

## **Hepatic fat content and bone mineral density in children with overweight/obesity**

**Running title:** Hepatic steatosis and bone health

Idoia Labayen<sup>1,2</sup>, Jonatan R. Ruiz<sup>3</sup>, Lide Arenaza<sup>1,2</sup>, María Medrano<sup>1,2</sup>, Ignacio Tobalina<sup>4</sup>, Luis Gracia-Marco<sup>3</sup>, Francisco B. Ortega<sup>3</sup>, Beatriz Rodriguez-Vigil<sup>5</sup>

<sup>1</sup>Department of Health Sciences, Public University of Navarra, Pamplona, Spain

<sup>2</sup>Institute for Innovation & Sustainable Development in Food Chain (IS-FOOD), Public University of Navarra, Pamplona, Spain

<sup>3</sup>PROFITH “PROmoting FITness and Health through physical activity Research Group, Department of Physical Education and Sports, Faculty of Sport Sciences, University of Granada, Spain.

<sup>4</sup>Department of Nuclear Medicine, University Hospital of Araba (HUA), Vitoria-Gasteiz, Spain

<sup>5</sup>Department of Magnetic Resonance Imaging, Osatek, University Hospital of Alava (HUA), Vitoria-Gasteiz, Spain.

### **AUTHOR FOR CORRESPONDENCE**

Idoia Labayen

Department of Health Sciences, Public University of Navarra

Avda. de Barañain, 31008 Pamplona

Phone: 01006: +34 948166133

E-mail: idoia.labayen@unavarra.es

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**DISCLOSURE**

The authors declare no conflict of interest.

## ABSTRACT

**Objectives:** To examine the influence of non-alcoholic fatty liver disease (NAFLD) and hepatic fat content on bone mineral density (BMD), and to investigate whether the relationship between NAFLD and BMD is independent of lifestyle factors related to BMD.

**Methods:** Hepatic fat content (magnetic resonance imaging), BMD, lean mass index, total and abdominal fat mass (dual-energy-X-ray absorptiometry), moderate to vigorous physical activity (MVPA) (accelerometry) and calcium and D vitamin intake (two 24h recalls) were measured in 115 children with overweight /obesity aged  $10.6\pm 1.1$  years old.

**Results:** Children with NAFLD had lower BMD than children without NAFLD regardless of sex, puberty stage, lean mass index, fat mass, MVPA, and calcium and D vitamin intake ( $0.89\pm 0.01$  g/cm<sup>2</sup> vs.  $0.93\pm 0.01$  g/cm<sup>2</sup>, for NAFLD and non-NAFLD, respectively,  $P<0.01$ ). Higher hepatic fat content was significantly associated with lower BMD regardless of confounders (adjusted  $P<0.05$ ).

**Conclusions:** Findings of the current study suggest that hepatic fat accumulation is associated with decreased BMD independently of adiposity, and regardless of lifestyle factors closely related to bone mineral accrual in children. These results may have implication in the clinical management of children with overweight/obesity given the high prevalence of pediatric NAFLD.

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## INTRODUCTION

Osteoporosis is a major public health concern that occurs mainly in the elderly(1). It is estimated that one third of fall-related deaths are due to low bone mineral density (BMD)(2). Achieving an optimal peak bone mass is considered the best protective factor against future osteoporosis and fracture(3). Childhood, particularly late childhood and peripubertal years, is a critical period for bone accretion since it contributes with a large proportion of the peak bone mass achieved by the end of skeletal development in the early adulthood(3,4). Lifestyle factors including physical activity and dietary habits, specifically dairy products consumption, and calcium and D vitamin intake, contribute up to 20% of the variation in peak bone mass(2,5). Recently, it has been reported an inverse associations of sedentary time and sedentary behaviors with BMD in children and adolescents(6,7).

Non-alcoholic fatty liver disease (NAFLD) is the leading cause of liver disease in children affecting up to one third of children with overweight or obesity(8). The clinical burden of NAFLD is not limited to liver associated morbidity and mortality, but NAFLD is also associated with elevated risk to develop type 2 diabetes and cardiovascular disease(9). Preliminary evidence suggests a link between NAFLD and decreased BMD(10,11). However, the majority of the studies have been conducted in Asian populations, and in adults(12) and adolescents(13,14), while the information in children is scarce(15, 16). Moreover, NAFLD was diagnosed using ultrasonography in the majority of the studies, and this methodology has limited repeatability and reproducibility and is highly operator-dependent. Finally, it has not been investigated whether the influence of NAFLD on BMD is independent of lifestyle factors closely associated with obesity and bone health, such as physical activity and sedentary time. Therefore, the aims of the current study were: (i) To compare BMD between

overweight/obese children with or without NAFLD; (ii) To examine the association of hepatic fat content with BMD; and (iii) To investigate whether the relationship between NAFLD and BMD is independent of lifestyle factors related to BMD such as physical activity, and calcium and D vitamin intake.

