Letter

Relationship of Non-Essential and Essential Metals with Vitamin D in a Chinese Early Adolescent Cohort

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Vitamin D deficiency (VDD) represents a significant nutritional concern among children and adolescents. The estimated prevalence of VDD in China is 46.8% in this population[1]. VDD during childhood and adolescence has been associated with the onset of various conditions, including acute respiratory infections, asthma, atopic dermatitis, and food allergies[2]. Multiple factors, including age, sun exposure, adiposity, and genetics, influence vitamin D levels[2,3]. Increasing attention has been directed toward understanding the environmental determinants that may influence vitamin D status. Given the potential of metallic pollutants to disrupt endocrine function and their ubiquity in the environment, investigating the effects of metal exposure on human vitamin D status, particularly in vulnerable populations, is imperative.

Metallic elements can be categorized into two types: hazardous heavy metals and trace essential elements. Exposure to toxic metals such as cadmium (Cd) and lead (Pb) can adversely affect the vitamin D status in children. However, essential trace elements, including copper (Cu), zinc (Zn), cobalt (Co), and manganese (Mn), are vital for supporting diverse metabolic and physiological functions. Few studies have explored the association between exposure to multiple metals and vitamin D levels in adolescents. Therefore, we conducted a study involving the early adolescent population to investigate the association between exposure to 12 metals and vitamin D levels. The metals investigated included non-essential metals [such as vanadium (V), chromium (Cr), nickel (Ni), Cd, Pb, and arsenic (As)] and essential metals [such as Mn, iron (Fe), Co, Cu, Zn, and molybdenum (Mo)]. These twelve metals were selected due to their widespread occurrence in the environment. We analyzed the correlations between individual metals, metal mixtures, and serum 25-hydroxyvitamin D [25(OH)D] concentrations. Furthermore, existing literature indicates that the effects of metal exposure vary between male and female adolescents[4]. In addition, the absorption and half-lives of certain metals exhibit sex-based differences. Therefore, exploring sex-related variations in the association between metal exposure and total serum 25(OH)D levels is important.

This study utilized the data from the Chinese Early Adolescent Cohort (CEAC); detailed information on the cohort is available in our previous publications[5]. The baseline survey (Wave 1) included all seventh-grade students from the selected school, excluding individuals diagnosed with chronic or organic diseases that could affect vitamin D metabolism, including inflammatory disorders, liver disease, and chronic renal disease, as well as those with a documented history of psychiatric conditions, specifically depression and anxiety. Follow-up surveys were conducted at one-year intervals, ending September 2021 (Wave 3). Participants with missing baseline serum metal concentrations, and serum 25(OH)D levels at wave 1 and wave 3 were excluded. The study protocol adhered to the principles outlined in the Declaration of Helsinki and was approved by the Ethics Committee of Anhui Medical University (Approval No. 20180083). Written informed consent was obtained from both parents and participants.

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The concentrations of 12 metals—including non-essential metals (V, Cr, Ni, Cd, Pb, and As) and essential metals (Mn, Fe, Co, Cu, Zn, and Mo)—in the serum specimens were simultaneously quantified using inductively coupled plasma mass spectrometry (ICP-MS; Perkin Elmer NexION 350X, Shelton, CT, USA). The analytical procedure followed the protocols highlighted in earlier studies[6]. The intra- and inter-assay variation coefficients were less than 20%. Detection frequencies exceeded 95% for all metals, and values below the limit of detection (LOD) were imputed as LOD divided by $\sqrt{2}$. Due to the markedly skewed distribution of metal concentration in the study population, a $\log_{10}$ transformation was applied to all 12 metals to approximate normality. Serum 25(OH)D levels (ng/mL) were measured using a direct competitive chemiluminescence immunoassay *via* the LIASON 25-OH vitamin D assay (TOTAL; DiaSorin, Inc.). VDD status was defined as a serum 25(OH)D concentration below 20 ng/mL[2].

