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Associations between serum calcium, 25(OH)D level and bone mineral density in adolescents

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Abstract

Backgrounds: It is important to improve our understanding of the roles of calcium and vitamin D in bone health for preventing osteoporosis. We aimed at exploring the associations between serum calcium, vitamin D level, and bone mineral density (BMD) in adolescents included in the National Health and Nutrition Examination Survey (NHANES) 2001–2006.

Methods: Weighted multivariate linear regression models were used to estimate the associations of serum calcium, 25(OH)D level with total BMD. Smooth curve fitting was used to explore the potential non-linear relationship.

Results: A total of 5990 individuals aged between 12 and 19 years were included in this study. The fully-adjusted model showed serum calcium positively correlated with total BMD. However, an inverted U-shaped relationship was found when we performed the smooth curve fitting method, and the inflection point was calculated at 9.6 mg/dL using the two-piecewise linear regression model. In contrast, there was a positive correlation between serum 25(OH)D and total BMD after adjusting for potential confounders.

Conclusions: The present study revealed a positive correlation between serum 25(OH)D level and total BMD, and an inverted U-shaped relationship between serum calcium and total BMD.

Keywords: Calcium, Vitamin D, Bone health, Adolescent, NHANES

Introduction

Osteoporosis is a global health problem that is reported to originate during childhood or adolescence [1]. Adolescence is a critical period of skeletal development and peak bone mass (PBM) may be reached in late adolescence [2]. Evidence indicates that when PBM increases by 5% during childhood and adolescence, the risk of osteoporotic fracture reduces by 40%, while when PBM increases by 10%, this risk decreases by 50% [3, 4]. Therefore, increased bone mass accumulation during this period is an effective way to maintain bone health in adulthood and prevent osteoporosis in older age [5].

Calcium is an essential nutrient for skeletal development and maintenance [6]. Calcium supplementation is recommended for improving bone health in older adults. However, the effect of calcium supplementation on bone mineral density (BMD) remains controversial [7], and it is uncertain whether an elevated serum calcium level is beneficial to bone health [8, 9]. Moreover, vitamin D is known to play an essential role in maintaining normocalcaemia, thus permitting normal skeletal mineralization [10]. In individuals with a low BMD or those at a high risk of osteoporotic fractures, calcium and vitamin D supplementation are suggested as adjuncts to osteoporosis therapies [11]. However, their effect on fracture risk is

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unclear [12]. Furthermore, whether serum calcium, and vitamin D independently correlate with BMD in the general population or certain population groups remains unclear [13, 14]. To the best of our knowledge, no large-sample study has been performed in adolescents. Moreover, the beneficial effect of vitamin D on bone health was reported to be associated with serum 25(OH)D level and not (1,25(OH)2D) [15], and serum 25(OH)D level has been used to assess vitamin D status [16, 17]. Therefore, in this study, we examined the associations of serum calcium, and 25(OH)D level with total BMD among US adolescents using samples from a database of a multiracial population.

Methods

Study population

As a nationally representative survey, the National Health and Nutrition Examination Survey (NHANES) collected health examination data from the non-institutionalized US population [18]. These data are released on a two-year cycle. We used the data from three cycles of NHANES 2001–2006 in this study. A total of 5990 individuals aged 12–19 years with available data on serum calcium, and 25(OH)D levels, and total BMD were included. The NHANES protocols were approved by the Institutional Review Board of the National Center for Health Statistics, and participants or their proxies (< 18 years) provided informed consent [19].

Variables

In this study, the dependent variable was total BMD, and the independent variables were serum calcium and 25(OH)D levels. All subjects included in the present study received dual-energy X-ray absorptiometry (DXA) total body scans. For the 2001–2006 cycles, total BMD was measured using the DXA scans obtained from a QDR-4500A fanbeam densitometer (Hologic, Inc., Bedford, Massachusetts) by trained and certified radiologic technologists. The Beckman Synchron LX20 (Beckman Coulter, Brea, CA) was used to determine serum calcium, and a radioimmunoassay kit (DiaSorin, Stillwater, Minnesota, USA) was used to determine serum 25(OH)D for the 2001–2006 cycles.

