HHS Public Access

Author manuscript

*Obesity (Silver Spring)*. Author manuscript; available in PMC 2022 March 01.

Published in final edited form as:

*Obesity (Silver Spring)*. 2021 March ; 29(3): 595–600. doi:10.1002/oby.23100.

# Association between liver fat and bone density is confounded by general and visceral adiposity in a community-based cohort

**Belinda T. Li, BA**[1], **Tracey G. Simon, MD, MPH**[2], **Na Wang, MA**[3], **Raymond T. Chung, MD**[2], **Kathleen E. Corey, MD, MPH**[2], **Laura E. Dichtel, MD**[4], **Elizabeth J. Samelson, PhD**[5], **Douglas P. Kiel, MD, MPH**[5], **Michelle T. Long, MD, MSc**[6]

[1]Boston University School of Medicine, Boston, MA, USA

[2]Liver Center, Gastroenterology Division, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

[3]Biostatistics & Epidemiology Data Analytics Center, School of Public Health, Boston University, Boston, MA, USA

[4]Neuroendocrine Unit, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

[5]Hebrew SeniorLife, Hinda and Arthur Marcus Institute for Aging Research, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

[6]Section of Gastroenterology, Boston Medical Center, Boston University School of Medicine, Boston, MA, USA

## Abstract

**Objective:** Non-alcoholic fatty liver disease (NAFLD) is associated with low bone mineral density (BMD); however, it is not known if early stage NAFLD may associate with BMD, after accounting for body mass index (BMI) or visceral adipose tissue (VAT).

**Methods:** Cross-sectional study of 3,462 Framingham Heart Study participants who underwent computed tomography (CT) measurement of liver fat, VAT volume, volumetric spine BMD, vertebral cross-sectional area (CSA), and vertebral compression strength. We excluded heavy alcohol consumers. We performed multivariable linear regression models to assess the association between NAFLD and volumetric BMD, CSA, and vertebral compression strength after accounting for covariates, including BMI or VAT.

**Results:** A total of 2,253 participants (mean age 51.2 ± 10.7 years; 51.1% women) were included. In multivariable-adjusted models, we observed positive associations between NAFLD and integral BMD, trabecular BMD, and vertebral compressive strength. However, results were attenuated and no longer significant after we additionally adjusted for BMI or VAT. We observed NAFLD to be weakly associated with a lower vertebral CSA in adjusted models.

**Correspondence**: Michelle T. Long, MD, MSc, Boston University School of Medicine, Section of Gastroenterology, 85 East Concord Street 7th Floor, Boston MA 02118, mtlong@bu.edu, Tel: (617) 638-8392, Fax: (617) 638-6529.

Conflicts of Interest:

TGS has served as a consultant to Aetion on work unrelated to this project.

The other authors have no conflicts to report.

**Conclusions:** In a community-based cohort, the associations between NAFLD and BMD and vertebral strength were confounded by BMI and VAT. However, NAFLD was associated with a reduced vertebral CSA in adjusted models.

### Keywords

NAFLD; bone mineral density; bone fracture; vertebral strength; osteoporosis

## Introduction

Osteoporosis is a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fracture.[1] It has long been known that advanced liver diseases, particularly cholestatic conditions and alcohol-related liver disease, are associated with hepatic osteodystrophy, a loss of skeletal mass that parallels postmenopausal or age-related bone loss.[2] Emerging data suggest that loss of bone mineral density (BMD) may also accompany early stages of non-alcoholic fatty liver disease (NAFLD), well before the development of cirrhosis.[3] NAFLD is strongly associated with obesity and often occurs in the setting of other cardiometabolic diseases, such as dyslipidemia and insulin resistance.[4] Traditionally, body weight has been considered protective against bone loss and osteoporotic fracture.[5] However, recent evidence has challenged this assumption, with some reports suggesting that specific regional adipose tissue depots, particularly visceral adipose tissue (VAT), may have an adverse effect upon BMD,[6,7] conferring an increased risk of osteoporotic fractures, particularly in patients with diabetes or dyslipidemia.[7,8] Metabolically-active pro-inflammatory cytokines[9] and adipokines[10] secreted by VAT may accelerate the metabolism of steroid hormones,[11] which may in turn compromise skeletal health.[12–16]

The extent to which early stage NAFLD may associate with loss of BMD, after accounting for body weight or VAT, is not definitively known. Several prior studies, mostly in Asia, have observed associations between low BMD and NAFLD.[2,17–21] In pediatric patients with biopsy-confirmed NAFLD, progressive histological NAFLD was associated with decreased BMD,[22] and in adults, case-control and cross-sectional studies using ultrasound-defined NAFLD have similarly shown an association between NAFLD and reductions in lumbar[3] as well as hip and femoral neck[23,24] BMD. However, a meta-analysis of 1,276 adults observed an inverse association with body mass index (BMI) and BMD, but no associations with NAFLD, though there was significant heterogeneity among included studies.[25]

The purpose of this study is to evaluate the association between NAFLD and BMD within a community-based cohort and to determine the role of BMI and VAT on this association after adjusting for cardiometabolic risk factors, including BMI and VAT.