The model encompassed several covariates, including sex, age, whether the participant was an only child (yes or no), family structure (nuclear family, single-parent family, large family, or others), father's education level (primary school and below, middle school, or senior high school and above), mother's education level (primary school and below, middle school, or senior high school and above), self-perceived family economic status (high, middle, or low), body mass index, physical activity, and place of residence (urban or rural)[2]. The baseline survey documented participants' engagement in intense physical activities lasting 20 min and moderate exercises continuing for 30 min in the previous week. The response options were coded as 0, 1–2, and ≥ 3 days.

Descriptive statistics were calculated for the serum 25(OH)D levels, 12 metals, and sociodemographic variables. The metal detection rates and geometric means were analyzed separately by sex. The analysis primarily examined both the cross-sectional and longitudinal links between metal exposure and vitamin D levels or status. Generalized linear regression models, logistic regression analysis, and Bayesian kernel machine regression (BKMR) models were employed to explore the relationships among individual metals, metal mixtures, and serum 25(OH)D concentrations. Details of the statistical analysis are presented in the Supplementary Materials.

Finally, a sample of 1,425 middle school students with a mean age of 12.49 years was included in the analysis. Univariate associations between potential confounders and serum 25(OH)D concentrations are displayed in Table 1. The detection rates and concentration distributions of the 12 metals in the serum samples are presented in Supplementary Table S1. The majority of metal concentrations exhibited low to moderate positive correlations (Supplementary Figure S1).

Table 2 presents the cross-sectional associations between individual metals and baseline 25(OH)D concentrations. In both the total sample and male subgroup, V and Cu were positively correlated with the serum 25(OH)D level, while Cr and Mn were negatively associated. In females, log-transformed As and Mo were positively associated with baseline 25(OH)D levels across both model types, and As and Mo demonstrated an inverse association with baseline VDD in the multi-metal adjusted model (Table 2 and Supplementary Table S2). In the analysis of incident VDD, V exhibited a significant inverse association with incident VDD in the overall population and among males, across both model types ($P < 0.05$, see Table 3). Moreover, aside from the pronounced negative associations between V and changes in 25(OH)D levels observed in the single-metal regression models, no other significant relationships were identified between baseline metal concentrations and changes in 25(OH)D levels (Supplementary Table S3).

The BKMR model identified no statistically significant associations between the non-essential and essential metal mixtures and initial serum 25(OH)D concentrations, VDD, subsequent VDD, or alterations in 25(OH)D concentrations (Supplementary Figure S2). Supplementary Figure S3 illustrates the estimated effects of individual metals on vitamin D-related outcomes based on the BKMR model for the entire sample. At fixed percentiles, serum Cu levels were positively associated with baseline serum 25(OH)D concentrations. When the remaining metals were set at the 50th and 75th percentiles, serum Mn levels were also negatively correlated with baseline serum 25(OH)D concentrations. When the other metals were fixed at the 50th and 75th percentiles, serum Cr levels were negatively associated with baseline serum 25(OH)D levels. Serum As levels were positively associated with baseline serum 25(OH)D levels at the 75th percentile. When other metals were fixed at the 50th and 75th percentiles, serum Cr was positively associated with baseline VDD, whereas serum As was inversely associated with baseline VDD. Additionally, serum V was negatively associated with incident VDD at the 25th and 50th percentiles. The univariate exposure-response curves of the BKMR model are presented in Supplementary Figure S4.

