Modification of vitamin B6 on the associations of blood lead levels and cardiovascular diseases in the US adults

Jia Wei,¹ John S Ji ^{1,2}**To cite:** Wei J, Ji JS.

Modification of vitamin B6 on the associations of blood lead levels and cardiovascular diseases in the US adults. *BMJ Nutrition, Prevention & Health* 2020;**3**:e000088. doi:10.1136/bmjnp-2020-000088

► Additional material is published online only. To view please visit the journal online (<http://dx.doi.org/10.1136/bmjnp-2020-000088>).

¹Environmental Research Center, Duke Kunshan University, Kunshan, China

²Nicholas School of the Environment, Duke University, Durham, North Carolina, USA

Correspondence to

Dr John S Ji, Duke Kunshan University, Kunshan 215316, China; john.ji@dukekunshan.edu.cn

Received 14 April 2020

Revised 12 July 2020

Accepted 14 July 2020

Published Online First

12 August 2020

© Author(s) (or their employer(s)) 2020. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

ABSTRACT

Background Cardiovascular disease (CVD) is a leading cause of death in the US population. Lead exposure is an important risk factor of CVDs, as is associated with elevated homocysteine level and oxidative stress. We aim to examine whether vitamin B6, which has been shown to reduce homocysteine level, can modify the relationship between blood lead and the risk of CVDs.

Methods Cross-sectional data on ever-report CVDs (congestive heart failure, coronary heart disease, angina pectoris, heart attack and stroke), blood lead level (BLL) and vitamin B6 in the form of plasma pyridoxal 5'-phosphate were obtained from US National Health and Nutrition Examination Survey 2005–2006 for adults ≥20 years old. The association between CVDs and quartiles of BLL was estimated using multivariate logistic regression models adjusted for demographics factors, lifestyle variables, stress variables, comorbidities and CVD biomarkers (C reactive protein, homocysteine, cholesterol) and was stratified by vitamin B6 deficiency level (<20 nmol/L) and median value of vitamin B6 (42.5 nmol/L).

Results Positive associations between BLL and CVDs only appeared in the vitamin B6 deficiency group, with quartile 2 to quartile 4 of BLL showing higher risk of CVDs (OR=3.1, 95% CI 0.9 to 10.6; OR=6.5, 95% CI 1.4 to 30.8; OR=5.5, 95% CI 1.4 to 21.7) compared with quartile 1. When stratified by median value of vitamin B6, a significant association between higher CVD risk with higher BLL was only observed in subjects with low vitamin B6 (p trend=0.004).

Conclusions Vitamin B6 could modify the association between BLL and CVDs, which suggests a potential value of vitamin B6 in influencing the effects of lead exposure on the cardiovascular system.

INTRODUCTION

Cardiovascular diseases (CVDs) are the leading causes of death in the US population.¹ The risk factors of CVDs include hypertension, obesity, diabetes and environmental toxicants such as lead (Pb).² Lead is a common heavy metal and exposure to lead has been linked to many acute or chronic diseases. Accumulating epidemiological studies have confirmed a hypertensive effect of lead that lead exposure is associated with elevated blood pressure, which is one

What this paper adds

- This study suggests that vitamin B6 level can modify the association between blood lead level and the risk of CVDs in US adults.
- The association between higher blood lead level and higher risk of CVDs was stronger among people with lower vitamin B6 level.
- Vitamin B6 may influence the susceptibility to the harmful effect of lead exposure on the human cardiovascular system.

of the mechanisms mediating the effect of lead on CVDs.³ Other studies also reported positive association between lead exposure and cardiovascular outcomes. For example, blood lead was found to be associated with left ventricular hypertrophy and peripheral arterial disease.^{4,5} Lead exposure can cause elevated homocysteine level, oxidative stress, endothelial injury and inflammation, which can promote hypertension and CVDs.⁶

Notably, micronutrients such as vitamin B6 are found to be involved in homocysteine metabolisms.⁷ Vitamin B6 is an essential coenzyme participating in a wide range of biochemical reactions and can only be obtained from the diet, such as fish, poultry, nuts, legumes and bananas.⁸ Pyridoxal 5'-phosphate (PLP) is the major derivatives of vitamin B6 and is considered as the indicator for vitamin B6 status.⁹ It functions when homocysteine is transforming to cystathionine and further to cysteine.⁸ Studies also found that vitamin B6 might be associated with CVDs.^{9–12} It is still unknown whether vitamin B6 has a potential influence on the association between lead exposure and CVDs.

Therefore, we aim to evaluate the association between lead exposure and CVD risk in a US population and the degree to which this association is modified by vitamin B6. We used data from the National Health and Nutrition Examination Surveys (NHANES), a nationally representative survey in the USA.

METHODS

Study population

NHANES is a series of national, population-based, cross-sectional surveys, conducted by the National Center for Health Statistics. The survey includes interviews, physical examinations and laboratory tests. NHANES examines a sample of about 10 000 persons each 2-year wave, which is selected to represent the non-institutionalised US population of all ages and races.¹³ All participants in the NHANES provided written informed consent.

In this analysis, data from the 2005–2006 wave was used because of data availability. We included adults aged 20 years and older. Among the 4979 participants, 4482 subjects with valid measures of cardiovascular outcomes, blood lead level (BLL) and vitamin B6 concentrations were included in the study.