## Methods

### Study sample

Participants were drawn from the Framingham Heart Study (FHS) Offspring and Third Generation cohorts who underwent computed tomography (CT) scanning between the years

2002–2005 as part of the multidetector CT substudy (n=3,462).[26] The multidetector CT scans provided measurements of liver fat and VAT volume and also included three-dimensional quantitative bone measures. Individuals were excluded if they had incomplete covariate data (n=14), had significant alcohol use defined as > 7 drinks per week for women and > 14 drinks per week for men (n=418), were missing alanine aminotransferase (ALT) (n=287), if the CT scans were not interpretable for liver attenuation or VAT (n=320), or if they were missing bone measurements (n=170), which yielded a total sample size of 2,253 participants (n=939 Offspring Cohort and n=1,317 Third Generation Cohort). All subjects provided written informed consent, and the study and protocol were approved by the institutional review boards at the Boston University Medical Center, Hebrew Senior Life, and Massachusetts General Hospital.

## CT measurement of visceral adipose tissue and liver fat

The multidetector CT scan cohort and protocol have been described in detail previously.[27–29] Briefly, the CT captured 5-mm thick slices (120 kVp, 400mA, gantry rotation time 500 ms, and table feed 3:1) covering 125 mm above S1 using an 8-slice multi-detector abdominal CT scanner (LightSpeed Ultra, General Electric, Milwaukee, WI). Participants were positioned supine and a calibration phantom (Image Analysis, Lexington, KY) was placed under each participant and was visualized on each image obtained. To identify pixels containing fat, an image display window of −195 to −45 Hounsfield Units (HU) and a window center of −120 HU was used. A single reader then manually traced the muscular abdominal wall, separating the VAT compartment from the subcutaneous adipose tissue compartment. Subsequently, VAT was quantified using a semiautomatic segmentation technique at a dedicated offline work station (Aquarius 3D Workstation; TeraRecon, San Mateo, CA) as described.[30] The correlation coefficients for VAT volume between 2 independent readers on a subset of 100 randomly selected participants were 0.992.

We quantified liver fat using the CT liver fat attenuation, which is described in detail elsewhere.[26] In brief, we measured the liver attenuation in HU in three areas from the liver and from an external phantom. We calculated the average fat HU divided by the phantom to create liver phantom ratios (LPR). We defined NAFLD as a LPR of ≤ 0.33, which has been shown to have a sensitivity of 70% and specificity of 98% for hepatic steatosis.[31]

## CT measurement of bone density, cross sectional area, and vertebral compression strength

Volumetric CT scans of the thoracic and lumbar spine were obtained in the multidetector CT scan protocol as previously described.[31] Vertebral integral, trabecular, and cortical volumetric bone density (vBMD; $g/cm^3$) and vertebral cross-sectional area (CSA; $cm^2$) were measured from the CT scan using a modification of published algorithms to assess multiple vertebral levels.[31,32] Briefly, the volume of interest for integral vBMD included the entire vertebral body (both cortical and trabecular compartments) but excluded the transverse and posterior processes. The volume of interest for trabecular vBMD measurements was an elliptical region in the L3 vertebra encompassing the anterior vertebral body, centered at the mid-vertebral level and encompassing 70% of the volume between vertebral endplates. We calculated the mean CSA of the mid-vertebral body from a central 10mm thick slice.

Vertebral compressive strength was estimated by using previously published algorithms.[31] Briefly, vertebral compressive strength is estimated as a linear combination of vBMD and CSA. The vertebral body is primarily loaded in compression and the vertebral body strength is related to its structural rigidity at the weakest cross section. Structural rigidity depends on bone size and bone elastic modulus, which is estimated using a previously published relationship that relates integral vBMD to elastic modulus. The elastic modulus ($N/cm^2$) is defined as follows: $-34.7 + 3230 \times$ integral vBMD.[33] Thus, we calculated the vertebral compressive strength using the following equation: vertebral strength (N) = $0.0068 \times$ elastic modulus $\times$ CSA.[34]

## Covariates and baseline measurements

At the FHS examination visits, routine medical history, physical examination, and laboratory evaluations were performed. For this study, covariates were evaluated at the Offspring exam 7 and Generation 3 exam 1. Self-reported data on smoking status and alcohol use was assessed on the basis of clinician-administrated questionnaires. Participants were considered current smokers if they had smoked at least one cigarette per day in the year preceding the FHS examination. Physical activity was assessed using the Framingham activity index, which quantifies activity based on a participant's reporting of the number of hours per day spent sedentary or at various activities, as well as the activity level.[35] Trained technicians used standard protocols for measuring weight, height, and waist circumference. BMI was defined as weight (kg)/height$^2$ ($m^2$). Diabetes was defined as a fasting plasma glucose ≥126 mg/dL or treatment with a hypoglycemic agent or insulin. Self-reported data on use of lipid lowering medications and menopausal status was also assessed on the basis of clinician-administered questionnaires. Post-menopausal status was defined as the cessation of menses for ≥ 1 year. This includes the following subcategories: natural menopause, surgical menopause or other cause of menopause, consistent with previous FHS analyses.[36,37]

## Statistical analysis

We performed sex-specific Pearson correlation coefficients to test the relationship between continuous LPR and vBMD, CSA, and vertebral compression strength. We then measured the association between NAFLD and vBMD, CSA, and vertebral compression strength by constructing multiple linear regression models with bone density as the dependent variable and NAFLD status as the primary independent variable. Continuous LPR was a secondary independent variable. We constructed a series of multivariable models: A multivariable model adjusted for age, sex, FHS Cohort, smoking status, alcohol intake (drinks/week), physical activity, diabetes, statin medication use, and estrogenic status (in women; comprising menopausal status and the current use of any systemic estrogen replacement therapy). Additional models individually added BMI or VAT to the multivariable model. We tested the correlation between VAT and BMI using Spearman correlations and we observed a strong correlation between VAT and BMI. We obtained the component of VAT volume that is not explained by BMI (i.e. VAT residuals) by regressing VAT volume on BMI and performed an additional model adding adjustment for BMI and VAT residuals to the multivariable model.

