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Association between nonalcoholic fatty liver disease and bone mineral density: Mendelian randomization and mediation analysis

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ABSTRACT

Background: Observational studies have reported significant association between non-alcoholic fatty liver disease (NAFLD) and bone mineral density (BMD), a critical indicator of bone health. We aimed to investigate whether NAFLD is a cause for changes in BMD.

Methods: We selected 29 independent SNPs as instrumental variables for NAFLD. A range of Mendelian randomization (MR) methods, namely the inverse variance-weighted (IVW) method, weighted-median, weighted-mode, and MR-Egger regression, were utilized to determine the causal effects of NAFLD on BMD. Two-step MR analysis was conducted to determine the mediating effect of fasting glucose, insulin, glycosylated hemoglobin, low-density cholesterol, and body-mass index on the association between NAFLD and BMD. False-discovery-rate (FDR) was used to correct for multiple testing bias.

Results: The IVW-method indicated a significantly inverse association between genetically predicted NAFLD and total body BMD ($\beta = -0.04$, 95 % CI -0.07 to −0.02, FDR = 0.010). Notably, the relationship was more pronounced in participants over 60 years of age ($\beta = -0.06$, 95 % CI -0.11 to −0.02, FDR = 0.030). Inverse associations were observed in other subpopulations and in site-specific BMD, though they were not statistically significant after correcting for multiple testing. We observed a significantly positive association between NAFLD and the risk of osteoporosis. Consistency in results was observed across multiple MR methods and in the repeated analysis. Fasting glucose, insulin, and glycosylated hemoglobin mediated 25.4 % (95 % CI 17.6–31.5 %), 18.9 % (12.0–24.9 %), and 27.9 % (19.9–36.7 %) of the effect of NAFLD on BMD, respectively.

Conclusion: Our findings underscore a probable causal negative link between NAFLD and BMD, indicating that NAFLD might detrimentally affect bone health, especially in older individuals.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) has emerged as a global health concern, affecting a substantial proportion of the population (Riazi et al., 2022). NAFLD encompasses a spectrum of liver conditions ranging from simple steatosis to nonalcoholic steatohepatitis, cirrhosis, and hepatocellular carcinoma. Beyond its hepatic manifestations, emerging evidence suggests that NAFLD may have systemic effects on various extrahepatic organs and metabolic processes (Mantovani et al., 2021; Liu et al., 2020; Simon et al., 2022; Liu et al., 2022). One area of interest is the potential association between NAFLD and bone mineral density (BMD), a critical measure of bone health.

Both NAFLD and low BMD are prevalent conditions with shared risk factors, including obesity, diabetes, insulin resistance, and dyslipidemia (Costa de Miranda et al., 2019; Cherif et al., 2018; Bugianesi et al., 2005; Montagnani et al., 2011). These common risk factors suggest a potential link between NAFLD and alterations in bone metabolism. Understanding this relationship is crucial for identifying individuals at risk of bone loss and fractures, enabling targeted interventions to mitigate such risks. Previous epidemiological studies have revealed a negative association between these two traits. For example, NAFLD is found to be associated with low BMD regardless of insulin resistance in Korean men (Ahn et al., 2018). Likewise, a meta-analysis suggested that the presence and severity of NAFLD are significantly associated with reduced whole-body BMD in children and adolescents (Mantovani et al., 2019). Although the consistent evidence, the observational design of these studies does not allow for proving causality.

To address the causal relationship between NAFLD and BMD, Mendelian randomization (MR) analysis provides a powerful approach. MR utilizes genetic variants as instrumental variables, taking advantage of

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their randomly allocated distribution during meiosis to assess causal relationships between an exposure (NAFLD) and an outcome (BMD) (Davey Smith and Hemani, 2014; Liu et al., 2023). MR analysis helps overcome limitations of traditional observational studies, such as confounding and reverse causality, by leveraging genetic variants that are associated with the exposure but not influenced by the outcome (Birney, 2022). There were several MR studies have been performed to investigate the association between NAFLD and BMD (or osteoporosis) (Liu et al., 2024; Huang et al., 2023; Zhou et al., 2023; Cui et al., 2023; Pei et al., 2024). To further understand the impact of NAFLD on BMD, in the current study, we analyzed the genetic associations between NAFLD and BMD in individuals of different ages and at various body sites. We also explored the potential mediators in the association between NAFLD and BMD. Understanding the causal relationship between these two conditions can provide valuable insights into the impact of NAFLD on bone health and potentially inform preventive strategies and interventions for individuals at risk of bone loss. Moreover, unraveling this association contributes to a broader understanding of the systemic consequences of NAFLD and sheds light on the intricate interplay between liver health and bone metabolism.