Exposure

In NHANES, whole blood lead (Pb) was measured by inductively coupled plasma mass spectrometry based on quadrupole ICP-MS technology. Vitamin B6 was measured in the form of plasma PLP by high-performance liquid chromatographic method. The methods for blood collection, sample transportation and storage and sample analysis were described in the laboratory procedure manual.^{14 15} The NHANES quality assurance and quality control protocols meet the 1988 Clinical Laboratory Improvement Act mandates.

Outcome

Cross-sectional data on self-reported CVDs was acquired through the question ‘Has a doctor or other health professional ever told [you/SP] that [you/s/he] ... had a XX?’. CVDs included congestive heart failure, coronary heart disease, angina pectoris, heart attack and stroke. All participants aged 20 years and older were eligible for the questions.

Covariate

In our analysis, we include a series of demographic, lifestyle, stress, comorbidities and CVD biomarker variables as covariates. Demographic variables included age, gender, ethnicity, education and family income. Age was categorised as 20–40, 40–60, 60–80 and over 80 years old. To avoid identifiability issue, NHANES indicates age as 85 years for all individuals >85 years of age. Race/ethnicity included Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black and other. Education was categorised into <9th grade, 9th–11th grade, high school graduate, some college or associate degree, college graduate and above. Family income was defined by family poverty-income ratio, which was divided using the eligibility cut-off points for the United States Department of Agriculture food assistance programmes into low-income (0.000–1.850), middle-income (1.851–3.500) and high-income (3.501+).¹⁶

Lifestyle variables included smoking, alcohol use, body mass index (BMI), physical activity and dietary

supplement use. Participants who smoked less than 100 cigarettes in life were defined as never smokers. If they smoked more than 100 cigarettes and reported current smoking behaviours, they were classified as current smokers. If they smoked more than 100 cigarettes but with no current smoking behaviours, they were classified as past smokers. Participants who had less than 12 alcoholic drinks in life were defined as never drinker and those who did not drink any alcohol over the past 12 months were past drinkers. If they consumed alcohol over the past 12 months, they were further classified into heavy drinker and light drinker based on whether they had more than one drink per day on average or not. BMI was classified into four groups: underweight (BMI<18.5), normal weight (18.5≤BMI<25), overweight (25≤BMI<30) and obesity (BMI≥30). Normal weight was used as the reference group. Physical activity level was calculated by adding the total time of household activity, transportation activity and leisure time activity per week. Participants who did not have any activities were classified as sedentary. The rest were divided into low, moderate and high activity levels by cutoffs at 140 min and 420 min per week. Dietary supplements were determined by the survey question ‘whether had supplements taken in the past month’.

Stress variables included sleep duration and depression. Sleep duration was divided into less than 7 hours, 7–8 hours and more than 8 hours. Seven to 8 hours was used as the reference group. Depression was ascertained through the Patient Health Questionnaire, a validated self-reported assessment based on the nine DSM-IV symptoms for depression. The nine symptoms questions were scored as ‘0’ (not at all), ‘1’ (several days), ‘2’ (more than half the days) and ‘3’ (nearly every day). Participants’ total score was then calculated by adding all scores up and was used to determine the severity of depression. Symptoms were classified as none (score 0–4), mild (score 5–9) and moderate-to-severe (score 10+) in our analysis.

History of hypertension and diabetes were considered as covariates since they are common comorbidities for CVD. In addition, CVD biomarkers including C reactive protein (CRP), homocysteine and cholesterol were also incorporated into our study.

Statistical analysis

BLLs were categorised into quartiles (Q1–Q4) by cut-off points at the 25th, 50th and 75th percentile values. Vitamin B6 was dichotomised into groups of deficiency (<20 nmol/L) and non-deficiency (≥20 nmol/L).¹⁷

To estimate the ORs of CVDs according to BLL, multivariate logistic regression analyses were performed using PROC SURVEYLOGISTIC statement in SAS with CVDs as the dependent variables. The ORs and 95% CIs for CVDs versus no CVDs were generated using unadjusted and adjusted regression models separately. Three different adjusted models were built: Model 1 adjusted for all the sociodemographic variables (age, gender, race, education and family income); Model 2 adjusted for sociodemographic variables and lifestyle variables (smoking, alcohol

use, BMI, physical activity and dietary supplement use); Model 3 further adjusted for stress variables, comorbidities and biomarkers (sleep, depression, hypertension, diabetes, CRP, homocysteine and cholesterol). All three biomarkers were log-transformed due to their skewed distribution. We then stratified the analysis by vitamin B6 deficiency and the median level of vitamin B6 to determine whether the associations differed depending on vitamin B6 levels. P value for trend between quartiles of BLL and vitamin B6 in each model were also calculated to demonstrate a dose-response association, and p value for interaction was reported for the effect modification of vitamin B6. Due to missingness in some of the covariates (ranging from 0.1% to 7.7%), samples with missing variables were deleted in the adjusted models, making the total sample size of each model to 4280 (Model 1), 3940 (Model 2) and 3884 (Model 3). Statistical analysis was carried out with SAS V.9.4 (SAS Institute, Cary, North Carolina, USA). NHANES assigned each individual a sample weight for the representation of the US Census civilian non-institutionalised population. It is a measure of the number of people in the population represented by the sample person. NHANES cluster design variables (SDMVSTRA, SDMVPSU) and a full sample 2-year medical examination weight WTMEC2YR were used in the analysis. In all analyses, the statistical significance level was set at $p < 0.05$.

