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Unraveling the role of serum metabolites in the relationship between plant-based diets and bone health in community-dwelling older adults

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ABSTRACT

The potential adverse effects of the plant-based dietary pattern on bone health have received widespread attention. However, the biological mechanisms underlying the adverse effects of plant-based diets on bone health remain incompletely understood. The objective of this study was to identify potential biomarkers between plant-based diets and bone loss utilizing metabolomic techniques in the Taizhou Imaging Study (TIS) (N = 788). Plant-based diet indexes (overall plant-based diet index (PDI), healthy plant-based diet index (hPDI), and unhealthy plant-based diet index (uPDI)) were calculated using the food frequency questionnaire, and bone mineral density (BMD) was measured using dual-energy X-ray absorptiometry. A multinomial logistic regression was used to explore the associations of plant-based diet indexes with bone loss. Furthermore, mediation analysis and exploratory factor analysis (EFA) were performed to explore the mediated effects of metabolites on the association of plant-based diets with BMD T-score. Our results showed that higher hPDI and uPDI were positively associated with bone loss. Moreover, nineteen metabolites were significantly associated with BMD T-score, among them, seven metabolites were associated with uPDI. Except for cholesterol esters in VLDL-1, the remaining six metabolites significantly mediated the negative association between uPDI and BMD T-score. Interestingly, we observed that the same six metabolites mediated the positive association between fresh fruit and BMD T-score. Collectively, our results support the deleterious effects of plant-based diets on bone health and discover the potential mediation effect of metabolites on the association of plant-based diets with bone loss. The findings offer valuable insights that could optimize dietary recommendations and interventions, contributing to alleviate the potential adverse effects associated with plant-based diets.

1. Introduction

Osteoporosis is a prevalent metabolic bone disorder accompanied by bone mass reduction and compromised bone microstructure, consequently elevating the susceptibility to fragility fractures (Compston et al., 2019; US Preventive Services Task Force et al., 2018). Recently, based on a large-scale multicenter survey, it has been reported that the prevalence of osteoporosis in individuals aged over 50 in China

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is 6.46 % for males and 29.13 % for females, leading to a heavy medical and economic burden (J. Wang et al., 2023). As the accelerated aging in China, the prevalence of osteoporosis is expected to further rise (L. Wang et al., 2021). Thus, the prevention of osteoporosis has emerged as a critical concern in public health. As a chronic disease, osteoporosis requires long-term management and treatment (Cotts and Cifu, 2018). However, the long duration of medication and concerns regarding side effects often lead to reduced patient compliance (Schattner, 2018). Given this context, prioritizing dietary factors, which represent easily modifiable risk factors, may present a more suitable approach to preventing and managing the disease.

In recent years, the popularity of a plant-based diet has been steadily increasing due to its potential health benefits and environmental sustainability aspects (Heianza et al., 2021; J. Kim et al., 2021; Thompson et al., 2023). The defining feature of this dietary pattern lies in its emphasis on increased consumption of plant-based foods while reducing the intake of animal-based foods (H. Li et al., 2022). While there is accumulating evidence of the promising potential of plant-based diets in reducing the risk of chronic diseases (e.g., cardiovascular diseases, type 2 diabetes), concerns persist regarding their impact on bone health (H. Kim et al., 2019; Satija et al., 2016). Several studies have suggested that adherence to a plant-based diet, particularly a strict vegetarian diet, may increase the risk of osteoporosis (Iguacel et al., 2019; Li et al., 2021a). This phenomenon may be associated with insufficient consumption of essential nutrients, such as calcium, vitamin D, vitamin B-12, protein, and n-3 fatty acids (Key et al., 2022; Muñoz-Garach et al., 2020). However, focusing solely on a macronutrient perspective is insufficient to gain a comprehensive understanding of the intricate mechanisms through which plant-based diets impact BMD. These mechanisms are likely to be closely connected to bone metabolism and the body's internal environment.

Metabolomics, as a fast-growing technology, has significantly contributed to advancing nutritional research by investigating diet-induced alterations in metabolic profiles (Wang et al., 2022b). Indeed, different dietary patterns (e.g., plant-based diet, DASH diet, and Western and prudent diets) have been demonstrated to exert a considerable influence on the concentrations of diverse metabolites, including but not limited to fatty acids, amino acids, and lipids (Chandler et al., 2020; Rebholz et al., 2018; Wang et al., 2022). These observed metabolic variations offer valuable insights for identifying potential target pathways for further exploration. Additionally, there was also a significant correlation between lipids and amino acids and BMD. A cross-sectional study of the U.S. population found that five lipids and seven amino acids were significantly associated with bone loss (Zhao et al., 2018). Moreover, Niu et al. showed an inverse correlation between HDL-C and BMD (Niu et al., 2021). These findings emphasize the importance of metabolites in influencing BMD.

Taken together, there is likely a strong relationship between plant-based diet, metabolites, and BMD. Nevertheless, the specific metabolites that mediate the association between plant-based diet and BMD remain to be determined. To address this gap, a cross-sectional study was conducted on a community-based population, employing the nuclear magnetic resonance platform for metabolomic profiling. We sought to identify metabolites associated with a plant-based diet and BMD in a Chinese elderly population. Furthermore, we aimed to evaluate the potential mediating effects of the identified metabolites on the association between plant-based diet and BMD. The present study may provide novel insights into the underlying biological processes linking a plant-based diet with bone loss.

2. Materials and methods

2.1. Study design and participants

The Taizhou Imaging Study is an ongoing cohort embedded in the Taizhou Longitudinal Study (Jiang et al., 2021). The primary objective of the TIS is to assess both risk factors and potential biomarkers associated with chronic diseases in the elderly Chinese population. This study adopts a cross-sectional design, and initially enrolled 904 individuals aged 55–65 years without a history of stroke, cancer, or other serious illnesses. Among them, 788 individuals had complete data on dietary intake, BMD, and metabolome, and they had no recorded history of fracture, thyroid disease, or non-menopausal status. The exclusion of individuals with a history of fractures, thyroid disease, and non-menopausal status to minimize potential bias introduced by confounding factors that could affect bone health. This is because previous skeletal injuries, hormonal imbalances related to thyroid disease, and changes in estrogen levels can affect bone metabolism. The criteria and process of exclusion are detailed in Fig. 1. All participants included in the TIS gave their written informed consent. The TIS was granted ethical approval by the Committee of the School of Life Sciences, Fudan University, and Fudan University Taizhou Institute of Health Sciences (institutional review board approval numbers 496 and B017, respectively).

