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Associations between multiple metals exposure and bone mineral density: a population-based study in U.S. children and adolescents

Jian Han[1†], Jiaqing Sun[1†], Lin Yuan[1], Luyao Lou[1] and Xiaofeng Jiang[1*]

Abstract

Aim This study examined the correlation between multi-metal exposure and bone mineral density (BMD) in U.S. children and adolescents.

Methods Data from 1,591 participants (aged 8–19) were analyzed using the National Health and Nutrition Examination Survey (NHANES) 2011–2016. We measured serum copper (Cu), selenium (Se), zinc (Zn), and blood lead (Pb), cadmium (Cd), mercury (Hg), manganese (Mn). Dual-energy X-ray absorptiometry assessed lumbar and total BMD. Advanced statistical approaches including weighted quantile sum (WQS) regression and bayesian kernel machine regression (BKMR) were employed to evaluate complex exposure interactions.

Results Blood Pb and serum Cu showed inverse associations with, while serum Se positively correlated with lumbar BMD (blood Pb: β: -0.013, serum Cu: β: -0.063, serum Se: 0.035) (all $P < 0.05$). The WQS index showed a significant association with both lumbar BMD(β = 0.019, $P < 0.05$) and total BMD (β = 0.019, $P < 0.001$). WQS analysis identified Cd, Se, and Hg as primary contributors to both lumbar and total BMD variations. BKMR models revealed nonlinear exposure–response relationships and synergistic effects between Cd and Mn.

Conclusion These findings highlight the importance of considering mixed metal exposures in bone health assessments, providing crucial insights for developing preventive strategies to protect skeletal development in pediatric populations.

Keywords Multiple metals exposure, Bone mineral density (BMD), Children and adolescents, Cross-sectional study

Introduction

Osteoporosis involves reduced bone density and mass, leading to greater bone fragility and an elevated risk of fractures [11]. Bones are dynamic tissues that provide structural support, anchor muscles, protect internal organs, and store calcium. Bone mineral density (BMD) serves as a critical indicator of skeletal health during childhood and adolescence, playing an essential role in bone growth and mineralization [27]. Individuals with low peak bone density during youth have an increased risk of developing early-onset osteoporosis and fragility fractures in adulthood [36]. By age 18, approximately 95% of bone size and musculoskeletal mass is achieved, emphasizing the crucial importance of childhood development in establishing a robust musculoskeletal system [2]. Multiple factors influence bone structure and quality, including genetic predisposition, organ function, chronic

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systemic diseases, medications, muscle disorders, and metabolic abnormalities [7]. Pediatric osteoporosis typically presents with three main clinical manifestations: recurrent fractures, skeletal deformities, and chronic back pain.

Research indicates that prolonged exposure to low-level cadmium(Cd) (urinary levels < 1 μg/g creatinine) is linked to growth retardation, osteoporosis, bone demineralization, bone loss, and a heightened risk of fractures [4, 14]. Blood lead(Pb) concentration exhibits a dose–response relationship with bone loss, with higher concentrations significantly increasing the odds ratio for bone loss [13]. An animal study demonstrated that chronic low-dose Pb exposure in male mice reduces bone density and trabecular bone volume, inhibiting bone formation and causing bone damage [39]. Numerous studies have linked high blood levels of Pb and Cd and decreased bone mineral density (BMD), potentially increasing the risk of fragility fractures. The accumulation of Pb and Cd in the bone tissue of osteoporosis patients could be related to bone metabolism disorders [38]. A study found no link between manganese(Mn) and bone or muscle quality [1], whereas a large-scale study (n = 9,732) identified a negative association between blood Mn levels and BMD, with the strongest effect observed in the femoral neck(S. [23, 24]). What is more, Li et al. demonstrated that long-term occupational Mn exposure increases osteoporosis risk in retired female workers [22]. Research indicates a positive correlation between lumbar spine BMD and dietary patterns high in antioxidant trace elements, such as copper(Cu) and Mn [17]. A study on elderly individuals revealed significantly higher concentrations of Mn, Cu, and zinc(Zn) in non-osteoporotic subjects compared to those with osteoporosis. Additionally, high bone Zn concentration negatively correlated with osteoporosis incidence(S. [23, 24]). A South Korean study analyzing hair selenium(Se) levels found that participants with lower Se concentrations exhibited higher levels of phosphorus, alkaline phosphatase, and osteocalcin, along with reduced bone density [30]. A study in Southeastern Australia demonstrated that low dietary intake of Cu and Se was independently associated with reduced BMD, specifically, the lowest quartile of Cu and Se intake was consistently linked to lower mean BMD across multiple skeletal sites [31]. Huang et al. found no correlation between osteoporosis and blood mercury(Hg) levels [16]. According to Kim et al., blood Hg is negatively correlated with femoral neck BMD in a Korean population [18].

Current studies primarily examine the link between BMD and exposure to specific metals. Multiple metal exposures have not been well studied in terms of their overall health effects. Nevertheless, individuals are often exposed to more than one metal at the same time. Besides, existing research has revealed complex metal contact patterns in real life by examining multi metal mixed exposures, which are associated with obesity and various chronic diseases in adults [51]. However, these studies mainly focused on specific populations such as pregnant women, residents around factories, and Native Americans, with limited sample sizes and lack of representativeness, severely limiting the extrapolation and universality of research findings to a wider population(Y. [23, 24]). Therefore, conducting this research has significant scientific significance and practical value. For assessing health effects associated with exposure to multiple metals, researchers commonly employ two statistical approaches: weighted quantile sum (WQS) regression and Bayesian kernel machine regression (BKMR). These two approaches have been applied to evaluate various health outcomes, including sarcopenia, cognitive function, psoriasis, and lung cancer [5, 15, 40, 50]. This study utilized logistic regression, WQS regression, and BKMR models to investigate the correlation between metal exposure and bone density in children and adolescent populations. We analyzed Cu, Se, and Zn in serum samples, alongside blood concentrations of Pb, Cd, Hg, and Mn.