We tested for interaction with age and sex. We also performed sensitivity analyses to adjust for VAT and height, weight, or height and weight in place of BMI. All p-values were two-sided and a $p<0.05$ was considered significant. Statistical analyses were performed using SAS version 9.4 for Windows (SAS Institute, Cary, NC).

## Results

### Study Sample Baseline Characteristics

The characteristics of the study participants are shown in Table 1. Of the 2,253 participants (mean ± standard deviation (SD) age 51.2 ± 10.7 years), 16.8% (n=379) had NAFLD, including 195 women and 184 men. At baseline, regardless of NAFLD status, participants were similar in many clinical variables, including age, smoking status, alcohol use, physical activity levels, menopause status, and hormone supplementation use. Participants with NAFLD had higher waist circumference, VAT volume, ALT, and more metabolic disease compared to those without NAFLD.

### Correlations Between Continuous Liver Fat and Volumetric Bone Measures

Liver fat was weakly, but significantly associated with vertebral CSA and the association was slightly weaker for women ($r = 0.020$) compared to men ($r = 0.077$) (Table 2). We also observed a weak negative correlation between liver fat and integral vBMD in women only ($r=-0.061$; $p=0.04$). We observed no significant correlations between liver fat and trabecular vBMD or vertebral strength.

### Multivariable-adjusted Linear Regression Analyses

In the multivariable-adjusted model, we observed associations between NAFLD and higher integral vBMD ($\beta= 0.006$, 95% CI 0.002 to 0.010), trabecular vBMD ($\beta = 0.005$, 95% CI 0.001 to 0.009), and vertebral compressive strength ($\beta = 1.188$, 95% CI 0.061 to 2.314) (Table 3). However, after adding BMI, VAT, or BMI and VAT residuals to the multivariable model, the associations with NAFLD and integral vBMD, trabecular vBMD, and vertebral compressive strength were no longer statistically significant.

In the multivariable models additionally adjusted for BMI, VAT, or BMI and VAT residuals, we observed associations between NAFLD and a significantly lower vertebral CSA. Compared to the multivariable model adjusting for BMI, the multivariable model adjusting for BMI and VAT residuals had a slightly attenuated effect, though there remained a statistically significant lower vertebral CSA (MV+ BMI model: $\beta = -0.243$, 95% CI −0.398 to −0.087 vs. MV + BMI and VAT residuals: $\beta=-0.203$, 95% CI −0.364 to −0.042).

When continuous liver fat (-LPR) was the independent variable in place of NAFLD, results were overall very similar (Table 3). We observed a statistically significant association between continuous liver fat and lower vertebral strength in the multivariable and BMI adjusted model ($\beta=-0.440$, 95% CI −0.867 to −0.014); however, the association was no longer significant after VAT residuals were added to the model ($\beta=-0.352$, 95% CI −0.797 to 0.092).

We performed secondary sensitivity analyses to explore different covariates in place of BMI. We adjusted for VAT and height, weight, and weight and height to explore different ways of measuring adiposity. The results were not significantly different for integral vBMD, trabecular vBMD, and vertebral compressive strength (Supplementary Table 1). For vertebral CSA, all models except multivariable + VAT + height were significant. We also considered ALT as the exposure variable, in place of liver fat. Overall, results were very similar, though slightly attenuated, compared with the primary models with NAFLD or continuous liver fat (Supplementary Table 2). Additionally, we further adjusted for ALT in our original analyses (Supplementary Table 3), and we found that the liver fat estimate by CT was still associated with vertebral CSA. The effect estimates were slightly different, but remained statistically significant.

## Discussion

### Principle findings

In this large, community-based sample of middle-aged to older adults, we observed significant associations between NAFLD and integral vBMD, trabecular vBMD and vertebral compressive strength in multivariable-adjusted models. However, these associations were attenuated and no longer statistically significant after accounting for BMI and VAT. Our findings support the hypothesis that associations between NAFLD and BMD and vertebral strength are confounded by general and visceral adiposity and the associations observed are likely not specific to liver fat. However, NAFLD was significantly associated with lower vertebral CSA and this association remained after we accounted for BMI and VAT. The association between NAFLD and lower vertebral CSA was weak and the clinical significance is not known. Our findings suggest that the possible increased risk of fracture in NAFLD relates to an imbalance of load distributed over a small vertebral CSA, though additional studies are needed. Our findings are also consistent with a recent systematic review of NAFLD, BMD, and fracture that found NAFLD to be associated with history of fracture but not with BMD, again suggesting the possibility of an effect of NAFLD on bone size contributions to fracture.[38]