The following variables were selected as potential confounders: age, gender, race/ethnicity, physical activity

Table 1 Participant characteristics

Characteristic	Boys (*n* = 3086)	Girls (*n* = 2904)	*P* value
Age, mean ± SD (years)	15.46 ± 2.26	15.35 ± 2.23	0.0551
Race/Ethnicity (%)			0.9754
Non-Hispanic White	62.87	63.15	
Non-Hispanic Black	14.47	14.48	
Mexican American	11.50	11.13	
Other race/ethnicity	11.16	11.25	
BMI, mean ± SD (kg/m^2)	23.31 ± 5.50	23.55 ± 5.78	0.1073
Income to poverty ratio, mean ± SD	2.64 ± 1.62	2.57 ± 1.62	0.0912
Physical activity (%)			< 0.0001
Sedentary	2.95	4.85	
Low	7.72	11.63	
Moderate	6.51	8.47	
High	22.05	16.20	
Not recorded	60.77	58.85	
Calcium supplementation (%)			< 0.0001
Not use	87.21	83.80	
< 0.4 g/d	10.94	12.99	
≥ 0.4 g/d	1.84	3.21	
Serum calcium, mean ± SD (mg/dL)	9.80 ± 0.30	9.64 ± 0.30	< 0.0001
Serum vitamin D, mean ± SD (ng/mL)	24.23 ± 8.63	23.32 ± 10.02	0.0002
Total BMD, mean ± SD (g/cm^2)	1.10 ± 0.14	1.06 ± 0.10	< 0.0001

Mean ± SD for continuous variables: *P* value was calculated by weighted linear regression model
% for categorical variables: *P* value was calculated by weighted chi-square test
Abbreviation: *BMI*, body mass index, *BMD* bone mineral density

Table 2 Associations of serum calcium, 25(OH)D with total bone mineral density

	Model 1 β (95% CI)	Model 2 β (95% CI)	Model 3 β (95% CI)
Serum calcium	−0.0157 (−0.0257, −0.0058)	−0.0003 (− 0.0083, 0.0077)	0.0084 (0.0007, 0.0160)
Serum calcium (quartile)			
Q1	Reference	Reference	Reference
Q2	−0.0065 (− 0.0163, 0.0033)	0.0072 (− 0.0003, 0.0148)	0.0088 (0.0017, 0.0160)
Q3	0.0008 (−0.0087, 0.0103)	0.0136 (0.0062, 0.0211)	0.0141 (0.0070, 0.0212)
Q4	−0.0144 (− 0.0233, − 0.0055)	0.0032 (− 0.0040, 0.0103)	0.0098 (0.0030, 0.0166)
P for trend	0.004	0.484	0.008
Serum 25(OH)D	−0.0005 (− 0.0009, − 0.0002)	0.0001 (− 0.0003, 0.0003)	0.0006 (0.0003, 0.0008)
Serum 25(OH)D (quartile)			
Q1	Reference	Reference	Reference
Q2	−0.0415 (− 0.0532, − 0.0298)	−0.0051 (− 0.0145, 0.0044)	0.0050 (− 0.0040, 0.0139)
Q3	−0.0475 (− 0.0581, − 0.0369)	0.0046 (− 0.0046, 0.0138)	0.0166 (0.0079, 0.0254)
Q4	−0.0384 (− 0.0483, − 0.0284)	0.0030 (− 0.0062, 0.0122)	0.0202 (0.0113, 0.0291)
P for trend	< 0.001	0.181	< 0.001

Model 1: no covariates were adjusted
Model 2: age, gender, race/ethnicity were adjusted
Model 3: age, gender, race/ethnicity, body mass index, income to poverty ratio, physical activity, and calcium supplementation use were adjusted

(based on suggested metabolic equivalent rank) [20], income to poverty ratio, body mass index, and calcium supplementation use. Detailed information on serum calcium, and 25(OH)D levels, total BMD, and other variables can be found at www.cdc.gov/nchs/nhanes/.