Materials and methods

Study population

This research employed data from NHANES, a national survey, utilizing a complex, multistage probability sampling design to evaluate the health and nutritional status of the U.S. population. The study combined NHANES survey data from three biennial cycles: 2011–2012, 2013–2014, and 2015–2016. Additionally, we excluded participants who did not have complete data on metal concentrations or other relevant covariates. The final analysis included 1591 participants aged 8–19.

Measurement of seven heavy metals

We assessed metal exposure levels through the measurement of Cd, Pb, Hg, Mn in whole blood, as well as Cu, Se, and Zn in serum. Blood Pb, Cd, Hg,Mn, and Se concentrations were analyzed using inductively coupled plasma mass spectrometry (ICP-MS). This multi-element analytical technique is based on quadrupole ICP-MS technology. Detailed information can be found at the following website https://wwwn.cdc.gov/Nchs/Data/Nhanes/Public/2011/DataFiles/PBCD_G.htm#Description_of_Laboratory_Methodology. Serum metal concentrations were quantified using inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS), which is a multi-element analytical technique capable of trace level elemental analysis. The isotopes measured by this method include Zn, Cu, and Se. Detailed information

about laboratory procedures can be found at https://wwwn.cdc.gov/Nchs/Data/Nhanes/Public/2011/DataFiles/CUSEZN_G.htm#Component_Description. When values were under the limit of detection(LOD) threshold, they were substituted using the calculation LOD divided by the square root of 2 during data preprocessing to address missing data.

Bone mineral density (outcomes)

Dual-energy X-ray absorptiometry(DXA) full-body scans were conducted using Hologic Discovery A densitometers (Hologic, Inc., Bedford, Massachusetts). Whole-body DXA scans cause minimal radiation exposure, with doses below 20 μSv. We analyzed all scans using Hologic APEX 4.0 software that incorporated the NHANES Body Composition Analysis option.

Other covariates

Based on prior studies, we identified potential covariates linked to significant predictors of blood heavy metals and BMD. Covariate data were gathered via home interviews, physical examinations, laboratory analyses, and standardized questionnaires. Covariates involved gender, age, race (Mexican Americans, other Hispanics, non-Hispanic Whites, non-Hispanic Blacks, non-Hispanic Asians,or other races) [9, 25], body mass index (BMI <25, 25–30, ≥ 30, kg/m^2) [45, 46],family income to poverty ratio [52] (PIR ≤ 1.3, 1.3–3.5 or >3.5),tobacco exposure (cotinine <0.05 ng/ml, 0.05–2.99 ng/ml or ≥ 3 ng/ml) [10], fasting duration [26] (<8 h or ≥ 8 h),physical activity [48],and the presence of high blood pressure or diabetes. The assessment of physical activity takes into account its frequency, time, and intensity, and calculates the weekly metabolic equivalent (MET) according to the Global Physical Activity Questionnaire(GPAQ) guidelines. Individuals with a MET of less than 500 per week are classified as sedentary, while those with 500–1000 min per week are considered moderate, and those exceeding 1000 min per week are categorized as high.

Statistical analysis

In this study, statistical analyses were executed utilizing R software (version 4.3.2). The threshold for statistically significant set at $P< 0.05$. Study participants were stratified by age into two groups: children (< 12 years) and adolescents (> =12 years). Group comparisons were conducted using Student's t-test for continuous variables and chi-square test for categorical variables. Shapiro–Wilk normality tests revealed that all blood/serum heavy metal concentrations were skewed. For subsequent analyses, we applied logarithmic transformation to address the non-normal distribution of these data. As a means of assessing the interaction between seven heavy metals, Pearson correlation coefficients were calculated for log-transformed concentrations of each metal.

We constructed generalized linear regression models using the'glm'package to examine the associations between individual blood/serum trace elements and both total and lumbar BMD. We employed the'gWQS'package to construct a WQS regression model, estimating the relationship between multi-metal exposure and bone density. This study calculated the WQS index by categorizing seven metals into quartiles.

We constructed a non-parametric BKMR model using the"bkmrhat"package to assess the joint effects of serum and blood trace elements on lumbar and total BMD. BKMR combines Bayesian inference with machine learning techniques, employing a Gaussian kernel function to iteratively model the exposure–response relationship. This methodology enables the detection of complex, nonlinear, and interactive effects among multiple serum and blood trace elements. Given the high correlation among the chemical substances, we employed a Markov chain Monte Carlo algorithm for hierarchical variable selection, using 10,000 iterations.

Results

Characteristics of participants

Figure 1 illustrates the participant selection process. The study comprised 1,591 participants, consisting of 592 children aged 8–11 and 999 adolescents aged 12–19. The demographic baseline data is shown in Table 1. The study found significant differences in BMI, cotinine levels, physical activity, fasting status, hypertension prevalence, total and lumbar BMD, as well as serum Cu, Se, Zn and blood Pb, Cd, Hg among the included children and adolescents (all $P< 0.05$). Compared to children, adolescents had higher proportion of hypertension prevalence, and higher levels of physical activity, lumbar BMD, and total BMD. Children exhibited elevated serum Cu and blood Pb levels compared to adolescents.