### In the context of the current literature

Our cross-sectional study builds upon prior studies on the association between NAFLD and BMD. In contrast to our study, other studies in more select populations observed a negative association between NAFLD and BMD,[3,17–22,24,39] even after controlling for BMI. It is possible that differences in sample populations may account for these apparently discrepant results. Prior studies were mostly performed in China or Korea and study participants had a lower mean BMI compared to participants in our US-based study.[3,19,20,24] BMI may more strongly influence BMD in a more obese population compared to a relatively lean population. Additionally, unmeasured confounding factors, such as dietary patterns and family history of osteoporosis, may differ by study setting and could possibly account for the discrepancy in study findings. Furthermore, the sample of one large Korean-based study was exclusively focused on postmenopausal women,[18] a population whose bone architecture is compromised at baseline.[40] Other studies included both women and men, but excluded women who were pre-menopausal.[20,24] In our community-based study, we included both

women and men and, regardless of NAFLD status, the majority of the women in our sample were pre-menopausal. It is possible that the association between NAFLD and BMD is limited to older adults who already have diminished bone architecture. We expanded the analysis of NAFLD and BMD by further adjusting for VAT, a variable that was not accounted for in prior studies. Thus, it is possible that the observed association between NAFLD and BMD may be driven by VAT and not specifically liver fat. Whereas prior studies assessed the relationship between NAFLD and BMD, our study is the first, to our knowledge, to also examine the relationship between NAFLD and vertebral strength and CSA. Though the association between NAFLD and vertebral strength was attenuated after accounting for BMI and VAT, the association with CSA remained significant even after accounting for general or visceral adiposity.

## Potential mechanisms

Several lines of evidence suggest that impairment of the growth hormone (GH) / insulin-like growth factor-1 (IGF-1) axis and VAT expansion may mediate bone loss in patients with NAFLD. First, the metabolically-active pro-inflammatory cytokines[9] and adipokines[10] secreted by VAT are thought to accelerate the metabolism of steroid hormones,[11] which may in turn compromise skeletal health.[12–16] Second, VAT has been associated with low serum IGF-1 levels, and in several recent cross-sectional reports, low circulating IGF-1 has been linked to progressive histological NAFLD.[41,42] Finally, within the general population, low circulating IGF-1 is also associated with decreased BMD scores and increased bone marrow fat.[43] To date, however, results have been conflicting, with some studies showing an association between central obesity and increased fracture risk,[44,45] and others reporting greater cortical and trabecular indices of bone strength, in patients with higher VAT volume.[6–8,44,45] Taken together, these data suggest that VAT may contribute to the relationship between NAFLD and BMD, and that this relationship may be modified by impaired GH/IGF-1 signaling.

## Strengths and limitations

The main strength of our investigation includes the use of a large, community-based cohort well phenotyped for both NAFLD, VAT, and BMD using objective measures. However, several limitations exist. Due to the cross-sectional nature of our study, we are not be able to infer causality in the observed relationships between NAFLD and BMD, vertebral compression strength or CSA. Consequently, future studies with serial radiographic and/or histological measurements evaluating changes in VAT and NAFLD severity over time as they relate to BMD, will be needed. Additionally, this study represents an observational analysis that is subject to residual confounding. Moreover, while it benefits from its large, community-based design, which enhances overall generalizability, it is nonetheless comprised of a primarily Caucasian population, and therefore future studies will be needed to validate our findings in other multi-ethnic populations. Finally, this study lacks hepatic histology, which remains the gold standard for the diagnosis of NAFLD. Given that CT only has 70% sensitivity, there is the possibility of misclassification of NAFLD, and the inclusion of people with NAFLD in the non-NAFLD group may decrease the ability to detect a relationship if one exists. Furthermore, although CT is well-validated and widely used for the diagnosis of steatosis, CT cannot reliably identify non-alcoholic steatohepatitis (NASH)

or assess fibrosis. Due to differences in the mechanisms of NAFLD compared to NASH with regard to liver function and systemic inflammation, results may not be generalizable to individuals with NASH or more advanced liver phenotypes. Thus, future studies will be needed in carefully-phenotyped NAFLD populations to determine whether the observed relationships from this study are related to NAFLD severity.

## Conclusion

In conclusion, we observed that the associations between NAFLD and BMD and vertebral strength were confounded by BMI and VAT; however, NAFLD was associated with low vertebral CSA in adjusted models. Additional studies are needed to confirm our findings and to determine if an imbalance of load distributed over a small vertebral CSA may contribute to the increased risk of fracture in NAFLD.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Grant Support

The Framingham Heart Study is supported by 75N92019D00031. Dr. Long is supported in part by the National Institute of Diabetes and Digestive and Kidney Diseases K23 DK113252, Gilead Sciences, the Doris Duke Charitable Foundation, the Boston University School of Medicine Department of Medicine Career Investment Award and the Boston University Clinical Translational Science Institute UL1 TR001430. Dr. Simon is supported in part by the National Institute of Diabetes and Digestive and Kidney Diseases K23 DK122104 and by the Harvard University Center for AIDS Research Career Development Award. Dr. Samelson is supported by AG041658.