The sex-stratified BKMR models (Supplementary Figures S5 and S6) did not identify any significant

Table 1. Univariable associations between potential confounders and serum 25(OH)D concentration

Variables		25 (OH)D level at wave 1			25 (OH)D level at wave 3		
	n (%)/M ± SD	M ± SD, ng/mL	*β* (95% *CI*)	*P*-value	M ± SD, ng/mL	*β* (95% *CI*)	*P*-value
Age, years	12.49 ± 0.48	–	−0.58 (−1.19 to 0.03)	0.063	–	0.53 (−0.47 to 1.55)	0.297
BMI, kg/m^2	19.21 ± 4.46	–	−0.12 (−0.19 to −0.15)	0.000	–	0.12 (−0.11 to 0.34)	0.085
Sex							
Males	865 (60.7)	23.90 ± 5.74	Reference		20.41 ± 5.96	Reference	
Female	560 (39.3)	22.04 ± 5.38	−1.89 (−2.45 to −1.26)	0.000	18.08 ± 5.40	−2.47 (−3.41 to −1.53)	0.000
Residence							
Rural	1,107 (77.7)	22.35 ± 5.85	Reference		18.83 ± 5.89	Reference	
Urban	318 (22.3)	23.40 ± 5.60	1.05 (0.35 to 1.76)	0.004	19.69 ± 5.84	0.84 (−0.30 to 1.98)	0.151
Only child							
Yes	232 (16.3)	23.25 ± 5.79	Reference		19.68 ± 6.27	Reference	
No	1193 (83.7)	23.15 ± 5.65	−0.10 (−0.90 to 0.69)	0.799	19.46 ± 5.78	0.88 (−0.43 to 2.19)	0.187
Family structure							
Nuclear family	655 (46.0)	22.78 ± 5.48	Reference		19.30 ± 5.82	Reference	
Single-parent family	227 (15.9)	23.49 ± 6.29	0.39 (−0.42 to 1.19)	0.345	19.57 ± 6.01	−0.50 (−1.88 to 0.87)	0.475
Large family	524 (36.8)	23.48 ± 5.66	0.49 (−0.11 to 1.11)	0.111	19.63 ± 5.84	−0.06 (−1.04 to 0.92)	0.900
Other	19 (1.3)	24.10 ± 4.00	0.95 (−1.62 to 3.51)	0.471	21.71 ± 5.55	2.51 (−1.13 to 6.15)	0.178
Self-perceived family economic status							
Low	159 (11.2)	23.55 ± 5.82	Reference		19.22 ± 5.47	Reference	
Medium	1,063 (74.6)	23.17 ± 5.72	−0.01 (−0.68 to 0.67)	0.983	19.60 ± 5.86	0.42 (−0.73 to 1.58)	0.473
High	203 (14.2)	22.88 ± 5.27	−0.33 (−1.18 to 0.51)	0.437	19.18 ± 6.12	−0.69 (−2.18 to 0.80)	0.364
Father’s education level							
Primary school and below	221 (15.5)	23.17 ± 5.83	Reference		19.14 ± 5.99	Reference	
Middle school	765 (53.7)	23.26 ± 5.53	0.21 (−0.38 to 0.80)	0.486	19.68 ± 5.81	0.88 (−0.07 to 1.83)	0.071
Senior high school and above	439 (30.8)	23.00 ± 5.83	−0.25 (−0.88 to 0.39)	0.449	19.36 ± 5.88	−0.93 (−1.97 to 0.10)	0.079
Mother’s education level							
Primary school and below	367 (25.8)	23.32 ± 5.86	Reference		19.49 ± 5.86	Reference	
Middle school	721 (50.6)	23.10 ± 5.64	−0.15 (−0.74 to 0.44)	0.626	19.45 ± 5.74	−0.19 (−1.14 to 0.76)	0.693
Senior high school and above	337 (23.6)	23.16 ± 5.73	−0.01 (−0.71 to 0.68)	0.974	19.62 ± 6.11	0.18 (−0.93 to 1.28)	0.756
Moderate−intensity Physical activity							
No	516 (36.2)	22.93 ± 5.47	Reference		19.02 ± 5.76	Reference	
1–2 days	590 (41.4)	23.01 ± 5.74	−0.27 (−0.87 to 0.33)	0.371	19.46 ± 5.60	−0.78 (−1.74 to 0.19)	0.115
≥ 3 days	319 (22.4)	23.85 ± 5.83	0.88 (0.17 to 1.58)	0.015	20.33 ± 6.39	1.42 (0.34 to 2.49)	0.010
High−intensity Physical activity							
No	568 (39.9)	23.19 ± 5.49	Reference		19.18 ± 5.41	Reference	
1–2 days	583 (40.9)	22.85 ± 5.85	−0.53 (−1.13 to 0.07)	0.083	19.37 ± 5.93	−0.28 (−1.26 to 0.69)	0.565
≥ 3 days	274 (19.2)	23.79 ± 5.62	0.77 (0.03 to 1.52)	0.042	20.42 ± 6.49	1.06 (−0.06 to 2.17)	0.063