Measurements of seven metals and their correlation

Table 2 summarizes the arithmetic mean, geometric mean, and distribution patterns of the seven metal concentrations. The levels of Mn, Cu, Se, and Zn were all completely detected. The detection frequency of the other three metals, were Pb 98.93%, Hg 75.24%, and Cd 59.59%, respectively. The blood concentrations ranked from highest to lowest were Mn, Pb, Hg, and Cd. The concentration of Se in serum was the highest, about 1.5 times higher than that of Zn.

The correlations between the concentrations of these seven metal substances is not obvious(r ranging from 0.01 to 0.22), as shown in detail in Fig. 2. The strongest

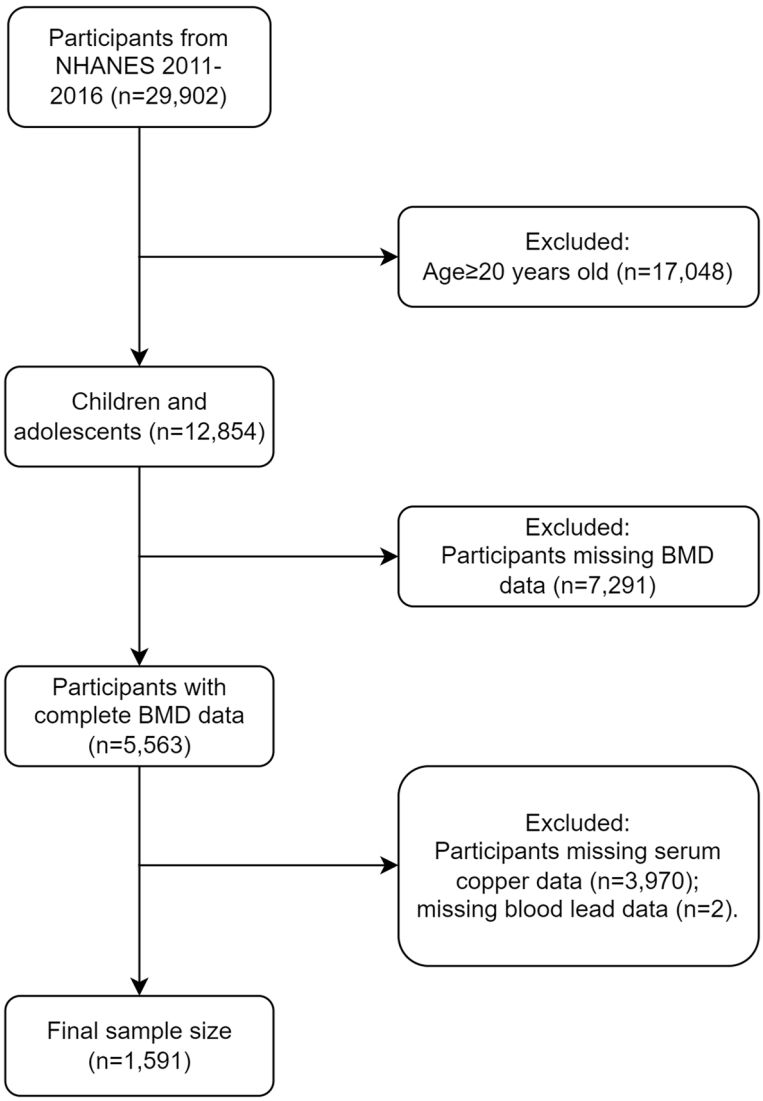

Fig. 1 Data filtering process diagram

correlation in this study was between serum Se and Zn, with a weak correlation ($r = 0.22$).

Linear regression analysis

Table 3 presents the results of the generalized linear regression analysis of the single metal model after controlling for confounding factors. Blood Pb, serum Cu, and serum Se were correlated with lumbar BMD (blood Pb: β: − 0.013, serum Cu: β: − 0.063, serum Se: 0.035) (all $P < 0.05$). In the multiple-metals model, blood Pb and serum Cu were negatively correlated (blood Pb: β: − 0.011, serum Cu: β: − 0.064) (all $P < 0.05$), and serum Se was positively correlated with lumbar BMD (β: 0.054, $P < 0.05$).

In the multivariate regression between a single metal and total BMD, blood Cd, blood Pb, blood Mn, and serum Cu were all negatively associated with total BMD (blood Cd: β: − 0.008, blood Pb: β: − 0.011, blood Mn: − 0.017, serum Cu: − 0.033) (all $P < 0.05$). In the multiple-metals model, blood Pb and serum Cu are correlated with total BMD. (Blood Pb: β: − 0.011 serum Cu: β: − 0.033), all $P < 0.05$).

WQS analysis

We employed WQS regression to examine the cumulative impact of seven heavy metals on lumbar and total BMD. This study utilized the WQS regression model to examine the effects of exposure to seven heavy metals on lumbar and total BMD. The WQS indices were

