Association between serum pyridoxal 5′-phosphate levels and all-cause, cardiovascular mortality, and cardiovascular disease in adults: a population-based cohort study

Chao Xuan [iD], Ru-Hua Liu, Cong Zhao, Jing Li, Ting-Ting Zhou,
Qing-Wu Tian and Guo-Wei He

Abstract

Background: The association between pyridoxal 5′-phosphate (PLP) and cardiovascular disease (CVD) remains a topic of discussion.

Objectives: This study aimed to explore the relationship between serum PLP levels and the incidence of all-cause mortality, cardiovascular mortality, and the risk of CVD among the US population.

Design: A population-based cohort study.

Methods: This study analyzed data from the National Health and Nutrition Examination Survey. Adjusted hazard ratios (HRs) and their corresponding 95% confidence intervals (CIs) were calculated using weighted Cox proportional hazards regression models to assess the risk associated with all-cause and cardiovascular mortality. Weighted binary logistic regression was utilized to assess the relationship between serum PLP levels and the risk of CVD. Nonlinear associations were evaluated using multivariable-adjusted restricted cubic splines.

Results: There were 2546 cases of all-cause mortality and 867 cases of cardiovascular mortality over a mean follow-up of 11.36 years. In the fully adjusted model, the adjusted HRs with 95% CIs for all-cause mortality associated with increases in serum PLP levels corresponding to the interquartile ranges were 0.83 (0.74–0.93), 0.71 (0.63–0.80), and 0.64 (0.56–0.74), respectively. Similarly, cardiovascular mortality decreased by 0.78 (0.62–0.97), 0.63 (0.49–0.81), and 0.62 (0.50–0.77) with each quartile increase in serum PLP levels. Higher serum PLP levels confer protection against CVD risk (odds ratio: 0.87, 95% CI: 0.79–0.96). Serum PLP levels showed nonlinear relationships with risk of all-cause mortality, cardiovascular mortality, and CVD.

Conclusion: The results of this study provide evidence that serum PLP serves as a protective factor against all-cause mortality, cardiovascular mortality, and CVD in US adults, with dose–response relationships.

Correspondence to:
Chao Xuan
Department of Clinical Laboratory, The Affiliated Hospital of Qingdao University, No. 1677, Wutai Mountain Road, Qingdao 266500, China
bio-x.c@hotmail.com;
cxuan@qdu.edu.cn

**Ru-Hua Liu
Jing Li
Ting-Ting Zhou
Qing-Wu Tian**
Department of Clinical Laboratory, The Affiliated Hospital of Qingdao University, Qingdao, China

Cong Zhao
Department of Cardiology, The Affiliated Hospital of Qingdao University, Qingdao, China

Guo-Wei He
Department of Cardiovascular Surgery, TEDA International Cardiovascular Hospital; Academy of Medical Sciences & Peking Union Medical College, Tianjin, China

Department of Surgery, Oregon Health and Science University, Portland, OR, USA

Plain language summary

Association between serum pyridoxal 5'-phosphate levels and all-cause, cardiovascular mortality, and cardiovascular disease in US adults

Why was the study done? The association between pyridoxal 5'-phosphate (PLP) and cardiovascular disease (CVD) remains a topic of discussion. This study aimed to explore

the relationship between serum PLP levels and the incidence of all-cause mortality, cardiovascular mortality, and the risk of CVD among the US population. What did the researchers do? This study used data from National Health and Nutrition Examination Survey (NHANES), applying statistical methods to see if there's a connection between the amount of PLP in the blood and health outcomes. What did the researchers find? The findings showed that over an average of 11 years, people with higher levels of PLP in their blood were less likely to die for any reason. They were also less likely to die from heart-related problems or to develop heart disease. The protection seemed to increase as PLP levels got higher. What do the findings mean? The results of this study provide evidence that serum PLP serves as a protective factor against all-cause mortality, cardiovascular mortality, and cardiovascular disease in US adults, with dose–response relationships.

Introduction

Vitamin B6 is a water-soluble vitamin that can be converted into the coenzyme pyridoxal 5′-phosphate (PLP) through metabolic conversion. Typically, "PLP" is used interchangeably with "Vitamin B6." Vitamin B6 plays a critical role in numerous metabolic functions and maintaining intracellular homeostasis, including serving as a coenzyme in amino acid metabolism, one-carbon metabolism, neurotransmitter metabolism, nucleotide synthesis, hemoglobin synthesis, gluconeogenesis, and glycogenolysis.[1] The endogenous synthesis of vitamin B6 is absent in humans, necessitating its acquisition from both naturally occurring and fortified food sources.[2] Although severe vitamin B6 deficiency is infrequent, there is a prevalence of suboptimal levels or mild deficiency.[3]

The involvement of vitamin B6 in the development and progression of cardiovascular disease (CVD) has been the subject of extensive scientific discourse over a prolonged period, with a variety of proposed mechanisms being put forth. Through its role as coenzyme in multiple reactions related to enzymes involved in amino acid pathways as well as lipid and carbohydrate metabolic processes, vitamin B6 appears to have a variety of cardioprotective effects.[4] Homocysteine is a recognized independent risk factor for CVD, particularly coronary heart disease (CAD) and stroke.[5,6] The essential enzymes in the transsulfuration pathway of homocysteine metabolism, cystathionine β-synthase and γ-cystathionase, require PLP as a cofactor. As a result, a vitamin B6 deficiency impedes cystathionine-β-synthase activity, leading to the accumulation of S-adenosylhomocysteine. Simultaneously, it promotes the release of homocysteine from cells, which eventually results in hyperhomocysteinemia and premature atherosclerosis and thrombosis.[7] Therefore, the current consensus is that vitamin B6's impact on total plasma homocysteine concentrations mediates its relationship with CVD. Additionally, vitamin B6 may participate in the entirety of atherosclerosis by impacting inflammation, lipid metabolism and synthesis, immune function, coagulation pathways, endothelial cell function, and antioxidant properties.[8–10]