2. Methods

2.1. GWAS of nonalcoholic fatty liver disease

So far, there were only a few genome-wide association studies (GWAS) that been performed for NAFLD, and were varied by NAFLD diagnostic criteria (e.g., liver biopsy, electronic health record [EHR], and biomarkers) (Vujkovic et al., 2022; Ghodsian et al., 2021; Anstee et al., 2020). In this study, we retrieved the GWAS summary information from study of Vujkovic et al. (Vujkovic et al., 2022), in which the NAFLD case was defined by chronic elevation of alanine aminotransferase (cALT) levels without other liver diseases. Specifically, the primary cALT phenotype was defined by: (1) elevated ALT $>$ 40 U/L for men or $>$ 30 U/L for women during at least two time points at least 6 months apart within a 2-year window at any point prior to enrollment and (2) exclusion of other causes of liver disease, chronic liver diseases or systemic conditions and/or alcohol use disorders. The control group was defined by having a normal ALT ($\leq$ 30 U/L for men, $\leq$ 20 U/L for women) and no apparent causes of liver disease or alcohol use disorder or related conditions (Vujkovic et al., 2022). In this GWAS, a total of 218,595 multiancestry subjects, of which 75.1 % were of European ancestry (68,725 NAFLD cases and 95,472 controls), were included. The identified significant signals were also validated in two independent cohorts: (1) a biopsy-determined NAFLD cohort including 7397 NAFLD cases and 56,785 controls and (2) an imaging-determined NAFLD cohort including 44,289 subjects.

Anstee et al. performed a GWAS for 1483 European NAFLD cases that were diagnosed by liver biopsy and 17,781 genetically matched controls (Anstee et al., 2020) (full summary data were available at GWAS-Catalog by identifier of GCST90011885). Besides, Ghodsian et al. performed a GWAS of EHR-documented NAFLD in participants of European ancestry (8434 cases and 770,180 controls) from four cohorts: The Electronic Medical Records and Genomics (eMERGE) network, the UK Biobank, the Estonian Biobank and FinnGen (Ghodsian et al., 2021) (full summary data were available at GWAS-Catalog by identifier of GCST008468).

2.2. GWAS of bone mineral density

Medina-Gomez et al. performed a GWAS for total-body BMD (TB-BMD) that measured by dual-energy X-ray absorptiometry in ~66,628 individuals from populations across America, Europe, and Australia (Medina-Gomez et al., 2018). The GWAS was performed for the overall population as well as in subgroups of individuals by age strata, defined by bins of 15 years (i.e., 0–15 years, 15–30 years, 30–45 years, 45–60 years, and 60 or more years). This is the first study to identify gene variants associated with TB-BMD across the lifespan and to investigate possible differences of genetic effects across age periods. The full GWAS summary data of the TB-BMD were available at GWAS-Catalog using identifiers of GCST005348, GCST005345, GCST005344, GCST005346, GCST005350, and GCST005349 for the whole population, people aged 0–15 years, 15–30 years, 30–45 years, 45–60 years, and 60 or more years, respectively. We also retrieved GWAS summary data of site-specific BMD (i.e., heel, forearm, and femoral neck BMD) from the GWAS-Catalog or IEU-Open GWAS database using identifiers of GCST006979, ieu-a-982, and GCST90013422, respectively. Moreover, to further validate the MR findings, we retrieved the GWAS summary data of osteoporosis using identifier of GCST90038656 from the GWAS-Catalog (Dönertaş et al., 2021).