## References:

1. Klibanski A, Adams-Campbell L, Bassford T, et al. Osteoporosis prevention, diagnosis, and therapy. In: Journal of the American Medical Association. Vol 285 American Medical Association; 2001:785–795. doi:10.1001/jama.285.6.785 [PubMed: 11176917]
2. Targher G, Lonardo A, Rossini M. Nonalcoholic fatty liver disease and decreased bone mineral density: is there a link? J Endocrinol Invest. 2015;38(8):817–825. doi:10.1007/s40618-015-0315-6 [PubMed: 26003827]
3. Moon S-S, Lee Y-S, Kim SW. Association of nonalcoholic fatty liver disease with low bone mass in postmenopausal women. Endocrine. 2012;42(2):423–429. doi:10.1007/s12020-012-9639-6 [PubMed: 22407492]
4. Speliotes EK, Massaro JM, Hoffmann U, et al. Fatty liver is associated with dyslipidemia and dysglycemia independent of visceral fat: the Framingham Heart Study. Hepatology. 2010;51(6):1979–1987. doi:10.1002/hep.23593 [PubMed: 20336705]
5. Edelstein SL, Barrett-connor E. Relation between body size and bone mineral density in elderly men and women. Am J Epidemiol. 1993;138(3):160–169. doi:10.1093/oxfordjournals.aje.a116842 [PubMed: 8356959]
6. Zhao LJ, Liu YJ, Liu PY, Hamilton J, Recker RR, Deng HW. Relationship of obesity with osteoporosis. J Clin Endocrinol Metab. 2007;92(5):1640–1646. doi:10.1210/jc.2006-0572 [PubMed: 17299077]
7. Hsu YH, Venners SA, Terwedow HA, et al. Relation of body composition, fat mass, and serum lipids to osteoporotic fractures and bone mineral density in Chinese men and women. Am J Clin Nutr. 2006;83(1):146–154. doi:10.1093/ajcn/83.1.146 [PubMed: 16400063]
8. Janghorbani M, Feskanich D, Willett WC, Hu F. Prospective study of diabetes and risk of hip fracture: The nurses' health study. Diabetes Care. 2006;29(7):1573–1578. doi:10.2337/dc06-0440 [PubMed: 16801581]

9. Pou KM, Massaro JM, Hoffmann U, et al. Visceral and subcutaneous adipose tissue volumes are cross-sectionally related to markers of inflammation and oxidative stress: the Framingham Heart Study. Circulation. 2007;116(11):1234–1241. doi:10.1161/CIRCULATIONAHA.107.710509 [PubMed: 17709633]
10. Jain SH, Massaro JM, Hoffmann U, et al. Cross-sectional associations between abdominal and thoracic adipose tissue compartments and adiponectin and resistin in the Framingham heart study. Diabetes Care. 2009;32(5):903–908. doi:10.2337/dc08-1733 [PubMed: 19223612]
11. Corbould AM, Bawden MJ, Lavranos TC, Rodgers RJ, Judd SJ. The effect of obesity on the ratio of type 3 17beta-hydroxysteroid dehydrogenase mRNA to cytochrome P450 aromatase mRNA in subcutaneous abdominal and intra-abdominal adipose tissue of women. Int J Obes Relat Metab Disord. 2002;26(2):165–175. doi:10.1038/sj.ijo.0801886 [PubMed: 11850747]
12. Campos RMS, de Piano A, da Silva PL, et al. The role of pro/anti-inflammatory adipokines on bone metabolism in NAFLD obese adolescents: effects of long-term interdisciplinary therapy. Endocrine. 2012;42(1):146–156. doi:10.1007/s12020-012-9613-3 [PubMed: 22315014]
13. Zou W, Hakim I, Tschoep K, Endres S, Bar-Shavit Z. Tumor necrosis factor-α mediates RANK ligand stimulation of osteoclast differentiation by an autocrine mechanism. J Cell Biochem. 2001;83(1):70–83. doi:10.1002/jcb.1202 [PubMed: 11500955]
14. Peterson CA, Heffernan ME. Serum tumor necrosis factor-alpha concentrations are negatively correlated with serum 25(OH)D concentrations in healthy women. J Inflamm (Lond). 2008;5:10. doi:10.1186/1476-9255-5-10 [PubMed: 18652680]
15. Zeadin MG, Butcher MK, Shaughnessy SG, Werstuck GH. Leptin promotes osteoblast differentiation and mineralization of primary cultures of vascular smooth muscle cells by inhibiting glycogen synthase kinase (GSK)-3β. Biochem Biophys Res Commun. 2012;425(4):924–930. doi:10.1016/j.bbrc.2012.08.011 [PubMed: 22906741]
16. DeMambro VE, Maile L, Wai C, et al. Insulin-like growth factor-binding protein-2 is required for osteoclast differentiation. J Bone Miner Res. 2012;27(2):390–400. doi:10.1002/jbmr.545 [PubMed: 22006816]
17. Shen Z, Cen L, Chen X, et al. Increased risk of low bone mineral density in patients with non-alcoholic fatty liver disease: A cohort study. Eur J Endocrinol. 2020;182(2):157–164. doi:10.1530/EJE-19-0699 [PubMed: 31770104]
18. Lee DY, Park JK, Hur KY, Um SH. Association between nonalcoholic fatty liver disease and bone mineral density in postmenopausal women. Climacteric. 2018;21(5):498–501. doi:10.1080/13697137.2018.1481380 [PubMed: 30122075]
19. Ahn SH, Seo DH, Kim SH, Nam MS, Hong S. The relationship between fatty liver index and bone mineral density in Koreans: KNHANES 2010–2011. Osteoporos Int. 2018;29(1):181–190. doi:10.1007/s00198-017-4257-z [PubMed: 29051986]
20. Kim G, Kim KJ, Rhee Y, Lim SK. Significant liver fibrosis assessed using liver transient elastography is independently associated with low bone mineral density in patients with non-alcoholic fatty liver disease. PLoS One. 2017;12(7). doi:10.1371/journal.pone.0182202
21. Lee SH, Yun JM, Kim SH, et al. Association between bone mineral density and nonalcoholic fatty liver disease in Korean adults. J Endocrinol Invest. 2016;39(11):1329–1336. doi:10.1007/s40618-016-0528-3 [PubMed: 27561910]
22. Purnak T, Beyazit Y, Ozaslan E, Efe C, Hayretci M. The evaluation of bone mineral density in patients with nonalcoholic fatty liver disease. Wien Klin Wochenschr. 2012;124(15–16):526–531. doi:10.1007/s00508-012-0211-4 [PubMed: 22850810]
23. Li M, Xu Y, Xu M, et al. Association between Nonalcoholic Fatty Liver Disease (NAFLD) and osteoporotic fracture in middle-aged and elderly Chinese. J Clin Endocrinol Metab. 2012;97(6):2033–2038. doi:10.1210/jc.2011-3010 [PubMed: 22466338]
24. Cui R, Sheng H, Rui X-F, et al. Low bone mineral density in chinese adults with nonalcoholic Fatty liver disease. Int J Endocrinol. 2013;2013:396545. doi:10.1155/2013/396545 [PubMed: 23983685]
25. Upala S, Sanguankeo A, Jaruvongvanich V. Association between nonalcoholic fatty liver disease and bone mineral density: A systematic review and meta-analysis. J Endocrinol Invest. 2015;38(8):931–932. doi:10.1007/s40618-015-0342-3 [PubMed: 26105975]