RESULTS

The overall weighted prevalence of CVDs in our study sample of US adults was 12.9% ($n=497$). The mean concentration (SE) of BLL was 1.75 (0.04) $\mu\text{g/dL}$. The mean concentration (SE) of vitamin B6 was 76.2 (2.6) nmol/L . The weighted prevalence of vitamin B6 deficiency was 12.9% ($n=691$).

As shown in [table 1](#), the percentages of adults who reported having CVDs differed significantly by covariates. As expected, the risk of CVDs increased significantly with age. CVDs showed a strong association with socioeconomic status. Higher educational level and family income were both strong protective factors of CVDs. Past smoking and drinking behaviours were both associated with a higher prevalence of CVDs ($\text{OR}=2.39$, 95% CI 1.85 to 3.09; $\text{OR}=1.94$, 95% CI 1.29 to 2.91). Less than 7-hour sleep and more than 8-hour sleep yielded higher odds of having CVDs than having 7–8-hour sleep ($\text{OR}=1.76$, 95% CI 1.39 to 2.24; $\text{OR}=2.44$, 95% CI 1.97 to 3.02). CVDs appeared more often among those with depression, hypertension and diabetes. Finally, the risk of CVDs increased significantly in higher levels of CRP (tertile 2/tertile 3 versus tertile 1: $\text{OR}=1.76$, 95% CI 1.39 to 2.24/ $\text{OR}=2.44$, 95% CI 1.97 to 3.02), and in higher levels of homocysteine (tertile 2/tertile 3 versus tertile 1: $\text{OR}=2.02$, 95% CI 1.14 to 3.57/ $\text{OR}=6.40$, 95% CI 4.32 to 9.50).

BLL and vitamin B6 concentrations also showed variations by covariates. BLL was higher in older adults, males, Mexican Americans and Blacks, smokers, people with

lower socioeconomic status, less than 7-hour sleep, hypertension, diabetes and higher homocysteine. Vitamin B6 concentration was higher in older adults, males, non-Hispanic whites, non-smokers, no depression, people with higher socioeconomic status, higher physical activity level, lower CRP and lower homocysteine (online supplementary table). The higher BLL was also associated with lower vitamin B6, with a coefficient $= -2.32$ ($p=0.03$).

In the overall analysis, CVDs were significantly associated with BLL in the crude model ([table 2](#)). Compared with Q1 (0.18–0.92 $\mu\text{g/dL}$), participants in Q2–Q4 (0.93–1.49; 1.50–2.37; 2.38–26.4 $\mu\text{g/dL}$) yielded significantly higher risk of having CVDs ($\text{OR}=2.4$, 95% CI 1.3 to 4.7/ $\text{OR}=4.7$, 95% CI 2.5 to 8.7/ $\text{OR}=6.8$, 95% CI 4.0 to 11.1). When adjusted for confounding variables in model 1 to model 3, the association was less significant, although higher quartiles of BLL still led to higher ORs of CVDs. The p value for trend was 0.02 in Model 1, 0.01 in Model 2 and 0.006 in Model 3, indicating a significant dose-response association between BLL and CVDs.

When we stratified the whole sample by vitamin B6 deficiency level ($<20 \text{ nmol/L}$), a positive association between quartiles of BLL and CVDs appeared in the deficiency group, but not in the normal group ([table 2](#)). In the normal group, a significant association between BLL and CVDs only appeared in the crude model (Q3 vs Q1: $\text{OR}=3.9$, 95% CI 1.9 to 7.7/Q4 vs Q1: $\text{OR}=5.7$, 95% CI 3.3 to 10.0), but not in the adjusted models, although the p value for trend indicated a higher risk of CVDs associated with higher BLL. In the deficiency group, significant associations remained in all models. In the fully adjusted model 3, compared with Q1, participants in Q3–Q4 had significantly higher risks of CVDs ($\text{OR}=6.5$, 95% CI 1.4 to 30.8/ $\text{OR}=5.5$, 95% CI 1.4 to 21.7). The p value for trend was not significant in the adjusted models, probably because participants in Q4 did not yield a higher risk than participants in Q3.

When we stratified the whole sample according to the median vitamin B6 level (42.5 nmol/L), similar results appeared. In the below-median group, participants in Q4 had significantly higher odds of CVDs in the adjusted model 3 ($\text{OR}=2.7$, 95% CI 1.1 to 7.2), but this association was not observed in the above-median group. What is more, a statistically significant dose-response association was observed in both groups in the crude model ($p \text{ trend} < 0.0001$), but was only among subjects with below-median vitamin B6 levels in the adjusted model ($p \text{ trend}=0.004$ in model 3) ([table 2](#)). The p value for interaction was significant in all models (Crude: $p=0.009$; Model 1: $p=0.01$; Model 2: $p=0.005$; Model 3: $p=0.003$), indicating an effect modification of vitamin B6 in the dose response association between BLL and CVDs ([table 2](#)).