Note. *CI*, confidence interval; BMI, body mass index; SD, standard deviation; M, mean.

relationships among the metal mixture, essential metal mixture, and vitamin D-related outcomes in either sex. In addition, a positive linear correlation was observed between the essential metal mixture and baseline serum 25(OH)D levels in females. This positive association was observed when the essential metal mixture exceeded the 60th percentile. When other metals were fixed at the 50th and 75th percentiles, serum Cr levels were negatively associated with baseline serum 25(OH)D concentrations but positively associated with VDD in males (Supplementary Figure S7). Serum As levels were also positively correlated with baseline serum 25(OH)D concentrations at the 75th percentile in both sexes (Supplementary Figures S7 and S8). When other metals were fixed at the 25th, 50th, and 75th

Table 2. Cross-sectional associations between individual metals with 25(OH)D levels at baseline

Metals	Total		Male		Female	
	β (95% *CI*)	*P*-value	*β* (95% *CI*)	*P*-value	*β* (95% *CI*)	*P*-value
Non-essential metals						
Single-metal model						
V	4.73 (0.84 to 8.61)	0.017	7.72 (2.61 to 12.84)	0.003	−0.02 (−5.99 to 5.96)	0.994
Cr	−3.26 (−7.29 to −0.78)	0.013	−5.71 (−11.07 to −0.35)	0.037	0.75 (−5.35 to 6.85)	0.809
Ni	0.26 (−0.69 to 1.21)	0.593	−0.07 (−1.31 to 1.17)	0.913	0.78 (−0.71 to 2.27)	0.304
Cd	1.09 (−0.17 to 2.36)	0.090	1.39 (−0.27 to 3.06)	0.101	0.60 (−1.34 to 2.54)	0.542
Pb	1.51 (−0.41 to 3.42)	0.123	1.47 (−1.03 to 3.98)	0.249	1.59 (−1.40 to 4.59)	0.296
As	0.67 (−0.43 to 1.77)	0.231	−0.08 (−1.52 to 1.35)	0.906	1.82 (0.10 to 3.53)	0.038
Multi-metal adjusted model						
V	6.02 (1.72 to 10.32)	0.006	10.51 (4.85 to 16.17)	0.000	−0.79 (−7.38 to 5.80)	0.814
Cr	−6.72 (−11.14 to −2.30)	0.003	−9.81 (−15.67 to −3.95)	0.001	−0.93 (−7.56 to 5.71)	0.784
Ni	0.39 (−0.57 to 1.36)	0.421	0.18 (−1.06 to 1.42)	0.778	0.45 (−1.06 to 1.97)	0.558
Cd	0.82 (−0.50 to 2.13)	0.222	1.04 (−0.69 to 2.76)	0.238	0.40 (−1.64 to 2.43)	0.703
Pb	1.66 (−0.48 to 3.81)	0.128	0.79 (−1.99 to 3.57)	0.578	3.02 (−0.34 to 6.38)	0.078
As	0.99 (−0.23 to 2.20)	0.111	−0.10 (−1.68 to 1.48)	0.905	2.49 (0.61 to 4.38)	0.010
Essential metals						
Single-metal model						
Mn	−2.02 (−3.64 to −0.39)	0.015	−2.92 (−5.03 to −0.80)	0.007	−0.33 (−2.88 to 2.23)	0.802
Fe	−0.32 (−2.20 to 1.56)	0.742	−0.86 (−3.35 to 1.63)	0.500	0.47 (−2.4 to 3.37)	0.751
Co	−1.86 (−3.68 to −0.03)	0.047	−1.91 (−4.34 to 0.51)	0.122	−1.98 (−4.77 to 0.81)	0.163
Cu	5.51 (1.69 to 9.31)	0.005	5.35 (0.61 to 10.09)	0.027	6.32 (−0.18 to 12.81)	0.057
Zn	3.07 (−2.43 to 8.57)	0.274	3.01 (−4.04 to 10.06)	0.402	2.52 (−6.42 to 11.45)	0.580
Mo	0.20 (−2.17 to 2.57)	0.868	−2.33 (−5.33 to 0.66)	0.126	5.38 (1.47 to 9.29)	0.007
Multi-metal adjusted model						
Mn	−2.38 (−4.17 to −0.59)	0.009	−3.00 (−5.32 to −0.68)	0.011	−1.95 (−4.79 to 0.88)	0.177
Fe	0.33 (−1.67 to 2.33)	0.743	0.63 (−2.04 to 3.30)	0.643	0.32 (−2.74 to 3.37)	0.839
Co	−1.30 (−3.17 to 0.57)	0.328	−1.03 (−3.55 to 1.50)	0.424	−2.18 (−5.03 to 0.66)	0.132
Cu	6.01 (2.11 to 9.92)	0.003	6.19 (1.30 to 11.09)	0.013	7.76 (1.21 to 14.31)	0.020
Zn	3.39 (−2.35 to 9.13)	0.246	3.14 (−4.18 to 10.47)	0.400	2.37 (−7.00 to 11.74)	0.620
Mo	1.41 (−1.09 to 3.90)	0.269	−0.79 (−3.93 to 2.36)	0.624	6.50 (2.36 to 10.63)	0.002