Statistical analysis

We used sample weights in all analyses according to the stratified, multistage probability sampling design. The *P*-value was calculated using the weighted chi-square test for categorical variables, and the weighted linear regression model for continuous variables. The weighted multivariate linear regression model was used to investigate whether serum calcium and 25(OH)D levels independently correlated with total BMD. We used generalized additive models and smooth curve fitting to explore potential non-linear relationships. We further calculated the inflection points using the two-piecewise linear regression model. All analyses were conducted using

Table 3 Total bone mineral density by quartiles of serum calcium, stratified by race/ethnicity and age

Quartiles of serum calcium	White	Black	Mexican American	Other race
	Total BMD g/cm^2 (95% Confidence Interval)			
12 to 15 years				
Lowest quartile	1.012 (0.994, 1.029)	1.069 (1.055, 1.083)	1.015 (1.000, 1.031)	1.015 (0.983, 1.047)
2nd	1.011 (0.996, 1.026)	1.068 (1.055, 1.081)	1.008 (0.995, 1.021)	1.024 (1.000, 1.048)
3rd	1.011 (0.998, 1.025)	1.065 (1.052, 1.077)	1.010 (0.998, 1.023)	1.027 (1.002, 1.052)
Highest quartile	1.006 (0.995, 1.017)	1.062 (1.052, 1.072)	0.994 (0.983, 1.005)	0.998 (0.978, 1.018)
P for trend	0.540	0.383	0.031	0.200
16 to 19 years				
Lowest quartile	1.132 (1.118, 1.146)	1.207 (1.195, 1.220)	1.120 (1.109, 1.131)	1.139 (1.116, 1.162)
2nd	1.137 (1.124, 1.151)	1.203 (1.190, 1.216)	1.117 (1.105, 1.128)	1.164 (1.139, 1.189)
3rd	1.140 (1.127, 1.152)	1.214 (1.202, 1.225)	1.117 (1.105, 1.129)	1.132 (1.108, 1.156)
Highest quartile	1.137 (1.126, 1.148)	1.207 (1.196, 1.219)	1.113 (1.102, 1.125)	1.152 (1.127, 1.176)
P for trend	0.598	0.722	0.485	0.883

Gender, body mass index, income poverty ratio, physical activity, and calcium supplement use were adjusted

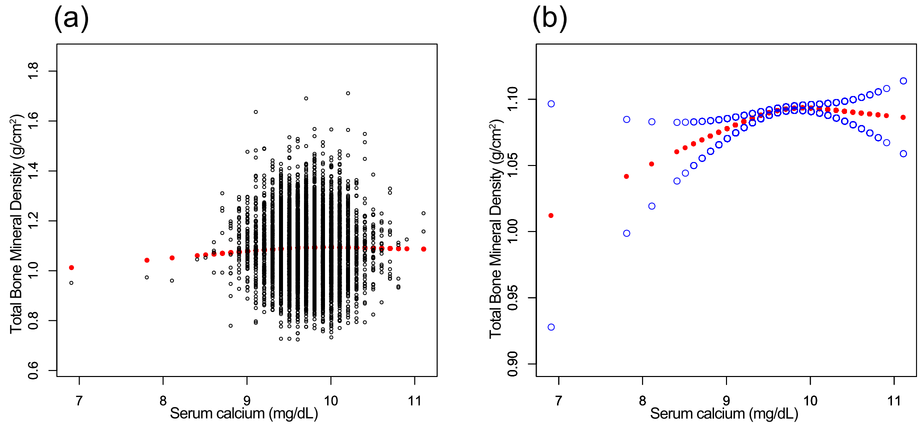

Fig. 1 The association between serum calcium and total bone mineral density. **a** Each black point represents a sample. **b** Solid rad line represents the smooth curve fit between variables. Blue bands represent the 95% of confidence interval from the fit. Adjusted for age, gender, race/ethnicity, income to poverty ratio, education, physical activity, body mass index, calcium supplementation use

R (version 3.5.3) and EmpowerStats software (http://www.empowerstats.com). P-value < 0.05 was considered statistically significant.

