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Association between dietary protein intake and bone mineral density based on NHANES 2011–2018

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This study examines the relationship between dietary protein intake and bone mineral density (BMD) using data from the 2011–2018 National Health and Nutrition Examination Survey (NHANES), addressing existing controversies in current evidence. This cross-sectional study included 16,775 participants. Dietary protein intake, the exposure variable, was collected with the use of two 24-h dietary recall methods and usual intake was assessed by the National Cancer Institute (NCI) method. While whole-body BMD, the outcome variable, was measured with dual-energy X-ray absorptiometry. Covariates included demographic, socioeconomic, and health factors. Weighted multivariable regression and generalized additive models were used for the association between dietary protein intake and BMD. After adjusting for covariates, a positive association was found between protein intake and BMD. Each additional gram of protein consumed was significantly associated with a BMD increase of 0.0003 g/cm^2 (95% CI 0.0001, 0.0004, $P = 0.0003$). Subgroup analysis by gender and ethnicity revealed significant positive correlations in women and Mexican Americans. Additionally, a saturation threshold effect was observed in women at 60.70 g/day and in non-Hispanic whites at 135.53 g/day, where the correlation was no longer significant beyond these thresholds. The study demonstrates a positive association between dietary protein intake and BMD, although this relationship is complex and nonlinear with varying effects across different populations. Specifically, positive correlation is only significant below a specific threshold level in some populations. These findings suggest the need for personalized dietary guidelines and provide important insights for clinical nutritional interventions and bone health management.

Keywords Dietary protein intake, Bone mineral density, NHANES, Cross-sectional study, Saturation threshold effect

The decline in bone mineral density (BMD) is a global health concern affecting people of all ages. According to the International Osteoporosis Foundation (IOF), approximately 200 million people worldwide suffer from osteoporosis, a condition that significantly increases fracture risk[1]. While BMD loss is often associated with older adults, it is increasingly recognized among younger populations, particularly as lifestyle and dietary habits change[2]. Reduced BMD leads to fragile bones and a higher likelihood of fractures, profoundly impacting individual health and quality of life[3].

Dietary protein intake plays a crucial role in maintaining normal body functions and health. Protein is a fundamental component of cells and is essential for the growth and repair of muscles, bones, and other tissues[4]. Recent studies suggest a strong link between dietary protein intake and the incidence and prognosis of various chronic diseases. For example, high-protein diets may reduce cardiovascular disease risk and have potential benefits in managing certain cancers and metabolic disorders[5,6]. However, excessive protein intake may adversely affect kidney function, especially in individuals with pre-existing kidney disease[7,8].

The relationship between dietary protein intake and BMD remains contentious. Some studies indicate a positive correlation between high protein intake and increased BMD, particularly in the lumbar spine and hips. For instance, research involving middle-aged and older adults found that higher protein consumption was

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positively associated with femoral neck and total hip BMD[9]. Conversely, other studies have found no significant link[10,11] or suggest that high protein intake might negatively affect bone health by increasing urinary calcium excretion[12]. These conflicting results may arise from differences in study populations, methodologies, and unaccounted confounding factors, highlighting the need for further research to clarify the true impact of protein intake on bone health.

This study aims to assess the association between daily dietary protein intake and BMD using data from the 2011–2018 National Health and Nutrition Examination Survey (NHANES) in the United States. Through this research, we hope to enhance understanding of the impact of dietary protein on bone health and provide scientific evidence for clinical practice and public health policy.

Materials and methods

Study population

This cross-sectional analysis was based on data from the 2011–2018 NHANES. NHANES is an ongoing national survey employing a complex, multistage, stratified probability sampling design to collect diet and health data from the U.S. population. Of the initial 39,156 participants, 17,874 had available BMD measurements. We excluded 1099 individuals with incomplete dietary protein intake data, resulting in a final sample of 16,775 participants. Given the complex, multistage probability sampling design of NHANES, which includes oversampling of certain population subgroups, it is essential to use appropriate weighting methods to ensure the results are representative. Therefore, we applied weighted analysis to explain the significant differences in the dataset. After applying survey weights, our study sample of 16,775 participants is representative of the U.S. population of approximately 157 million. Figure 1 illustrates the sample selection process. A comparison of the key characteristics between the included and excluded populations is presented in Supplementary Table 1. No significant differences were observed in age, gender distribution, race, education level, income-to-poverty ratio, or BMI (all $P > 0.05$), indicating that the selection process did not introduce substantial bias.

Variables

The exposure variable, daily dietary protein intake, was collected using a 24-h dietary recall method. Trained nutritionists conducted two non-consecutive 24-h dietary interviews using the Automated Multiple-Pass Method (AMPM) software from the U.S. Department of Agriculture (USDA)[13]. AMPM is designed to provide an effective and accurate method for collecting intake data in large-scale national surveys. It includes a range

