias for the eligible studies).

### *Effect of NAFLD on BMD Z scores*

The distribution of studies by estimate of the association between NAFLD and BMD Z scores at various skeletal sites is plotted in **Figure 2**. Six cross-sectional studies or case-control studies (involving a total of 525 overweight or obese children/adolescents) provided data suitable for the pooled primary analysis (14-19).

As reported in the forest plot, there was a significant difference in BMD Z scores between children/adolescents with and without NAFLD, i.e. a significantly lower BMD Z score in those with NAFLD than in non-steatotic controls (pooled WMD: -0.48, 95% CI -0.74 to -0.21;  $I^2=55.5\%$ ). This result was consistent for BMD Z scores at different skeletal sites: whole body BMD (n=5 studies; pooled WMD: -0.31, 95% CI -0.57 to -0.05;  $I^2=29\%$ ) and lumbar BMD (n=2 studies; pooled WMD: -0.79, 95% CI -1.06 to -0.51;  $I^2=0\%$ ). As specified in Table 1, for most of the eligible studies the WMD was independent of common clinical risk factors (such as age, sex, race/ethnicity, BMI and others).

As shown in **supplementary Figure 1**, the Egger's regression test did not show any statistically significant asymmetry of the funnel plots ( $p=0.31$ ), thus suggesting that publication bias was unlikely, although it should be noted that the numbers of included studies was relatively small.

### *Subgroup analyses and meta-regressions*

Sensitivity analyses were carried out for exploring possible sources of heterogeneity across the included studies (14-19).

When the comparison was stratified by study country, the observed differences in BMD Z scores between children/adolescents with and without NAFLD were consistent for all studies but they were statistically significant especially for studies performed in the Europe (**supplementary Figure 2**).

When the comparison was stratified by the methods used for diagnosing NAFLD, the significant differences in BMD Z scores observed between children/adolescents with and without NAFLD were consistent for all studies included, although they appeared to be stronger for those studies that used magnetic resonance imaging or biopsy for diagnosing NAFLD (**supplementary Figure 3**).

We also tested the possibility of excessive influence of individual studies by using an influence test that eliminated each of the included studies one at a time. Exclusion of each of the eligible studies from the analysis had no significant effect on the pooled WMD in BMD Z scores observed between subjects with and without NAFLD (**supplementary Figure 4**).

Stratifying the eligible studies by increasing absolute values of BMD Z score, the significant differences in BMD Z scores observed between children/adolescents with and without NAFLD were consistent for all studies included (**supplementary Figure 5**).

Finally, in **supplementary Figure 6** we reported the results of univariable meta-regression analyses of the eligible studies, showing a borderline inverse association of WMD with female sex, but not with age and BMI.

#### *Effect of the histological severity of NAFLD on whole-body BMD Z scores*

The distribution of studies by estimate of the association between the histological severity of NAFLD and whole-body BMD Z score is plotted in **Figure 3**. Four cross-sectional studies or case-control studies (involving a total of 184 United States and Italian overweight/obese adolescents with NASH or no-NASH on liver histology) provided data suitable for the pooled analysis (15,17,20,21). No longitudinal studies were available for this analysis.

Overall, the meta-analysis showed a significant difference in whole-body BMD Z scores between adolescents with and without histologically confirmed NASH, i.e. a significantly lower BMD Z scores in those with NASH than in those with no-NASH (pooled WMD: -0.27, 95% CI -0.40 to -0.13;

$I^2=0\%$ ). As specified in Table 1, for most of these studies the WMD was independent of age, sex, race/ethnicity, BMI, waist circumference or homeostasis model assessment-insulin resistance.

However, it should be noted that the overall sample size was small and no studies involving Asian individuals were available for this latter analysis, thus limiting the generalizability of these findings to other ethnicities.