DISCUSSION

Our study aims to explore the modifying effect of vitamin B6 on the association between BLL and CVDs, which has not been conducted before. We found that the association

Table 1 Baseline characteristics and prevalence of CVDs by covariates (weighted)

Characteristics	N (%)	CVD (%)	No CVD (%)	Crude OR (95% CI)	P value
Overall	4482(100)	497 (12.9)	3985 (87.1)		
Age (years)					
20–40	1697 (37.1)	15 (0.8)	1682 (99.2)	1(ref)	
40–60	1377 (39.7)	102 (6.4)	1275 (93.6)	8.16 (4.55 to 14.64)	<0.0001
60–80	1081 (19.0)	251 (22.0)	830 (78.0)	33.59 (20.72 to 54.44)	<0.0001
80+	327 (4.2)	129 (40.6)	198 (59.4)	81.33 (44.61 to 148.25)	<0.0001
Gender					
Female	2330 (52.0)	214 (8.2)	2116 (91.8)	1 (ref)	
Male	2152 (48.0)	283 (9.3)	1869 (90.7)	1.16 (0.89 to 1.51)	0.3
Ethnicity					
Mexican American	911 (8.0)	53 (4.4)	858 (95.6)	0.43 (0.32 to 0.58)	<0.0001
Other Hispanic	136 (3.3)	6 (3.6)	130 (96.4)	0.35 (0.13 to 0.90)	0.03
White (non-Hispanic)	2263 (72.5)	306 (9.6)	1957 (90.4)	1 (ref)	
Black (non-Hispanic)	999 (11.1)	120 (9.2)	879 (90.9)	0.95 (0.70 to 1.29)	0.7
Other	173 (5.1)	12 (5.6)	161 (94.4)	0.56 (0.29 to 1.09)	0.09
Education					
Less than 9th grade	553 (6.4)	85 (14.8)	468 (85.2)	1 (ref)	
9–11th grade	691 (11.2)	89 (12.3)	602 (87.7)	0.81 (0.58 to 1.13)	0.2
High school graduate	1056 (24.8)	120 (9.2)	936 (90.8)	0.58 (0.38 to 0.89)	0.01
Some college or associate's degree	1283 (31.4)	136 (8.6)	1147 (91.4)	0.54 (0.37 to 0.80)	0.002
College graduate and above	894 (26.2)	67 (5.5)	827 (94.5)	0.34 (0.22 to 0.53)	<0.0001
N/A	5				
Family PIR					
0–1.85	1668 (27.1)	220 (12.1)	1448 (87.9)	1 (ref)	
1.86–3.50	1158 (28.2)	137 (9.8)	1021 (90.2)	0.79 (0.56 to 1.13)	0.2
>3.50	1455 (44.7)	104 (5.3)	1351 (94.7)	0.41 (0.31 to 0.53)	<0.0001
N/A	201				
Smoking					
Never smoker	2358 (51.1)	192 (6.4)	2166 (93.6)	1 (ref)	
Past smoker	1138 (25.0)	210 (14.1)	928 (85.9)	2.39 (1.85 to 3.09)	<0.0001
Current smoker	984 (23.9)	95 (8.1)	889 (91.9)	1.27 (0.99 to 1.63)	0.06
N/A	2				
Alcohol use					
Never drinker	584 (11.1)	64 (9.7)	520 (90.3)	1 (ref)	
Past drinker	891 (17.4)	183 (17.3)	708 (82.7)	1.94 (1.29 to 2.91)	0.001
Current drinker light	912 (24.2)	105 (8.6)	807 (91.4)	0.87 (0.60 to 1.27)	0.5
Current drinker heavy	1775 (47.3)	112 (5.2)	1663 (94.8)	0.51 (0.36 to 0.72)	0.0001
N/A	320				
BMI					
Underweight	72 (1.7)	7 (8.1)	65 (91.9)	1.71 (0.61 to 4.80)	0.3
Normal	1260 (31.0)	94 (4.9)	1166 (95.1)	1 (ref)	
Overweight	1516 (33.9)	169 (9.4)	1347 (90.6)	2.01 (1.61 to 2.51)	<0.0001
Obesity	1561 (34.4)	198 (10.9)	1363 (89.1)	2.37 (1.88 to 3.00)	<0.0001
N/A	73				
Activity per week (hours)					
Sedentary	820 (13.6)	165 (18.0)	665 (82.0)	3.48 (2.60 to 4.65)	<0.0001
Low	1256 (26.5)	133 (8.2)	1123 (91.8)	1.42 (1.13 to 1.78)	0.003
Moderate	1227 (30.1)	113 (7.7)	1114 (92.3)	1.32 (0.83 to 2.11)	0.2