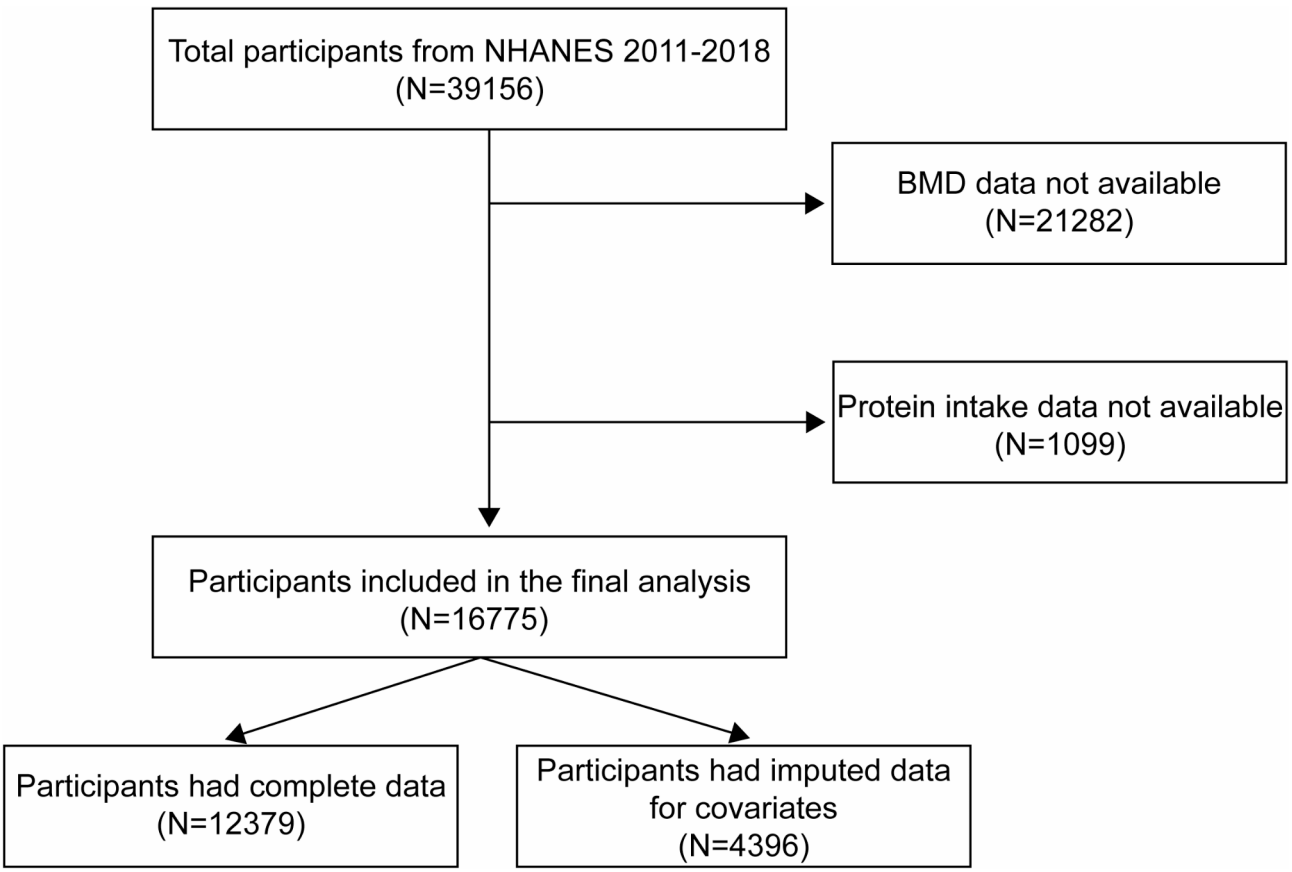

Fig. 1. Flowchart of participant selection. NHANES, National Health and Nutrition Examination Survey; BMD, Bone Mineral Density.

of standardized food-specific questions and possible response options. Validated in a large study, AMPM has proven to be an effective tool for accurately collecting group-level energy intake data[14]. Protein intake was calculated using the USDA Food Composition Database and adjusted for total energy intake. To account for intra-individual variation between the two 24-h recalls, we applied the National Cancer Institute (NCI) method, which is designed to estimate usual dietary intake while accounting for day-to-day variation in consumption. This method helps to better estimate the distribution of usual intake in the population by considering both the individual's average intake and the day-to-day variation in intake.

The outcome variable, total body BMD, was measured using dual-energy X-ray absorptiometry (DXA) with a Hologic QDR-4500 A scanner (Hologic, Inc., Bedford, Massachusetts). Certified radiology technicians conducted all DXA scans, which were quality-controlled by a central DXA coordination center.

Covariates included age, gender, race/ethnicity, education level, poverty-income ratio, body mass index (BMI), smoking status, moderate physical activity, serum calcium and phosphorus levels, serum uric acid, and dietary calcium intake. Demographic information was assessed using a standardized questionnaire (Sample Person Questionnaire). BMI was calculated by dividing weight (in kilograms) by height (in meters) squared, then rounding to one decimal place. The measurement of weight and height was carried out by professionals who had undergone standardized training using calibrated equipment: weight was measured using a digital weighing scale, and height was measured using a height measuring instrument when standing. Serum calcium and phosphorus levels were measured using the Roche Cobas 6000 analyzer, while serum uric acid levels were determined by colorimetric assay. Moderate physical activity was assessed through a standardized questionnaire that asked participants whether they engaged in activities, such as exercise, fitness, or recreational activities, that caused a slight increase in breathing or heart rate for at least 10 min during a typical week (yes/no). Although this binary approach simplifies analysis, we acknowledge that it may not fully capture an individual's overall physical activity level. The selection of these covariates was based on a causal inference framework and validated using a directed acyclic graph (DAG) (www.dagitty.net/dags.html). The DAG illustrates the causal pathways between protein intake and bone mineral density, as well as potential confounding factors and mediating variables (Supplementary Fig. 1). Equipment calibration was performed by the health technicians and verified by supervisory staff. The questionnaire used to collect covariate information was administered by trained interviewers through the computer-assisted personal interview (CAPI) system. This system is programmed with built-in consistency checks to minimize data entry errors. After data collection, NHANES field office staff reviewed the interview data to ensure the accuracy and completeness of the selected items. Detailed information on sample design and related analysis issues is available at https://wwwn.cdc.gov/nchs/nhanes/analyticguidelines.aspx.