Results

The weighted characteristics of the study population according to gender are presented in Table 1. Most of the participants were racially/ethnically classified as "non-Hispanic white". A total of 3086 boys and 2904 girls were included in this study: For boys and girls, respectively, the mean age was 15.46 ± 2.26 and 15.35 ± 2.23 years, mean serum calcium was 9.80 ± 0.30 mg/dL and 9.64 ± 0.30 mg/dL, mean serum 25(OH)D was 24.23 ± 8.63 ng/mL and 23.32 ± 10.02 ng/mL, and mean total BMD was 1.10 ± 0.14 g/cm^2 and 1.06 ± 0.10 g/cm^2.

Association between serum calcium level and total BMD

There was a negative correlation between the serum calcium level and total BMD in the unadjusted model [− 0.0157 (− 0.0257, − 0.0058)] (Table 2), while after adjusting for all potential confounders, a positive correlation was found [0.0084 (0.0007, 0.0160)]. Compared to participants with the lowest serum calcium level in Q1, participants in other groups had a higher total BMD. The results of total BMD by quartiles of the serum calcium level, stratified by race/ethnicity and age, are shown in Table 3. Furthermore, we found an inverted U-shaped relationship between the serum calcium level and total BMD using the smooth curve fitting method (Fig. 1). We subsequently calculated that the inflection point was 9.6 mg/dL using the two-piecewise linear regression model (Table 4). In the subgroup analysis stratified by gender and race/ethnicity, this inverted U-shaped relationship existed in boys, Whites and Blacks (Figs. 3 and 4), whereas the serum calcium level positively correlated with total BMD in girls, Mexican Americans and other race/ethnicity. In addition, we calculated that the inflection points were 9.6 mg/dL in boys and Whites, and 9.2 mg/dL in Blacks.

Association between serum 25(OH)D level and total BMD

The serum 25(OH)D level negatively correlated with total BMD in the unadjusted model [0.0005 (− 0.0009, − 0.0002)] (Table 2). This association became positive after adjusting for all potential confounders [0.0006 (0.0003, 0.0008)] (Table 2, Fig. 2). Compared to participants with the lowest serum 25(OH)D level in Q1, participants in the other groups had a higher total BMD. The trend test remained significant ($P < 0.001$). The results of total BMD by quartiles of serum 25(OH)D, stratified by race/ethnicity and age. Are shown in Table 5. In the subgroup analysis stratified by gender and race/ethnicity, we found a U-shaped relationship in boys, and an inverted U-shaped relationship in girls and Whites (Figs. 3 and 4). The results of the inflection points are shown in Table 4.

Discussion

The aim of this study was to explore whether there are independent correlations between serum calcium, and 25(OH)D levels and total BMD among adolescents aged 12–19 years. The results showed that serum 25(OH)D was positively correlated with total BMD, and the relationship of serum calcium with total BMD assumed an inverted U-shaped (inflection point: 9.6 mg/dL).

Calcium plays an important role in many biological systems, most notably in bones. Thus, it is essential to

Table 4 Threshold effect analysis of serum calcium and 25(OH)D on total bone mineral density by using two-piecewise linear regression