Note. V, Vanadium; Cr, Chromium; Mn, Manganese; Fe, Iron; Co, Cobalt; Ni, Nickel; Cu, Copper; Zn, Zinc; As, Arsenic; Mo, Molybdenum; Cd, Cadmium; Pb, Lead; *CI*, confidence interval.

percentiles, serum V levels were negatively associated with incident VDD in males (Supplementary Figure S7). As demonstrated in Supplementary Figures S7, when other metals were fixed at the 25th and 50th percentiles, the serum Cu was positively associated with the baseline serum 25(OH)D concentrations in males. In females, serum Cu levels were positively correlated with baseline serum 25(OH)D concentrations at the 50th and 75th percentiles. Serum Mo levels were positively associated with baseline serum 25(OH)D concentrations and inversely associated with VDD in females (Supplementary Figure S8). The univariate exposure-response curves of the BKMR model are

Table 3. Logistic regression models of the relationship between single serum metal concentrations at baseline and incident vitamin D deficiency at follow-up

Metals	Overall		Male		Female	
	OR (95% *CI*)	*P*-value	*OR* (95% *CI*)	*P*-value	*OR* (95% *CI*)	*P*-value
Non-essential metals						
Single-metal model						
V	0.12 (0.02 to 0.64)	0.014	0.05 (0.01 to 0.41)	0.006	0.65 (0.04 to 11.73)	0.767
Cr	0.34 (0.05 to 2.20)	0.260	1.04 (0.10 to 11.42)	0.975	0.04 (0.01 to 1.00)	0.053
Ni	1.06 (0.69 to 1.62)	0.787	1.34 (0.80 to 2.26)	0.273	0.64 (0.29 to 1.34)	0.237
Cd	0.674 (0.39 to 1.16)	0.157	0.81 (0.40 to 1.62)	0.545	0.42 (0.16 to 1.10)	0.082
Pb	0.71 (0.29 to 1.68)	0.430	1.34 (0.45 to 3.95)	0.595	0.16 (0.03 to 0.75)	0.021
As	0.94 (0.588 to 1.53)	0.801	1.02 (0.56 to 1.87)	0.949	1.00 (0.43 to 2.34)	0.998
Multi-metal adjusted model						
V	0.15 (0.02 to 0.97)	0.046	0.02 (0.01 to 0.28)	0.003	2.10 (0.09 to 50.80)	0.649