Continued

Table 1 Continued

Characteristics	N (%)	CVD (%)	No CVD (%)	Crude OR (95% CI)	P value
High	1179 (29.8)	86 (6.0)	1093 (94.0)	1(ref)	
Supplement use					
Yes	2266 (54.3)	261 (9.1)	2005 (90.9)	1.11 (0.95 to 1.30)	0.2
No	2213 (45.7)	235 (8.3)	1978 (91.7)	1(ref)	
N/A	3				
Sleep					
Less than 7 hours	1684 (35.6)	221 (10.6)	1463 (89.4)	1.76 (1.39 to 2.24)	<0.0001
7–8 hours	2424 (57.2)	216 (7.0)	2208 (93.0)	1 (ref)	
8+ hours	364 (7.2)	55 (12.0)	309 (88.0)	2.44 (1.97 to 3.02)	<0.0001
N/A	10				
Depression					
No	3276 (80.1)	322 (7.6)	2954 (92.4)	1 (ref)	
Mild	595 (14.3)	74 (10.7)	521 (89.3)	1.46 (1.10 to 1.94)	0.008
Moderate–severe	266 (5.6)	55 (16.3)	211 (83.7)	2.39 (1.69 to 3.38)	<0.0001
N/A	345				
Hypertension					
Yes	1740 (36.3)	378 (17.7)	1362 (82.3)	5.67 (4.39 to 7.29)	<0.0001
No	2742 (63.7)	119 (3.7)	2623 (96.3)	1(ref)	
N/A	5				
Diabetes					
Yes	533 (9.5)	168 (27.9)	365 (72.1)	5.36 (4.14 to 6.95)	<0.0001
No	3949 (90.5)	329 (6.7)	3620 (93.3)	1(ref)	
N/A	5				
C reactive protein					
Tertile 1	1539 (39.0)	111 (5.5)	1428 (94.5)	1 (ref)	
Tertile 2	1484 (33.0)	173 (9.3)	1311 (90.7)	1.76 (1.39 to 2.24)	<0.0001
Tertile 3	1454 (28.0)	211 (12.4)	1243 (87.6)	2.44 (1.97 to 3.02)	<0.0001
N/A	5				
Homocysteine					
Tertile 1	1489 (31.7)	52 (3.2)	1437 (96.8)	1 (ref)	
Tertile 2	1486 (37.2)	116 (6.2)	1370 (93.8)	2.02 (1.14 to 3.57)	0.02
Tertile 3	1486 (31.2)	1486 (17.3)	1159 (82.7)	6.40 (4.32 to 9.50)	<0.0001
N/A	21				
Cholesterol					
Tertile 1	1527 (33.1)	235 (11.9)	1292 (88.1)	1 (ref)	
Tertile 2	1450 (34.0)	146 (8.0)	1304 (92.0)	0.64 (0.46 to 0.91)	0.01
Tertile 3	1491 (32.9)	110 (5.8)	1381 (94.2)	0.46 (0.32 to 0.67)	<0.0001
N/A	14				

Bold texts indicate significant p value, defined as $p < 0.05$.

BMI, body mass index; CVD, cardiovascular disease; PIR, poverty income ratio.

between lead and CVDs differed depending on vitamin B6 levels, and the association was stronger among participants with low vitamin B6 concentrations.

Many believe that lead exposure has a profound effect on human cardiovascular systems by acting on various markers. Studies found that some clinical markers in cardiovascular systems were associated with BLL, including blood pressure, high-density lipoprotein cholesterol, CRP, low-density lipoprotein cholesterol and

homocysteine.^{18–20} For example, a cross-sectional study in Baltimore revealed that homocysteine level increased 0.35 $\mu\text{mol/L}$ with 1.0 $\mu\text{g/dL}$ increase in blood lead.²¹ Similarly, an Asian cross-sectional study found an increase of 1 $\mu\text{g/dL}$ in blood lead was associated with an increase of 0.05 $\mu\text{mol/L}$ in homocysteine.²² A prospective cohort study in Korea also found that BLLs were positively associated with plasma homocysteine levels and was modified by genetic polymorphism.²³ Elevated homocysteine

Table 2 ORs and 95% CIs for CVDs according to BLL stratified by vitamin B6 deficiency and median value of vitamin B6