Statistical analysis

All participants had complete data on both BMD and dietary protein intake. For the covariates with missing data, we applied the multiple imputation (MI) method, based on the Markov-chainMonte Carlo method in the SAS Ml procedure, to address the issue. This approach minimized the risk of selection bias that could arise from excluding participants with incomplete data. Group differences in categorical variables were assessed using Rao-Scott chi-square tests, and continuous variables were evaluated with weighted linear regression models. Categorical variables were represented as proportions [95% confidence interval (CI)], continuous variables were described using mean values plus or minus standard deviation (SD). We explored the relationship between BMD and protein intake using weighted multivariable regression models and conducted subgroup analyses via stratified multivariable regression. To explore the potential nonlinear relationship between BMD and protein intake, we used a generalized additive model (GAM) with smoothing splines. We chose GAM for its flexibility in modeling complex relationships without assuming a specific functional form. This approach aligned with our goal of detecting potential threshold effects in the protein-BMD relationship. Combining these methods allowed us to account for the complexity of the NHANES survey design while capturing subtle patterns in the data. Nonlinear relationships were assessed with the use of a generalized additive model of cubic splines with a degree-of-freedom setting based on generalized cross-validation, that is, restricted cubic splines with three knots at the 10th, 50th, and 90th percentiles of protein intake. After nonlinearity was detected, the inflection point was determined by calculating the first and second derivatives of the smooth curve by piecewise regression analysis. We examined threshold effects of the relationship between BMD and protein intake primarily using two-piecewise linear regression models. The significance of the threshold effect was determined using the log-likelihood ratio test, with $P < 0.05$ considered statistically significant. All analyses were performed using R software version 4.3.1 (R Foundation, Vienna, Austria) and EmpowerStats version 4.0 (X&Y Solutions, Inc., Boston, MA), with a P value of < 0.05 considered statistically significant.

Results

As shown in Table 1, the study population ($N = 16,775$) was divided into four groups based on daily protein intake: Q1 (< 62.89 g), Q2 (62.90–74.94 g), Q3 (74.95–89.78 g), and Q4 (> 89.79 g). With increasing protein intake, participants' age gradually increased (Q1: 23.58 ± 15.51 years vs. Q4: 34.60 ± 13.58 years), and the proportion of males rose significantly (Q1: 18.65% vs. Q4: 87.04%). Regarding racial composition, the proportion of Mexican Americans was higher in the high-protein group (Q4: 34.81%), whereas non-Hispanic Whites were more prevalent in the low-protein group (Q1: 27.26%). Educational attainment showed an interesting pattern: participants in the high-protein group had the highest proportion with some college education or an associate degree (Q4: 53.37%), while those in the low-protein group had the highest proportion with a college degree or higher (Q1: 61.89%). Additionally, as protein intake increased, participants' income-to-poverty ratio (Q1: 2.02 ± 1.54 vs. Q4: 2.59 ± 1.67), BMI (Q1: 25.27 ± 7.47 vs. Q4: 27.73 ± 6.64 kg/m^2), uric acid levels (Q1: 284.13 ± 72.12 vs. Q4: 341.68 ± 78.75 μmol/L), and calcium intake (Q1: 686.32 ± 352.63 vs. Q4: 1395.20 ± 677.36 mg/day) all showed an