Due to the intricate functions of vitamin B6 and the variability in study populations, establishing a causal connection between the vitamin and the risk of CVD becomes challenging. Despite the fact that numerous case–control and prospective studies have linked low plasma vitamin B6 levels to an increased risk of CVD and have provided evidence that vitamin B6 supplements may provide CVD protection, it is important to take into account conflicting results from other studies.[11–13] He and co-workers conducted a prospective study involving 43,732 American men aged 40–75, all free from CVD and diabetes at baseline. Participants were followed from 1986 to 2000. After 14 years of
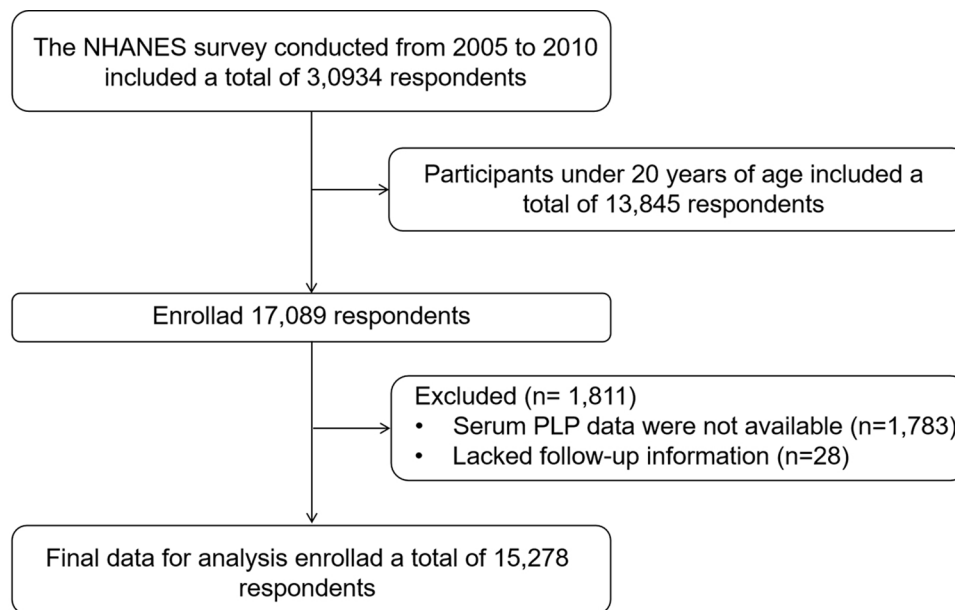
Figure 1. Flow-chart of the study. Eligible participants and those included in the analyses of the associations between serum pyridoxal 5'-phosphate levels and all-cause, cardiovascular mortality, and cardiovascular disease in adults.

follow-up, the study found no association between vitamin B6 intake and the risk of ischemic or hemorrhagic stroke.[14] Similarly, Zhang et al., using data from the UK Biobank, found no significant link between the intake of vitamins B6 and the risk of cardiovascular events, stroke, myocardial infarction, or cardiovascular mortality.[15] However, a Japanese cohort study that included 58,730 Asians aged 40–79 found that, over a 14-year follow-up, higher vitamin B6 intake was associated with a reduced risk of cardiovascular mortality.[16] Additionally, a large prospective cohort study of American women, followed for 14 years, found a graded association between higher vitamin B6 intake and a lower risk of CAD.[17] The National Health and Nutrition Examination Survey (NHANES) evaluated plasma PLP concentrations in sampled populations across the United States during three consecutive cycles from 2005 to 2010. The Linked Mortality File (LMF) was updated in June 2023 and contains mortality tracking data through December 31, 2019. The publicly available data from this survey afforded us the chance to explore the updated correlations between plasma PLP levels and nationwide all-cause mortality, cardiovascular mortality, and CVD risk in the United States.

Methods

Study design and data source

Data extracted from three NHANES survey cycles spanning 2005–2010, with each 2-year period constituting a cycle, were integrated with the mortality data sourced from the National Death Index until December 31, 2019, to establish the foundation for this population-based cohort study. The NHANES survey conducted from 2005 to 2010 included a total of 30,934 respondents. After excluding individuals for whom serum PLP data were not available ($n = 1783$), were under 20 years of age ($n = 13,845$), and lacked follow-up information ($n = 28$), the analysis comprised a final cohort of 15,278 participants (Figure 1).

Vitamin B6 measurement

The National Center for Environmental Health handled the processing, preservation, and delivery of serum samples to the Division of Laboratory Sciences (NCEH). The Center operates under the auspices of the CDC and its primary mission is to perform comprehensive analyses. Comprehensive details regarding sample collection and processing

procedures are available in the Laboratory Technologist section of the NHANES manual.[18]

The analysis serum was prepared by combining the sample with a 5% solution of metaphosphoric acid in a 1:1 proportion, resulting in the precipitation of proteins. After performing vortex mixing and centrifugation, the sample supernatant was combined with approximately equal volumes of dichloromethane in order to extract lipids. The resulting mixture was subjected to another round of vortex mixing and centrifugation. The topmost layer of the sample is subsequently filtered using a syringe to enable high-performance liquid chromatography (HPLC) analysis. The measurement of the PLP forms of vitamin B6 was conducted using reversed-phase HPLC coupled with fluorescence detection. The fluorescence detection involved excitation at a wavelength of 325 nm and emission at a wavelength of 425 nm. To enhance the signal of PLP, a post-column introduction of sodium chlorite derivatization reagents was integrated into the HPLC system. The quantification process relied on the determination of analyte peak areas, which were estimated by interpolating values from a five-point calibration curve derived from aqueous standards.

Ascertaining the outcome

The vital statuses of participants were identified up until December 31, 2019.[19] The study focused on mortality associated with CVD and all-cause mortality. The 2019 Public-Use Linked Mortality Files reveal the primary causes of death among NHANES participants. These include diseases of heart, malignant neoplasms, chronic lower respiratory, accidents (unintentional injuries), cerebrovascular diseases, Alzheimer's disease, diabetes mellitus, nephrosis, and all other causes (residual). Cardiovascular mortality was recorded as deaths resulting from heart diseases or stroke which adhered to the International Statistical Classification of Diseases.