2.3. Selection of instrumental variables

As shown in Fig. 1A, a valid genetic instrumental variable (IV) should comply with three assumptions: (1) significantly associated with the exposure (i.e., NAFLD); (2) not associated with the outcome (i.e., BMD) conditional on the exposure and confounders; and (3) not associated with any confounder of the exposure-outcome association (Burgess and Thompson, 2017). In the current study, we retrieved the significant signals from NAFLD GWAS in Vujkovic et al.'s study (Vujkovic et al., 2022). Overall, after clumping process, a total of 55 eligible SNPs reaching conventional threshold of GWAS *P*-value (5×10^{-8}) were identified in European Americans in the primary analysis. We selected 29 of the 55 SNPs that with a minor allele frequency $>$ 1 % and were validated in the biopsy-determined NAFLD cohort as the genetic IVs (Table 1). To ensure the validity of the IVs, we calculated the F-statistics to assess the strength of the association between IVs and NAFLD using the following Eq. (Zhu et al., 2022):

$$F = \frac{R^2/k}{(1-R^2)/(n-k-1)}$$

where R^2 is the proportion of phenotype that can be explained by the genetic information, k is the number of instruments used in the model, and n is the sample size. A F-statistics $>$10 indicates the suitability of IVs, namely, meeting the first assumption of MR analysis (Sanderson et al., 2021).

2.4. Mendelian randomization analysis

As displayed in Fig. 1B, we constructed a flowchart to conduct MR analysis step by step. First, we harmonized the GWAS summary data of NAFLD and of BMD using the selected IVs as matching index. Second, we used Cochran's Q test in inverse variance weighted (IVW) method to detect the between-SNP heterogeneity. Third, we used MR-Egger regression to test the horizontal pleiotropy. We selected the primary MR method as follows:

(1) if neither horizontal pleiotropy nor heterogeneity was detected, use fixed-effect IVW.

(2) if no horizontal pleiotropy but heterogeneity, use weighted median method (Verbanck et al., 2018).

(3) if horizontal pleiotropy was detected, use MR-Egger regression (Burgess and Thompson, 2017).

The above procedure was performed separately for BMD that measured in different populations (i.e., the whole population, people aged 0–15 years, 15–30 years, 30–45 years, 45–60 years, and $\geq$ 60 years). We calculated the statistical power for MR analyses using mRnd website (https://shiny.cnsgenomics.com/mRnd/) (Brion et al., 2013). We also performed a leave-one-out analysis to identify outliers. To further validate the MR estimates, we used genetic IVs of NAFLD that retrieved from GWAS of Anstee et al. and Ghodsian et al. to repeat the MR analysis procedure (Supplementary Tables S1–2).

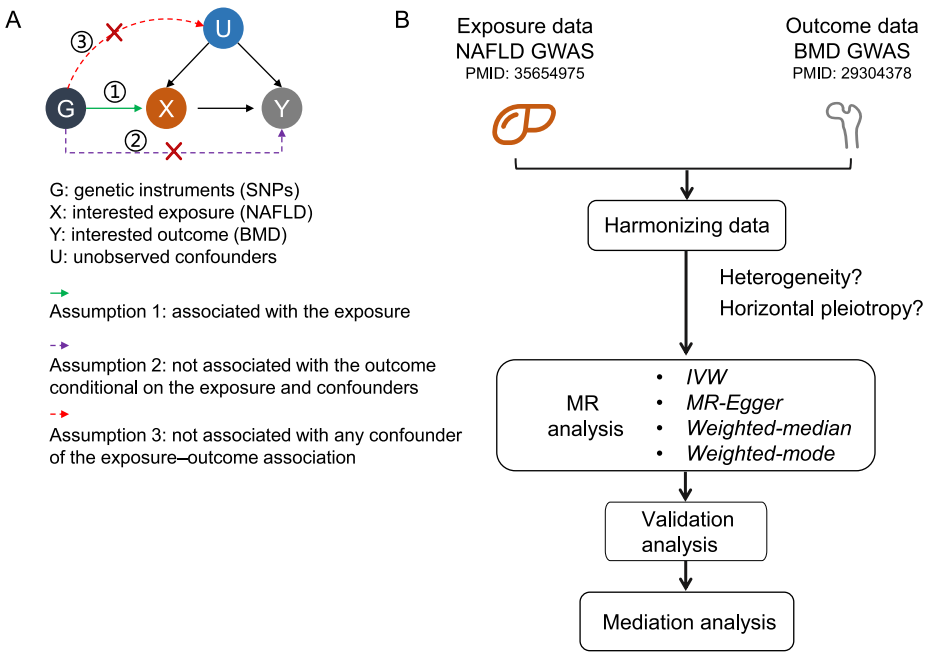

Fig. 1. Study flowchart. A, the schematic plot of Mendelian randomization analysis; B, procedure for performing Mendelian randomization analysis in this study.