2.2. Bone mineral density assessment

A dual-energy X-ray absorptiometry (Lunar DPX NT-400157; GE Healthcare, Madison, WI, United States) was employed for the assessment of BMD at the lumbar spine (L1-L4), total hip, and the femoral neck (Lv et al., 2022). BMD is measured by an experienced physician using the same instrument, and strictly following the manufacturer's guidelines for equipment usage (Jiang et al., 2018). According to the lowest BMD T-score among the three measurement sites, the 788 participants were categorized into three groups: normal (BMD T-score > -1 SD), osteopenia (-2.5 SD $<$ BMD T-score ≤ -1 SD), and osteoporosis (BMD T-score ≤ -2.5 SD) (Kanis et al., 1994). Similarly, the BMD T-score used in the subsequent analyses was the lowest BMD T-score for the three sites.

2.3. Plant-based diet index

Dietary consumption data was obtained from a standard food frequency questionnaire to reflect the dietary habits of the participants in the year leading up to the survey. In the present study, 12 individual food items were employed to evaluate the plant-based dietary pattern. The plant-based diet index was proposed and developed by Satija et al. They discerned distinctions between healthy and unhealthy plant-based foods based on existing knowledge regarding the association of these foods with various chronic diseases (cardiovascular disease, diabetes, cancer, etc.) and intermediate states (obesity, hypertension, lipids, inflammation) (Satija et al., 2016). Furthermore, given the high consumption frequency of preserved plant food within the Chinese elderly population and the substantiated adverse health effects observed in numerous studies, we considered the incorporation of this food group into the category of unhealthy plant-based foods (Ma et al., 2022; Yoo et al., 2020). Specifically, we classified the 12 individual food items into three groups, including healthy plant-based foods (whole grains, fresh fruits, fresh vegetables, legumes), unhealthy plant-based foods (refined grains, sweets, and preserved plant food), and animal-based foods (dairy products, eggs, seafood, meat, and processed animal food). In accordance with the dietary pattern grading system established by Satija et al., we assigned either positive or reverse scores to each food item (Satija et al., 2016). Positive scores of 5 were assigned to the highest quintile of intake, while a score of 1 was designated for the lowest quintile. Conversely, reverse scores of 5 were allocated to the lowest quintile of intake, with a score of 1 attributed to the highest quintile. The detailed scoring rules (positive or reverse scoring) for different food items are shown in Supplementary Table 1. Ultimately, the three plant-based diet indexes (PDI, hPDI, and uPDI) were derived through the summation of scores from 12 food groups for each participant.

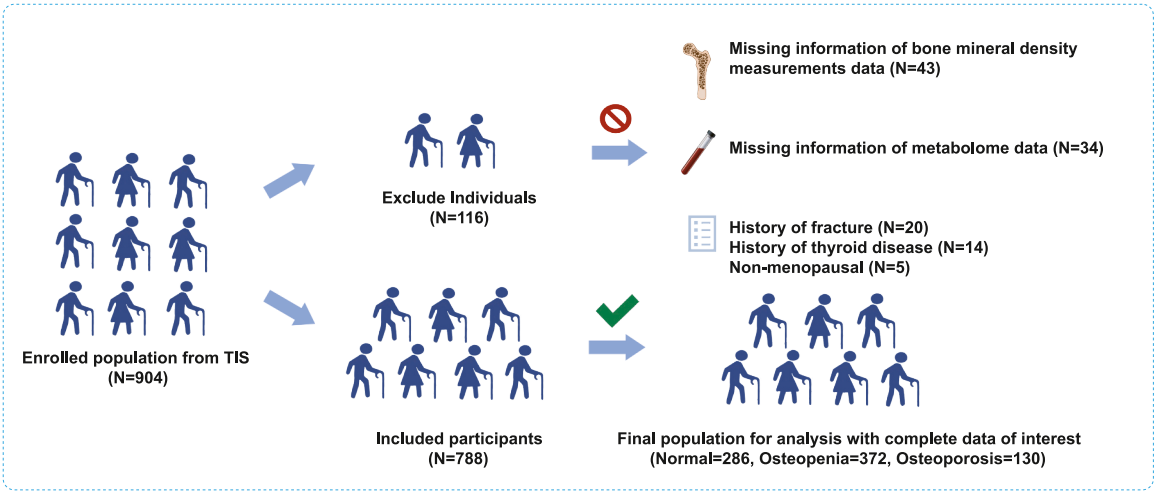

Fig. 1. Flow diagram of participant inclusion and exclusion. Abbreviations: TIS, Taizhou Imaging Study.

2.4. Metabolome information

We collected venous blood samples from these participants for metabolomics analysis. Non-targeted serum metabolomic analysis was conducted using a high-throughput nuclear magnetic resonance platform, which enabled the generation of absolute concentrations and calculated ratios for approximately 350 metabolic features, including lipids, lipoproteins, amino acids, and other small molecule metabolites (Jiang et al., 2021). The detections followed a standardized procedure and were obtained using parameters consistent with prior studies (Jiang et al., 2021; Jiménez et al., 2018). Each sample underwent acquisition of three 1D spectra: the NOESYPR1D sequence, the Carr-Purcell-Meibom-Gill sequence, and the diffusion-edited spectrum. Furthermore, pooled samples were used to gather a set of 2D spectra for spectral assignment. Subsequently, all spectra underwent manual baseline and phase correction using TOPSPIN (v3.6.0, Bruker Biospin, Germany). In the present study, exclusively absolute quantitative metabolic concentrations were employed for the analysis. Moreover, metabolites that met any of the following criteria were excluded from the analysis: (i) metabolite data with 20 % or more missing values; (ii) insufficient serum sample volume; (iii) serum sample with severe chylemia. Half of the minimum detection value was imputed for metabolites with missing values of less than 20 %. Finally, 181 absolute quantitative metabolic features were inverse-normally transformed to standardize Z-scores for subsequent analyses. See Supplementary Table 2 for the full names of the metabolites.

2.5. Covariates

A detailed questionnaire, administered by interviewers, was employed for the acquisition of demographics (age and sex) and lifestyle factors (smoking status, alcohol consumption, tea habits, and physical activity). Smoking was characterized as current tobacco use for at least six months prior to the survey. Alcohol consumption was considered to be drinking at least once weekly, while tea consumption was considered to be drinking at least one cup daily, both in the six months prior to the survey. Physical activity was defined as at least 1–3 times per month or more. Waist-to-Hip Ratio (WHR) was calculated as the waist circumferences divided by hip circumferences. The diagnostic criteria for hypertension included systolic blood pressure $\geq$140 mmHg and/or diastolic blood pressure $\geq$90 mmHg, self-reported hypertension, or current use of antihypertensive medications. For diabetes diagnosis, we considered a fasting blood glucose level of $\geq$7.0 mmol/L, self-reported diabetes, or current use of antidiabetic medications or insulin. Regarding hyperlipidemia, the diagnostic criteria encompassed a total cholesterol level $\geq$5.2 mmol/L and/or triglycerides $\geq$1.7 mmol/L, self-reported hyperlipidemia, or current use of lipid-lowering medications.