26. Speliotes EK, Massaro JM, Hoffmann U, et al. Liver fat is reproducibly measured using computed tomography in the Framingham Heart Study. J Gastroenterol Hepatol. 2008;23(6):894–899. doi:10.1111/j.1440-1746.2008.05420.x [PubMed: 18565021]
27. Arase Y, Suzuki F, Ikeda K, Kumada H, Tsuji H, Kobayashi T. Multivariate analysis of risk factors for the development of type 2 diabetes in nonalcoholic fatty liver disease. J Gastroenterol. 2009;44(10):1064–1070. doi:10.1007/s00535-009-0091-1 [PubMed: 19533014]
28. Adams LA, Lymp JF, St. Sauver J, et al. The natural history of nonalcoholic fatty liver disease: A population-based cohort study. Gastroenterology. 2005;129(1):113–121. doi:10.1053/j.gastro.2005.04.014 [PubMed: 16012941]
29. Söderberg C, Stål P, Askling J, et al. Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. Hepatology. 2010;51(2):595–602. doi:10.1002/hep.23314 [PubMed: 20014114]
30. Fox CS, Maurovich-Horvat P, Meigs JB, et al. Abdominal Visceral and Subcutaneous Adipose Tissue Compartments. Circulation. 2007;116(1):39–48. doi:10.1161/circulationaha.106.675355 [PubMed: 17576866]
31. Samelson EJ, Christiansen BA, Demissie S, et al. QCT measures of bone strength at the thoracic and lumbar spine: the Framingham Study. J Bone Miner Res. 2012;27(3):654–663. doi:10.1002/jbmr.1482 [PubMed: 22143959]
32. Lang T, LeBlanc A, Evans H, Lu Y, Genant H, Yu A. Cortical and trabecular bone mineral loss from the spine and hip in long-duration spaceflight. J Bone Miner Res. 2004;19(6):1006–1012. doi:10.1359/JBMR.040307 [PubMed: 15125798]
33. Kopperdahl DL, Morgan EF, Keaveny TM. Quantitative computed tomography estimates of the mechanical properties of human vertebral trabecular bone. J Orthop Res. 2002;20(4):801–805. doi:10.1016/S0736-0266(01)00185-1 [PubMed: 12168670]
34. Bruno AG, Broe KE, Zhang X, et al. Vertebral size, bone density, and strength in men and women matched for age and areal spine BMD. J Bone Miner Res. 2014;29(3):562–569. doi:10.1002/jbmr.2067 [PubMed: 23955966]
35. Kannel WB, Sorlie P. Some health benefits of physical activity. The Framingham Study. Arch Intern Med. 1979;139(8):857–861. http://www.ncbi.nlm.nih.gov/pubmed/464698. Accessed March 3, 2020. [PubMed: 464698]
36. Lisabeth LD, Beiser AS, Brown DL, Murabito JM, Kelly-Hayes M, Wolf PA. Age at natural menopause and risk of ischemic stroke the framingham heart study. Stroke. 2009;40(4):1044–1049. doi:10.1161/STROKEAHA.108.542993 [PubMed: 19233935]
37. Yanes LL, Reckelhoff JF. Postmenopausal hypertension. Am J Hypertens. 2011;24(7):740–749. doi:10.1038/ajh.2011.71 [PubMed: 21509049]
38. Mantovani A, Dauriz M, Gatti D, et al. Systematic review with meta-analysis: non-alcoholic fatty liver disease is associated with a history of osteoporotic fractures but not with low bone mineral density. Aliment Pharmacol Ther. 2019;49(4):375–388. doi:10.1111/apt.15087 [PubMed: 30600540]
39. Umehar T Nonalcoholic fatty liver disease with elevated alanine aminotransferase levels is negatively associated with bone mineral density: Crosssectional study in U.S. adults. PLoS One. 2018;13(6). doi:10.1371/journal.pone.0197900
40. Karlamangla AS, Burnett-Bowie SAM, Crandall CJ. Bone Health During the Menopause Transition and Beyond. Obstet Gynecol Clin North Am. 2018;45(4):695–708. doi:10.1016/j.ogc.2018.07.012 [PubMed: 30401551]
41. Ajmera V, Perito ER, Bass NM, et al. Novel plasma biomarkers associated with liver disease severity in adults with nonalcoholic fatty liver disease. Hepatology. 2017;65(1):65–77. doi:10.1002/hep.28776 [PubMed: 27532276]
42. Dichtel LE, Corey KE, Misdraji J, et al. The Association Between IGF-1 Levels and the Histologic Severity of Nonalcoholic Fatty Liver Disease. Clin Transl Gastroenterol. 2017;8(1):e217. doi:10.1038/ctg.2016.72 [PubMed: 28125073]
43. Bredella MA, Torriani M, Ghomi RH, et al. Vertebral bone marrow fat is positively associated with visceral fat and inversely associated with IGF-1 in obese women. Obesity (Silver Spring). 2011;19(1):49–53. doi:10.1038/oby.2010.106 [PubMed: 20467419]