Table 1
Instrumental variables for NAFLD.

SNP	CHR	Position(hg37)	Effect allele	Other allele	EAF	Beta	SE	*P* value
rs1497406	1	16,505,320	A	G	0.426	−0.0420	0.0074	1.46E-08
rs79598313	1	27,284,913	T	C	0.0225	0.1796	0.0247	3.37E-13
rs74816838	1	161,643,560	T	C	0.1116	0.0871	0.0128	1.09E-11
rs1337101	1	219,726,100	T	G	0.3000	−0.0509	0.0080	1.95E-10
rs2642438	1	220,970,028	A	G	0.2946	−0.0786	0.0080	8.04E-23
rs848559	2	36,694,497	T	A	0.1509	−0.0610	0.0106	7.59E-09
rs13409360	2	113,838,102	A	G	0.3971	−0.0601	0.0076	1.95E-15
rs6717858	2	165,539,661	C	T	0.3995	−0.0506	0.0075	2.04E-11
rs73024760	2	169,885,122	T	C	0.0375	0.1073	0.0196	4.64E-08
rs2138157	2	227,103,717	A	C	0.3532	−0.0643	0.0076	3.49E-17
rs4684847	3	12,386,337	T	C	0.1226	−0.0716	0.0111	1.11E-10
rs9833411	3	142,640,398	A	T	0.3569	−0.0478	0.0078	1.08E-09
rs71633358	4	88,183,817	C	T	0.2807	−0.0902	0.0085	1.89E-26
rs4734654	8	103,669,991	G	A	0.3620	−0.0517	0.0077	1.48E-11
rs2954038	8	126,507,389	C	A	0.3063	0.1394	0.0079	2.12E-70
rs7041363	9	117,146,043	G	C	0.4909	−0.1351	0.0075	1.12E-71
rs10883451	10	101,924,418	C	T	0.4766	−0.1607	0.0073	3.60E-106
rs2792751	10	113,940,329	T	C	0.2925	0.0721	0.0080	1.28E-19
rs11601507	11	5,701,074	A	C	0.0729	0.0884	0.0139	2.16E-10
rs4919741	12	53,272,920	A	G	0.3439	−0.0570	0.0077	1.72E-13
rs11621792	14	24,871,926	T	C	0.4530	0.0422	0.0074	1.11E-08
rs28929474	14	94,844,947	T	C	0.0178	0.4809	0.0281	1.03E-65
rs55868793	15	73,956,856	G	T	0.4077	0.0591	0.0076	8.92E-15
rs1801689	17	64,210,580	C	A	0.0317	0.1755	0.0206	1.39E-17
rs58542926	19	19,379,549	T	C	0.0749	0.2219	0.0137	2.13E-59
rs429358	19	45,411,941	C	T	0.1386	−0.0943	0.0106	4.72E-19
rs2207132	20	39,142,516	A	G	0.0293	0.1890	0.0291	7.96E-11
rs132665	22	36,564,170	G	A	0.1544	−0.0691	0.0103	1.84E-11
rs738409	22	44,324,727	G	C	0.2281	0.2691	0.0086	2.84E-213

EAF, effect allele frequency; CHR, chromosome; SE, standard error.