Cr	0.78 (0.10 to 5.96)	0.812	3.32 (0.24 to 45.82)	0.371	0.07 (0.01 to 2.02)	0.119
Ni	1.01 (0.65 to 1.56)	0.962	1.17 (0.69 to 2.01)	0.561	0.73 (0.33 to 1.60)	0.426
Cd	0.75 (0.42 to 1.33)	0.323	0.85 (0.41 to 1.77)	0.667	0.60 (0.21 to 1.56)	0.273
Pb	0.95 (0.35 to 2.54)	0.912	2.10 (0.61 to 7.25)	0.241	0.19 (0.03 to 1.08)	0.061
As	1.03 (0.60 to 1.78)	0.910	1.35 (0.68 to 2.68)	0.384	0.84 (0.32 to 2.16)	0.712
Essential metals						
Single-metal model						
Mn	0.92 (0.44 to 1.92)	0.828	1.71 (0.69 to 4.23)	0.243	0.29 (0.08 to 1.07)	0.064
Fe	0.81 (0.35 to 1.87)	0.622	1.21 (0.41 to 3.57)	0.734	0.49 (0.12 to 1.95)	0.312
Co	1.23 (0.55 to 2.76)	0.616	1.86 (0.68 to 5.16)	0.230	0.57 (0.14 to 2.30)	0.429
Cu	1.09 (0.20 to 5.82)	0.923	1.30 (0.18 to 9.52)	0.795	0.62 (0.02 to 16.85)	0.777
Zn	6.13 (0.49 to 77.65)	0.160	10.87 (0.49 to 249.49)	0.133	0.94 (0.01 to 93.16)	0.977
Mo	0.93 (0.32 to 2.71)	0.889	0.96 (0.26 to 3.53)	0.948	1.10 (0.15 to 8.13)	0.924
Multi-metal adjusted model						
Mn	0.87 (0.39 to 1.95)	0.739	1.56 (0.58 to 4.20)	0.380	0.28 (0.07 to 1.19)	0.085
Fe	0.70 (0.28 to 1.71)	0.432	0.87 (0.27 to 2.82)	0.817	0.59 (0.13 to 2.64)	0.492
Co	1.30 (0.57 to 3.00)	0.531	1.84 (0.64 to 5.34)	0.259	0.61 (0.15 to 2.56)	0.502
Cu	0.92 (0.17 to 5.09)	0.923	1.23 (0.16 to 9.69)	0.846	0.62 (0.02 to 17.25)	0.780
Zn	9.02 (0.64 to 127.95)	0.104	9.30 (0.36 to 243.96)	0.181	4.14 (0.03 to 538.05)	0.567
Mo	1.04 (0.34 to 3.21)	0.946	0.82 (0.21 to 3.25)	0.777	2.22 (0.27 to 18.21)	0.458

Note. V, Vanadium; Cr, Chromium; Mn, Manganese; Fe, Iron; Co, Cobalt; Ni, Nickel; Cu, Copper; Zn, Zinc; As, Arsenic; Mo, Molybdenum; Cd, Cadmium; Pb, Lead; *OR*, odds ratio; *CI*, confidence interval illustrated in Supplementary Figures S9 and S10.