Table 1 Participant Characteristics, NHANES 2011–2016

Variables	Total ($n = 1,591$)	Age		P
		< 12 years old ($n = 592$)	≥ 12 years old ($n = 999$)	
Gender, n (%)				0.826
Female	777 (48.84)	287 (48.48)	490 (49.05)	
Male	814 (51.16)	305 (51.52)	509 (50.95)	
Race, n (%)				0.18
Mexican American	363 (22.82)	146 (24.66)	217 (21.72)	
Non-Hispanic Black	373 (23.44)	128 (21.62)	245 (24.52)	
Non-Hispanic White	413 (25.96)	161 (27.20)	252 (25.23)	
Other Hispanic	178 (11.19)	71 (11.99)	107 (10.71)	
Other Race	264 (16.59)	86 (14.53)	178 (17.82)	
BMI category, n (%)				< 0.001
< 25 kg/m2	1165 (73.22)	519 (87.67)	646 (64.66)	
25–30 kg/m2	239 (15.02)	47 (7.94)	192 (19.22)	
≥ 30 kg/m2	187 (11.75)	26 (4.39)	161 (16.12)	
PIR, n (%)				0.084
< 1.3	704 (44.25)	279 (47.13)	425 (42.54)	
1.3–3.5	598 (37.59)	202 (34.12)	396 (39.64)	
> 3.5	289 (18.16)	111 (18.75)	178 (17.82)	
Cotinine category, n (%)				< 0.001
< 0.05 ng/ml	919 (57.76)	359 (60.64)	560 (56.06)	
0.05–2.99 ng/ml	526 (33.06)	215 (36.32)	311 (31.13)	
≥ 3 ng/ml	146 (9.18)	18 (3.04)	128 (12.81)	
Physical activity, n (%)				< 0.001
Sedentary Insufficient	818 (51.41)	592 (100.00)	226 (22.62)	
Moderate	101 (6.35)	0 (0.00)	101 (10.11)	
High	672 (42.24)	0 (0.00)	672 (67.27)	
Fasting status, n (%)				< 0.001
< 8 h	967 (60.78)	451 (76.18)	516 (51.65)	
≥ 8 h	624 (39.22)	141 (23.82)	483 (48.35)	
Hypertension, n (%)				< 0.001
No	1519 (95.47)	589 (99.49)	930 (93.09)	
Yes	72 (4.53)	3 (0.51)	69 (6.91)	
Diabetes, n (%)				0.105
No	1580 (99.31)	591 (99.83)	989 (99.00)	
Yes	11 (0.69)	1 (0.17)	10 (1.00)	
Total BMD (g/cm2,Mean ± SD)	0.95 ± 0.16	0.81 ± 0.09	1.04 ± 0.12	< 0.001
Lumbar BMD (g/cm2,Mean ± SD)	0.87 ± 0.19	0.70 ± 0.10	0.97 ± 0.16	< 0.001
Pb (μg/L,Mean ± SD)	0.67 ± 0.68	0.72 ± 0.61	0.64 ± 0.72	0.016
Cd (μg/L,Mean ± SD)	0.17 ± 0.21	0.12 ± 0.06	0.21 ± 0.26	< 0.001
Hg (μg/L,Mean ± SD)	0.63 ± 0.85	0.56 ± 0.76	0.66 ± 0.89	0.015
Mn (μg/L,Mean ± SD)	10.88 ± 3.76	10.86 ± 3.89	10.90 ± 3.68	0.871
Cu (μg/L,Mean ± SD)	112.80 ± 26.05	120.42 ± 21.75	108.28 ± 27.32	< 0.001
Se (μg/L,Mean ± SD)	122.13 ± 14.92	117.91 ± 12.91	124.63 ± 15.47	< 0.001
Zn (μg/L,Mean ± SD)	82.57 ± 14.71	81.31 ± 13.76	83.32 ± 15.21	0.008

NHANES National health and Nutrition Examination Survey, *BMI* body mass index, *PIR* Family income to poverty ratio, *BMD* bone mineral density, *SD* Standard Deviation

Table 2 Heavy metal concentrations in blood and serum samples from the study population

Variables (unit)	Detection frequency	GM	Mean	Percentile				
				5 th	25 th	50 th	75 th	95 th
Pb (μg/L)	98.93%	0.55	0.67	0.24	0.39	0.53	0.75	1.47
Cd (μg/L)	59.59%	0.14	0.17	0.07	0.10	0.12	0.19	0.39
Hg (μg/L)	75.24%	0.42	0.63	0.18	0.20	0.37	0.66	2.09
Mn (μg/L)	100%	10.34	10.88	6.21	8.39	10.25	12.68	17.395
Cu (μg/L)	100%	110.04	112.8	78.00	95.10	110.10	126.20	155.70
Se (μg/L)	100%	121.25	122.13	100.50	112.20	120.60	130.80	147.35
Zn (μg/L)	100%	81.32	82.57	61.30	72.40	81.40	91.30	107.90

GM Geometric mean

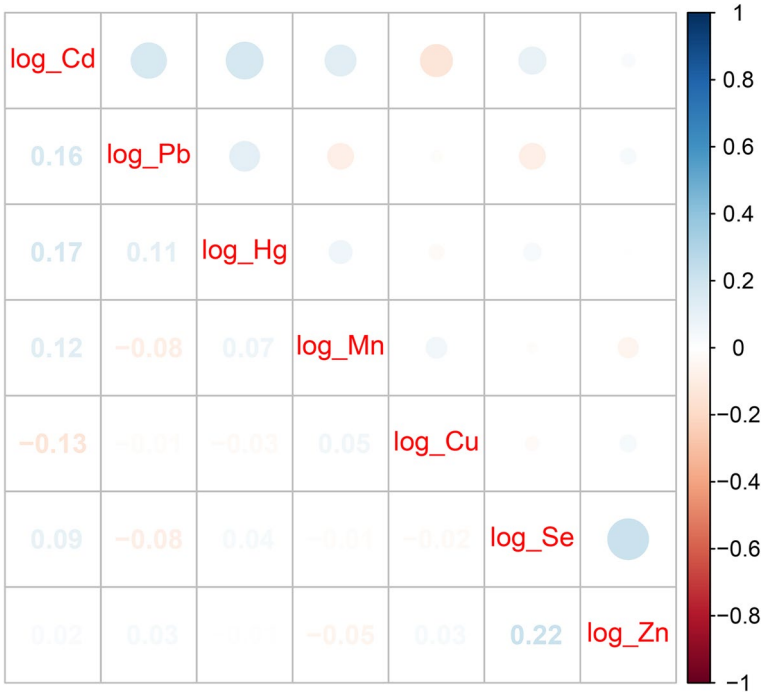

Fig. 2 Pairwise pearson correlations between blood and serum concentrations of the seven heavy metals within the population