CVDs were diagnosed using responses from a standardized questionnaire about health condition diagnoses during home-based interviews. CVDs comprised CAD, angina/angina pectoris, heart attack, congestive heart failure, and stroke.[20]

Covariates

Demographic data were collected to obtain information on the sociodemographic characteristics, including gender, age, race/ethnicity, family poverty income ratio (PIR), marital status, and educational levels. To determine participants' smoking status, serum cotinine levels were analyzed and categorized accordingly: less than the limit of detection (LOD), LOD ~10 and >10 ng/mL. Body mass index (BMI) data were collected from the examination results. The levels of C-reactive protein (CRP), alanine aminotransferase (ALT), glucose (GLU), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), total cholesterol (TCHO), serum creatinine (SCr), and PLP were obtained from laboratory data. Hypertension was defined as self-reported doctor-diagnosed high blood pressure, prescribed hypertension medication, and blood pressure of ≥140/90 mmHg (averaged over multiple assessments). Diabetes mellitus (DM) was defined using the following criteria: clinical diagnosis made by healthcare professionals, current use of insulin, fasting blood glucose concentration equal to or exceeding 7.0 mmol/L, a 2-h postprandial glucose concentration equal to or exceeding 11.1 mmol/L, and/or a glycated hemoglobin level equal to or exceeding 6.5%.

Statistical analysis

The variance in all NHANES surveys conducted between 2005 and 2010 was estimated using Taylor series linearization and sample weights, in accordance with National Center for Health Statistics (NCHS) guidelines, to ensure the accuracy of national estimates. Demographic data files provided masked variance units.[21]

All statistical analyses were conducted using the Stata Statistical Software (version 16.0; StataCorp, College Station, TX, USA) and R (version 4.3.2). Continuous variables were presented as mean ± standard errors (SE), whereas categorical variables were presented as proportions and 95% confidence intervals (CIs). Chi-square tests were used to analyze categorical variables. One-way analysis of variance was used to compare means between three or more unrelated groups, and the independent sample t-test was used to compare two sample means from unrelated groups. The least significant difference method was implemented for post-hoc testing with multiple comparisons. Spearman's correlation analysis assessed the correlations between serum PLP levels and covariates. The findings were visually conveyed in a heatmap. We

categorized serum pyridoxal 5′-phosphate (PLP) levels into four quartiles: Q1 (≤25.8 nmol/L), Q2 (25.8–42.95 nmol/L), Q3 (42.95–76.00 nmol/L), and Q4 (>76.00 nmol/L). Hazard ratios (HRs) and 95% CIs were estimated to assess all-cause and cardiovascular mortality across the different quartiles of PLP levels. This was achieved through the use of weighted Cox proportional hazard regression models. Further investigation into any possible correlation between serum PLP levels and CVD risk was done using binary logistic regression analysis. The outcomes were presented as odds ratios (ORs) and their corresponding 95% CIs. Four statistical models were created. Model 1 was adjusted for age, sex, race/ethnicity, educational level, family PIR, and marital status. Model 2 was subsequently adjusted for BMI, serum cotinine levels, and the presence of hypertension and DM. Lastly, Model 3 was additionally adjusted for serum levels of CRP, TCHO, ALT, GLU, TG, SCr, and HDL-C. A multivariable-adjusted restricted cubic spline with four knots was used to clarify the dose–response relationship. Nonlinearity was assessed using the likelihood ratio test. To counteract the reduction in sample size resulting from missing covariates, multiple imputation was conducted for missing variables. Furthermore, stratified analyses were carried out based on gender. Statistical significance was defined as a two-tailed *p*-value less than 0.05.

Results

The study included of 15,278 adult participants, with a weighted average age of 49.56 years and a SE of 0.15, of whom 7416 were men (weighted proportion: 48.18%), including 1202 participants with CVD. Table 1 shows the baseline demographic and clinical parameters of the participants in the survival, all-cause mortality, and cardiovascular mortality groups. In comparison to the survival population (69.73 ± 0.74 nmol/L), the all-cause mortality population (62.07 ± 1.59 nmol/L, $p < 0.001$) and the cardiovascular mortality population (63.53 ± 2.94 nmol/L, $p = 0.035$) exhibited notably decreased levels of serum PLP. The heat map of Spearman's correlation coefficients between serum PLP levels and covariates is shown in Figure 2. The baseline demographic and clinical parameters among participants in none-CVD and CVD group are listed in Table 2. The CVD group had significantly lower serum PLP levels (62.03 ± 2.20 nmol/L)

than the control group (70.05 ± 0.76 nmol/L, $p = 0.002$).

During a median follow-up of 11.36 years (95% CI: 11.23–11.49), a total of 2546 cases of all-cause mortality were recorded, among which 867 cases were attributed to cardiovascular mortality. The weighted Cox regression analysis classified participants into four groups, determined by the interquartile range of serum PLP levels. The low-concentration groups were used as the reference categories. Following adjustment for multiple variables, a notable correlation emerged, indicating that increased serum PLP levels are associated with a diminished risk of all-cause mortality (Table 3). The HRs with 95% CIs for different quartiles of serum PLP levels were as follows: 1.00 (reference), 0.83 (0.74–0.93), 0.71 (0.63–0.80), and 0.64 (0.56–0.74). The weighted Cox proportional regression models that were adjusted for covariates were used to document the association between serum PLP levels and cardiovascular mortality. The results of these models are presented in Table 4. Each quartile increase in serum PLP levels was associated with a reduction in cardiovascular mortality of 22.00% (HR: 0.78, 95% CI: 0.62–0.97), 37.00% (HR: 0.63, 95% CI: 0.49–0.81), and 38.00% (HR: 0.62, 95% CI: 0.50–0.77), respectively. The dose–response analysis revealed a significant nonlinear correlation between serum PLP levels and both all-cause mortality ($p_{\text{nonlinearity}} = 0.0006$) and cardiovascular mortality ($p_{\text{nonlinearity}} = 0.0091$), as demonstrated by the restricted cubic spline (Figure 3). Significant associations were observed between serum PLP levels falling below 42.95 and 43.86 nmol/L with an elevated risk of all-cause mortality and cardiovascular mortality, respectively.

The relationship between serum PLP levels and the risk of CVD was further analyzed by weighted binary logistic regression and the results are listed in Table 5. For each quartile of elevated serum PLP levels, the risk of total CVD (OR: 0.87, 95% CI: 0.79–0.96), congestive heart failure (OR: 0.85, 95% CI: 0.75–0.97), CAD (OR: 0.86, 95% CI: 0.75–0.99), heart attack (OR: 0.84, 95% CI: 0.75–0.94), and stroke (OR: 0.85, 95% CI: 0.75–0.96) was significantly reduced. The inflection point of the significant nonlinear relationship ($p_{\text{nonlinearity}} < 0.0001$) between serum PLP levels and CVD risk was identified as 43.86 nmol/L by the restricted cubic spline (Figure 4).