2.6. Statistical analysis

In the present study, continuous variables were expressed as mean (standard deviation), and group distinctions were assessed through analysis of variance, while categorical variables were exhibited as frequency (percentage) and compared using the chi-square test. Three models of multinomial logistic regression were used to estimate the odds ratios (ORs) and 95 % confidence intervals (95 % CIs) for the associations between three plant-based diet indexes and bone loss. Model 1 was adjusted for age and sex, Model 2 was further adjusted for smoking status, alcohol consumption, tea consumption, and physical activity, and Model 3 was further adjusted for diabetes, hypertension, and hyperlipidemia. Furthermore, linear regression was applied to assess the relationship between metabolites and plant-based diet indexes and BMD T-score. The false-discovery rate (FDR) correction was employed to adjust for multiple testing within the regression models. Moreover, EFA (maximum likelihood method) was conducted to decrease the dimensionality of the metabolites, revealing metabolite profiles and aiding interpretation. The potential factors were selected depending on the breakpoint of the scree plot and their capacity for interpretation. A varimax rotation was performed on the factor loading matrices, with the obtained factor loadings reflecting the relationship between each metabolite and the relevant potential factor. Finally, the potential mediating effects of single and mixed metabolites (presented as EFA-metabolites) on the associations of plant-based diet indexes with BMD T-score were evaluated by applying mediation models.

We also performed several sensitivity analyses to ensure the robustness of the analysis results. Considering the impact of dietary supplements on BMD, we also adjusted for them in the models as potential confounders, including vitamins, calcium, iron, and zinc. Furthermore, an assessment of sensitivity was conducted by applying the R package “medsens” to evaluate the robustness of the mediation effect and to examine any potential breach of the assumption of references (Smith and VanderWeele, 2019).

The R software (version 4.2.0) was used for all statistical analyses, with $P < 0.05$ being considered statistically significant for all two-tailed tests.

3. Results

3.1. Sample characteristics

The study included a total of 788 participants from the Taizhou Imaging Study, of whom 372 (47.21 %) were categorized as osteopenia, and 130 (16.50 %) as osteoporosis. The demographic characteristics, lifestyle factors, and medical history of the study population are shown in Table 1. The three groups (normal BMD, osteopenia, and osteoporosis) were significantly different in sex, WHR, smoking status, tea and alcohol consumption, and hypertension ($P < 0.05$).

3.2. Associations between plant-based diet indexes and bone loss

Table 2 displays the associations between the three plant-based diet indexes and bone loss by multiple logistic regression. In model 3, the highest quintile of hPDI (OR = 2.20; 95 % CI: 1.20 to 4.03; P_{trend} = 0.030) and uPDI (OR = 2.19; 95 % CI: 1.18 to 4.07; P_{trend} = 0.006) increased the risk of osteopenia compared to the lowest quintile. In addition, the quantile 5 of uPDI (OR = 2.91; 95 % CI: 1.27 to 6.64; P_{trend} = 0.025) was positively associated with osteoporosis compared to quantile 1. Additionally, we found no significant association between PDI and bone loss in our study. In the sensitivity analysis, we further adjusted for potential confounders, such as the intake of dietary supplements, and the results remained consistent and robust (Supplementary Table 3).

3.3. Associations between metabolites and BMD

We investigated metabolites that differed significantly among the three BMD status groups using ANOVA. Supplementary Table 4 presents 28 significant metabolites between groups (FDR-adjusted $P < 0.05$), comprising 20 lipids (14 high-density lipoprotein (HDL) subfractions and 6 very-low-density lipoprotein (VLDL) subfractions), 5 amino acids, and 3 other small molecule metabolites. Subsequently, we further analyzed the association between these 28 significant metabolites and BMD by linear regression (Fig. 2). We found that 13 HDL subfractions (H–CH, H0PL, H1CH, H2CH, H3CH, H3PL, H2A1, H0CE, H1CE, H2CE, H3CE, H0LP, and H3LP) were negatively associated with BMD, while 5 VLDL subfractions (V1CH, V1FC, V1PL, V1CE, V1LP) and an amino acid (valine) were positively associated with BMD (FDR-adjusted $P < 0.05$).

3.4. Associations between plant-based diet indexes and metabolites

We further examined the association between these 19 metabolites and two plant-based diet indexes (continuous variable). Among these 19 metabolites, seven were significantly associated with uPDI (3 HDL subfractions, 3 VLDL subfractions, and valine), while none showed a significant association with hPDI (Fig. 3). Specifically, V1CE (β = −0.014; 95 % CI: 0.024 to −0.004), V1CH (β = −0.013; 95 % CI: 0.023 to −0.004), V1FC (β = −0.013; 95 % CI: 0.022 to −0.003) and valine (β = −0.016; 95 % CI: 0.026 to −0.005) had negative associations with uPDI, while H0CE (β = 0.015; 95 % CI: 0.004 to 0.026), H2A1 (β = 0.014; 95 % CI: 0.003 to 0.025) and H–CH (β = 0.013; 95 % CI: 0.003 to 0.024) had positive associations with uPDI (FDR-adjusted $P < 0.05$).

3.5. Mediation analyses

Additionally, mediation analyses were conducted to assess the potential mediational effects of metabolites on the associations of uPDI with BMD T-score (Fig. 4A). For HDL subfractions, the mediated proportion of H0CE, H2A1, and H–CH were 8.80 %, 13.80 %, and 7.65 % on the associations of uPDI with BMD T-score respectively ($P < 0.05$). For VLDL subfractions, V1CH and V1FC had significant mediated effects on the associations of uPDI with BMD T-score, and the proportion of mediation was 7.76 % and 9.27 % respectively ($P < 0.05$). Furthermore,

Table 1
Baseline characteristics of the study participants.