statistically correlated with lumbar and total BMD. According to the adjusted model, the WQS index showed a significant association with both lumbar BMD($\beta = 0.019$, $P < 0.05$) and total BMD ($\beta = 0.019$, $P < 0.001$). The details were provided in Table 4.

Table 5 presents the estimated metal weights for each WQS index. In the relationship between WQS mixed variables and lumbar BMD, the weighted values of serum Se, blood Cd and blood Hg account for 43%, 38%, and 15%, respectively. In the relationship between WQS mixed variables and total BMD, the weighted values of blood Cd, serum Se, and blood Hg account for 43%, 29%, and 18%, respectively. Serum Cu had the least impact on both lumbar and total BMD.

BKMR analysis

The BKMR model assessed the collective effect of metal exposure on lumbar and total BMDs. The overall effect is decreasing compared to the 50 th percentile. Individuals exposed to metal levels below the 50 th percentile exhibited an inverse relationship with both lumbar and total BMD. Refer to Fig. 3 for detailed information.

Figure 4 shows the trend of exposure–response for seven metal substances. An inverse relationship was

Table 3 Linear regression analysis of the association between seven heavy metals and lumbar and total BMD

	Lumbar BMD			Total BMD		
	β	95%CI	*P* value	β	95%CI	*P* value
Single-metal model						
Cd(μg/L)	− 0.009	− 0.02,0.002	0.098	− 0.008	− 0.017,0	0.044
Pb(μg/L)	− 0.013	− 0.023,− 0.002	0.021	− 0.011	− 0.02,− 0.003	0.006
Hg(μg/L)	− 0.003	− 0.011,0.004	0.405	0	− 0.006,0.005	0.898
Mn(μg/L)	− 0.014	− 0.033,0.005	0.14	− 0.017	− 0.032,− 0.003	0.019
Cu(μg/L)	− 0.063	− 0.09,− 0.036	< 0.001	− 0.033	− 0.054,− 0.012	0.002
Se(μg/L)	0.035	− 0.26,0.33	0.025	0.002	− 0.035,0.039	0.911
Zn(μg/L)	− 0.024	− 0.059,0.01	0.168	0.005	− 0.022,0.032	0.711
Multiple-metals model						
Cd(μg/L)	− 0.005	− 0.016,0.006	0.381	− 0.005	− 0.013,0.004	0.255
Pb(μg/L)	− 0.011	− 0.022,0	0.049	− 0.011	− 0.019,− 0.003	0.011
Hg(μg/L)	− 0.001	− 0.008,0.006	0.799	0.002	− 0.007,0.01	0.558
Mn(μg/L)	− 0.01	− 0.029,0.009	0.319	− 0.014	− 0.029,0	0.056
Cu(μg/L)	− 0.064	− 0.091,− 0.037	< 0.001	− 0.033	− 0.054,− 0.012	0.002
Se(μg/L)	0.054	0.005,0.104	0.032	0.003	− 0.0350.041	0.883
Zn(μg/L)	− 0.026	− 0.061,0.01	0.16	0.008	− 0.019,0.036	0.568

All models were adjusted for gender, race, age, body mass index, family income to poverty ratio, cotinine category, fasting status, physical activity, hypertension and diabetes

BMD bone mineral density

Table 4 Relationship between the weighted quantile sum regression index and BMDs

Outcome	β	95%CI	*P* value
Total BMD			
Model1	0.058	0.046–0.071	< 0.001
Model2	0.019	0.008–0.029	< 0.001
Lumbar BMD			
Model1	0.069	0.054–0.083	< 0.001
Model2	0.019	0.007–0.031	0.002

Model 1: unadjusted

Model 2: adjusted for gender, race, age, body mass index, family income to poverty ratio, cotinine category, fasting status, physical activity, hypertension and diabetes

BMD bone mineral density

Table 5 Weights of the seven metals in the weighted quantile sum model

mix_name	Lumbar BMD:mean_weight	Total BMD:mean_weight
Cd	0.383	0.434
Se	0.432	0.289
Hg	0.148	0.182
Zn	0.0255	0.0794
Pb	0.00422	0.0124
Mn	0.00689	0.00315
Cu	0	0

BMD bone mineral density

observed between serum Zn and lumbar BMD, but a flat relationship with total BMD. Blood Pb and Mn showed a negative correlation with lumbar BMD. Blood Cd, Hg and serum Se showed a positive correlation with lumbar BMD. For total BMD, there is a negative association between blood Pb and Mn, whereas positive associated with blood Hg and serum Se. At low concentrations, blood Cd correlates positively with total BMD, but at high concentrations, it correlates negatively.

We conducted further exploration into the interactions among metals, which were shown in Fig. 5. The study demonstrated that with fixation of Mn concentrations at 25, 50, and 75 percentiles in blood, and other metals at 50 percentiles, the slope of the relationship between blood Cd concentration and lumbar/total BMD would vary with changes in Mn concentration, indicating the presence of an interaction between Mn and Cd.