44. Compston JE, Watts NB, Chapurlat R, et al. Obesity is not protective against fracture in postmenopausal women: Glow. Am J Med. 2011;124(11):1043–1050. doi:10.1016/j.amjmed.2011.06.013 [PubMed: 22017783]

45. Laslett LL, Just Nee Foley SJ, Quinn SJ, Winzenberg TM, Jones G. Excess body fat is associated with higher risk of vertebral deformities in older women but not in men: a cross-sectional study. Osteoporos Int. 2012;23(1):67–74. doi:10.1007/s00198-011-1741-8 [PubMed: 21850547]

## Study Importance Questions

**What is already known about this subject?**

- Non-alcoholic fatty liver disease (NAFLD) is strongly associated with obesity and often occurs in the setting of other cardiometabolic diseases, such as dyslipidemia and insulin resistance.
- NAFLD is associated with the loss of bone mineral density (BMD) and bone fractures in prior studies.

**What are the new findings in your manuscript?**

- We observed that the associations between NAFLD and BMD and vertebral strength were confounded by body mass index (BMI) and visceral adipose tissue (VAT).
- NAFLD was associated with low vertebral cross-sectional area (CSA), even after adjusting for BMI and VAT

**How might your results change the direction of research or the focus of clinical practice?**

- Additional studies are needed to confirm our findings and to determine if an imbalance of load distributed over a small vertebral CSA may contribute to the increased risk of fracture in NAFLD.

**Table 1.**

Characteristics of Framingham Heart Study participants (N=2,253), according to the presence of NAFLD

| Variable | NAFLD N=379 | Non-NAFLD N=1,874 | Overall N=2,253 |
|---|---|---|---|
| Age, years, mean (SD) | 52.8 (10.9) | 50.9 (10.6) | 51.2 (10.7) |
| Women, % | 195 (44.6) | 982 (52.4) | 1151 (51.1) |
| Cohort, N (%) | | | |
| Offspring | 177 (46.7) | 759 (40.5) | 936 (41.5) |
| Smoking, current (%) | 38 (10.0) | 191 (10.2) | 229 (19.6) |
| Alcohol (drinks / week), mean (SD) | 3.1 (3.9) | 3.0 (3.4) | 3.0 (3.5) |
| Physical activity (Framingham activity index), mean (SD) | 36.8 (6.5) | 37.5 (6.9) | 37.4 (6.8) |
| Weight, kg, mean (SD) | 90.3 (18.5) | 77.2 (16.2) | 79.4 (17.3) |
| Height, cm, mean (SD) | 169.9 (9.3) | 169.5 (9.6) | 169.6 (9.6) |
| BMI, kg/m$^2$, mean (SD) | 31.8 (5.7) | 26.8 (4.8) | 27.5 (5.2) |
| Waist circumference (cm) | 41.8 (5.6) | 37.1 (5.3) | 37.9 (5.6) |
| VAT volume, cm$^3$, mean (SD) | 2623 (1055) | 1566 (908) | 1744 (1014) |
| Liver phantom ratio, mean (SD) | 0.27 (0.06) | 0.37 (0.02) | 0.36 (0.05) |
| ALT, U/L, mean (SD) | 31.5 (20.2) | 22.7 (13.7) | 24.2 (15.4) |
| Diabetes, N (%) | 51 (13.5) | 73 (3.9) | 124 (5.5) |
| Post-menopausal status, N (%) | 104 (27.4) | 487 (26.0) | 591 (26.2) |
| Use of hormone supplementation, N (%) | 37 (9.8) | 189 (10.0) | 226 (10.0) |
| Use of lipid lowering medications, N (%) | 71 (18.7) | 257 (13.7) | 328 (14.6) |
| Integral BMD, g/cm$^3$, mean (SD) | 0.189 (0.039) | 0.187 (0.041) | 0.187 (0.041) |
| Trabecular BMD, g/cm$^3$, mean (SD) | 0.142 (0.041) | 0.142 (0.043) | 0.142 (0.042) |
| Vertebral CSA, cm$^2$, mean (SD) | 11.3 (1.6) | 11.3 (1.7) | 11.3 (1.7) |
| Vertebral compressive strength, N, mean (SD) | 44.5 (12.0) | 43.7 (12.4) | 43.8 (12.3) |