Characteristic	Overall	Normal	Osteopenia	Osteoporosis	*P*-value
Participants, n	788	286	372	130	
Demographic characteristics					
Age, years	59.84 (3.15)	59.55 (3.15)	59.98 (3.12)	60.10 (3.21)	0.138
Female, n (%)	437 (55.5)	96 (33.6)	226 (60.8)	115 (88.5)	<**0.001**
WHR, mean (SD)	0.91 (0.06)	0.92 (0.07)	0.90 (0.06)	0.90 (0.07)	**0.001**
Lifestyle characteristics					
Physical activity, n (%)	123 (15.6)	53 (18.5)	54 (14.5)	16 (12.3)	0.195
Smoker, n (%)	111 (14.1)	58 (20.4)	49 (13.2)	4 (3.1)	<**0.001**
Tea consumption, n (%)	188 (24.1)	103 (36.3)	73 (19.9)	12 (9.2)	<**0.001**
Alcohol consumption, n (%)	224 (28.6)	115 (40.4)	100 (27.0)	9 (7.0)	<**0.001**
History of disease					
History of diabetes, n (%)	80 (10.5)	36 (12.9)	35 (9.8)	9 (7.1)	0.180
History of hypertension, n (%)	398 (53.1)	161 (59.2)	177 (49.9)	60 (48.8)	**0.039**
History of dyslipidemia, n (%)	429 (56.2)	153 (54.8)	209 (58.2)	67 (53.2)	0.530
Plant-based diet index					
hPDI, n (%)					**0.002**
Q1 (26–36)	213 (27.0)	93 (32.5)	95 (25.5)	25 (19.2)	
Q2 (37–38)	128 (16.2)	46 (16.1)	61 (16.4)	21 (16.2)	
Q3 (39–41)	188 (23.9)	76 (26.6)	82 (22.0)	30 (23.1)	
Q4 (42–43)	135 (17.1)	47 (16.4)	61 (16.4)	27 (20.8)	
Q5 (44–50)	124 (15.7)	24 (8.4)	73 (19.6)	27 (20.8)	
PDI, n (%)					**0.007**
Q1 (22–32)	182 (23.1)	75 (26.2)	86 (23.1)	21 (16.2)	
Q2 (33–34)	153 (19.4)	71 (24.8)	63 (16.9)	19 (14.6)	
Q3 (35–36)	143 (18.1)	48 (16.8)	66 (17.7)	29 (22.3)	
Q4 (37–39)	179 (22.7)	59 (20.6)	85 (22.8)	35 (26.9)	
Q5 (40–49)	131 (16.6)	33 (11.5)	72 (19.4)	26 (20.0)	
uPDI, n (%)					**0.006**
Q1 (17–30)	185 (23.5)	84 (29.4)	76 (20.4)	25 (19.2)	
Q2 (31–34)	167 (21.2)	69 (24.1)	70 (18.8)	28 (21.5)	
Q3 (35–37)	129 (16.4)	38 (13.3)	70 (18.8)	21 (16.2)	
Q4 (38–42)	178 (22.6)	64 (22.4)	87 (23.4)	27 (20.8)	
Q5 (42–55)	129 (16.4)	31 (10.8)	69 (18.5)	29 (22.3)	

Missing rates were 0.06 % for marital status, 7.74 % for WHR, 0.38 % for smoke status, 0.63 % for alcohol consumption, 1.02 % for tea consumption, 3.17 % for history of diabetes, 4.82 % for history of hypertension, 3.05 % for history of dyslipidemia. Group distinctions were assessed through analysis of variance (continuous variables) and the chi-square test (categorical variables). A statistically significant difference was defined as $P < 0.05$ and data with *P*-value below 0.05 are presented in bold type. Abbreviations: WHR, Waist-to-Hip Ratio; PDI, overall plant-based diet index; hPDI, healthy plant-based diet index; uPDI, unhealthy plant-based diet index.

Table 2
ORs and 95 % CIs for bone loss, according to quintiles for hPDI, PDI, and uPDI.

	Osteopenia vs Normal			Osteoporosis vs Normal		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
hPDI						
Q1	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
Q2	1.03 (0.63,1.70)	0.99 (0.58,1.68)	1.03 (0.60,1.76)	1.06 (0.51,2.20)	1.03 (0.47,2.27)	1.06 (0.48,2.34)
Q3	0.92 (0.59,1.43)	0.98 (0.62,1.58)	1.03 (0.64,1.66)	1.04 (0.54,2.02)	1.03 (0.51,2.09)	1.06 (0.52,2.18)
Q4	1.06 (0.64,1.73)	0.95 (0.56,1.61)	1.02 (0.60,1.73)	1.43 (0.71,2.89)	1.31 (0.61,2.79)	1.21 (0.56,2.64)
Q5	2.13 (1.21,3.75) **	2.16 (1.18,3.94) **	2.20 (1.20,4.03) **	2.08 (0.98,4.44)	1.83 (0.82,4.09)	1.83 (0.81,4.10)
P for trend	0.059	0.081	0.030*	0.095	0.237	0.126
PDI						
Q1	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
Q2	0.67 (0.41,1.08)	0.67 (0.40,1.11)	0.69 (0.41,1.17)	0.68 (0.32,1.45)	0.78 (0.34,1.78)	0.79 (0.35,1.80)
Q3	1.01 (0.61,1.66)	1.03 (0.60,1.76)	1.03 (0.60,1.78)	1.49 (0.72,3.09)	1.63 (0.74,3.59)	1.58 (0.72,3.50)
Q4	0.89 (0.55,1.44)	0.81 (0.48,1.35)	0.80 (0.48,1.35)	1.05 (0.52,2.10)	1.03 (0.49,2.19)	0.92 (0.43,1.96)
Q5	1.37 (0.80,2.36)	1.48 (0.84,2.62)	1.46 (0.82,2.59)	1.43 (0.67,3.08)	1.60 (0.71,3.61)	1.45 (0.63,3.32)
P for trend	0.330	0.341	0.079	0.393	0.405	0.102
uPDI						
Q1	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
Q2	1.15 (0.72,1.84)	1.06 (0.64,1.75)	1.00 (0.60,1.67)	1.42 (0.72,2.79)	1.43 (0.69,2.98)	1.44 (0.68,3.05)
Q3	2.10 (1.25,3.53) **	1.81 (1.05,3.10) *	1.90 (1.09,3.29) *	1.96 (0.93,4.15)	1.78 (0.81,3.93)	2.01 (0.89,4.50)
Q4	1.48 (0.93,2.36)	1.27 (0.77,2.08)	1.40 (0.85,2.31)	1.38 (0.70,2.72)	1.10 (0.52,2.33)	1.33 (0.62,2.86)
Q5	2.08 (1.21,3.58) **	2.13 (1.15,3.93) **	2.19 (1.18,4.07) **	2.28 (1.11,4.71) *	2.49 (1.10,5.60) *	2.91 (1.27,6.64) **
P for trend	0.004**	0.018*	0.006**	0.040*	0.092	0.025*

Note: Model 1: adjusted for age and sex; Model 2: Model 1 plus WHR, physical exercise, smoking status, alcohol consumption, and tea consumption; Model 3: Model 2 plus history of diabetes, history of hypertension, and history of dyslipidemia. The median value of each quintile was taken as a continuous variable and entered into the model to obtain *P* for trend. The significance threshold was set at $*P < 0.05$ and $**P < 0.01$; Abbreviations: OR, odds ratio; 95 % CI, 95 % confidence interval; PDI, overall plant-based diet index; hPDI, healthy plant-based diet index; uPDI, unhealthy plant-based diet index.