## DISCUSSION

The main and novel findings of our systematic review and meta-analysis of eight observational, cross-sectional or case-control studies are as follows: (1) children or adolescents with imaging-defined or biopsy-proven NAFLD had lower whole-body or lumbar BMD Z scores than their counterparts without NAFLD (pooled WMD: -0.48, 95% CI -0.74 to -0.21;  $I^2=55.5\%$ ); (2) these results were consistent in most subgroups considered; and (3) a significant difference in whole-body BMD Z scores between children and adolescents with and without histologically confirmed NASH was also observed (pooled WMD: -0.27, 95% CI -0.40 to -0.13;  $I^2=0\%$ ). Notably, in most of the eligible studies the aforementioned WMDs in BMD Z scores between the two groups were independent of common clinical risk factors for low BMD (e.g., age, sex, race/ethnicity and BMI).

To our knowledge, this is the first systematic review and meta-analysis specifically aimed at quantifying the magnitude of the association between pediatric NAFLD and BMD Z scores.

The finding of a significant, graded relationship between the histological severity of NAFLD and whole-body BMD Z scores is particularly relevant in view of the disease burden that NAFLD represents and the potential impact on healthcare resources needed to survey and manage these patients adequately. It is known that osteoporosis and osteomalacia are two frequent complications in adult patients with cirrhosis, especially in those with alcoholic cirrhosis or advanced cholestatic liver disease (29,30). However, since our results are based on four cross-sectional studies (involving a total of 184 overweight/obese adolescents with biopsy-proven NASH or no-NASH), we believe that this question remains currently unresolved, and further larger

studies are needed in order to prove whether the histological severity of NAFLD adversely affects bone health in childhood.

Until now, the practice guidelines of the European and United States societies of pediatric gastroenterology and hepatology do not recommend to measure BMD Z score in children and adolescents with NAFLD (31-33). The findings of our meta-analysis support the view that pediatric NAFLD is significantly associated with low whole-body BMD Z scores (irrespective of age, sex, race/ethnicity and BMI), and may also have clinical implications for the management of children with overweight or obesity. Indeed, these findings suggest that overweight/obese adolescents with NAFLD should be screened for low BMD (given their increased risk of developing osteoporosis and pathologic fractures in adulthood); and that NAFLD should be looked for in all overweight/obese adolescents with low BMD, given that these subjects are at higher risk of NASH. However, unlike adult individuals in whom the bone volume does not change over time, a child's bones grow and model over time, with the growth of individual bones not occurring uniformly in three dimensions. It is also noteworthy that sufficient population-based data correlating BMD measures with fracture risk in healthy children and adolescents are currently unavailable (13). Currently, lifestyle modifications to improve diet and increase physical activity are recommended as the first-line treatment for all children and adolescents with NAFLD (32,33). Based on the findings of our meta-analysis, we believe that another good reason to further reinforce the utility of these non-pharmacological treatments for NAFLD is that increased physical activity in children with NAFLD not only has positive metabolic effects on the liver but may also exert beneficial effects on bone health.

Although the pathophysiological inter-relationships between NAFLD and low BMD are not well understood, there is now emerging evidence of biological plausibility that NAFLD may increase long-term risk of osteoporosis (10,34,35). Indeed, NAFLD (especially NASH with varying amounts of liver fibrosis) exacerbates insulin resistance and causes the release of a variety of pro-inflammatory cytokines (e.g., interleukin-6, tumor necrosis factor- $\alpha$ ) and bone-influencing molecules (such as osteoprotegerin, osteocalcin, sclerostin, dickkopf-related protein-1 or procollagen type 1 N-terminal propeptide), which may promote bone fragility and susceptibility to fracture over time (10,34-36). A recent study has also reported that lumbar spine bone marrow fat content (as measured by magnetic resonance imaging) is associated with increased hepatic fat

content, independent of age, sex, BMI and abdominal visceral fat mass in children with known or suspected NAFLD (28). However, more research is needed to better elucidate the complex pathophysiological links between NAFLD and low BMD.