Table 1. Baseline demographic and clinical parameters among participants in survival, all-cause mortality, and cardiovascular mortality group.

Variable	All (*n* = 15278)	Survival (*n* = 12732)	All-cause mortality (*n* = 2546)	Cardiovascular mortality (*n* = 867)	p_1*	p_2**
Age (years)	49.56 ± 0.15	45.50 ± 0.14	69.87 ± 0.25	71.64 ± 0.39	<0.001	<0.001
Gender, male (%)	48.18 (47.48–48.89)	47.61 (46.79–48.42)	52.34 (50.23–54.45)	51.86 (47.77–55.92)	<0.001	0.055
Race/ethnicity (%)						
Mexican American	8.13 (6.51–10.1)	8.78 (7.06–10.87)	3.37 (2.36–4.8)	3.54 (2.22–5.61)	<0.001	<0.001
Other Hispanic	4.42 (3.28–5.93)	4.78 (3.55–6.40)	1.79 (1.12–2.85)	2.42 (1.27–4.54)		
Non-Hispanic White	70.6 (66.65–74.26)	69.24 (65.24–72.97)	80.45 (76.24–84.07)	78.84 (73.74–83.17)		
Non-Hispanic Black	10.86 (9.13–12.87)	10.89 (9.17–12.88)	10.67 (8.47–13.37)	11.77 (8.91–15.38)		
Other race	6.00 (5.00–7.17)	6.31 (5.26–7.55)	3.71 (2.65–5.16)	3.43 (2.14–5.47)		
Education (%)						
<High school	18.75 (17.24–20.37)	17.11 (15.67–18.65)	30.73 (27.53–34.12)	32.3 (28.5–36.35)	<0.001	<0.001
High school/GED	24.21 (22.94–25.52)	23.73 (22.37–25.15)	27.66 (25.58–29.85)	27.86 (24.64–31.33)		
>High school	57.04 (54.73–59.32)	59.16 (56.93–61.36)	41.61 (37.92–45.40)	39.84 (34.8–45.1)		
Marital status (%)						
Divorced/widowed/separated	18.36 (17.51–19.23)	15.42 (14.57–16.32)	39.66 (37.52–41.84)	44.45 (40.28–48.7)	<0.001	<0.001
Married/unmarried couple	65.12 (63.49–66.73)	66.81 (65.1–68.48)	52.83 (50.13–55.51)	48.68 (44.23–53.14)		
Never married	16.52 (15.21–17.92)	17.76 (16.39–19.22)	7.51 (6.01–9.35)	6.87 (4.66–10.04)		
Family PIR						
<1	13.42 (12.38–14.53)	13.17 (12.08–14.34)	15.24 (13.5–17.16)	15.93 (13.23–19.06)	0.026	0.058
≥1	86.58 (85.47–87.62)	86.83 (85.66–87.92)	84.76 (82.84–86.5)	84.07 (80.94–86.77)		
BMI (kg/m^2)	29.01 ± 0.05	29.06 ± 0.06	28.71 ± 0.13	29.16 ± 0.23	0.016	0.672
Hypertension (%)	35.52 (34.15–36.92)	31.12 (29.72–32.55)	67.56 (65.35–69.69)	75.11 (70.69–79.07)	<0.001	<0.001
Diabetes (%)	12.17 (11.34–13.04)	9.64 (8.96–10.36)	30.56 (28.47–32.74)	38.48 (34.89–42.2)	<0.001	<0.001
Cotinine (ng/mL)						
<LOD	19.98 (18.39–21.67)	19.91 (18.18–21.76)	20.52 (18.59–22.59)	22.05 (18.61–25.93)	0.486	0.023
LOD-10	53.46 (51.78–55.13)	53.69 (51.85–55.52)	51.81 (49.28–54.33)	56.72 (52.08–61.24)		
>10	26.55 (25.08–28.08)	26.40 (24.81–28.06)	27.67 (25.42–30.03)	21.23 (18.25–24.55)		
CRP (mg/dL)	0.445 ± 0.007	0.409 ± 0.007	0.626 ± 0.024	0.602 ± 0.036	<0.001	<0.001
TCHO (mmol/L)	5.10 ± 0.01	5.13 ± 0.01	4.94 ± 0.02	4.94 ± 0.04	<0.001	<0.001
HDL-C (mmol/L)	1.374 ± 0.003	1.373 ± 0.004	1.377 ± 0.009	1.361 ± 0.015	0.705	0.390

(Continued)

Table 1. (Continued)

Variable	All (*n* = 15278)	Survival (*n* = 12732)	All-cause mortality (*n* = 2546)	Cardiovascular mortality (*n* = 867)	p_1*	p_2**
TG (mmol/L)	1.18 ± 0.01	1.78 ± 0.01	1.79 ± 0.02	1.82 ± 0.04	0.662	0.322
ALT (U/L)	25.67 ± 0.16	26.15 ± 0.18	23.25 ± 0.34	22.49 ± 0.50	<0.001	<0.001
GLU (mmol/L)	5.65 ± 0.02	5.50 ± 0.02	6.37 ± 0.05	6.72 ± 0.10	<0.001	<0.001
SCr (μmol/L)	80.33 ± 0.30	76.62 ± 0.27	98.90 ± 1.14	102.16 ± 2.06	<0.001	<0.001
PLP (nmol/L)	68.46 ± 0.67	69.73 ± 0.74	62.07 ± 1.59	63.53 ± 2.94	<0.001	0.035