Protein intake (g/day)	Total (*N* = 16775)	Q1 (< 62.89, *N* = 4193)	Q2 (62.90-74.94, *N* = 4193)	Q3 (74.95–89.78, *N* = 4192)	Q4 (> 89.79, *N* = 4197)	*P* value
Age (years)	29.22 ± 15.73	23.58 ± 15.51	27.50 ± 16.07	31.20 ± 15.44	34.60 ± 13.58	< 0.001
Gender (%)						< 0.001
Men	50.37(49.62, 51.21)	18.65(17.56, 19.74)	37.04(35.60, 38.48)	58.73(57.24, 60.22)	87.04(85.98, 88.10)	
Women	49.63(48.88, 50.38)	81.35(80.26, 82.44)	62.96(61.52, 64.40)	41.27(39.78, 42.76)	12.96(11.90, 14.02)	
Race (%)						< 0.001
Mexican American	32.29 (31.59, 32.98)	29.55 (28.26, 30.84)	32.36 (31.03, 33.69)	32.44 (31.10, 33.78)	34.81 (33.44, 36.18)	
Non-hispanic white	22.30 (21.73, 22.87)	27.26 (25.99, 28.52)	23.47 (22.21, 24.72)	20.80 (19.54, 22.06)	17.66 (16.43, 18.88)	
Non-hispanic black	17.34 (16.79, 17.90)	15.10 (13.99, 16.21)	16.98 (15.79, 18.17)	17.70 (16.55, 18.85)	19.56 (18.39, 20.73)	
Other race	28.08 (27.38, 28.78)	28.09 (27.01, 29.17)	27.19 (26.06, 28.32)	29.06 (27.88, 30.25)	27.97 (26.82, 29.12)	
Education level (%)						< 0.001
Less than high school	11.01 (10.51, 11.51)	8.51 (7.56, 9.46)	9.87 (8.97, 10.77)	11.98 (11.03, 12.93)	13.68 (12.68, 14.68)	
High school	13.48 (12.98, 13.98)	10.97 (9.97, 11.97)	12.81 (11.81, 13.81)	12.95 (12.00, 13.90)	17.18 (16.03, 18.33)	
Some college or AA degree	37.01 (36.31, 37.71)	18.63 (17.28, 19.98)	31.96 (30.51, 33.41)	44.06 (42.56, 45.56)	53.37 (51.87, 54.87)	
College graduate or above	38.50 (37.80, 39.20)	61.89 (60.34, 63.44)	45.36 (43.86, 46.86)	31.01 (29.56, 32.46)	15.77 (14.42, 17.12)	
Moderate physical activity (%)						0.001
Yes	46.00 (45.20, 46.80)	42.88 (41.18, 44.58)	46.26 (44.56, 47.96)	46.58 (44.93, 48.23)	47.58 (45.98, 49.18)	
No	54.00 (53.20, 54.80)	57.12 (55.42, 58.82)	53.74 (52.04, 55.44)	53.42 (51.77, 55.07)	52.42 (50.82, 54.02)	
Income to poverty ratio	2.33 ± 1.63	2.02 ± 1.54	2.27 ± 1.60	2.45 ± 1.64	2.59 ± 1.67	< 0.001
BMI (kg/m^2)	26.49 ± 7.33	25.27 ± 7.47	25.96 ± 7.61	27.01 ± 7.33	27.73 ± 6.64	< 0.001
Smoked at least 100 cigarettes in life (%)						< 0.001
Yes	37.45 (36.55, 38.35)	34.86 (32.91, 36.81)	35.37 (33.47, 37.27)	36.04 (34.29, 37.79)	41.42 (39.72, 43.12)	
No	62.55 (61.65, 63.45)	65.14 (63.19, 67.09)	64.63 (62.73, 66.53)	63.96 (62.21, 65.71)	58.58 (56.88, 60.28)	
Serum calcium (mmol/L)	2.36 ± 0.09	2.36 ± 0.09	2.35 ± 0.09	2.35 ± 0.09	2.36 ± 0.08	< 0.001
Serum phosphorus (mmol/L)	1.25 ± 0.21	1.28 ± 0.21	1.27 ± 0.22	1.25 ± 0.22	1.23 ± 0.20	< 0.001
Uric acid (μmol/L)	312.19 ± 80.62	284.13 ± 72.12	296.07 ± 76.25	318.07 ± 81.61	341.68 ± 78.75	< 0.001
Calcium intake (mg/day)	1024.33 ± 557.51	686.32 ± 352.63	932.64 ± 402.10	1082.83 ± 484.43	1395.20 ± 677.36	< 0.001
Total BMD (g/cm^2)	1.05 ± 0.15	0.99 ± 0.15	1.02 ± 0.15	1.06 ± 0.14	1.12 ± 0.13	< 0.001

Table 1. Weighted characteristics of the study population based on dietary protein intake quartiles. Mean ± SD for continuous variables: the *P* value was calculated by the weighted linear regression model. % (95% CI) for categorical variables: the *P* value was calculated by the Rao-Scott chi-square test.

upward trend. Notably, total bone mineral density also increased with higher protein intake (Q1: 0.99 ± 0.15 vs. Q4: 1.12 ± 0.13 g/cm^2). All these differences were statistically significant ($P < 0.001$).

Table 2 presents the results of the multivariable linear regression analysis. In the unadjusted Model 1, each additional 1 g/day of protein intake was associated with a 0.0021 g/cm^2 increase in BMD (95% CI 0.0020, 0.0022, $P < 0.0001$). This association weakened but remained significant after adjusting for age, sex, and race in Model 2 (β = 0.0015, 95% CI 0.0014, 0.0016, $P < 0.0001$). Further adjustment for education, economic factors, and health behaviors in Model 3 reduced the association but maintained statistical significance (β = 0.0003, 95% CI 0.0001, 0.0004, $P = 0.0003$). Grouping analysis by protein intake revealed that higher intake groups (Q2 to Q4) showed greater BMD increases across all models compared to the lowest intake group (Q1). Specifically, in Model 3, Q4 exhibits a 0.0123 g/cm^2 BMD increase compared to Q1 (95% CI 0.0043, 0.0203, $P = 0.0025$). The trend analysis ($P = 0.0001$) indicated a significant dose-response relationship between increased protein intake and BMD improvement. According to Table 2, subgroup analysis by sex and race/ethnicity in Model 3 showed that the positive correlation between protein intake and BMD remained significant for women (β = 0.0003, 95% CI 0.0001, 0.0006, $P = 0.0036$) and Mexican Americans (β = 0.0003, 95% CI 0.0001, 0.0005, $P = 0.0116$).

Figures 2 and 3 and 4 depict smoothed curve fits and generalized additive models, indicating a non-linear relationship between protein intake and BMD. Further threshold effect analysis (Table 3) revealed a saturation effect between protein intake and total BMD in females and non-Hispanic whites. In females, a threshold effect was evident (log-likelihood ratio $P = 0.0005$). Below a protein intake of 60.70 g/day, each additional gram of protein consumed was significantly associated with a BMD increase of 0.0005 g/cm^2 (95% CI 0.0002, 0.0008, $P = 0.0004$). Above this threshold, the association was no longer significant (β = 0.0000, 95% CI − 0.0001, 0.0001, $P = 0.9879$). Similarly, for non-Hispanic whites, a significant threshold effect (log-likelihood ratio $P = 0.0328$) was observed at 135.53 g/day. Below this level, each 1 g/day increase in protein intake was associated with a BMD increase of 0.0002 g/cm^2 (95% CI 0.0000, 0.0004, $P = 0.0397$). Beyond the threshold, the association was not significant (β = -0.0001, 95% CI − 0.0005, 0.0004, $P = 0.7835$).