	Adjusted ß (95% CI), *p*-value
Serum calcium	
Total	
Fitting by standard linear model	0.0084 (0.0007, 0.0160) 0.0317
Fitting by two-piecewise linear model	
Inflection point	9.6
Serum calcium < 9.6 (mg/dL)	0.0348 (0.0165, 0.0530) 0.0002
Serum calcium > 9.6 (mg/dL)	−0.0051 (− 0.0164, 0.0063) 0.3824
Log likelihood ratio	0.002
Boy	
Fitting by standard linear model	0.0034 (−0.0076, 0.0143) 0.5446
Fitting by two-piecewise linear model	
Inflection point	9.6
Serum calcium < 9.6 (mg/dL)	0.0447 (0.0128, 0.0765) 0.0060
Serum calcium > 9.6 (mg/dL)	−0.0109 (− 0.0260, 0.0042) 0.1564
Log likelihood ratio	0.007
White	
Fitting by standard linear model	0.0129 (−0.0017, 0.0276) 0.0843
Fitting by two-piecewise linear model	
Inflection point	9.6
Serum calcium < 9.6 (mg/dL)	0.0465 (0.0091, 0.0839) 0.0148
Serum calcium > 9.6 (mg/dL)	−0.0025 (− 0.0240, 0.0191) 0.8218
Log likelihood ratio	0.055
Black	
Fitting by standard linear model	0.0053 (−0.0080, 0.0187) 0.4353
Fitting by two-piecewise linear model	
Inflection point	9.2
Serum calcium < 9.2 (mg/dL)	0.1074 (0.0200, 0.1949) 0.0161
Serum calcium > 9.2 (mg/dL)	−0.0011 (− 0.0155, 0.0133) 0.8780
Log likelihood ratio	0.020
Serum 25(OH)D	
Boy	
Fitting by standard linear model	0.0013 (0.0009, 0.0018) < 0.0001
Fitting by two-piecewise linear model	
Inflection point	24
Serum 25(OH)D < 24(ng/mL)	0.0024 (0.0015, 0.0034) < 0.0001
Serum 25(OH)D > 24(ng/mL)	0.0007 (0.0000, 0.0014) 0.0400
Log likelihood ratio	0.008
Girl	
Fitting by standard linear model	0.0003 (−0.0000, 0.0007) 0.0565
Fitting by two-piecewise linear model	
Inflection point	26
Serum 25(OH)D < 26(ng/mL)	0.0011 (0.0005, 0.0018) 0.0006
Serum 25(OH)D > 26(ng/mL)	−0.0003 (− 0.0008, 0.0003) 0.3218

Table 4 Threshold effect analysis of serum calcium and 25(OH)D on total bone mineral density by using two-piecewise linear regression *(Continued)*

	Adjusted ß (95% CI), *p*-value
Log likelihood ratio	0.004
White	
Fitting by standard linear model	0.0003 (−0.0002, 0.0009) 0.1945
Fitting by two-piecewise linear model	
Inflection point	23
Serum 25(OH)D < 23(ng/mL)	0.0035 (0.0018, 0.0052) < 0.0001
Serum 25(OH)D > 23(ng/mL)	−0.0005 (− 0.0011, 0.0002) 0.1629
Log likelihood ratio	< 0.001

age, gender, race/ethnicity, body mass index, income to poverty ratio, physical activity, and calcium supplementation use were adjusted
In the analysis for boy/girl, white/black, the model is not adjusted for gender or race/ethnicity, respectively

ensure adequate calcium intake throughout life for building and maintaining bones [21]. However, a lower-than-recommended calcium intake has been reported for European and Brazilian populations [22, 23]. Several trials have suggested that supplementing by oral calcium salts is notably associated with bone attenuation [24, 25]. Some recent studies showed either no significant correlation or a negative association between serum calcium level with BMD [8, 9, 13]; our results suggested an inverted U-shaped relationship in adolescents aged 12–19 years. There are some biological explanations for the findings of this study. It was found that a high calcium intake or an intake exceeding the optimal level, could reduce osteoblast differentiation and mineralization in vitro [26, 27] and in some animals [28]. Thus, there may be a narrow range of optimal calcium levels that promote bone growth, whereas elevated calcium levels may have deleterious effects on bone. The above evidence suggested that calcium intake is required to improve BMD in subjects with low serum calcium levels.

It has been shown that a low serum 25(OH)D level has an adverse effect on bone health [29]. Several cross-sectional studies reported a positive association between serum 25(OH)D level and BMD in adults [30–32]. Vitamin D deficiency has been a global health problem in the general population, with a distinct lack of data in children and adolescents [33]. Recent studies showed that hypovitaminosis D is a common disease in children, and studies in an adult population regarding the aetiology of osteoporosis have somewhat linked the evidence to vitamin D deficiency during childhood and adolescence [34]. The results of a cross-sectional study of 100 Indian healthy school-age children showed that the serum 25(OH)D level positively related to BMD [35].