ALT, alanine aminotransferase; BMI, body mass index; CRP, C-reactive protein; GED, General Educational Development; GLU, glucose; HDL-C, high density lipoprotein cholesterol; LOD, the limit of detection; PIR, poverty income ratio; PLP, pyridoxal 5′-phosphate; SCr, serum creatinine; TCHO, total cholesterol; TG, triglycerides.
*p Values for comparisons of variables between Survival and All-Cause Mortality groups.
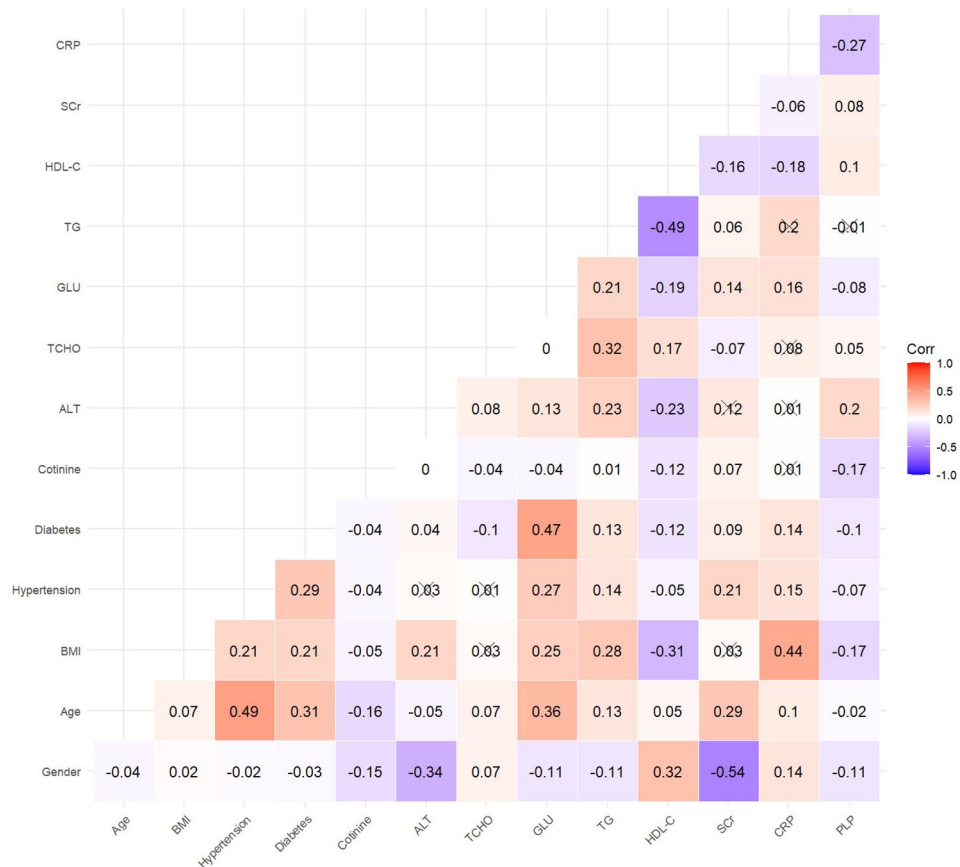**p Values for comparisons of variables between Survival and Cardiovascular Mortality groups.

Figure 2. Spearman's correlation matrix of serum PLP in the study. The values of correlation coefficient contained in a matrix are represented as colors and an "×" indicates that the correlation was not significant ($p > 0.05$).
PLP, pyridoxal 5′-phosphate.

Table 2. Baseline demographic and clinical parameters among participants in none-cardiovascular disease and cardiovascular disease group.

Variable	None-CVD (*n* = 12397)	CVD (*n* = 1202)	*p*
Age (years)	45.46 ± 0.15	64.81 ± 0.39	<0.001
Gender, male (%)	47.26 (46.48–48.05)	55.18 (51.45–58.85)	<0.001
Race/ethnicity (%)			
Mexican American	8.83 (7.13–10.89)	4.75 (3.31–6.78)	<0.001
Other Hispanic	4.81 (3.58–6.45)	2.80 (1.81–4.30)	
Non-Hispanic White	69.25 (65.24–72.99)	75.21 (70.6–79.3)	
Non-Hispanic Black	10.81 (9.07–12.83)	12.57 (10.37–15.15)	
Other race	6.30 (5.21–7.58)	4.68 (3.36–6.47)	
Education (%)			
<High school	17.09 (15.66–18.61)	27.09 (23.93–30.51)	<0.001
High school/GED	23.88 (22.53–25.29)	24.26 (20.69–28.22)	
>High school	59.03 (56.83–61.20)	48.65 (43.66–53.66)	
Marital status (%)			
Divorced/Widowed/Separated	15.68 (14.78–16.61)	30.45 (27.36–33.73)	<0.001
Married/Unmarried couple	66.27 (64.50–67.99)	62.92 (59.17–66.51)	
Never married	18.06 (16.65–19.55)	6.63 (4.88–8.95)	
Family PIR			
<1	13.06 (12.01–14.17)	16.52 (14.01–19.38)	0.002
≥1	86.94 (85.83–87.99)	83.48 (80.62–85.99)	
BMI (kg/m^2)	28.92 ± 0.06	30.62 ± 0.21	<0.001
Hypertension (%)	30.04 (28.66–31.46)	74.43 (70.89–77.67)	<0.001
Diabetes (%)	9.15 (8.48–9.87)	34.95 (31.37–38.7)	<0.001
Cotinine (ng/mL)			
<LOD	19.96 (18.24–21.80)	20.56 (17.56–23.91)	0.633
LOD-10	53.77 (51.92–55.62)	54.45 (51.29–57.58)	
>10	26.27 (24.65–27.94)	24.99 (22.21–28.00)	
CRP (mg/dL)	0.407 ± 0.007	0.570 ± 0.028	<0.001
TCHO (mmol/L)	1.76 ± 0.01	1.95 ± 0.05	<0.001

(Continued)

Table 2. (Continued)

Variable	None-CVD (*n* = 12397)	CVD (*n* = 1202)	*p*
HDL-C (mmol/L)	1.381 ± 0.004	1.288 ± 0.011	<0.001
TG (mmol/L)	5.16 ± 0.01	4.77 ± 0.03	<0.001
ALT (U/L)	26.05 ± 0.17	24.51 ± 0.76	0.049
GLU (mmol/L)	5.49 ± 0.02	6.50 ± 0.08	<0.001
SCr (µmol/L)	76.49 ± 0.26	96.40 ± 1.74	<0.001
PLP (nmol/L)	70.05 ± 0.76	62.03 ± 2.20	0.002

ALT, alanine aminotransferase; BMI, body mass index; CRP, C-reactive protein; CVD, cardiovascular disease; GED, General Educational Development; HDL-C, high density lipoprotein cholesterol; GLU, glucose; LOD, The limit of detection; PIR, poverty income ratio; PLP, pyridoxal 5′-phosphate; SCr, serum creatinine; TCHO, total cholesterol; TG, triglycerides.

Table 3. Hazard ratios for all-cause mortality of all participants, stratified by serum PLP levels quartiles.