Lead (ug/dL) Quartile Range	Total		Crude		Model 1		Model 2		Model 3	
	CVD (n (%))	No CVD (n (%))	OR (95% CI)	P value	P trend	OR (95% CI)	P value	P trend	OR (95% CI)	P value
Overall										
Q1: 0.18-0.92	39 (3.4)	1096 (96.6)	1 (ref)		<0.0001	1 (ref)		0.02	1 (ref)	
Q2: 0.93-1.49	85 (7.6)	1029 (92.4)	2.4 (1.3 to 4.7)	0.008		0.9 (0.5 to 1.7)	0.8		1.1 (0.6 to 2.0)	0.9
Q3: 1.50-2.36	160 (14.3)	957 (85.7)	4.7 (2.5 to 8.7)	<0.0001		1.3 (0.7 to 2.4)	0.3		1.6 (0.9 to 2.9)	0.1
Q4: 2.37-26.4	213 (19.1)	903 (80.9)	6.8 (4.0 to 11.1)	<0.0001		1.3 (0.9 to 2.0)	0.2		1.7 (1.1 to 2.5)	0.01
Stratified by vitamin B6 deficiency (plasma PLP<20nmol/L)										
Vitamin B6-normal										
Q1: 0.18-0.92	32 (3.3)	930 (96.7)	1 (ref)		<0.0001	1 (ref)		0.02	1 (ref)	
Q2: 0.93-1.49	64 (6.7)	892 (93.3)	2.0 (1.0 to 4.0)	0.0600		0.7 (0.3 to 1.4)	0.3		0.8 (0.4 to 1.8)	0.7
Q3: 1.50-2.36	119 (12.5)	831 (87.5)	3.9 (1.9 to 7.7)	0.0001		1.0 (0.5 to 1.9)	0.9		1.2 (0.6 to 2.3)	0.6
Q4: 2.37-26.4	161 (17.4)	762 (82.6)	5.7 (3.3 to 10.0)	<0.0001		1.0 (0.6 to 1.7)	0.8		1.4 (1.0 to 2.1)	0.08
Vitamin B6-deficiency										
Q1: 0.18-0.92	7 (4.0)	166 (96.0)	1 (ref)		0.0002	1 (ref)		0.2	1 (ref)	
Q2: 0.93-1.49	21 (13.3)	137 (86.7)	6.9 (2.7 to 17.9)	<0.0001		2.6 (1.2 to 5.9)	0.02		3.1 (0.9 to 10.6)	0.07
Q3: 1.50-2.36	41 (24.5)	126 (75.5)	11.8 (5.0 to 28.1)	<0.0001		3.4 (1.3 to 9.0)	0.01		6.5 (1.4 to 30.8)	0.02
Q4: 2.37-26.4	52 (26.9)	141 (73.1)	14.4 (4.9 to 42.2)	<0.0001		3.5 (1.1 to 11.3)	0.04		5.5 (1.4 to 21.7)	0.01
P value for interaction*					0.06			0.2		0.3
Stratified by median value of vitamin B6 deficiency (plasma PLP=42.5 mol/L)										
Vitamin B6≥Median										
Q1: 0.18-0.92	16 (2.9)	539 (97.1)	1 (ref)		<0.0001	1 (ref)		0.9	1 (ref)	
Q2: 0.93-1.49	37 (6.4)	545 (93.6)	2.5 (1.1 to 5.8)	0.03		0.9 (0.4 to 1.8)	0.8		1.0 (0.4 to 2.4)	0.9
Q3: 1.50-2.36	58 (9.8)	534 (90.2)	3.9 (1.9 to 8.3)	0.0004		1.1 (0.5 to 2.3)	0.8		1.1 (0.5 to 2.6)	0.8
Q4: 2.37-26.4	70 (13.6)	445 (86.4)	5.3 (2.7 to 10.7)	<0.0001		1.0 (0.5 to 1.9)	0.9		1.0 (0.5 to 2.3)	0.9
Vitamin B6<Median										
Q1: 0.18-0.92	23 (4.0)	557 (96.0)	1 (ref)		<0.0001	1 (ref)		0.02	1 (ref)	
Q2: 0.93-1.49	48 (9.0)	484 (91.0)	2.4 (1.1 to 5.5)	0.03		1.0 (0.4 to 2.2)	0.9		1.2 (0.4 to 3.2)	0.7
Q3: 1.50-2.36	102 (19.4)	423 (80.6)	5.6 (2.4 to 13.0)	<0.0001		1.6 (0.7 to 3.5)	0.2		2.1 (0.7 to 6.3)	0.2
Q4: 2.37-26.4	143 (23.8)	458 (76.2)	7.8 (3.7 to 16.4)	<0.0001		1.7 (0.8 to 3.4)	0.1		2.7 (1.1 to 7.2)	0.04
P value for interaction*					0.009			0.01		0.005

Model 1: adjusted for all the sociodemographic variables (age, gender, race, education, family income).

Model 2: further adjusted for lifestyle variables (smoking, alcohol use, BMI, activity level, dietary supplement).

Model 3: further adjusted for stress variables (smoking, comorbidities, and biomarkers (sleep, depression, hypertension, diabetes, CRP, homocysteine, cholesterol)).

Bold texts indicate significant p value, defined as p < 0.05.

P value for interaction was calculated by adding and testing the significance of the interaction term (vitamin B6 deficiency BLL/ median vitamin B6 *BLL) in the overall regression model.

BLL, blood lead level; BMI, body mass index; CRP, C reactive protein; CVD, cardiovascular disease; PLP, pyridoxal 5'-phosphate.

is an independent risk factor for CVDs,²⁴ suggesting that homocysteine could be a mechanism that underlies the effects of lead on the cardiovascular systems.

The modifying effect of vitamin B6 on the association between lead exposure and CVDs found in our study suggests that higher serum vitamin B6 concentration may influence the harmful effect of lead exposure on the cardiovascular system. Similarly, a few studies examined the modifying effect of micronutrients on the association of BLL with homocysteine levels. A longitudinal study using data from the VA Normative Aging Study revealed a positive association between lead exposure and homocysteine concentrations and further recovered that this association was stronger among participants with below-median intakes of vitamin B6 and folate.²⁵ Another cohort of premenopausal women also reported that higher BLL was associated with increased homocysteine but this association was only significant in the lower three quartiles of vitamin B6, vitamin B12 and folate consumption.²⁶ A NHANES study found a positive association between BLL and homocysteine only among participants with low levels of folate or vitamin B6 (below-median).⁷ These studies suggest that among people who have been exposed to lead, higher consumptions of B-vitamins may mitigate the effect of lead on homocysteine.²⁵ Also, lead may biologically interact with folate, vitamin B6 and vitamin B12.⁷ Considering lead exposure is a risk factor of elevated homocysteine by disturbing essential enzymes in homocysteine metabolism, and B-vitamins are also involved in the pathways of homocysteine metabolisms, a potential role of homocysteine can be established in the interaction between lead exposure, vitamin B6 and CVDs.