In this study, we observed an inverse association between serum V levels and 25(OH)D concentrations in males in the cross-sectional analysis. In addition, baseline serum V was inversely associated with both incident VDD and longitudinal changes in 25(OH)D across the two waves. This finding suggests a nonlinear relationship between V and 25(OH)D, as supported by the BKMR analysis. Significant positive cross-sectional associations were observed between serum Mo and 25(OH)D levels in females. In a study involving 512 adolescents (with an average blood lead concentration of 46 μg/L), a positive association was observed between serum 25(OH)D levels and urinary Mo and Tl; meanwhile, no significant association was noted between 25(OH)D concentrations and either Pb or Cd[7]. Cross-sectional association was observed between serum As and 25(OH)D levels. Previous reports have indicated that serum 25(OH)D concentrations are negatively correlated with urinary As levels[7]. Among females, median serum As concentrations were 2.52 μg/L (interquartile range = 2.70 [P_{25}–P_{75}: 1.53–4.23]), and the observed positive correlation may indicate a low-dose potential protective effect.

This study identified a positive link between serum Cu levels and 25(OH)D concentrations in both sexes. Furthermore, Cu homeostasis is essential, and the dose-response relationship between Cu levels and health outcomes follows a U-shaped pattern. The positive association between Cu and vitamin D levels may reflect only the left segment of the U-shaped curve. The absence of a longitudinal association between the 11 metals and vitamin D levels or status in this study may be attributed to the extended interval between follow-up and baseline. The biological half-life of the prohormone 25(OH)D is approximately 2–3 weeks[3]. In addition, most metals, including Mn and As, are primarily excreted via the serum, reflecting exposures within the preceding hours or days. The short half-lives of both metals and vitamin D suggest that metals may exert cross-sectional or short-term effects on vitamin D levels, a notion supported by the findings of the cross-sectional analysis in this study.

A male-specific negative association was observed between serum Cr and Mn concentrations and reduced 25 (OH) D levels. Studies have reported that Cr and Mn are positively associated with liver function biomarkers, indicating that heavy metal exposure may affect D levels by impacting liver function[8]. Furthermore, metals, such as As, Pb, Hg, and Cd, individually and in combination, have been reported to correlate with renal function parameters in adolescents aged 12–18 years[9]. However, the biological mechanisms underlying the association between metal exposure and elevated 25(OH)D levels remain unclear. Given that 25(OH)D is strongly influenced by sun exposure, metal concentrations may serve as proxies for environmental exposure during outdoor activities, which could, in turn, contribute to higher 25(OH)D levels. In addition, sex-specific differences in the association between heavy metal exposure and vitamin D may be attributable to variations in gene expression, dietary patterns, behavioral habits, and the duration of outdoor activities between females and males[10]. This was partly confirmed by the sex differences between moderate-(at least one in last 7 days, males: 68.1% *vs*. females: 57.1%, P < 0.001) and high-intensity physical activity (at least one in last 7 days, males: 66.9% *vs*. females: 49.6%, P < 0.001) in this study.

Nevertheless, this study has certain limitations that warrant consideration. First, several factors potentially influencing vitamin D status—such as genetic background, dietary vitamin D intake, smoking status, quantified sun exposure, and pubertal status—were not assessed. Second, given the short biological half-lives of some metals, relying on the concentration of a single measurement to estimate metal exposure levels may result in misclassification. Finally, this study was conducted among students within a narrow age range at a single school in one city, limiting the generalizability of the findings to other age groups or populations with different levels of environmental metal exposure.

Overall, this study suggests that some metals influence serum vitamin D levels in a sex-specific manner. This study underscores the importance of minimizing environmental metal exposure to prevent VDD in adolescents, thereby supporting overall adolescent health. However, this was only a preliminary analysis. Future studies should employ longitudinal designs with repeated measurements of both metals and vitamin D, along with evaluations of calcium and phosphorus metabolism, to further uncover the potential health impacts of metal exposure.

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Competing Interests The authors declare no competing interests.

Ethics This study was approved by the Biomedicine Ethics Committee of Anhui Medical University (approval no. 20180083). All participants provided informed consent before participating in the study.

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