	Quartiles of serum PLP levels (nmol/L)			
	Q1	Q2	Q3	Q4
Crude	1.00	0.57 (0.5–0.65)	0.46 (0.41–0.52)	0.52 (0.45–0.6)
p		<0.001	<0.001	<0.001
Model 1	1.00	0.74 (0.65–0.84)	0.6 (0.53–0.67)	0.54 (0.48–0.61)
p		<0.001	<0.001	<0.001
Model 2	1.00	0.78 (0.69–0.89)	0.66 (0.59–0.74)	0.61 (0.54–0.69)
p		<0.001	<0.001	<0.001
Model 3	1.00	0.83 (0.74–0.93)	0.71 (0.63–0.80)	0.64 (0.56–0.74)
p		<0.001	<0.001	0.002

Model 1 was adjusted for age, sex, race/ethnicity, educational level, family PIR, and marital status. Model 2 was subsequently adjusted for BMI, serum cotinine levels, and the presence of hypertension and DM. Lastly, Model 3 was additionally adjusted for serum levels of CRP, TCHO, ALT, GLU, TG, SCr, and HDL-C.
ALT, alanine aminotransferase; BMI, body mass index; CRP, C-reactive protein; DM, diabetes mellitus; GLU, glucose; HDL-C, high density lipoprotein cholesterol; SCr, serum creatinine; TCHO, total cholesterol; TG, triglycerides; Q, quartile.

Discussions

According to the findings of the population-based cohort study, serum PLP levels exhibited a dose–response relationship and had a significant impact on the risk of mortality from all causes, cardiovascular mortality, and CVD.

Vitamin B6 is a group of water-soluble complexes consisting of pyridoxine, pyridoxamine, pyramidal, and their 5′ phosphates. PLP is the predominant coenzyme form of plasma vitamin B6 and the most sensitive indicator of tissue vitamin B6 status. PLP serves as a cofactor in over 160 enzymatic reactions, playing a role in the anabolism and synthesis of amino acids, as well as in processes such as carbohydrate and lipid metabolism, one-carbon metabolism, heme synthesis, and neurotransmitter production. In addition, vitamin B6 can also chelate metal ions and scavenge oxygen free radicals.[22] The relationship between vitamin B6 and health has been of great interest since its discovery in 1932.

Table 4. Hazard ratios for cardiovascular mortality of all participants, stratified by serum PLP levels quartiles.

	Quartiles of serum PLP levels (nmol/L)			
	Q1	Q2	Q3	Q4
Crude	1.00	0.50 (0.39–0.65)	0.40 (0.32–0.51)	0.48 (0.38–0.60)
p		<0.001	<0.001	<0.001
Model 1	1.00	0.68 (0.54–0.85)	0.52 (0.40–0.67)	0.50 (0.41–0.61)
p		0.001	<0.001	<0.001
Model 2	1.00	0.73 (0.58–0.91)	0.58 (0.45–0.74)	0.57 (0.47–0.70)
p		0.006	<0.001	<0.001
Model 3	1.00	0.78 (0.62–0.97)	0.63 (0.49–0.81)	0.62 (0.50–0.77)
p		0.025	0.001	<0.001

Model 1 was adjusted for age, sex, race/ethnicity, educational level, family PIR, and marital status. Model 2 was subsequently adjusted for BMI, serum cotinine levels, and the presence of hypertension and DM. Lastly, Model 3 was additionally adjusted for serum levels of CRP, TCHO, ALT, GLU, TG, SCr, and HDL-C.
ALT, alanine aminotransferase; BMI, body mass index; CRP, C-reactive protein; DM, diabetes mellitus; GLU, glucose; HDL-C, high density lipoprotein cholesterol; PIR, poverty income ratio; PLP, pyridoxal 5′-phosphate; Q, quartile; SCr, serum creatinine; TCHO, total cholesterol; TG, triglycerides.
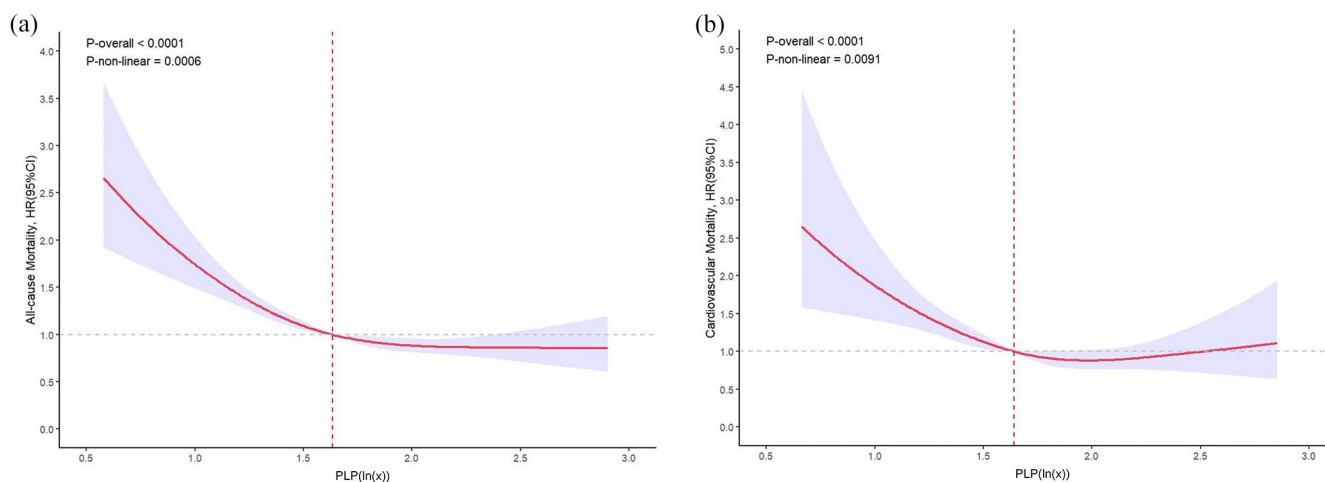