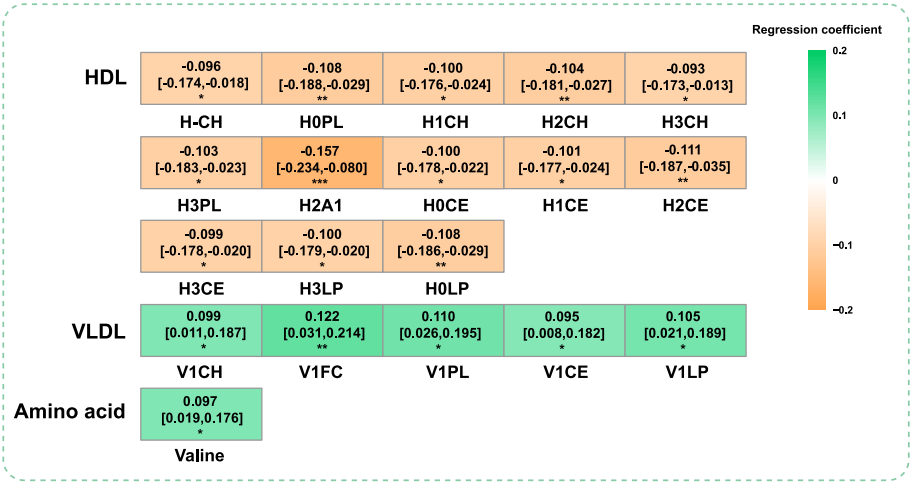

Fig. 2. Multivariate regression for effect of serum metabolites on BMD T-score. The regression coefficient (β) for the respective metabolite along with its associated 95 % CI was displayed in each box. All models were multivariate adjusted for age, sex, WHR, physical exercise, smoking status, alcohol consumption, tea consumption, history of diabetes, history of hypertension, and history of dyslipidemia. The significance threshold was set at $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$. Abbreviations: BMD, bone mineral density; HDL, high-density lipoprotein; VLDL, very-low-density lipoprotein; WHR, waist-to-Hip Ratio; 95 % CI, 95 % confidence interval.

valine mediated the associations of uPDI with BMD T-score with an 8.88 % proportion of mediation ($P < 0.05$). Sensitivity analysis on mediation effects demonstrated the robustness of the aforementioned mediation analysis results to potential unmeasured confounding variables (Supplementary Table 5).

Moreover, we employed EFA to classify these six metabolites showing mediating effects into three distinct groups, explaining 83 % of the total variance (Fig. 4B). The HDL-EFA (explained 43 % of the total variance) exhibited loadings on H–CH, H2A1, and H0CE, while the VLDL-EFA (explained 32 % of the total variance) displayed loadings on V1CH and V1FC. Meanwhile, the Val-EFA (explained 8 % of the total variance) was associated with valine. In addition, the results of the mediation analysis show that HDL-EFA and VLDL-EFA significantly mediated the associations of uPDI with BMD T-score with a mediation percentage of 9.04 % and 8.05 %, respectively ($P < 0.05$) (Fig. 4C).

3.6. Associations between individual food items, metabolites, and BMD

We also conducted analyses to investigate the associations between 12 individual food items with metabolites and the BMD T-score. The linear regression analysis revealed significant positive relationships between fruits (β = 0.37; 95 % CI: 0.12 to 0.61), sweets (β = 0.45; 95 % CI: 0.14 to 0.77), and seafood (β = 0.40; 95 % CI: 0.17 to 0.63) intake with BMD T-score among the 12 individual food items assessed (Supplementary Fig. 1). Additionally, we conducted further analysis to explore the association between these three individual food groups and the six metabolites exhibiting mediating effects. In particular, the highest quintile of fruit consumption (compared to quintile 1) was

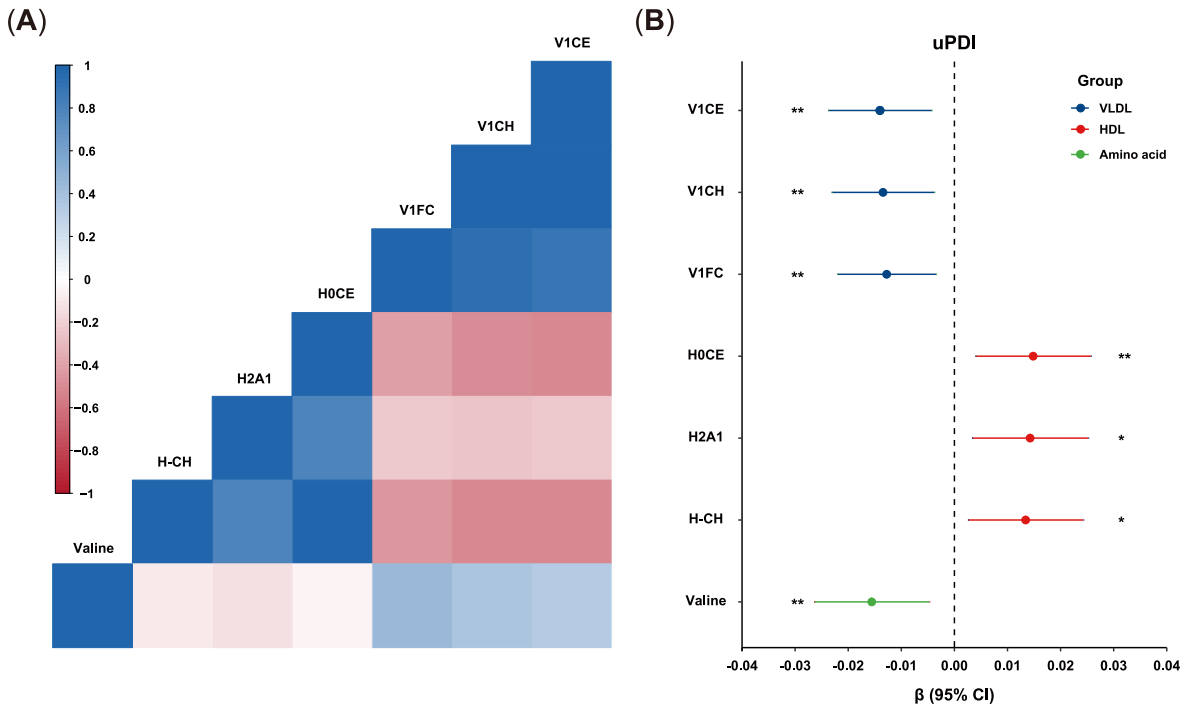

Fig. 3. (A) The correlation between serum metabolites. (B) Multivariate regression for effect of uPDI on serum metabolites. All models were multivariate adjusted for age, sex, WHR, physical exercise, smoking status, alcohol consumption, tea consumption, history of diabetes, history of hypertension, and history of dyslipidemia. Heatmap showing the interrelationship of the seven metabolites. The Benjamini-Hochberg method was used to adjust *P* values for multiple testing. The significance threshold was set at $^{*}P < 0.05$ and $^{**}P < 0.01$. Abbreviations: BMD, bone mineral density; HDL, high-density lipoprotein; uPDI, unhealthy plant-based diet index; VLDL, very-low-density lipoprotein; WHR, waist-to-Hip Ratio; 95 % CI, 95 % confidence interval.