## MATERIALS AND METHODS

### *Participants*

The **Prevention of Diabetes in Kids** (PREDIKID) project is a randomized clinical trial conducted in Vitoria-Gasteiz (Spain), whose main aim is to examine the effect of a family-based multidisciplinary intervention program on insulin resistance syndrome in prediabetic children (ClinicalTrials.gov ID: NCT03027726). For the current purpose, baseline data from 115 children with overweight/obesity aged between 8.5 and 12 years old participating in the PREDIKID study were included. The protocol of the study was reviewed and approved by the Ethic Committee of Clinical Investigation of Euskadi (PI2014045). Participants were recruited in the Pediatric Endocrinology Unit of the University Hospital of Araba and in Primary Care Clinics by pediatricians. All parents or legal guardians signed an informed consent and all the children gave their assent before being enrolled in the study.

### *Measurements*

#### *Physical examination*

Anthropometrical measurements such as body mass (SECA 760), height (SECA 220), and waist circumference (SECA 200) were measured in duplicate following standard protocols. Children were classified according to the body mass index (BMI) international cut-off values as overweight or obese(17). Pubertal stage was recorded by a pediatrician according to Tanner and Whitehouse(18).

#### *Hepatic fat and body composition*

Hepatic fat fraction was measured by magnetic resonance imaging (MRI) proton density fat fraction using a 1.5T system (MAGNETOM Avanto, SiemensHealthcare, Erlangen, Germany) equipped with a phased-array surface coil and a spine array coil and using the software package by Siemens Medical System (version syngo.MR B17A)<sup>(19,20)</sup>. The

detailed protocol of the measurement and calculations has been published elsewhere <sup>(21)</sup>. Thereafter, children were categorized into two groups according to the presence or absence of NAFLD ( $\geq 5.5\%$  or  $< 5.5\%$  of hepatic fat, respectively)<sup>(22)</sup>.

BMD, bone mineral content (BMC), lean mass (LM) and total and abdominal fat mass (FM), and were determined by dual energy X-ray absorptiometry (DXA) (HOLOGIC, QDR 4500W). All DXA scans and subsequent in-software analyses were completed by the same researcher. BMD z-score data were also obtained from the Hologic Discovery software according to the reference values for age, sex and ethnicity proposed by the National Health and Nutrition Examination Survey (NHANES 1999-2004). Abdominal adiposity was assessed at three different regions, R1, R2, and R3 using an extended research model. A rectangle was drawn on the digital scan image to establish every region. All had the lower horizontal border on the top of the iliac crest. For R1, the upper border was established parallel to the end of the lowest rib. The upper border of R2 was parallel to the junction of the T12 and L1 vertebrae, and that for R3 was parallel to the middle of the costo-vertebrae articulation of the last rib. The lateral sides were adjusted to include the maximal amount of abdominal tissue within each region. The coefficient of variation was not determined in the present study. However, precision studies in paediatric population have shown DXA's coefficient of variations of 0.96% for BMD in children (23).

#### *Physical activity, sedentary time and dietary habits assessment*

Physical activity (counts/min), time spent in moderate to vigorous physical activity (MVPA, min/day), and sedentary time (min/day) were objectively measured by accelerometry using validated thresholds for children to classify raw counts per minute into intensities of physical activity(24). Participants wore the accelerometer on the non-dominant wrist (ActiSleep, Actigraph, Pensacola, Florida) for 7 consecutive days and

completed a diary log to register the non-wear accelerometer time and in and out bed hours.

Dairy products consumption (milk, cheese and yoghurts), calcium (mg/day) and D vitamin ( $\mu\text{g}/\text{day}$ ) intake, as well as other dietary variables were assessed by two non-consecutive 24h recalls within a period of seven days assisted by an experienced nutritionist. Photographs of different food portions and servings were shown to participants to have a better estimation of the consumed amounts. Nutritional records were obtained using the EasyDiet software.