Previous literature has found that suboptimal level or mild deficiency of vitamin B6 could be associated with a higher risk of many chronic diseases, including CVDs.²⁷ For example, in a multicentre case-control study in Europe, vitamin B6 concentrations were significantly lower in patients with vascular disease than in healthy controls.²⁸ Another case-control study in the USA found a strong independent association between low PLP and stroke.²⁹ Underlying mechanisms may involve homocysteine metabolisms, in which the synthesis of cystathionine requires vitamin B6 in the form of PLP.³⁰ Vitamin B6 was also inversely related to inflammatory markers such as CRP and fibrinogen,⁹ which may induce a higher risk of CVDs.

However, there has not been a consensus on the effect of vitamin B6 supplementation on the prevention or treatment of CVDs. A Japan study showed that vitamin B6 supplement has the potential to reduce the risk of coronary heart disease and myocardial infarction.³¹ However, some large-scale clinical trials reported negative results on the effect of Vitamin B6 in reducing cardiovascular risk in humans.³² For example, the Heart Outcomes Prevention Evaluation 2 Study found no effect of vitamin B6 supplementation in reducing the risk of major cardiovascular events in patients with vascular disease.³³ The Norwegian Vitamin trial reported no association between vitamin B6

supplement and benefit for myocardial infarction and stroke.³⁴ These negative results may suggest that vitamin B6 does not work well in patients with CVDs but cannot preclude a protective effect in primary prevention.¹¹ It was also pointed out that some neglected confounding factors and inappropriate designs may implicate the negative evidence in previous studies.³⁵ Therefore, further studies are needed to find the role of vitamin B6 in CVD prevention and the optimal dose and combination.

We found a dose-response association between CVDs and BLL among the whole sample before and after adjustments. This is in consistent with current literature, which has widely recognised a positive association of lead exposure with CVDs.³ For example, in a prospective cohort study, men with increased BLLs had a higher risk of future ischaemic heart disease (IHD).³⁶ A Korean cohort study found that IHD, cerebrovascular disease, angina pectoris and cerebral infarction all showed a positive relationship with BLLs.³⁶ Another NHANES study found a decline in lead exposure was associated with reductions in cardiovascular mortality in US adults.³⁷

Our study is a large-scale population-based study using a nationally representative database in the USA. We built several models adjusting for a wide range of covariates. We target the interaction between BLL, vitamin B6 and CVDs, which has not been done before. However, our study has several limitations. First, because of the cross-sectional design of this study, we cannot determine the direction of causality. Second, the CVD outcome data were obtained by self-report questionnaires rather than clinical diagnosis. Thus, it was subject to recall bias and inaccurate self-perception. Third, considering population-based studies are unable to infer the potential mechanisms behind, future studies can focus on more cardiovascular biomarkers and genetic polymorphisms.

CONCLUSION

We found a modifying effect of vitamin B6 on the association between blood lead concentrations and the risk of CVDs in US adults. The association is only significant among people with lower vitamin B6 levels, suggesting that higher vitamin B6 concentration may influence susceptibility to the harmful effect of lead exposure on the human cardiovascular system. This study implicates a potential public health value of diet and micronutrients on reducing the influence of heavy metal exposure.

Twitter John S Ji @profjohnji

Contributors JW and JSJ designed the study. JW had access to raw data and conducted statistical analysis. JW and JSJ interpreted the results. All authors contributed to manuscript and approved of the final version.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available in a public, open access repository. The data that support the findings of this study are openly available in National Health and Nutrition Examination Survey at <https://wwwn.cdc.gov/nchs/nhanes/Default.aspx>.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