Our systematic review and meta-analysis has some important limitations (strictly inherent to the nature of the included studies) that should be mentioned. First, no longitudinal studies were available in the literature for this meta-analysis, and the cross-sectional design of the eligible studies limits our ability for establishing causal or temporal relationships between NAFLD and BMD. Second, although there is a relatively low heterogeneity in the pooled primary analysis ( $I^2=55.5\%$ ), the sample size of the meta-analysis is relatively small ( $n=625$ ), and the overall quality of the included studies is not consistently high (with a medium-high risk of bias according to the NOS scale of the available studies as shown in supplementary Tables 1-3). Third, most of the eligible studies have reported incomplete adjustments for established risk factors for low BMD (such as physical activity, pubertal stage, serum 25-hydroxyvitamin D<sub>3</sub> concentrations or vitamin D/calcium intakes), especially during the late childhood and peripubertal years, which is a critical period for bone accretion (13,36,37). In particular, we believe that the lack of data on serum vitamin D<sub>3</sub> concentrations or vitamin D supplementation is one of the most important weaknesses of the included studies, given that low vitamin D<sub>3</sub> is a risk factor for osteomalacia and osteoporosis both in adults and in adolescents (36,38). Finally, it should also be remembered that some diagnostic challenges might affect BMD measurement and its interpretation in obese children (e.g., greater precision error, difficulty in positioning, and effects of increased lean and fat tissue on bone health outcomes) (39). Thus, future research is needed to address these issues for improving bone health assessment in obese children.

Despite the aforementioned limitations, our meta-analysis has also important strengths. We believe that the topic of this meta-analysis is clinically relevant, given the conflicting literature on the risk of low BMD in pediatric NAFLD and the emerging data regarding the underlying biological mechanisms that link NAFLD with osteoporosis and risk of fractures. As discussed previously, this meta-analysis provides the most comprehensive and updated assessment on the association between NAFLD and BMD in the pediatric population. Moreover, we have used standardized and adjusted risk estimates from all eligible studies to allow consistent combination of estimates across studies. Finally, selective reporting bias of studies was not a major concern in our analyses,

as our comprehensive search have made it unlikely that any published report was missed, whilst visual inspection of the funnel plot and the Egger's regression test did not reveal any significant publication bias (although the interpretation of the Egger's regression test should be viewed cautiously because the number of studies included was relatively low).

In conclusion, the results of this first systematic review and meta-analysis of observational studies show that the presence and severity of NAFLD is significantly associated with low whole-body BMD Z scores in overweight or obese children and adolescents. However, the cross-sectional design of the eligible studies does not allow for establishing temporality and causality of the observed associations. Further large and well-designed prospective studies to confirm these findings should be undertaken, and mechanistic studies to better understand the link between NAFLD, low BMD and long-term fracture risk in middle adulthood are also warranted.

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## FIGURE LEGENDS

**Figure 1.** The PRISMA flow diagram of the meta-analysis.

**Figure 2.** Forest plot of comparison of bone mineral density Z scores (z-BMD) measured by dual energy X-ray absorptiometry at different skeletal sites (i.e., whole body or lumbar spine) between NAFLD and control groups. The effect size was expressed as weighted mean difference (WMD) and 95% confidence interval for all eligible studies (n=6 studies included).

**Figure 3.** Forest plot of comparison of whole-body bone mineral density Z scores (z-BMD) between subjects with non-alcoholic steatohepatitis (NASH) and no-NASH detected by liver histology. The effect size was expressed as weighted mean difference (WMD) and 95% confidence interval for all eligible studies (n=4 studies included).

**Supplementary Figure S1.** Funnel plot of standard errors by weighted mean difference (WMD) in BMD Z scores between NAFLD and control groups. *P*-value=0.31 by the Egger's regression test.

**Supplementary Figure S2.** Forest plot of comparison of BMD Z scores (z-BMD) between NAFLD and control groups, stratified by study country. The effect size was expressed as weighted mean difference (WMD) and 95% confidence interval for all studies.

**Supplementary Figure S3.** Forest plot of comparison of BMD Z scores (z-BMD) between NAFLD and control groups, stratified by the methods used for diagnosis of NAFLD. The effect size was expressed as weighted mean difference (WMD) and 95% confidence interval for all studies.

**Supplementary Figure S4.** Meta-analysis estimates, given named study is omitted. The effect size was expressed as weighted mean difference (WMD) and 95% confidence interval for all studies.

**Supplementary Figure S5.** Forest plot of comparison of BMD Z scores (z-BMD) between NAFLD and control groups, stratified by increasing absolute values of BMD Z scores. The effect size was expressed as weighted mean difference (WMD) and 95% confidence interval for all studies.