#### *Statistical analyses*

Unadjusted differences in descriptive characteristics between children with or without NAFLD were compared using independent t-test or Chi-squared test, as appropriate. Differences in BMD between children with or without NAFLD were examined by analysis of covariance (ANCOVA) adjusting by sex, puberty stage and lean mass index (Model 1), MVPA (Model 2: model 1 + MVPA), calcium and D vitamin intake (Model 3: model 2 + calcium and D vitamin intake), and total or abdominal FM (Model 4: model 3 + FM). Interactions by sex were explored including interaction terms into the models; as there were no significant interactions ( $P_s > 0.1$ ), the analyses were performed for boys and girls together.

The associations of hepatic fat content with BMD and BMD Z-score were examined using regression analyses controlling for sex, puberty stage and lean mass index (Model 1), and additionally for fat mass (Model 2) and MVPA and calcium and D vitamin intake (Model 3).

Interactions by sex were explored including interaction terms into the models; as there were no significant interactions ( $P_s > 0.1$ ), the analyses were performed for boys

and girls together. All statistical analyses were conducted using SPSS Statistics 21.0 (IBM, Armonk, NY) and the level of significance was set at  $\alpha = 0.05$ .

## RESULTS

The descriptive characteristics of the participants in the study are shown in **Table 1**. It was observed that there were no significant differences in dairy products consumption, calcium and D vitamin intake, physical activity, time spent in MVPA and sedentary behaviors between children with or without NAFLD. Children with NAFLD had higher amounts of total and abdominal adiposity ( $P < 0.01$ ).

The results showed that overweight/obese children with NAFLD had lower whole body BMD ( $\approx 4.3\%$ ,  $P < 0.05$ , Table 1) and BMD Z-score ( $\approx 32\%$ ,  $P < 0.05$ , Table 1). The differences in BMD between children with and without NAFLD were independent of sex, puberty stage, and total lean mass ( $P < 0.01$ , Model 1, **Figure 1**), MVPA ( $P < 0.01$ , Model 2, Figure 1), dietary intake of Ca and D vitamin ( $P < 0.01$ , Model 3, Figure 1), and total body fat ( $P < 0.01$ , Model 4 Figure 1). When abdominal fat was entered into the model instead of total body fat the results did not substantially change ( $0.900 \pm 0.009$  vs.  $0.926 \pm 0.006$  g/cm<sup>2</sup>,  $P = 0.019$ , for children with and without NAFLD, respectively).

Higher hepatic fat content was significantly associated with lower BMD ( $P < 0.01$ ) and BMD z-score regardless of age, puberty stage and LMI ( $P < 0.05$ , **Table 2**). The relationships of BMD persisted after further controlling fat mass (Model 2,  $P < 0.05$ , Table 2) and for MVPA and dietary intake of calcium and D vitamin (Model 3,  $P < 0.05$ , Table 2). The association of hepatic fat with BMD z-score showed the same pattern, but the strength of the association was attenuated after controlling for fat mass ( $P < 0.06$ , Model 2, Table 2), and lifestyle factors ( $P < 0.1$ , Model 3, Table 2).

## DISCUSSION

Findings from the current study show that BMD is lower in overweight/obese children with NAFLD compared to those without hepatic steatosis measured by MRI. Moreover, our results also show for the first time that the negative relationship between hepatic fat accumulation and BMD is independent of total and abdominal fat content, and lifestyle factors. These findings suggest that excess liver fat may play a pathogenic role on bone metabolism in children with overweight/obesity.

Several studies have previously reported decreased BMD in pediatric populations with NAFLD(13–15,25), which concurs with our findings. Pirgon et al.(13) observed that lumbar spine BMD was lower in the group of adolescents with obesity and NAFLD, than either in participants with obesity but without NAFLD or in lean adolescents. However, NAFLD was diagnosed as having high levels of transaminases. Pardee et al.(14)conducted a case-control study of 38 adolescents with biopsy-proven NAFLD and 38 controls without hepatic steatosis, who were matched in age ( $13\pm 2$  years old), sex and BMI ( $31\pm 5$  kg/m<sup>2</sup>). They observed that adolescents with NAFLD had significantly lower whole body BMD. In contrast, Chang et al.(25) reported no difference in age-matched BMD in 94 Korean children and adolescents with hepatic steatosis and non-alcoholic steatohepatitis (NASH), compared to healthy controls. Of note, that NAFLD was diagnosed with ultrasonography, and the presence of NASH as having high transaminase levels. In a well conducted study, Pacifico et al.(15) found that lumbar spine and whole body BMD decreased significantly according to liver histological staging evaluated with MRI and biopsy in 88 children and adolescents aged  $12.5 \pm 1.8$  years old. Interestingly, the authors observed that the relationship between hepatic fat content and BMD was independent of total body fat, in agreement with our findings. Our study adds to the current knowledge that the inverse relationship between

hepatic fat with BMD is present before adolescence and that the deleterious influence of hepatic fat content on BMD is irrespective not only of whole body adiposity, but also of abdominal fat and lifestyle factors related to bone accrual.