To investigate the influence of sex and race on the relationship between protein intake and BMD, we conducted stratified analyses by these factors (Supplementary Table 2). In both unadjusted and partially adjusted models, all sex-race subgroups demonstrated significant positive associations between protein intake and BMD. After full adjustment for confounding factors (Model 3), non-Hispanic White women showed a significant

	Model 1 β (95% CI) *P* value	Model 2 β (95% CI) *P* value	Model 3 β (95% CI) *P* value
Protein intake (g/day)	0.0021 (0.0020, 0.0022) < 0.0001	0.0015 (0.0014, 0.0016) < 0.0001	0.0003 (0.0001, 0.0004) 0.0003
Protein intake categories			
Q1 (< 62.89 g/day)	Reference	Reference	Reference
Q2 (62.90–74.94 g/day)	0.0332 (0.0273, 0.0392) < 0.0001	0.0201 (0.0145, 0.0256) < 0.0001	0.0085 (0.0020, 0.0151) 0.0109
Q3 (74.95–89.78 g/day)	0.0674 (0.0616, 0.0732) < 0.0001	0.0416 (0.0358, 0.0473) < 0.0001	0.0065 (-0.0003, 0.0133) 0.0621
Q4 (> 89.79 g/day)	0.1186 (0.1129, 0.1242) < 0.0001	0.0807 (0.0743, 0.0870) < 0.0001	0.0123 (0.0043, 0.0203) 0.0025
P for trend	< 0.0001	< 0.0001	0.0001
Subgroup analysis stratified by sex			
Men	0.0021 (0.0019, 0.0022) < 0.0001	0.0015 (0.0014, 0.0017) < 0.0001	0.0002 (-0.0000, 0.0004) 0.0754
Women	0.0018 (0.0016, 0.0019) < 0.0001	0.0013 (0.0011, 0.0015) < 0.0001	0.0003 (0.0001, 0.0006) 0.0036
Subgroup analysis stratified by race/ethnicity			
Mexican American	0.0023 (0.0021, 0.0024) < 0.0001	0.0017 (0.0015, 0.0018) < 0.0001	0.0003 (0.0001, 0.0005) 0.0116
Non-hispanic white	0.0021 (0.0018, 0.0023) < 0.0001	0.0012 (0.0010, 0.0015) < 0.0001	0.0003 (-0.0000, 0.0007) 0.0986
Non-hispanic black	0.0021 (0.0019, 0.0023) < 0.0001	0.0012 (0.0010, 0.0015) < 0.0001	0.0002 (-0.0002, 0.0005) 0.3295
Other race	0.0021 (0.0019, 0.0022) < 0.0001	0.0013 (0.0011, 0.0015) < 0.0001	0.0002 (-0.0000, 0.0004) 0.0914

Table 2. Multivariable regression to assess the association of protein intake (g/day) with total BMD (g/cm^2). Model 1: no covariates were adjusted. Model 2: age, gender, and race were adjusted. Model 3: age, gender, race, education level, income to poverty ratio, body mass index, moderate physical activity, smoking behavior, and serum calcium, serum phosphorus, uric acid and calcium intake were adjusted. In the subgroup analysis stratified by sex and race, the model is not adjusted for sex and race, respectively.

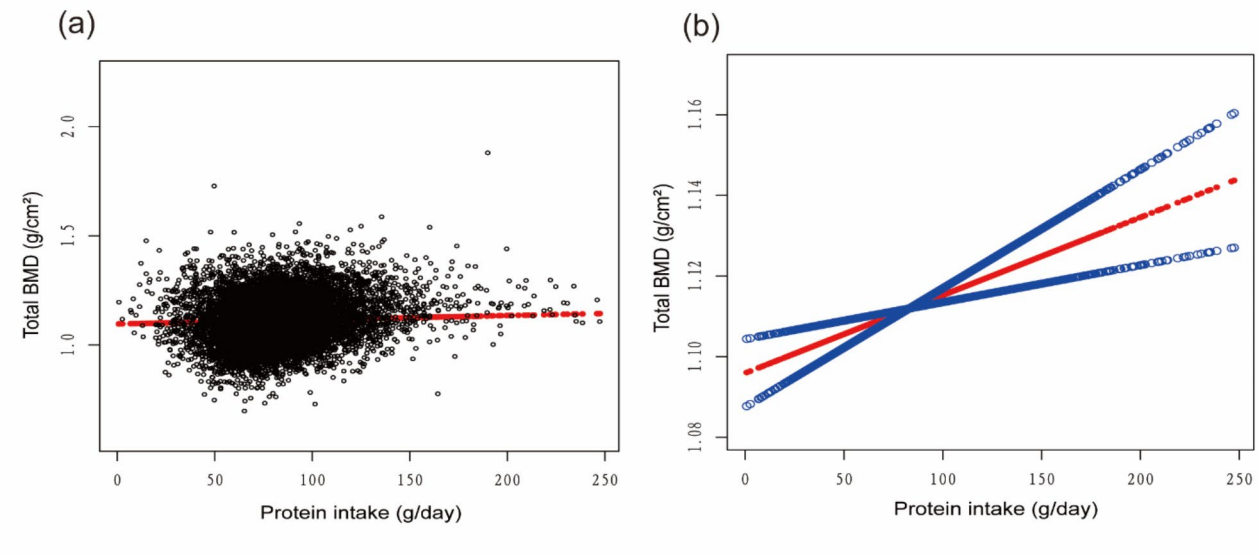

Fig. 2. The association between protein intake and total BMD. (**a**) Each black point represents a sample. (**b**) Solid rad line represents the smooth curve fit between variables. Blue bands represent the 95% of confidence interval from the fit. Age, gender, race, education level, income to poverty ratio, body mass index, moderate physical activity, smoking behavior, and serum calcium, serum phosphorus, uric acid and calcium intake were adjusted.

positive association (β = 0.0006, 95% CI 0.0000–0.0011, *P* = 0.0321). These findings provide important evidence for developing population-specific nutritional recommendations. For instance, non-Hispanic White women may require protein intake above the recommended daily allowance to maintain bone density.