Discussion

This study investigated the associations between BMD and seven metals (Pb, Cd, Hg, Mn, Cu, Se, and Zn) in children and adolescents. It is a large nationally representative sample data of 1591 participants aged 8–19,

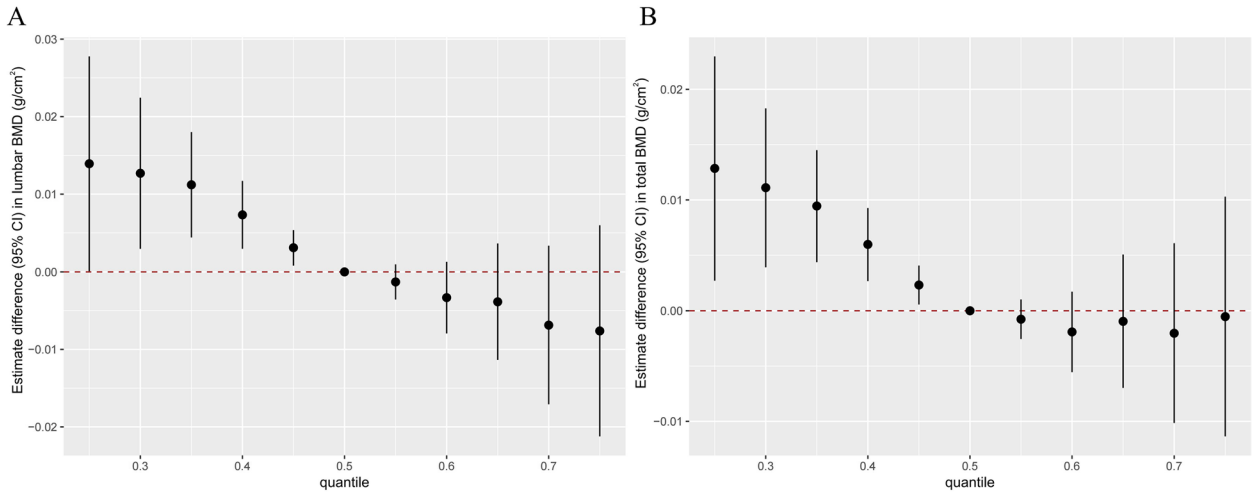

Fig. 3 The lumbar BMD (**A**) and total BMD (**B**) after exposure to seven metals are presented using the bayesian kernel machine regression model. The vertical axis depicts the estimated changes in BMD associated with exposure to seven metals at specific percentiles, as indicated on the X-axis, relative to their respective median values. Models were adjusted for gender, race, age, body mass index, family income to poverty ratio, cotinine category, fasting status, physical activity, hypertension and diabetes. BMD, bone mineral density

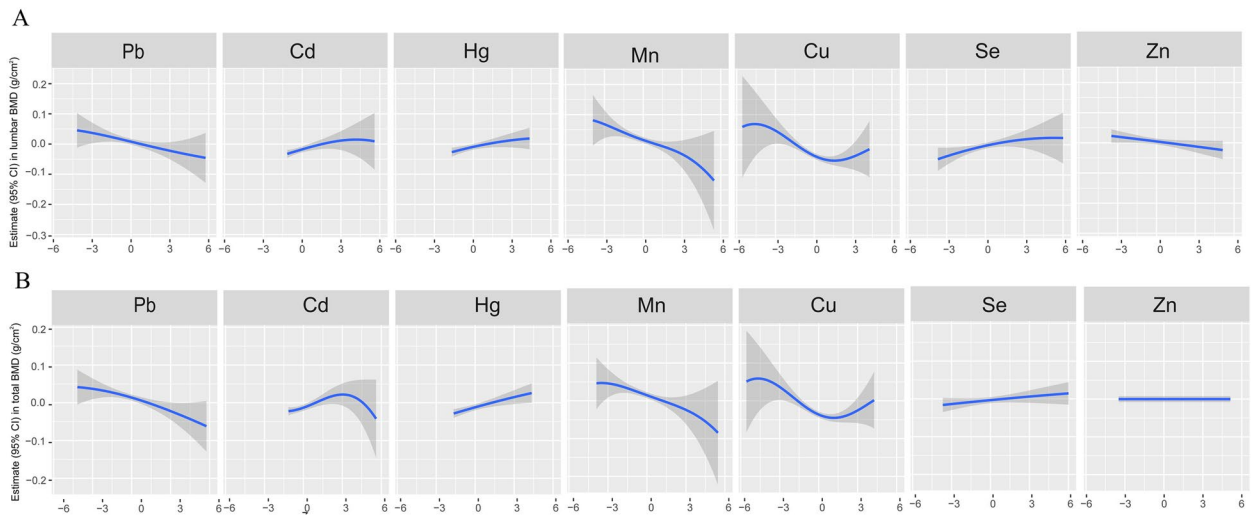

Fig. 4 Univariate correlation analysis (95% CI) between lumbar BMD (**A**) and total BMD (**B**) in relation to selected metal concentrations, with other metal concentrations held constant at their median values. h(Z) represents the association between chemicals and a latent continuous variable related to binary obesity outcomes. The results were assessed by the bayesian kernel machine regression model adjusted for gender, race, age, body mass index, family income to poverty ratio, cotinine category, fasting status, physical activity, hypertension and diabetes. BMD, bone mineral density

derived from NHANES 2011–2016. We employed multiple analytical approaches including linear regression, WQS regression, and BKMR. Our findings indicate that blood Pb and serum Cu are inversely related to lumbar and total BMD, whereas serum Se is positively correlated with lumbar BMD. WQS regression analysis revealed significant associations between metal co-exposure and decreased lumbar and total BMD values. The primary contributors to these associations were identified as Mn, Pb, and Se. BKMR analysis further confirmed complex, non-linear relationships between metal mixtures and BMD outcomes.