significantly associated with all six metabolites (Supplementary Table 6). More specifically, there was a negative association between fruit consumption and H0CE (β = −0.38; 95 % CI: 0.61 to −0.15), H2A1 (β = −0.32; 95 % CI: 0.55 to −0.09), and H–CH (β = −0.33; 95 % CI: 0.56 to −0.10), whereas there was a positive association with V1CH (β = 0.30; 95 % CI: 0.09 to 0.50), V1FC (β = 0.25; 95 % CI: 0.05 to 0.45) and valine (β = 0.27; 95 % CI: 0.03 to 0.50).

Meanwhile, the results of the mediation analysis showed that the six metabolites (H0CE, H2A1, H–CH, V1CH, V1FC, and valine) mediated the association between fruit consumption and BMD T-score, and the proportion of mediation was 13.67 %, 16.56 %, 11.67 %, 9.63 %, 9.25 %, and 8.03 %, respectively ($P < 0.05$) (Fig. 5A). Similarly, the mediated proportion of the HDL-EFA and VLDL-EFA were 13.60 % and 9.82 % on the associations of uPDI with BMD T-score respectively ($P < 0.05$) (Fig. 5B).

4. Discussion

In this study, we have focused on exploring the intricate relationship between plant-based dietary pattern, serum metabolites, and bone health among the Chinese elderly population. First, the two plant-based diet indexes (hPDI and uPDI) consistently identified a plant-based diet as a risk factor for bone loss. Additionally, six serum metabolites (H0CE, H2A1, H–CH, V1CH, V1FC, and valine) were implicated as mediators for the associations of uPDI with BMD T-score. Meanwhile, these six serum metabolites were also found to significantly mediate the association between fresh fruit intake and BMD T-score.

Our results are consistent with several cohort studies that have suggested a potential detrimental effect of a plant-based dietary pattern on bone health (Ghadiri et al., 2022; Webster et al., 2023; Y. Zheng et al., 2023). In previous investigations, we observed a positive association between hPDI and osteopenia in a US population (NHANES), as well as a positive association between uPDI and osteoporosis (Y. Zheng et al., 2023). Similarly, Ghadiri et al. also found a significant association between higher uPDI and lower BMD in the Iranian population (Ghadiri et al., 2022). Furthermore, a recent prospective study of the UK Biobank population showed that vegetarians exhibited a significantly elevated risk of hip fractures compared to individuals who regularly consumed meat (Webster et al., 2023). To explain the adverse effects of a plant-based dietary pattern, potential mechanisms have been proposed. An investigation based on the Iranian elderly population demonstrated that adherence to a higher uPDI was associated with lower osteocalcin levels (Shahinfar et al., 2021). Osteocalcin plays an active role in intricately regulating the processes of bone remodeling and mineralization, particularly in promoting the maturation and activity of osteoblasts (Komori, 2020). Additionally, from a nutrient intake perspective, individuals strictly following a plant-based dietary pattern typically consume lower amounts of protein, vitamin D, and vitamin B12, all of which are important nutrients for maintaining bone and muscle health (Webster et al., 2022, 2023). Overall, our results contribute additional evidence to support the potentially adverse effects of a plant-based dietary pattern on bone health.

Mechanistically, the focus of our research interests is metabolomics as numerous studies have increasingly emphasized the impact of diet on metabolic processes (Chandler et al., 2020; Liu et al., 2023; Playdon et al., 2017; Rebholz et al., 2018). Our results suggested that adherence to higher uPDI was associated with lower levels of VLDL subfractions, and valine and higher levels of HDL subfractions. In an Iranian population cohort study, a positive association was observed between uPDI and levels of HDL-C (Amini et al., 2021). Moreover, Li et al. found that a plant-based diet was associated with biosynthesis pathways of branched-chain amino acids (L-isoleucine and L-valine) and lipid biosynthesis (Li et al., 2021b). From an individual food group perspective, a higher uPDI indicated a greater intake of preserved plant foods, sweets, and refined grains. This dietary pattern, is characterized by high carbohydrate, sugar, and salt content, may contribute to glucose disorders, lipid and lipoprotein abnormalities, and the initiation of systemic inflammation and oxidative stress (Crimarco et al., 2022; Suliga et al.,