Discussion

Our study uses large-scale NHANES data from 2011 to 2018 to conduct a cross-sectional analysis, providing new insights into the relationship between protein intake and BMD. We identified population-specific associations between dietary protein intake and BMD, with threshold effects that vary by gender and race. The threshold for women (60.70 g/d) aligns closely with the current dietary recommendations, which suggest 0.8 g/kg/d.

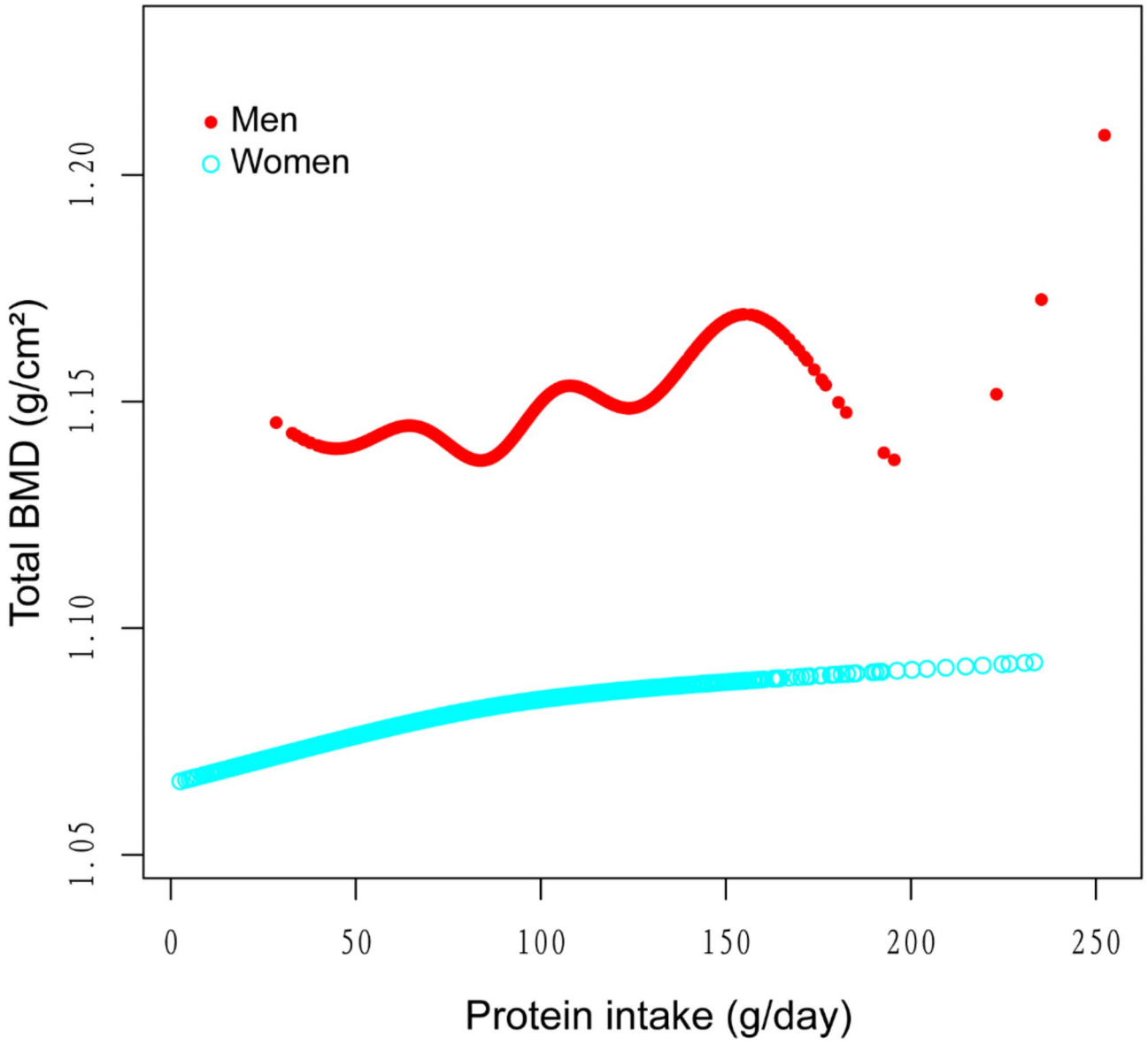

Fig. 3. The association between protein intake and total BMD stratified by gender. Age, race, education level, income to poverty ratio, body mass index, moderate physical activity, smoking behavior, and serum calcium, serum phosphorus, uric acid and calcium intake were adjusted.

This corresponds to approximately 48–56 g/d for women weighing between 60 and 70 kg. The slightly higher threshold in our study may reflect the additional protein needed for optimal bone health, compared to general health maintenance. For non-Hispanic White individuals, the threshold is significantly higher (135.53 g/d). This may be due to several factors: (1) different dietary patterns, with higher animal protein intake in this population; (2) genetic differences in protein metabolism and bone formation; and (3) variations in body composition and metabolic characteristics among different ethnic groups. Although the effect size appears small, it could have long-term clinical significance considering the slow progression of osteoporosis[15,16]. These findings provide essential insights for dietary guidelines and osteoporosis prevention strategies.