We also performed a two-step MR analysis to examine the potential mediating effect of fasting glucose, insulin, glycosylated hemoglobin (HbA1c), low-density cholesterol (LDL), and body-mass index (BMI) on the association between NAFLD and BMD (Carter et al., 2021). The mediating factors chosen for our analysis were selected due to their established associations with both NAFLD and bone health (Ensrud, 2017; Younossi et al., 2018). In this analysis, the primary technique we employed to determine NAFLD's impact on each mediator was IVW or the weighted-median method. To calculate the indirect effect, we utilized the "product of coefficients" strategy. In simpler terms, we first determined the causal relationship of NAFLD to potential mediators by using SNPs (IV1) for genetic prediction of NAFLD. Following that, we used SNPs (IV2) specific to potential mediators for their genetic prediction and to deduce their causal influence on BMD. It's worth noting that the SNP sets IV1 and IV2 are distinct. The GWAS summary data of the mediators are shown in Supplementary Table S3.

False discovery rate (FDR) were used to correct multiple testing bias. An FDR < 0.05 indicated statistical significance and supported strong

evidence of a causal relationship. Associations with $P < 0.05$ but FDR > 0.05 were regarded as suggestive evidence of association. All statistical analyses were implemented using R program (v 4.1.1). *TwoSampleMR* package (v 0.5.6) was used for MR analysis.

3. Results

3.1. Instrumental variables for NAFLD

In the primary analysis, we utilized 29 SNPs as IVs for NAFLD, with mean F-statistics ranging from 139.8 to 175.6 across different analysis scenarios, indicating a low probability of weak instrument bias. Significant between-SNP heterogeneity was only observed in the MR analysis of BMD measured in all age groups (Table 2). MR-Egger regression intercept test revealed significant horizontal pleiotropy for BMD in individuals aged 15–30 years, but not in the whole population or other subpopulations (Table 2). The current IVs provided sufficient statistical power (> 90 %) to detect minor (effect size [i.e., beta coefficient] between 0.01 and 0.05 or between −0.01 and − 0.05) or moderate (effect size >0.05 or < −0.05) associations between NAFLD and BMD (Table 2).

3.2. Association between NAFLD and BMD

The IVW-method suggested that genetically predicted NAFLD was significantly associated with a low level of tobal body BMD ($\beta = -0.04$, 95 % CI -0.07, −0.02, $P = 0.002$, FDR = 0.010) (Fig. 2). This inverse association was more evident among people aged over 60 years ($\beta = -0.06$, 95 % CI -0.11, −0.02, $P = 0.006$, FDR = 0.030). We also detected an inverse association between genetically predicted NAFLD and BMD in other subpopulations, although the effect sizes were either statistically non-significant or suggestive (Fig. 2). For example, in people aged 30–45 years, IVW-method revealed that genetically predicted NAFLD was negatively associated with BMD, with an estimate of β of −0.07 (95 % CI -0.14, 0, $P = 0.048$, FDR = 0.095). MR estimates from other three methods (i.e., weighted-median, weighted-mode, and MR-Egger regression) were consistent with that of the IVW-method in directions, although the estimates were statistically non-significant (Figs. 2 and Supplementary Fig. S1). Leave-one-out analysis did not detect any significant outliers (Supplementary Fig. S2).

3.3. Validation analyses

Only four SNPs were selected as the IVs for NAFLD accoridng to Anstee et al.'s and Ghodsian et al.'s study, respectively (Supplementary Tables S1–2). Based on the IVs of biopsy-determined NAFLD, the IVW-method suggested a significant inverse association between NAFLD and BMD in the entire population ($\beta = -0.021$, $P = 0.016$) (Supplementary Table S4). However, no significant associations were detected in other MR analyses. MR analyses on site-specific BMD also suggested negative associations between genetically-predicted NAFLD and BMD, although the MR estimates were not statistically significant (Supplementary Table S5). We detected a significantly positive association between NAFLD and the risk of osteoporosis, which was consistent acorss different MR approaches and GWAS datasets (Supplementary Table S6).

3.4. Mediation analysis

We discovered a significant positive association between NAFLD and all five mediators, with the effect sizes ranging from 0.15 for insulin to 0.74 for BMI (refer to Supplementary Table S7). However, a significant negative association was only observed between three diabetes-related biomarkers and BMD. The two-step MR analysis indicated that fasting glucose, insulin, and HbA1c mediated 25.4 % (95 % CI 17.6–31.5 %), 18.9 % (12.0–24.9 %), and 27.9 % (19.9–36.7 %) of the association between NAFLD and BMD, respectively (Supplementary Table S7).