Figure 3. Restrictive spline curve showing the effect of serum PLP levels on (a) all-cause mortality and (b) cardiovascular mortality. Red line and blue transparent area represent HR and 95% CI, respectively. (a) The inflection point of the curve for the effect of serum PLP levels on all-cause mortality was at 42.95 nmol/L. The risk of all-cause mortality was significantly increased when serum PLP levels were less than 42.71 nmol/L and decreased when serum PLP levels were between 43.87 and 238.63 nmol/L. (b) The inflection point of the curve for the effect of serum PLP levels on cardiovascular mortality was at 43.86 nmol/L. The risk of cardiovascular mortality was significantly increased when serum PLP levels were less than 42.76 nmol/L and decreased when serum PLP levels were between 43.86 and 48.54 nmol/L. Data was adjusted for gender, age, race/ethnicity, educational attainment, marital status, family PIR, BMI, waist circumference, heart rates, serum cotinine levels, presence of diabetes and hypertension, serum levels of CRP, TCHO, ALT, GLU, TG, SCr, and HDL-C.
ALT, alanine aminotransferase; BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; DM, diabetes mellitus; GED, General Educational Development; GLU, glucose; HDL-C, high density lipoprotein cholesterol; LOD, the limit of detection; PIR, poverty income ratio; PLP, pyridoxal 5′-phosphate; Q, quartile; SCr, serum creatinine; TCHO, total cholesterol; TG, triglycerides.

Significant vitamin B6 deficiency is primarily determined by identifiable clinical signs and symptoms, including dermatitis, rash, paresthesia, anemia, and even seizures. The definition of vitamin B6 deficiency does not have a widely accepted consensus. This is because the cut-off point for plasma PLP levels is still up for debate. Some believe it should be 20 nmol/L, while some studies have suggested that a borderline state of vitamin B6 deficiency is reached when plasma PLP levels are below 30 nmol/L.[23,24] With the exception of those living in extreme poverty, significant vitamin B6 deficiency is exceedingly rare. However, recent studies have demonstrated that not only does a significant deficiency in vitamin B6 increase the risk of developing specific chronic illnesses, but even mild deficiencies may also be linked to an increased risk of developing such conditions.[25] These chronic diseases primarily encompass DM, CVD, arthritis, chronic inflammatory bowel diseases, and cancer.[26–29]

The status of CVD as the "number one killer" of humans is unassailable. In the 1940s and 1950s, animal studies demonstrated that a diet deficient in vitamin B6 could promote the development of atherosclerosis.[30] Subsequently, a great deal of research has been conducted into the mechanisms of this process, and a number of candidate mechanisms have been proposed. Theoretically, vitamin B6 deficiency affects the activity of cystathionine-beta-synthase, γ-cystathionase, and serine hydroxymethyltransferase, and homocysteine is not metabolized and accumulates in and out of cells, ultimately leading to hyperhomocysteinemia and atherosclerosis progression. However, recent studies have not consistently supported this correlation.[31–33] Another hypothesis is that there is a close relationship between vitamin B6 deficiency and inflammatory processes. It has been suggested that vitamin B6 has antioxidant properties and that vitamin B6 deficiency leads to mitochondrial membrane disruption, accumulation of superoxide radicals, and lipid peroxidation, resulting in inflammatory processes that ultimately lead to CVD.[34] Population-based cohort studies have indicated a significant negative correlation between reduced plasma PLP levels and plasma CRP levels.[35] In this study, we also observed a notable inverse relationship between serum PLP levels and CRP, as demonstrated in Figure 1 ($rs = -0.27$, $p < 0.05$). Furthermore, several studies have specifically examined the correlation between vitamin B6 and various aspects of

Table 5. Associations between serum PLP levels and risk of total and individual CVD.

	OR (95% CI)	*p*
CVD		
Crude	0.78 (0.72–0.85)	<0.001
Model 1	0.78 (0.72–0.85)	<0.001
Model 2	0.85 (0.78–0.93)	0.001
Model 3	0.87 (0.79–0.96)	0.006
Congestive heart failure		
Crude	0.70 (0.62–0.78)	<0.001
Model 1	0.74 (0.66–0.84)	<0.001
Model 2	0.82 (0.72–0.93)	0.003
Model 3	0.85 (0.75–0.97)	0.016
Coronary artery disease		
Crude	0.81 (0.72–0.92)	0.001
Model 1	0.79 (0.70–0.90)	0.001
Model 2	0.86 (0.75–0.98)	0.021
Model 3	0.86 (0.75–0.99)	0.038
Angina/Angina pectoris		
Crude	0.87 (0.77–0.97)	0.017
Model 1	0.87 (0.78–0.98)	0.018
Model 2	0.97 (0.87–1.08)	0.580
Model 3	1.00 (0.89–1.12)	0.990
Heart attack		
Crude	0.72 (0.65–0.79)	<0.001
Model 1	0.75 (0.68–0.82)	<0.001
Model 2	0.82 (0.74–0.91)	<0.001
Model 3	0.84 (0.75–0.94)	0.003
Stroke		
Crude	0.72 (0.64–0.82)	<0.001
Model 1	0.77 (0.68–0.87)	<0.001
Model 2	0.83 (0.73–0.93)	0.002
Model 3	0.85 (0.75–0.96)	0.011