Our findings align with some previous studies while differing from others. Groenendijk et al.[9] found a positive correlation between high protein intake and greater BMD in adults over 65 years through a meta-analysis of cohort studies and a randomized controlled trial from different countries, mainly including Australia, the United States, China and Finland, bolstering our findings. Similarly, Weaver et al.[17]reported that higher protein intake groups exhibited 1.8-6.0% higher hip and lumbar spine BMD compared to lower intake groups. However, our study's larger sample size and broader age range enhance result generalizability.

Conversely, some studies suggest a negative or non-significant association between protein intake and BMD. For example, Sahni et al.[18] observed no significant correlation in the Framingham Offspring Cohort. Differences in study design and populations may contribute to these disparities. Wright et al.[19] also found inconsistent results regarding protein intake and bone health during weight loss in their meta-analysis. These contrasting

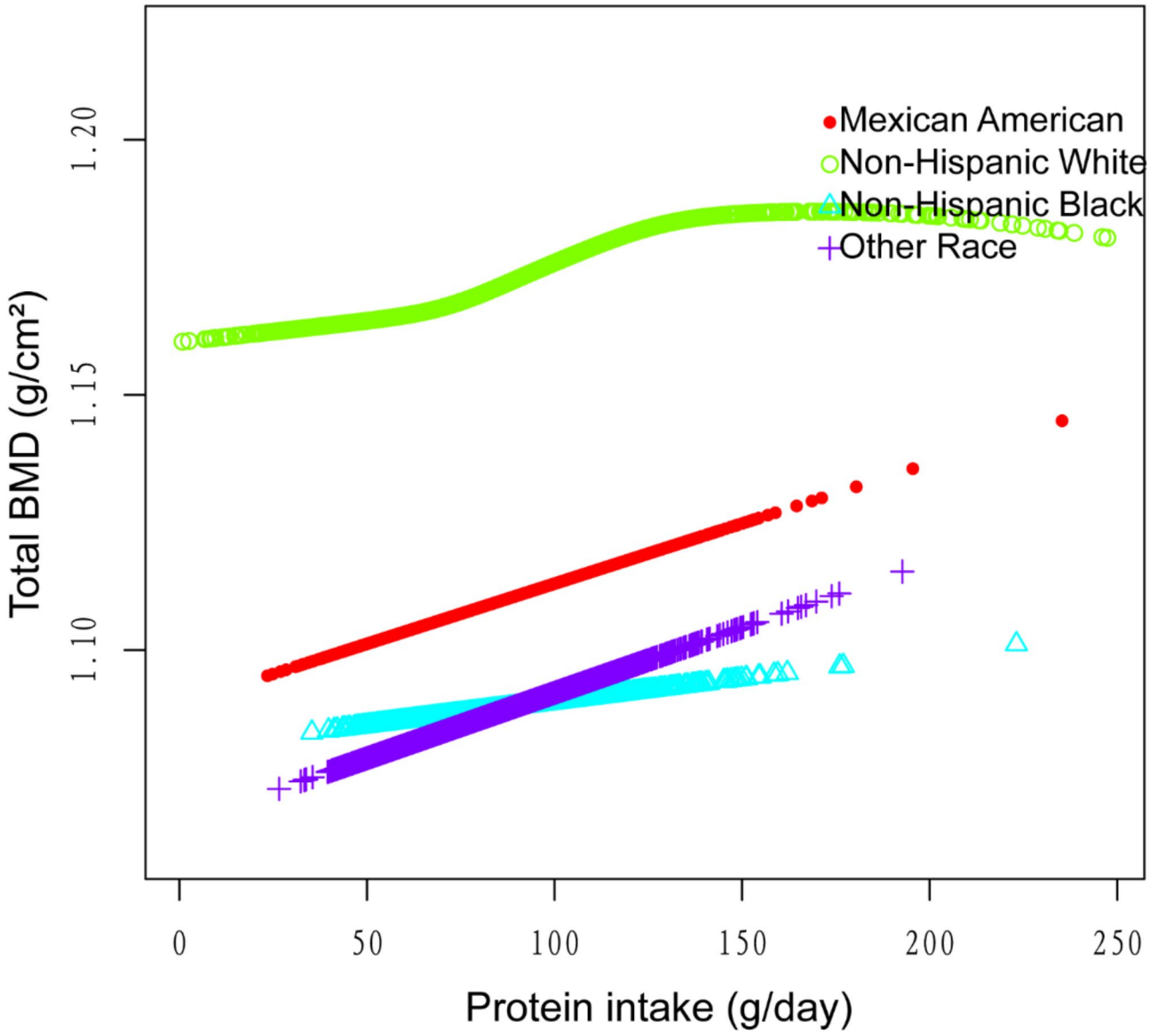

Fig. 4. The association between protein intake and total BMD stratified by race. Age, gender, education level, income to poverty ratio, body mass index, moderate physical activity, smoking behavior, and serum calcium, serum phosphorus, uric acid and calcium intake were adjusted.

results underscore the complexity of the relationship between protein intake and bone health, emphasized by our subgroup analysis highlighting threshold effects in women and non-Hispanic whites, an aspect not previously reported. This suggests a more intricate interaction, possibly influenced by factors like hormonal status or genetic predisposition[20].