4. Discussion

The present study aimed to investigate the potential causal relationship between NAFLD and BMD using MR analysis. The results of our analysis revealed a significant negative causal association between NAFLD and BMD, particularly in individuals aged over 60 years. These findings add to the existing body of literature indicating a detrimental impact of NAFLD on bone health.

The observed negative association between NAFLD and BMD in our study is consistent with previous epidemiological studies, most of which have consistently reported an inverse relationship between NAFLD and BMD (Xie and Liu, 2022; Lee et al., 2016; Lee et al., 2018). However, there were also observational studies that reported a null or even a positive association between NAFLD and BMD (Ciardullo et al., 2021; Li et al., 2022; Sung et al., 2020). The inconsistency in observational studies might be ascribed to many reasons, including samll sample size, different measurements for NAFLD and BMD, and underadjustment or overadjustment for confounders. In this case, MR analysis leveraging genetic information that are exempt from envrionmental confounders is a good complement for observational studies. Thus, the findings of previous epidemiological studies, along with our MR analysis, strengthen the evidence supporting the negative association between NAFLD and BMD.

Our findings were largely consistent with previous MR studies, some of which used data similar to ours (Liu et al., 2024; Huang et al., 2023; Zhou et al., 2023; Cui et al., 2023; Pei et al., 2024). However, unlike previous MR analyses, we expanded our analyses to include individuals of different ages and validated our main findings using different data sources. These analyses further strengthen the evidence for the detrimental effect of NAFLD on bone health. Several plausible explanations

Table 2
Statistics of Mendelian randomization analysis for NAFLD and total body bone mineral density.

Exposures	Outcomes	No. of IV	F-statistics	Between-SNP heterogeneity		Horizontal pleiotropy		Statistical power to detect effect size < −0.05 or > 0.05 (%)	Statistical power to detect effect size between 0.01 and 0.05 or between −0.01 and − 0.05(%)
				Q-value	P value	Egger-intercept	P value		
NAFLD	BMD (all ages)	28	175.6	53.1	**0.002**	0.0038	0.273	97	90
	BMD (0–15 years)	26	139.8	32.9	0.133	0.0031	0.634	97	90
	BMD (15–30 years)	26	139.8	36.4	0.066	0.0252	**0.027**	97	90
	BMD (30–45 years)	26	139.8	27.1	0.349	0.0075	0.256	97	90
	BMD (45–60 years)	28	175.6	35.9	0.118	0.0041	0.443	97	90
	BMD (over 60 years)	28	175.6	34.3	0.159	−0.0002	0.965	97	90

NAFLD, nonalcoholic fatty liver disease; BMD, body mineral density.