ORCID iD

John S Ji <http://orcid.org/0000-0002-5002-118X>

REFERENCES

- Visioli F, Hagen TM. Nutritional strategies for healthy cardiovascular aging: focus on micronutrients. *Pharmacol Res* 2007;55:199–206.
- Sharma P, Purohit P. Lead exposure exacerbates cardiovascular risk. *Indian J Clin Biochem* 2014;29:117–8.
- Navas-Acien A, Guallar E, Silbergeld EK, et al. Lead exposure and cardiovascular disease—a systematic review. *Environ Health Perspect* 2007;115:472–82.
- Schwartz J, Lead SJ. Lead, blood pressure, and cardiovascular disease in men and women. *Environ Health Perspect* 1991;91:71–5.
- Navas-Acien A, Selvin E, Sharrett AR, et al. Lead, cadmium, smoking, and increased risk of peripheral arterial disease. *Circulation* 2004;109:3196–201.
- Vaziri ND. Mechanisms of lead-induced hypertension and cardiovascular disease. *Am J Physiol Heart Circ Physiol* 2008;295:H454–65.
- Lee Y-M, Lee M-K, Bae S-G, et al. Association of homocysteine levels with blood lead levels and micronutrients in the US general population. *J Prev Med Public Health* 2012;45:387–93.
- Fratoni V, Brandi ML, vitamins B. B vitamins, homocysteine and bone health. *Nutrients* 2015;7:2176–92.
- Lotto V, Choi S-W, Friso S. Vitamin B6: a challenging link between nutrition and inflammation in CVD. *Br J Nutr* 2011;106:183–95.
- Righetti M. Protective effect of vitamin B therapy on bone and cardiovascular disease. *Recent Pat Cardiovasc Drug Discov* 2009;4:37–44.
- Friso S, Lotto V, Corrocher R, et al. Vitamin B6 and cardiovascular disease. *Subcell Biochem* 2012;56:265–90.
- Spinneker A, Sola R, Lemmen V, et al. Vitamin B6 status, deficiency and its consequences—an overview. *Nutr Hosp* 2007;22:7–24.
- CDC. About the National health and nutrition examination survey (introduction), 2017. Available: https://www.cdc.gov/nchs/nhanes/about_nhanes.htm [Accessed 10 Mar 2019].
- CDC. Laboratory procedure Manual-Lead cadmium mercury, 2008. Available: https://wwwn.cdc.gov/nchs/data/nhanes/2005-2006/labmethods/pbcd_d_met_lead_cadmium.pdf
- CDC. Laboratory Procedure Manual-Vitamin B6, 2010. Available: https://wwwn.cdc.gov/nchs/data/nhanes/2005-2006/labmethods/vitb6_d_met.pdf
- CDC. Analytic and reporting guidelines: the third National health and nutrition examination survey, NHANES III (1988–94). Hyattsville, Maryland: National Center for Health Statistics, 1996.
- IoMScotSEoDR I. Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline: national academies press (US), 1998.
- Obeng-Gyasi E, Armijos RX, Weigel MM, et al. Cardiovascular-Related outcomes in U.S. adults exposed to lead. *Int J Environ Res Public Health* 2018;15. doi:10.3390/ijerph15040759. [Epub ahead of print: 15 Apr 2018].
- Xu C, Shu Y, Fu Z, et al. Associations between lead concentrations and cardiovascular risk factors in U.S. adolescents. *Sci Rep* 2017;7:9121.
- Cai R, Zheng Y-F, Bu J-G, et al. Effects of blood lead and cadmium levels on homocysteine level in plasma. *Eur Rev Med Pharmacol Sci* 2017;21:162–6.
- Schafer JH, Glass TA, Bressler J, et al. Blood lead is a predictor of homocysteine levels in a population-based study of older adults. *Environ Health Perspect* 2005;113:31–5.
- Chia SE, Ali SM, Lee BL, et al. Association of blood lead and homocysteine levels among lead exposed subjects in Vietnam and Singapore. *Occup Environ Med* 2007;64:688–93.
- Kim K-N, Lee M-R, Lim Y-H, et al. Blood lead levels, iron metabolism gene polymorphisms and homocysteine: a gene-environment interaction study. *Occup Environ Med* 2017;74:899–904.
- Homocysteine Studies Collaboration. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. *JAMA* 2002;288:2015–22.
- Bakulski KM, Park SK, Weisskopf MG, et al. Lead exposure, B vitamins, and plasma homocysteine in men 55 years of age and older: the Va normative aging study. *Environ Health Perspect* 2014;122:1066–74.
- Pollack AZ, Mumford SL, Sjaarda L, et al. Blood lead, cadmium and mercury in relation to homocysteine and C-reactive protein in women of reproductive age: a panel study. *Environ Health* 2017;16:84.
- Fairfield KM, Fletcher RH. Vitamins for chronic disease prevention in adults: scientific review. *JAMA* 2002;287:3116–26.
- Robinson K, Arheart K, Refsum H, et al. Low circulating folate and vitamin B6 concentrations: risk factors for stroke, peripheral vascular disease, and coronary artery disease. European COMAC group. *Circulation* 1998;97:437–43.
- Kelly PJ, Shih VE, Kistler JP, et al. Low vitamin B6 but not homocyst(e)ine is associated with increased risk of stroke and transient ischemic attack in the era of folic acid grain fortification. *Stroke* 2003;34:e51–4.
- Miller JW, Ribaya-Mercado JD, Russell RM, et al. Effect of vitamin B-6 deficiency on fasting plasma homocysteine concentrations. *Am J Clin Nutr* 1992;55:1154–60.
- Ishihara J, Iso H, Inoue M, et al. Intake of folate, vitamin B6 and vitamin B12 and the risk of CHD: the Japan public health Center-Based prospective study cohort I. *J Am Coll Nutr* 2008;27:127–36.
- Ntaios G, Savopoulos C, Grekas D, et al. The controversial role of B-vitamins in cardiovascular risk: an update. *Arch Cardiovasc Dis* 2009;102:847–54.
- Lonn E, Yusuf S, Arnold MJ, et al. Homocysteine lowering with folic acid and B vitamins in vascular disease. *N Engl J Med* 2006;354:1567–77.
- Bönaa KH, Njølstad I, Ueland PM, et al. Homocysteine lowering and cardiovascular events after acute myocardial infarction. *N Engl J Med* 2006;354:1578–88.
- Debreceeni B, Debreceeni L. Why do homocysteine-lowering B vitamin and antioxidant E vitamin supplementations appear to be ineffective in the prevention of cardiovascular diseases? *Cardiovasc Ther* 2012;30:227–33.
- Jain NB, Potula V, Schwartz J, et al. Lead levels and ischemic heart disease in a prospective study of middle-aged and elderly men: the Va normative aging study. *Environ Health Perspect* 2007;115:871–5.
- Ruiz-Hernandez A, Navas-Acien A, Pastor-Barriuso R, et al. Declining exposures to lead and cadmium contribute to explaining the reduction of cardiovascular mortality in the US population, 1988–2004. *Int J Epidemiol* 2017;46:1903–12.