Pb and Se exposure implications

Multivariate regression analysis revealed negative associations between blood Pb and BMD, aligning with previous research findings. Hsieh et al. revealed a notable inverse correlation between blood Pb levels and BMD,

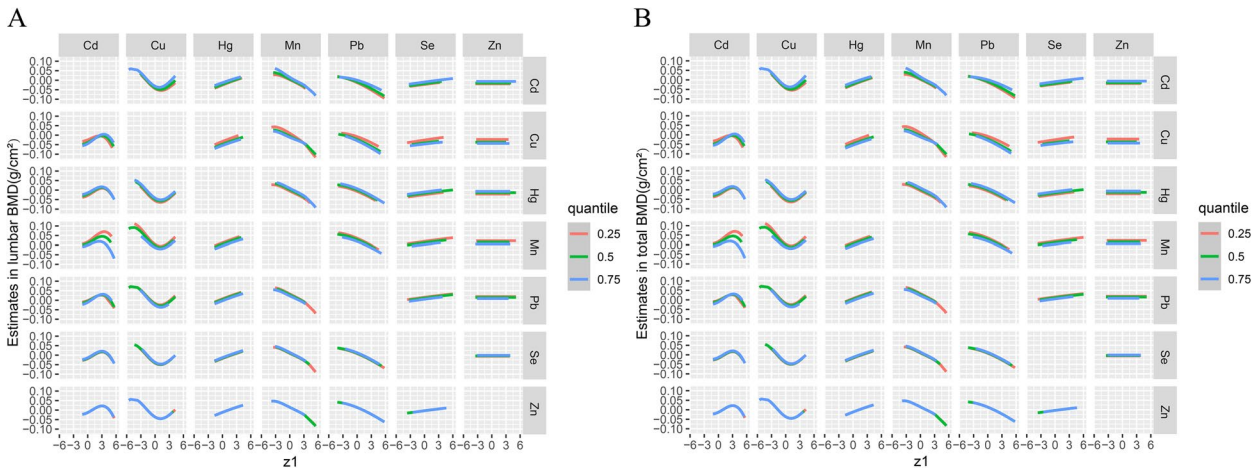

Fig. 5 Bivariate exposure–response relationships between metals and BMD. **A** and **B** illustrate the distribution of lumbar and total BMD against metal concentrations at the 25 th, 50 th, and 75 th percentiles. Other metals were set at their median values. All models were adjusted for gender, race, age, body mass index, family income to poverty ratio, cotinine category, fasting status, physical activity, hypertension and diabetes. BMD, bone mineral density

particularly in the femoral neck, in a cross-sectional study of 2,932 Taiwanese adults [13].

In contrast to prior studies, we identified a positive association between serum Se levels and BMD. This result is consistent with previous findings of Park et al.,who reported a correlation between decreased Se levels in hair and lower BMD in a sample of 126 postmenopausal Korean women [30]. The physiological function of Se is mainly mediated by selenoproteins [35], which have antioxidant activity and are known to maintain redox cell balance, prevent oxidative stress caused by reactive oxygen species, regulate inflammation and bone cell proliferation and differentiation [41], and prevent oxidative stress-induced bone loss [49].

Cu and BMD

Previous research on Cu and BMD was limited to the adult population. For example, Lin et al.'s large-scale study (n = 9,732) found an inverse relationship between serum Cu concentrations and BMD across multiple skeletal sites(S. [23, 24]). Recent studies indicate that individuals with Cu levels in the highest quartile have a higher incidence of fractures, particularly among adult males, suggesting that excessive Cu exposure may adversely affect bone health [34]. Meta-analyses have identified a direct link between Wilson's disease, marked by systemic Cu overload, and bone-related issues, such as reduced bone density, osteoporosis, and elevated fracture risk, affecting both children and middle-aged populations [6]. Our study extends these findings by examining these relationships specifically in children and adolescents, utilizing a nationally representative sample from NHANES.

The relationship between serum Cu and BMD varies across models, exhibiting non-linearity in the mixed exposure model. The discovery emphasizes the importance of considering mixed exposure and non-linear relationships when evaluating the impact of metal exposure on bone health.

Cu plays a role in angiogenesis and osteogenesis during bone regeneration and repair, promoting these processes through the regulation of mitochondrial oxidative stress [47]. While, Cu, serving as a crucial cofactor for lysyl oxidase, plays an indispensable role in the enzymatic cross-linking and maturation of collagen fibers during the cross-linking process, whereby its depletion or dysfunction can result in compromised bone structural integrity [44]. In addition, Cu is a cofactor for many enzymes in collagen synthesis, and its deficiency may lead to bone metabolism disorders and promote the development of osteoporosis [21]. The non-linear relationship between Cu and bone density likely results from multiple factors. Cu exhibits dose-dependent effects on bone cells: low concentrations enhance osteoblast viability and proliferation, whereas high doses induce cytotoxicity [20, 29, 37]. However, excessive Cu generates reactive oxygen species, induces lipid peroxidation, disrupts bone metabolism, and compromises the structural integrity of both osteoblasts and osteoclasts [8]. Although Cu deficiency is rare, it can also lead to decreased bone strength, impaired bone formation and growth, reduced bone mineralization, decreased ossification of growth centers, and impaired cartilage integrity [8]. Therefore, maintaining an optimal Cu concentration is crucial for bone health, as both deficiency and excess can negatively impact

bone metabolism and cellular function. Genetic factors significantly influence the association between copper and bone density. Various genetic loci may modulate an individual's sensitivity to copper and its effects on bone health [32]