**Supplementary Figure S6.** Univariable meta-regression analyses. A meta-analysis of the association of age (panel A), percentage of girls (panel B), and body mass index [BMI] (panel C) with weighted mean difference (WMD) in BMD Z scores between NAFLD and control groups.

**Table 1.** Principal cross-sectional and case-control studies examining the association between NAFLD (as detected by imaging or histology) and bone mineral density in predominantly overweight or obese children and adolescents.

Author, Year (Ref.)	Study Design, Sample Size, Population Characteristics	Diagnosis of NAFLD, Prevalence of NAFLD	Skeletal Sites examined on DEXA	Outcome Measures; Mean±SD BMD Z-score in no-NAFLD vs. NAFLD group	Covariate Adjustment(s)	Main Findings
<b>Pirgon O <i>et al.</i> 2011 (14)</b>	Case-control study of 42 Turkish obese adolescents with NAFLD (19 boys and 23 girls; mean age: 12.5 years, mean BMI 30.2 kg/m <sup>2</sup> ), 40 obese children without NAFLD (18 boys and 22 girls; mean age: 12.5 years, mean BMI 27.8 kg/m <sup>2</sup> ), and 30 lean, healthy children (15 boys and 15 girls; mean age: 12.3 years, mean BMI 20 kg/m <sup>2</sup> ); all these children had a pubertal Tanner stage ≥2	Ultrasonography; 42 subjects had NAFLD	Lumbar spine BMD	Lumbar BMD Z-score (mean±SD): 1.37±1.04 vs. 1.02±0.9 vs. 0.56±0.3, p<0.05 Lumbar BMD (mean±SD): 1.09±0.1 vs. 1.03±0.1 vs. 1.03±0.14 g/cm <sup>2</sup> in lean children, in obese children without NAFLD and in those with NAFLD, respectively	Age, sex, BMI, lipids, HOMA-IR score	Obese adolescents with NAFLD had lower BMD Z-score than lean controls or non-NAFLD obese adolescents
<b>Pardee PE <i>et al.</i> 2012 (15)</b>	Case-control study of 38 United States obese adolescents with biopsy-proven NAFLD (seen at the San Diego children Hospital) and 38 control adolescents without steatosis who were matched for age, sex, race/ethnicity and body weight (selected from the NHANES 1999-2004) (66 boys and 10 girls; mean age 13 years, BMI 31 kg/m <sup>2</sup> )	Biopsy; 38 subjects had NAFLD	Whole body BMD	Whole body BMD Z-score (mean; range): 0.48 (-2 to +2.2) vs. -1.98 (-4 to +1), p<0.001 in controls and NAFLD patients. Adolescents with NASH had a significantly lower whole body BMD Z-score (-2.37; range: -4.5 to 0) than NAFLD adolescents without NASH (-1.58; range: -4 to 0.5)	Age, sex, race/ethnicity, height, weight	Obese adolescents with NAFLD had lower BMD Z-score than obese children without NAFLD.
<b>Campos RMS <i>et al.</i> 2012 (16)</b>	Cross-sectional study of 40 Brazilian post-puberty severely obese adolescents (mean age 17 years, mean BMI 37.5 kg/m <sup>2</sup> )	Ultrasonography; 18 (45%) subjects had NAFLD	Whole body BMD	Whole body BMD Z-score (mean±SD): 1.6±1.0 vs. 1.5±1.0 Whole body BMD (mean±SD): 1.2±0.1 vs. 1.2±0.1 g/cm <sup>2</sup>	Age, sex (all subjects were in post-pubertal stage)	No significant differences were found in BMD Z-scores between the two groups
<b>Pacifico L <i>et al.</i> 2013 (17)</b>	Case-control study of 44 Italian obese adolescents with NAFLD and 44 non-steatotic controls matched for age, sex, pubertal stage and BMI-SDS score (48 boys and 40 girls; mean age 12.5 years, BMI-SDS 2.19)	Magnetic resonance imaging; liver biopsy was also performed in 35 (80%) adolescents with NAFLD; 44 adolescents had NAFLD	Whole body and lumbar spine BMD	Whole body BMD Z-score (mean±95%CI): 1.95 (1.67-2.10) vs. 1.55 (1.23-1.87), p=0.06 Lumbar BMD Z-score (mean±95%CI): 1.29 (0.95-1.63) vs. 0.55 (0.23-0.86), p<0.01. Adolescents with NASH	Age, sex, pubertal stage, BMI-SDS score	Obese adolescents with NAFLD had lower lumbar spine BMD Z-scores than those without NAFLD