SD: standard deviation; BMI: body mass index; VAT: visceral adipose tissue; ALT: alanine transaminase; BMD: bone mineral density; CSA: cross-sectional area

**Table 2.**

Pearson correlation coefficients between liver fat and bone mineral density, vertebral cross-sectional area and vertebral compressive strength, by sex

| | Pearson Correlation Coefficients | | | | | |
|---|---|---|---|---|---|---|
| | Women | | Men | | Overall | |
| **Outcomes** | **r** | **p-value** | **r** | **p-value** | **r** | **p-value** |
| L3 integral vBMD ($g/cm^3$) | −0.061 | 0.04 | 0.003 | 0.93 | −0.028 | 0.18 |
| L3 trabecular vBMD ($g/cm^3$) | −0.036 | 0.22 | 0.033 | 0.28 | −0.00014 | 0.99 |
| L3 cross-sectional area ($cm^2$) | 0.020 | 0.50 | 0.077 | 0.01 | 0.052 | 0.01 |
| Vertebral compressive strength (N) | −0.042 | 0.15 | 0.033 | 0.28 | 0.009 | 0.67 |

vBMD: volumetric bone mineral density

**Table 3.**

Multiple linear regression models for the association of NAFLD or continuous liver fat with bone mineral density, vertebral cross-sectional area, and vertebral compressive strength.

| Bone microarchitecture measures | Models | NAFLD | | Continuous liver fat* | |
|---|---|---|---|---|---|
| | | ß [95% CI] | p-value | ß [95% CI] | p-value |
| Integral BMD (g/cm$^3$) | MV | 0.006 (0.002, 0.010) | 0.002 | 0.001 (0.000, 0.003) | 0.06 |
| | MV + BMI | 0.002 (−0.002, 0.006) | 0.29 | 0.000 (−0.002, 0.001) | 0.83 |
| | MV + VAT | 0.003 (−0.001, 0.007) | 0.16 | 0.000 (−0.002, 0.002) | 1.00 |
| | MV + BMI + VAT residuals | 0.003 (−0.002, 0.007) | 0.22 | 0.000 (−0.002, 0.001) | 0.95 |
| Trabecular BMD (g/cm$^3$) | MV | 0.005 (0.001, 0.009) | 0.01 | 0.001 (−0.001, 0.002) | 0.30 |
| | MV + BMI | 0.001 (−0.003, 0.005) | 0.70 | −0.001 (−0.002, 0.001) | 0.30 |
| | MV + VAT | 0.002 (−0.002, 0.007) | 0.27 | 0.000 (−0.002, 0.001) | 0.66 |
| | MV + BMI + VAT residuals | 0.002 (−0.002, 0.006) | 0.35 | 0.000 (−0.002, 0.001) | 0.61 |
| Vertebral CSA (cm$^2$) | MV | −0.083 (−0.233, −0.067) | 0.28 | −0.038 (−0.094, 0.018) | 0.18 |
| | MV + BMI | −0.243 (−0.398, −0.087) | 0.002 | −0.096 (−0.154, −0.038) | 0.001 |
| | MV + VAT | −0.187 (−0.350, −0.025) | 0.02 | −0.079 (−0.139, −0.018) | 0.01 |
| | MV + BMI + VAT residuals | −0.203 (−0.364, −0.042) | 0.01 | −0.081 (−0.141, −0.021) | 0.008 |
| Vertebral compressive strength (N) | MV | 1.188 (0.061, 2.314) | 0.04 | 0.199 (−0.219, 0.618) | 0.35 |
| | MV + BMI | −0.528 (−1.681, 0.625) | 0.37 | −0.440 (−0.867, −0.014) | 0.04 |
| | MV + VAT | −0.094 (−1.309, 1.122) | 0.88 | −0.330 (−0.782, 0.122) | 0.15 |
| | MV + BMI + VAT residuals | −0.250 (−1.446, 0.946) | 0.68 | −0.352 (−0.797, 0.092) | 0.12 |

NAFLD: non-alcoholic fatty liver disease; BMD: bone mineral density; CSA: cross-sectional area; MV: multivariable model; BMI: body mass index; VAT: visceral adipose tissue

MV: age, sex, cohort, smoking, alcohol (drinks/week), physical activity, diabetes, lipid lowering medication use, menopausal status and current use of estrogen supplementation

*Models with continuous liver fat are modeled per standard deviation decrease in LPR (increase in liver fat).