Cd-Mn synergistic effect

Cd, a common environmental pollutant, is toxic to multiple organs, significantly affecting bone tissue. Long-term exposure to low levels of Cd is linked to a higher risk of osteoporosis and fractures [42]. Mn, an essential trace element, accumulates in various tissues, particularly in bones. However, its precise role in osteoporosis and bone density regulation remains unclear [43]. The potential synergistic interaction between Cd and Mn may amplify their detrimental effects on bone health. Specifically, cadmium exposure elevates bone resorption markers, while manganese potentially exacerbates this effect by interfering with calcium absorption and metabolism [12, 43]. Furthermore, both cadmium and manganese may contribute to bone damage by modulating the expression of metallothionein, a crucial protein in metal homeostasis. Metallothionein performs several vital cellular functions, including regulation of metal ion concentrations [19], participation in antioxidant reactions [3] and protection against heavy metal toxicity [33].

Cd and Mn exhibit a synergistic effect on bone density, with elevated Mn exposure potentially exacerbating Cd-induced toxicity in bone tissue. This interaction may stem from the antagonistic relationship between Cd and Mn [28], potentially disrupting elemental homeostasis and consequently affecting bone health. Previous studies have suggested that Cd exposure may have a negative impact on the levels of Mn in cartilage, possibly exacerbating cartilage degeneration due to a decrease in proteoglycans and glycosaminoglycans (key components of the extracellular matrix of articular cartilage cells) [28]. The Cd-Mn interaction may impair calcium absorption and metabolism, thereby altering bone mineral density. Chronic exposure to elevated levels of Cd and Mn may intensify these effects, potentially accelerating articular cartilage degeneration and increasing the risk of bone and joint disorders, including osteoarthritis and osteoporosis. Despite these observations, direct evidence elucidating the mechanisms by which high Mn exposure enhances Cd-induced toxicity on bone density remains limited. Further investigations are warranted to delineate the Cd-Mn interaction mechanisms and their cumulative impact on skeletal health. Future studies should incorporate quantitative analyses of bone density alterations under varying Cd and Mn concentrations, coupled with biomarker assessments, to elucidate the mechanistic pathways through which these elements influence skeletal integrity.

Strengths and limitations

This study offers several notable strengths. First, our study utilizes a large, representative sample from the NHANES, encompassing a diverse range of American children and adolescents. Second, for the assessment of exposure to metals we used blood samples, this approach eliminates recall bias associated with dietary intake assessments. Despite these strengths, our study has several limitations. It is difficult to build causal relationships between metal exposure and bone health due to the cross-sectional design of the study. Prospective studies are necessary to confirm and extend our findings. Second, our analysis excluded children under 8 years old due to incomplete data for this age group. It is necessary to conduct further research to fill this knowledge gap regarding metal exposure and bone density in children under the age of 8. We applied the same parameters of adults to determine the weight status of children and adolescents. Future research should adopt the more rigorous WHO 2006 standards, utilizing BMI z-scores or percentiles for classification. Then, we did not account for certain dietary and genetic factors, which may influence the results, despite adjusting for numerous potential confounders. Consequently, residual confounding from unmeasured variables may introduce some bias into our findings. Finally, we have not yet been able to determine the specific threshold for metal exposure, which is a limitation worthy of attention. The determination of this threshold has important guiding significance for clinical practice and the formulation of public health policies.

Policy recommendations and intervention measures

Our findings suggest implementing a comprehensive intervention framework to mitigate metal exposure risks in pediatric populations. Key policy recommendations include enhancing regulatory standards for industrial emissions and environmental metal pollutants, coupled with strengthening drinking water quality surveillance systems. Clinical interventions should integrate metal exposure screening into pediatric health examinations, with prompt intervention protocols and evidence-based nutritional support for high-risk individuals. Community-based initiatives should emphasize public health education and promote evidence-based protective strategies among families to minimize metal exposure risks. Additionally, we propose establishing an integrated bone health surveillance network to facilitate systematic monitoring and early identification of vulnerable populations.

Conclusion

Our investigation revealed inverse correlations between blood Pb and serum Cu levels and BMD in children and adolescents. Conversely, serum Se demonstrated a positive correlation with lumbar spine bone density. Further analysis using WQS regression and BKMR models revealed the complex nonlinear effects of Cu on bone density in various metal exposures, particularly the synergistic effect of Cd and Mn. Furthermore, future studies are required to establish causal relationships. Our study provides important insights into how environmental metal exposure impacts bone health in children and adolescents. These insights can inform the development of prevention strategies and public health policies aimed at safeguarding bone development in this vulnerable population. In summary, this study emphasized the significance of evaluating multi-metal exposure effects on bone health, providing the foundation for further research and intervention measures.

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Clinical trial number
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Authors' contributions
Lin Yuan and Luyao Lou contributed to the study conception and design. Data collection and analysis were performed by Jian Han and Xiaofeng Jiang. The first draft of the manuscript was written by Jiaqing Sun, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability
Our data is sourced from the NHANES database(https://www.cdc.gov/nchs/nhanes/).

Declarations

Ethics approval and consent to participate
This study was conducted in accordance with the Declaration of Helsinki and received approval from the Ethics Review Board of the National Center for Health Statistics. All participants provided written informed consent, and for participants under 16 years of age, consent was obtained from their parents or legal guardians.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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