				(n=20) had a lower lumbar spine BMD Z-score than those (n=15) without NASH [0.27 (-0.17-0.71) vs. 0.75 (0.13-1.39); p<0.05] as well as a lower whole body BMD Z-score [1.38 (0.89-1.17) vs. 1.93 (1.32-2.36); p<0.05]		
<b>Chang EJ et al. 2015 (18)</b>	Cross-sectional study of 94 South Korean obese children (66 boys and 28 girls; mean age 11 years, mean BMI 25.5 kg/m <sup>2</sup> )	Ultrasonography and serum liver enzymes; 15 had hepatic steatosis alone, 47 had hepatic steatosis and elevated aminotransferases (i.e. suspected NASH) and 32 healthy controls	Whole body BMD	Whole body BMD Z-score (median; range): 0.6 (1.3-1.5) vs. 0.5 (1.2-1.9) vs. 0.6 (1.3-3.0) Whole body BMD (median; range): 1.0 (0.8-1.2) vs. 1.0 (0.9-1.2) vs. 1.0 (0.8-1.9) g/cm <sup>2</sup> in controls, patients with steatosis alone and those with suspected NASH, respectively	Age, sex, BMI	No significant differences were found in BMD Z-scores among the three groups
<b>Labayen I et al. 2018 (19)</b>	Randomized controlled trial of 115 Spanish overweight or obese children (51 boys and 64 girls; mean age 10.6 years, mean BMI 25.5 kg/m <sup>2</sup> )	Magnetic resonance imaging; 41 (35.6%) subjects had NAFLD	Whole body BMD	Whole body BMD Z-score (mean±SD): 1.39±0.9 vs. 0.94±1.1, p<0.01 Whole body BMD (mean±SD): 0.93±0.06 vs. 0.89±0.05 g/cm <sup>2</sup> , p<0.01	Sex, pubertal stage, total lean and fat masses, vitamin D/calcium intakes	Overweight/obese children with NAFLD had lower whole-body BMD Z-scores than those without NAFLD
<b>Nobili V et al. 2014 (20)</b>	Cross-sectional study of 73 Italian consecutive overweight or obese adolescents with biopsy-proven NAFLD and elevated serum liver enzymes (47 boys and 26 girls; median age 13 years, median BMI 31.3 kg/m <sup>2</sup> )	Biopsy; all subjects had NAFLD (no control group was available)	Whole body BMD	Whole body BMD Z-score (mean±SD): -1.01±0.2 vs. -1.25±0.4, p<0.01; in patients with no-NASH (n=24) and those with NASH (n=49), respectively	Sex, age, BMI, waist circumference, HOMA-IR score	Overweight/obese adolescents with biopsy-proven NASH had lower BMD Z scores than those with no-NASH
<b>Mosca A et al. 2018 (21)</b>	Cross-sectional study of 34 Italian overweight or obese adolescents with biopsy-proven NAFLD and elevated serum liver enzymes (18 boys and 16 girls; mean age 13.8 years, mean BMI 27 kg/m <sup>2</sup> ); these children had a pubertal Tanner stage ≥2	Biopsy; all subjects had NAFLD (no control group was available)	Whole body BMD	Whole body BMD Z-score (mean±SD): 1.4±1.2 vs. 0.8±1.0, p<0.05; in patients with no-NASH (n=9) and those with NASH (n=25), respectively	Age, BMI, waist circumference	Overweight/obese adolescents with biopsy-proven NASH had lower BMD Z scores than those with no-NASH

**Abbreviations:** BMD, bone mineral density; BMI, body mass index; CI, confidence interval; DEXA, dual-energy X-ray absorptiometry; HOMA-IR, homeostasis model assessment-insulin resistance; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; SDS, standard deviation score.