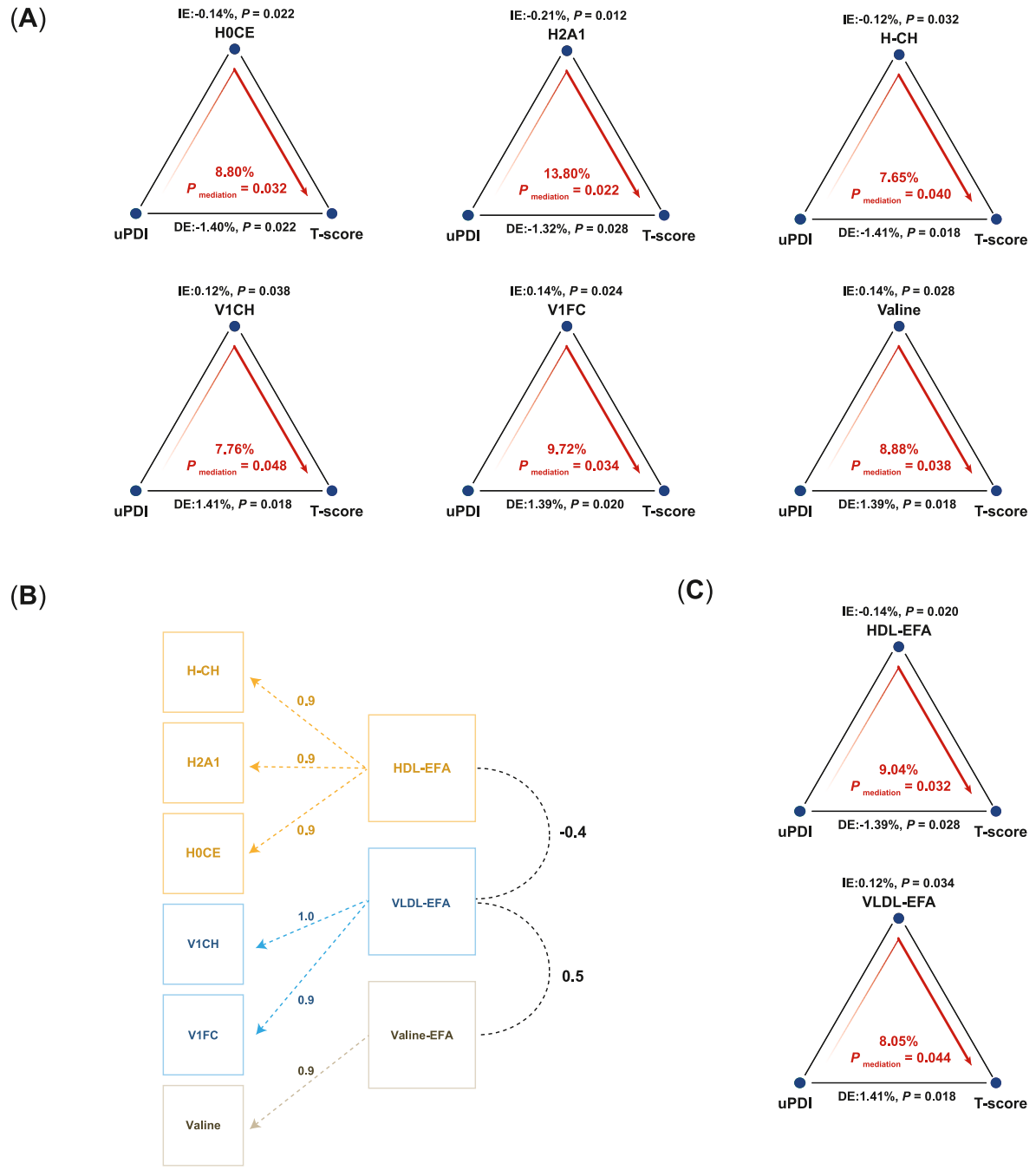

Fig. 4. (A) The mediation effects of the six candidate metabolites on the association between uPDI and BMD T-score. (B) Rotated factor loadings for the six candidate metabolites contributing to each metabolic feature. (C) The mediation effects of the HDL-EFA and VLDL-EFA on the association between uPDI and BMD T-score. Each percentage number in red represents the size of the mediated proportion. The numbers in the EFA represent the strength of association between the two variables. All models were multivariate adjusted for age, sex, WHR, physical exercise, smoking status, alcohol consumption, tea consumption, history of diabetes, history of hypertension, and history of dyslipidemia. The Benjamini-Hochberg method was used to adjust *P* values for multiple testing. Abbreviations: BMD, bone mineral density; DE, direct effect; EFA, exploratory factor analysis; IE, indirect effect; uPDI, unhealthy plant-based diet index; WHR, waist-to-Hip Ratio. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2022).

Alternatively, many studies have highlighted the potential influence of metabolites on BMD regulation. In our study, the metabolites displayed significant associations with BMD were primarily centered around HDL, VLDL, and valine, suggesting the potential influence of metabolites on BMD regulation. Accumulated epidemiological evidence has indicated a significant correlation between elevated levels of HDL and decreased BMD (Maghbooli et al., 2018; Tang et al., 2021; Zhang et al., 2020). It has been suggested that HDL may influence BMD through modulation of sex hormones (Tang et al., 2021; Wang et al., 2022a). Additionally, recent studies have shown that HDL-C can negatively affect osteogenic differentiation by removing oxysterols from peripheral tissues, further suggesting a role in bone health (Y. Yang et al., 2018). Secondly, two studies conducted within a Chinese population have supported the beneficial impact of VLDL on bone health (Lv et al., 2022; You et al., 2014). Moreover, investigations conducted using two separate cohorts (UK Biobank and MrOS) have revealed a positive correlation between valine levels and BMD at the femoral neck (Grahnemo