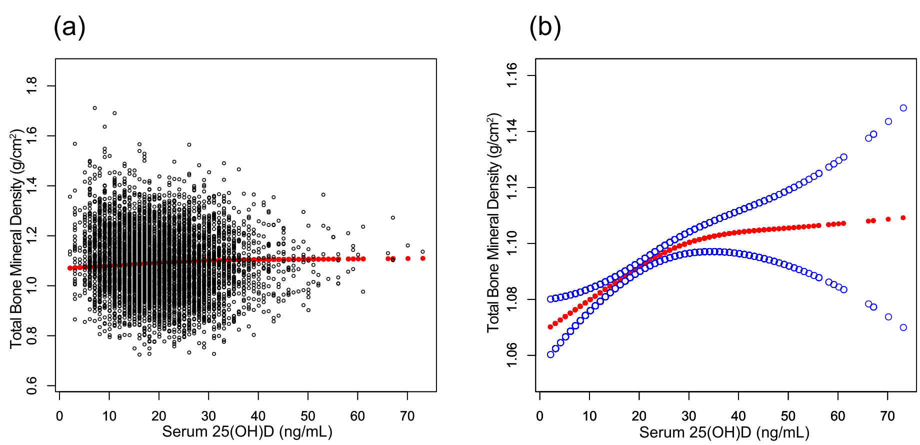

Fig. 2 The association between serum 25(OH)D level and total bone mineral density. **a** Each black point represents a sample. **b** Solid rad line represents the smooth curve fit between variables. Blue bands represent the 95% of confidence interval from the fit. Adjusted for age, gender, race/ethnicity, income to poverty ratio, education, physical activity, body mass index, calcium supplementation use

Table 5 Total bone mineral density by quartiles of serum 25(OH)D level, stratified by race/ethnicity and age

Quartiles of 25(OH)D level	White	Black	Mexican American	Other race
	Total BMD g/cm^2 (95% Confidence Interval)			
12 to 15 years				
Lowest quartile	0.975 (0.929, 1.022)	1.064 (1.054, 1.074)	0.996 (0.979, 1.013)	1.027 (0.996, 1.058)
2nd	0.994 (0.973, 1.015)	1.068 (1.056, 1.079)	0.994 (0.982, 1.007)	0.993 (0.970, 1.016)
3rd	1.013 (1.001, 1.025)	1.061 (1.048, 1.075)	1.012 (1.002, 1.023)	1.000 (0.981, 1.020)
Highest quartile	1.011 (1.002, 1.020)	1.071 (1.052, 1.090)	1.014 (1.000, 1.027)	1.050 (1.026, 1.074)
P for trend	0.134	0.780	0.024	0.079
16 to 19 years				
Lowest quartile	1.106 (1.073, 1.139)	1.212 (1.204, 1.220)	1.107 (1.094, 1.120)	1.135 (1.107, 1.162)
2nd	1.126 (1.106, 1.146)	1.203 (1.191, 1.215)	1.109 (1.098, 1.119)	1.153 (1.128, 1.178)
3rd	1.127 (1.115, 1.140)	1.198 (1.182, 1.215)	1.115 (1.104, 1.125)	1.136 (1.115, 1.157)
Highest quartile	1.144 (1.136, 1.152)	1.210 (1.188, 1.232)	1.140 (1.128, 1.152)	1.160 (1.136, 1.185)
P for trend	0.003	0.262	< 0.001	0.347

Gender, body mass index, income poverty ratio, physical activity, and calcium supplement use were adjusted

Our results also showed a positive association between the serum 25(OH)D level and total BMD. We performed subgroup analyses to describe the data in more detail following the STROBE guidelines [36]. A non-linear relationship between the 25(OH)D level and total BMD was found, and the inflection points were detected. These findings suggest a potentially optimal serum vitamin D level for BMD, while elevated 25(OH)D levels (beyond the turning point) may lead to a lower BMD in girls and Whites. The heterogeneity among these studies, including differences in participant selection, study size, study design, and controlled confounders, may provide a possible explanation for these conflicting conclusions.

To the best of our knowledge, this is the largest study investigating the relationships between serum calcium, and 25(OH)D levels and BMD in adolescents, and this representative sample of a multiracial population may be used as a general investigation of the whole population. Some limitations are worth noting. First, accurate data on puberty status were not included in our study. Although age was adjusted as a covariate in this study, the possible bias related to the unmeasured puberty status remained a limitation. Second, due to its cross-sectional design, this research had less power regarding the determination of causal relationships between serum calcium, and 25(OH)D levels and BMD.