The pathophysiological mechanisms underlying the interplay between NAFLD and BMD are unknown. Insulin resistance (13) and chronic inflammation(10,15), specially elevated levels of CRP that is exclusively produced by the liver, have been proposed as key contributors to low BMD in individuals with NAFLD. In addition, experimental evidence supports a complex network between the bone, the liver and adipose tissue regulating bone remodeling, liver metabolism and glucose homeostasis through the secretion of pro/anti-inflammatory cytokines and hormones (26). On the other hand, physical activity(27), particularly MVPA(28,29), has positive effects on bone metabolism and bone accrual in childhood and adolescence. Sedentary behavior, on the contrary, is related to higher risk of cardiovascular disease and mortality(30), while its influence on bone health is controversial(6). In fact, the majority of the studies objectively measuring sedentary time found no relationship with BMC or BMD in pediatric populations, which concurs with our findings(6,28). It should be noted that the association of hepatic fat content with BMD was independent of physical activity in the current report.

Healthy and adequate dietary habits are important to bone mineral accrual during the development of peak bone mass, and influence lifelong structural strength of the skeleton and prevention of osteoporosis(4,31). Particularly, adequate intakes of dietary calcium and D vitamin, and dairy food consumption that provide many of the nutrients that are important for BMD. In the current study, there were no significant differences in any of the dietary intake variables between children with or without NAFLD. Also, we did not observe significant associations of dietary variables with BMD. However, it

should be highlighted that all the children participating in this study were overweight/obese, and that dietary intakes of calcium and dairy products consumption were below the recommendations in the whole sample. In any case, the difference in BMD between children with or without NAFLD did not substantially change after taking into account dietary variables.

The associations of weight status and body composition with BMD in pediatric population is controversial. The majority of the studies show that children with overweight or obesity have higher BMD than their normal-weight peers (32). However, the association between BMI and BMD seems to be explained by the lean mass (33). In this regard, the influence of weight status or BMI on BMD is due to direct mechanical impact on the bone, while obesity as an excess of adiposity, has a negative impact on BMD. Gallego-Suárez et al. observed that adiposity was negatively associated with whole body BMD and lumbar spine BMD in a representative sample of the US pediatric children aged 8-18 years old) (32).

Strengths of the current study are the methodologies used. Likewise, hepatic fat content was measured by MRI using a six-echo acquisition with advanced signal analysis that provides a more accurate liver fat estimation; BMC and BMD were evaluated by DXA; and physical activity and sedentary time were assessed by accelerometry. The limitations of the present study are associated with its cross-sectional design which does not permit to determine any causality between hepatic fat accumulation and BMD. In addition, although our sample size is larger than in other previous studies, the interpretation of the findings may be influenced by its relatively small size. Finally, plasma D vitamin levels were not measured in the current study.

In conclusion, findings of the current study suggest that hepatic fat accumulation is associated with decreased BMD independently of total and abdominal adiposity, and

regardless of lifestyle factors closely related to bone mineral accrual in children. These results may have implication in the clinical management of children with overweight/obesity given the high prevalence of pediatric NAFLD.

### **CONFLICT OF INTEREST**

There is no conflict of interest.

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**Author contribution:** IL designed the study, analyzed the data and drafted the manuscript. IL takes full responsibility for the integrity of the data analyses. LA, MM, BRV and IT collected the data and critically revised the manuscript. IL, JRR, FOB and LGM participated in the interpretation of the results. All the authors critically revised the manuscript for important intellectual content and approved the final version of the submitted manuscript.

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**FIGURE LEGENDS**

**Figure 1.** Bone mineral density in children with or without non-alcoholic fatty liver disease (NAFLD). Model 1: Analyses were adjusted with sex, puberty stage and total lean mass; Model 2: Model 1 was further adjusted with moderate to vigorous physical activity; Model 3: Model 2 was additionally adjusted with calcium and D vitamin intake; Model 4: Model 3 was additionally adjusted with total body fat. Values are means  $\pm$  SEM.