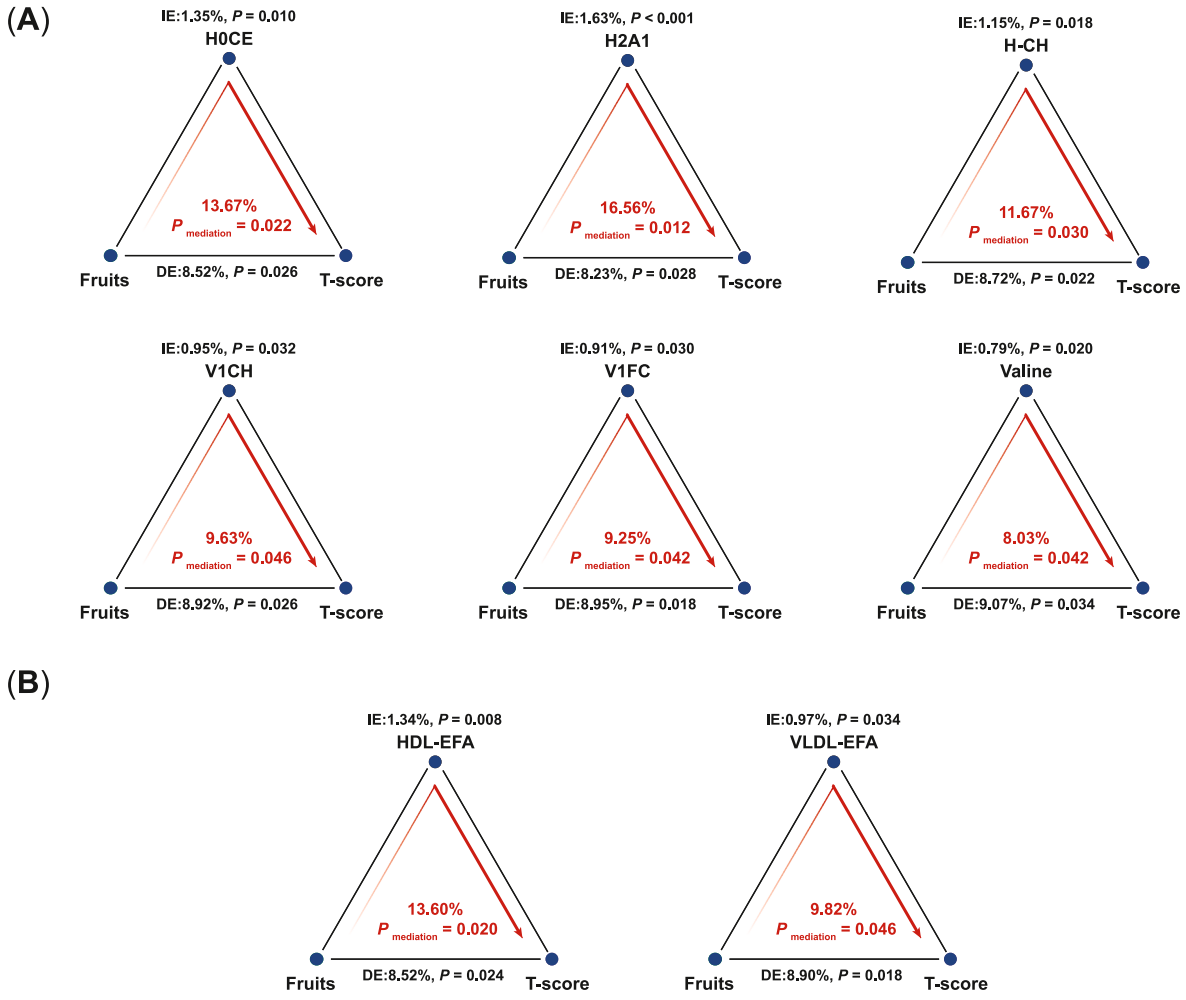

Fig. 5. The mediation effects of (A) the six candidate metabolites, (B) HDL-EFA, and VLDL-EFA on the association between fruit consumption and BMD T-score. Each percentage number in red represents the size of the mediated proportion. All models were multivariate adjusted for age, sex, WHR, physical exercise, smoking status, alcohol consumption, tea consumption, history of diabetes, history of hypertension, and history of dyslipidemia. Abbreviations: BMD, bone mineral density; DE, the estimate of the direct effect; IE, the estimate of the indirect effect; WHR, waist-to-Hip Ratio. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

et al., 2023). Meanwhile, a Mendelian randomization study also identified a significant effect of valine on BMD (Cui et al., 2021). Further investigations have demonstrated that the absence of the L-type amino acid transporter LAT1 in osteoblasts results in heightened activity (Ozaki et al., 2019). Notably, valine, is one of the amino acids transported intracellularly by LAT1, has the capability to activate the mTORC1 target, a pivotal stimulator of bone formation (Grahnemo et al., 2023; Meng et al., 2020). These findings collectively suggest a potentially beneficial impact of these metabolites on bone health.

Based on the aforementioned results, mediation analyses were further conducted, revealing six serum metabolites that significantly mediated the negative association between uPDI and BMD T-score. Multiple studies have demonstrated a connection between changes in lipid metabolites and the development of an inflammatory microenvironment, which can affect the differentiation and function of osteoblasts, potentially contributing to bone loss (Komatsu and Takayanagi, 2022; Wang et al., 2022a; N. Yang and Liu, 2021; L. Zheng et al., 2021). This may provide a plausible potential explanation for serum metabolites playing a role in mediating the association between an unhealthy plant-based diet and BMD T-score. Interestingly, we observed that the same six metabolites significantly mediated the positive association between fresh fruit and BMD T-score, highlighting the potential significance of fresh fruit in plant-based dietary patterns. Fresh fruits, especially those rich in flavonoids, are widely acknowledged for their antioxidant and anti-inflammatory properties, which aligns with the aforementioned inflammatory mechanisms (Maleki et al., 2019; Martiniakova et al., 2022; Rana et al., 2022). However, the interesting observation and potential explanation warrant validation through broader population cohort.

Currently, few studies have examined the potential mediating effects of serum metabolites on the association between plant-based diet and BMD T-score based on a community-based population. The current study provides unique and timely evidence that may contribute to revealing the underlying biological processes linking a plant-based diet with bone loss. There are several strengths to the present study. First, the study population in TIS was predominantly aged 55–65 years old, a critical stage encompassing the transition from bone loss to osteoporosis. Furthermore, the reliability and robustness of the results were enhanced by rigorously controlling for numerous confounding factors and conducting sensitivity analyses. However, it is important to carefully consider several limitations associated with this study. First, the cross-sectional design precludes establish a causal relationship between a plant-based diet and bone loss. Second, the dietary information was collected through questionnaire interviews, which might not comprehensively reflect the typical dietary patterns of individuals and could introduce subjective bias in the diet assessment. Third, our results are restricted to the Chinese elderly population and lack of external validity assessment. Therefore, further research is warranted to validate these

findings in diverse populations and ascertain their generalizability.

5. Conclusion

In conclusion, our study provides evidence for the mediating role of serum metabolites between uPDI and BMD T-score, revealing the potential biological processes in the adverse effects of an unhealthy plant-based diet on BMD. Furthermore, our results emphasize the importance of fresh fruit consumption in preserving bone health in a middle-aged and older population. From a perspective of public health, our findings may offer a theoretical foundation and valuable reference for guiding the dietary habits of individuals at elevated risk for osteoporosis. Nevertheless, to gain a more comprehensive understanding of the intricate interplay between plant-based diets, serum metabolites, and bone health, it is imperative to conduct longitudinal and repeated collections of metabolomics and BMD data. As the TIS is an ongoing study, future follow-up data will furnish vital corroborative evidence for this type of research.

Ethics approval and consent to participate

This study received approval by the Ethics Committee of the School of Life Sciences, Fudan University, and Fudan University Taizhou Institute of Health Sciences (institutional review board approval number: 496 and B017, respectively).

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CRediT authorship contribution statement

Yi Zheng: participated in the study design, Formal analysis, interpreted, Writing – original draft, Formal analysis, contributed to, Writing – review & editing. **Ningxin Gao:** participated in the study design, Formal analysis, interpreted, Writing – original draft. **Yucan Li:** recruited the study participants, supported. **Min Fan:** recruited the study participants, supported. **Weizhong Tian:** recruited the study participants, supported. **Yanfeng Jiang:** recruited the study participants, supported. **Yingzhe Wang:** recruited the study participants, supported. **Mei Cui:** recruited the study participants, supported. **Chen Suo:** recruited the study participants, supported. **Tiejun Zhang:** recruited the study participants, supported. **Li Jin:** Supervision, provided funding support. **Kelin Xu:** participated in the study design, Formal analysis, interpreted, Writing – original draft, provided funding support. **Xingdong Chen:** Supervision, provided funding support, All authors have read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Abbreviations

ACME	average causal mediated effect
BMD	bone mineral density
EFA	exploratory factor analysis
FDR	false-discovery rate
HDL	high-density lipoprotein
hPDI	healthy plant-based diet index
OR	odds ratio
PDI	overall plant-based diet index
TIS	Taizhou Imaging Study
uPDI	unhealthy plant-based diet index
VLDL	very-low-density lipoprotein
WHR	waist-to-Hip Ratio
95 % CI	95 % confidence interval

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2024.100687.

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