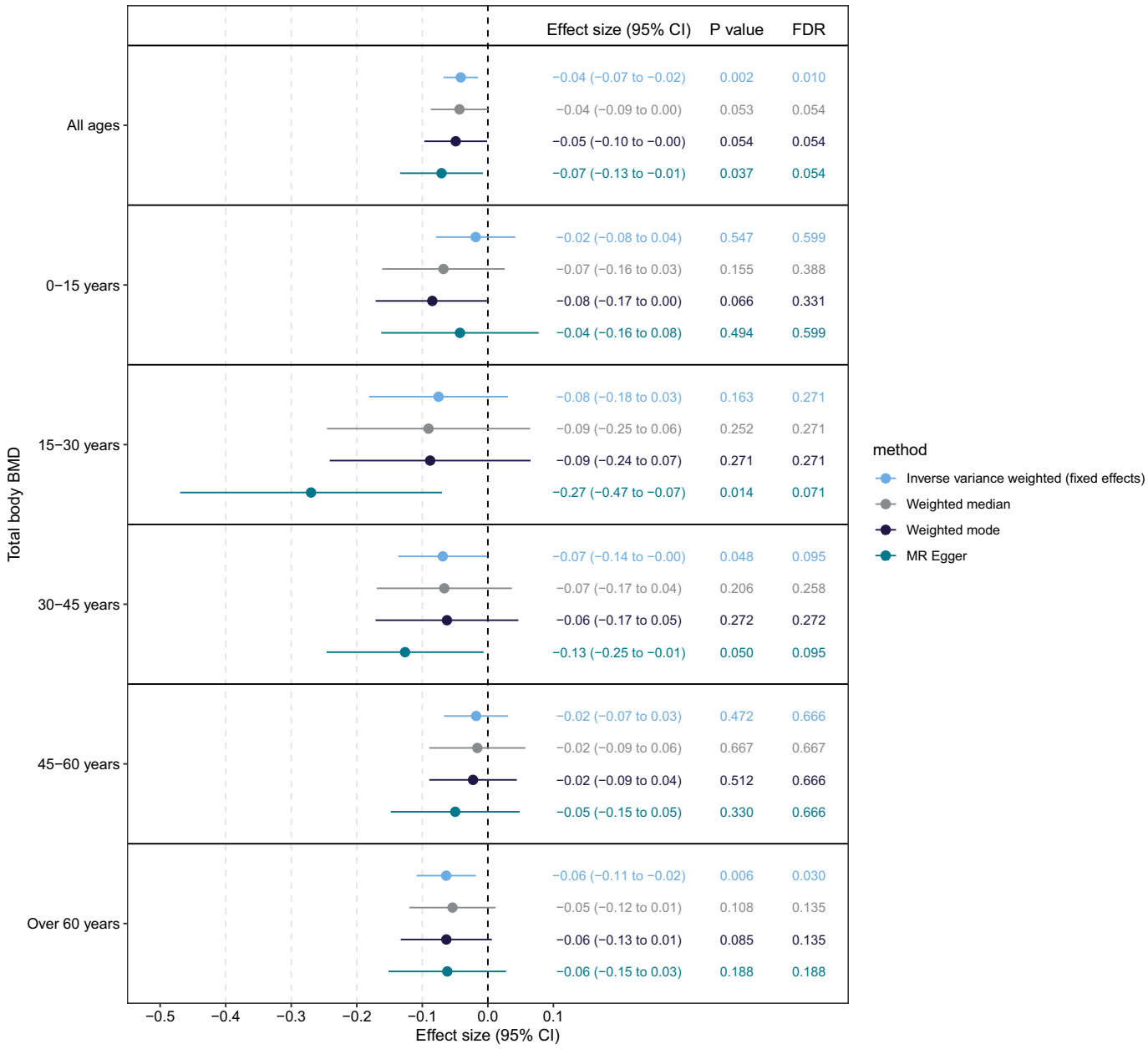

Fig. 2. The association between nonalcoholic fatty liver disease and bone mineral density according to Mendelian randomization analysis.

can be proposed to elucidate the underlying mechanisms of the negative association between NAFLD and BMD, although the full mechanisms have not been elucidated. NAFLD is frequently coexisted with metabolic abnormalities (Marchesini et al., 2005), such as obesity, insulin resistance, and dyslipidemia, which may contribute to disturbances in bone metabolism and ultimately result in reduced BMD (Sun et al., 2019; Targher et al., 2015). Obesity, for instance, is known to exert mechanical stress on bones, leading to increased bone resorption and decreased bone formation (Hou et al., 2020). Insulin resistance and dyslipidemia may also disrupt the delicate balance between bone resorption and formation, thereby affecting bone health (Zhou et al., 2021).

Furthermore, chronic inflammation and oxidative stress associated with NAFLD might negatively impact bone health (Filip et al., 2018). Inflammation can increase the production of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), which have been shown to stimulate bone resorption and inhibit bone formation (Amarasekara et al., 2015). Oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) production and antioxidant defenses, can also impair bone remodeling and lead to decreased BMD (Bonaccorsi et al., 2018). These inflammatory and oxidative processes, commonly observed in NAFLD (Monserrat-Mesquida et al., 2020), could contribute to the observed negative association with BMD. Another potential mechanism linking NAFLD and decreased BMD is alterations in adipokine secretion. In NAFLD, adipokines, such as adiponectin and leptin, secretion patterns are often disrupted, which have been proposed to play a role in bone metabolism (Deng and Scherer, 2010). Moreover, liver dysfunction in NAFLD may affect the metabolism of vitamin D, a crucial regulator of calcium homeostasis and bone mineralization (Filip et al., 2018). Vitamin D deficiency has been linked to decreased BMD and increased risk of osteoporosis. Impaired vitamin D metabolism in NAFLD could lead to inadequate calcium absorption and utilization, further compromising bone health.