Model 1 was adjusted for age, sex, race/ethnicity, educational level, family PIR, and marital status. Model 2 was subsequently adjusted for BMI, serum cotinine levels, and the presence of hypertension and DM. Lastly, Model 3 was additionally adjusted for serum levels of CRP, TCHO, ALT, GLU, TG, SCr, and HDL-C.
ALT, alanine aminotransferase; BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; CRP, C-reactive protein; DM, diabetes mellitus; GED, General Educational Development; GLU, glucose; HDL-C, high density lipoprotein cholesterol; PIR, poverty income ratio; LOD, the limit of detection; OR, odds ratio; PLP, pyridoxal 5'-phosphate; SCr, serum creatinine; TCHO, total cholesterol; TG, triglycerides.
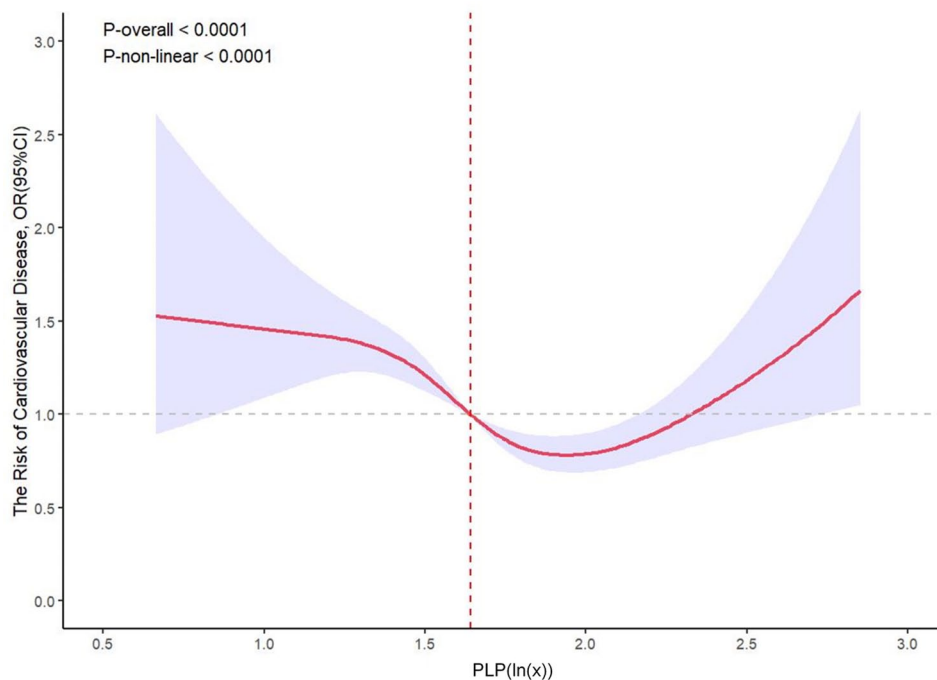
Figure 4. Restrictive spline curve showing the effect of serum PLP levels on CVD risk. Red line and blue transparent area represent HR and 95% CI, respectively. The inflection point of the curve for the effect of serum PLP levels on all-cause mortality was 43.86 nmol/L. The risk of CVD was significantly increased with serum PLP levels below 43.86 nmol/L and decreased with serum PLP levels between 43.86 and 144.30 nmol/L. Data was adjusted for gender, age, race/ethnicity, educational attainment, marital status, family PIR, BMI, waist circumference, heart rates, serum cotinine levels, presence of diabetes and hypertension, serum levels of CRP, TCHO, ALT, GLU, TG, SCr, and HDL-C.
ALT, alanine aminotransferase; BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; CRP, C-reactive protein; DM, diabetes mellitus; GED, General Educational Development; GLU, glucose; HDL-C, high density lipoprotein cholesterol; PIR, poverty income ratio; LOD, the limit of detection; PLP, pyridoxal 5′-phosphate; SCr, serum creatinine; TCHO, total cholesterol; TG, triglycerides.

cholesterol metabolism, fatty acid composition, immune function, and coagulation pathway.

The relationship between plasma vitamin B6 levels, dietary intake of vitamin B6, and the risk of CVD has been studied extensively. According to the findings of the majority of these studies, low levels of vitamin B6 in the plasma are a risk factor for CVD, and taking a moderate amount of vitamin B6 supplements can significantly reduce the risk of CVD.[4] However, the cardiovascular protective effects of vitamin B6 have not been confirmed in all studies. In some studies, the plasma PLP levels in the group with CVD were found to be lower than those in the control group; however, the difference was not found to be statistically significant. There was only a slight association between vitamin B6 and CVD in other studies.[12–14] This may be due to the fact that CVD is etiologically multifactorial, influenced by both genetic and environmental factors. Currently, the specific mechanism by which vitamin B6 contributes to atherosclerosis development is not fully understood, and the determination of plasma PLP levels indicative of vitamin B6 deficiency is yet to be defined. It is very challenging to establish a cause-and-effect relationship between vitamin B6 and the risk of CVD.

Our study is among the few that explore the link between PLP levels and the risk of CVD, all-cause mortality, and cardiovascular mortality based on the NHANES program. Compared to two earlier NHANES studies,[36,37] our research utilized updated Public-Use Linked Mortality Files. This update resulted in an increase of total deaths from 1666 to 2546 and CVD deaths from 290 to 867 in the study population. Moreover,

our study is the first to analyze the dose–response relationships. In this study, we analyzed data from 15,278 adults with an average age of 49.56 years, followed for a median of 11.36 years. Our findings revealed that for each quartile increase in serum PLP levels, all-cause mortality decreased by 17%, 29%, and 36%, while cardiovascular mortality decreased by 22%, 37%, and 38%. The optimal serum PLP levels for predicting both all-cause and cardiovascular mortality was approximately 43.0 nmol/L. Additionally, we observed a 13% reduction in CVD risk for each quartile increase in PLP levels. Our analysis may revealed a concerning prevalence of vitamin B6 deficiency. Using cutoff points of 20 and 30 nmol/L, we found that 15.1% and 31.8% of the population, respectively, were deficient in vitamin B6. These findings suggest that vitamin B6 deficiency is not limited to regions of extreme poverty but may be a widespread global issue.

It is imperative to acknowledge the remaining limitations of this study. First, this study did not include information not on the daily diet. The fact that the content of vitamin B6 precursors in foods varies considerably, as does the efficiency of individual conversion of vitamin B6 precursors, poses a challenge for quasi-deterministic and quantitative determination of active vitamin B6 levels. Second, due to the incomplete data on serum homocysteine levels provided by NHANES, this study did not investigate the correlation between serum vitamin B6 levels and homocysteine levels. Third, these findings were based on the US adult population, which may limit their generalizability to other demographic groups. Finally, residual and unmeasured confounding factors cannot be completely ruled out.

Conclusion

In a representative sample of adults in the United States, it was observed that increased concentrations of PLP in the serum correlated with a decreased risk of CVD, all-causes and cardiovascular mortality. Additionally, a dose–response relationship was evident in the findings. The precise physiological functions of vitamin B6 remain incompletely understood. Consequently, caution should be exercised when interpreting the effects observed in epidemiological studies. These findings need to be confirmed by future studies.

Declarations

Author contributions
Chao Xuan: Data curation; Formal analysis; Investigation; Methodology; Writing – original draft.

Ru-Hua Liu: Formal analysis; Investigation; Writing – review & editing.

Cong Zhao: Formal analysis; Writing – review & editing.

Jing Li: Investigation; Writing – review & editing.

Ting-Ting Zhou: Investigation; Writing – review & editing.

Qing-Wu Tian: Formal analysis; Writing – review & editing.

Guo-Wei He: Methodology; Writing – review & editing.

Competing interests
The authors declare that there is no conflict of interest.

ORCID iD
Chao Xuan https://orcid.org/0000-0001-8273-0178

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