Mechanistically, protein intake may influence BMD through various pathways. Protein can stimulate bone formation by enhancing insulin-like growth factor 1 (IGF-1) production and helps maintain serum calcium levels, thus reducing bone resorption[9,21]. High-quality protein intake has been shown to improve calcium absorption and muscle strength, benefiting bone health[17]. However, excessive protein intake might increase body acid load, leading to a compensatory release of calcium from bones as a buffer, potentially contributing to osteoporosis. These interactions might explain the nonlinear relationship and threshold effects we observed.

Our study's clinical value lies in its potential to inform personalized dietary guidelines and osteoporosis prevention strategies. Findings reveal a complex relationship between protein intake and BMD, with distinct threshold effects observed in women and non-Hispanic whites. Clinicians might need to adjust protein intake recommendations based on an individual's sex and racial background. For women, ensuring a daily protein intake of about 62 g is crucial, whereas non-Hispanic whites may require considering a higher threshold. However, further prospective studies and intervention trials are needed to validate these findings before formulating specific clinical guidelines[22]. Future research could focus on exploring how different protein sources impact BMD and investigating the interactions between protein intake and other nutrients on bone health[23].

Total BMD	β (95% CI) *P* value
Women	
Inflection point	60.70
Protein intake < 61.88 (g/day)	0.0005 (0.0002, 0.0008) 0.0004
Protein intake > 61.88(g/day)	0.0000 (− 0.0001, 0.0001) 0.9879
Log likelihood ratio	0.005
Non-hispanic white	
Inflection point	135.53
Protein intake < 142.78 (g/day)	0.0002 (0.0000, 0.0004) 0.0397
Protein intake > 142.78(g/day)	− 0.0001 (− 0.0005, 0.0004) 0.7835
Log likelihood ratio	0.0328

Table 3. Threshold effect analysis of protein intake (g/day) on total BMD (g/cm^2) using the two-piecewise linear regression model. Age, gender, race, education level, income to poverty ratio, body mass index, moderate physical activity, smoking behavior, and serum calcium, serum phosphorus, uric acid and calcium intake were adjusted. In the subgroup analysis stratified by sex and race, the model is not adjusted for sex and race, respectively.

Our study has several strengths, enhancing result reliability and generalizability. Firstly, we used data from NHANES from 2011 to 2018, which used a complex, multistage, stratified probability sampling design to ensure representative representation of the U.S. population. It provides the latest evidence from a large, representative population to address existing literature disputes. We employed advanced data analysis techniques, including weighted variance estimation, generalized additive models, and smoothing curve fitting to unveil the complex nonlinear relationship between protein intake and BMD. By calculating inflection points using recursive techniques and applying segmented linear regression models, we precisely quantified threshold effects, providing accurate estimates of optimal protein intake ranges. We accounted for a broad range of potential confounders, including demographics, socioeconomic status, lifestyle, and biochemical markers, greatly enhancing the credibility of our findings. Finally, detailed subgroup analyses revealed gender and racial influences on the protein intake-BMD relationship. Our methodological innovations in analyzing nonlinear relationships and threshold effects provide a robust framework for future nutritional epidemiology studies, while our findings offer practical guidance for personalized dietary recommendations in clinical settings.

Despite these strengths, certain limitations must be considered. Although selection bias may affect the generalizability of the results due to the exclusion of individuals with incomplete dietary protein intake data, our analysis showed that the included and excluded populations had similar demographic characteristics. This suggests that the impact of selection bias on our findings is likely minimal. Although the complex sampling design of the NHANES ensures representative representation of the US population, the findings may not be directly generalizable to populations in other countries because of differences in dietary patterns, genetic background, and environmental factors. As a cross-sectional observational study, we can only identify associations, not causal relationships. Though we controlled for various confounders, unmeasured or unknown factors like genetic influences, hormonal levels, or medication use may affect results. Additionally, some methodological limitations should be noted. The dichotomous classification of physical activity (yes/no) may not fully capture the complexity of physical activity patterns, potentially leading to residual confounding. Lastly, although we collected high-quality dietary data using two 24-h dietary recalls and applied the NCI method to estimate usual dietary intake, there remains a slight risk of undetected hidden outliers. These could be due to factors such as recall bias or reporting errors, which may affect the accuracy of the dietary data. Despite these limitations, the study provides essential insights into dietary protein intake and bone density, directing future research.

Conclusions

Our study highlights the complex relationship between dietary protein intake and BMD, particularly in women and non-Hispanic white populations. Significant positive correlations were identified, with noteworthy saturation thresholds. In women, protein intake below 60.70 g/day was beneficial for increasing BMD, whereas, for non-Hispanic whites, a higher threshold of 135.53 g/day was observed. These findings suggest that dietary protein intake has varying effects on bone health across different groups, revealing the necessity for personalized dietary guidelines. This research offers crucial insights for clinical nutritional interventions and advances our understanding of dietary impacts on bone health. Future investigations should focus on the interplay between protein source and other nutrients on BMD to bolster these findings further.

Data availability

The datasets generated and analysed during the current study are available in the NHANES repository, http://www.cdc.gov/nchs/nhanes/.

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Author contributions

ZZ and XC participated in the conception and design of the experiments; XC and YF performed most of the experiments; ZZ and YF analyzed and interpreted the data; XC drafted the paper and ZZ critically revised it for intellectual content. All authors read and approved the final manuscript. And that all authors agree to be accountable for all aspects of the work.

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Declarations

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

The NHANES data used in this study are publicly accessible and anonymized. The original NHANES research protocol received approval from the Research Ethics Review Board of the National Center for Health Statistics (NCHS) at the Centers for Disease Control and Prevention (CDC). All participants provided written informed consent, and the study adhered strictly to the ethical principles of the Declaration of Helsinki.

Additional information

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