Interestingly, the mediation analysis underscored the importance of glucose metabolism and its potential role in bone health, particularly in the context of NAFLD (Holloway-Kew et al., 2019; Sheu et al., 2023).

The potential mechanisms behind this could include direct effects of elevated glucose or insulin on bone cells, or the secondary effects of hyperglycemia and hyperinsulinemia on other factors influencing bone health (Sheu et al., 2023). The strong mediating effect of HbA1c, a long-term marker of glucose control, underscores the possibility that chronic exposure to dysregulated glucose metabolism may be particularly detrimental to bone health. While fasting glucose, insulin, and HbA1c had pronounced mediating effects, no significant mediation was observed for BMI and LDL. This finding is somewhat counterintuitive, given the established links between obesity (often measured by BMI) and both NAFLD and bone health (Fassio et al., 2018; Turcotte et al., 2021). It suggests that the relationship between NAFLD and BMD may not be directly driven by adiposity or cholesterol dysregulation, at least as captured by these metrics in our study. The absence of a significant mediating effect of LDL hints that lipid metabolism might not play a central role in linking NAFLD to bone mineral density. By selecting BMI, insulin, glucose, LDL, and HbA1c as mediators, our study aims to capture critical aspects of the metabolic interplay between NAFLD and bone health. These factors are well-documented in the literature for their roles in both conditions, and their inclusion allows for a robust analysis of the mechanisms underlying this relationship. Future research should continue to explore additional mediators, such as inflammatory markers, vitamin D levels, and lifestyle factors, to further elucidate the complex interactions at play.

Despite the significant findings, several limitations should be acknowledged. Firstly, the use of GWAS summary data from different studies for NAFLD and BMD introduces potential heterogeneity and bias. For example, the NAFLD GWAS was performed in adults without age limitation, whereas the BMD GWAS for subpopulations were performed in age-specific populations. Although efforts were made to select appropriate instrumental variables for MR analysis, there may still be unaccounted confounders or pleiotropic effects that could affect the results. Therefore, caution should be exercised in interpreting the causality inferred from the MR analysis. Additionally, while our previous focus was on total body BMD, this study did not find significant negative associations between NAFLD and site-specific BMD. Future investigations should consider evaluating the association between NAFLD and site-specific BMD measures to better understand the impact on different regions of the skeleton. Finally, while our study provides valuable insights into the relationship between NAFLD and BMD in a European population, we recognize that these findings may not be directly applicable to other ethnic groups. Ethnic variations in genetic, environmental, and lifestyle factors can significantly influence both NAFLD and bone health outcomes.

In conclusion, our MR analysis provides evidence supporting a causal negative association between NAFLD and BMD, indicating that NAFLD may have detrimental effects on bone health. Shared risk factors, including obesity, insulin resistance, and dyslipidemia, as well as underlying mechanisms such as chronic inflammation, oxidative stress, alterations in adipokine secretion, and impaired vitamin D metabolism, may contribute to this association. These findings highlight the importance of considering bone health in individuals with NAFLD and suggest the need for further research to validate these findings and investigate the underlying mechanisms. Understanding the interplay between NAFLD and bone health is crucial for developing preventive strategies and interventions to mitigate bone loss in this population.

Abbreviations

NAFLD	nonalcoholic fatty liver disease
BMD	bone mineral density
MR	Mendelian randomization
IVW	inverse variance weighted model
GWAS	genome-wide association study

Declaration of competing interest

Minzhe Zheng, Junxiang Xu, and Zongxian Feng declare that they have no conflict of interest.

Data availability

The GWAS summary data used in this study were available on GWAS-Catalog (details see Method section).

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CReDiT authorship contribution statement

Study conception: MZ; Data analyses: MZ and JX; Data illustration: MZ and JX; Manuscript draft: MZ; Manuscript revision: JX and ZF. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

The data used in this study were retrieved from previous studies. Therefore, ethical approval was not required.

Consent for publication

Not applicable.

Publisher's note

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bonr.2024.101785.

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