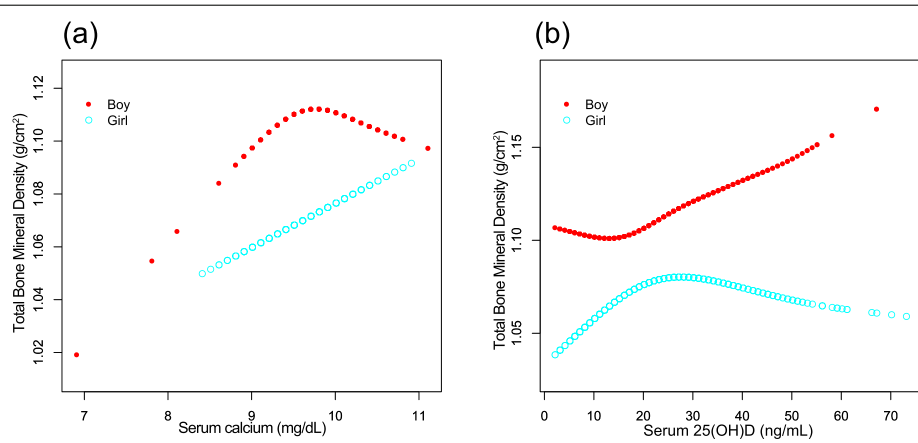

Fig. 3 The associations between serum calcium (**a**), serum 25(OH)D (**b**) and total bone mineral density stratified by gender. Adjusted for age, race/ethnicity, income to poverty ratio, education, physical activity, body mass index, calcium supplementation use

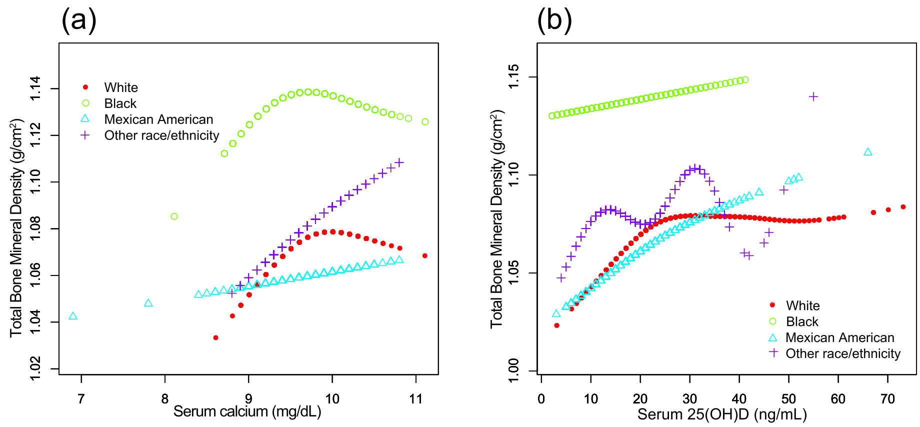

Fig. 4 The associations between serum calcium (**a**), serum 25(OH)D (**b**) and total bone mineral density stratified by race/ethnicity. Adjusted for age, gender, income to poverty ratio, education, physical activity, body mass index, calcium supplementation use

Conclusions

We found that serum calcium level had an inverted U-shaped relationship with total BMD, while serum 25(OH)D level positively correlated with total BMD. Further studies are needed to assess whether increased serum 25 (OH) D or calcium levels may have a beneficial effect on BMD in adolescents with low serum 25 (OH) D or calcium levels.

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Authors' contributions

KYP, RLT, and XYC contributed to data collection, analysis and writing of the manuscript. ZXZ contributed to study design and writing of the manuscript. The authors read and approved the final manuscript.

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Availability of data and materials

The survey data are publicly available on the internet for data users and researchers throughout the world (www.cdc.gov/nchs/nhanes/).

Declarations

Ethics approval and consent to participate

The ethics review board of the National Center for Health Statistics approved all NHANES protocols and written informed consents were obtained from all participants or their proxies (< 18 years).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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