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ORIGINAL ARTICLE

Elevated Blood Selenium Level has a Non-Linear Association with Risk of Anemia in U.S.A. Adults: Data from the NHANES 2011-2016

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SUMMARY

Background: **Only a few epidemiological studies have reported the association between blood selenium and the prevalence of anemia. To date, the evidence is limited and inconsistent.**
Methods: **We enrolled 9,335 participants (≥ 20 years) who participated in the National Health and Nutrition Examination Survey (NHANES) from 2011 to 2016 to assess the link between blood selenium and the risk of anemia. Multivariate logistic regression analysis and a generalized additive model (GAM) was applied to assess the relationship between blood selenium and anemia risk.**
Results: **We found a significant adverse association between blood selenium and the prevalence of anemia after adjusting for all potential covariates (OR = 0.98, 95% CI: 0.97, 0.98, p < 0.001). After a sequence of sensitive analyses, the conclusion remains stable (p for trend < 0.001). However, a non-linear relationship was detected based on GAM. We calculated a turning point of 205.89 μg/L using a two-piecewise linear regression model.**
Conclusions: **When blood selenium level is lower than 205.89 μg/L, blood selenium level is inversely associated with the risk of anemia. Our results provide a new strategy to reduce the risk of anemia.**
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KEYWORDS

blood selenium, anemia, non-linear, NHANES database

INTRODUCTION

Anemia is becoming a serious medical issue in the USA [1], which has markedly increased from 1.0% to 1.9% from 2003 to 2012 [1]. It is characterized in the presence of hemoglobin (Hb) values below 120 g/L for females and 130 g/L for males based on the World Health Organization (WHO) diagnostic criteria. Reports have been found that anemia status more likely links to higher mortality, cardiovascular diseases, dysfunctions, and disability [2-4]. Similarly, investigations showed that anemia was also a risk factor that could damage peripheral nervous system development, respiratory apparatus, and endocrine systems [5]. Therefore, anemia poses a significant challenge to world health.
Selenium (Se) is a trace element that is involved in the regulation of redox metabolic equilibrium, endocrine

metabolism, inflammation, antioxidant stress, and glutathione peroxidase activity [6,7]. Recent studies revealed that selenium was linked to the increased risk of type 2 diabetes (T2D) [8], prostate cancer [9], and non-alcoholic fatty liver disease [10]. These investigations indicated that both too low or too high concentration of selenium can be harmful [6]. However, epidemiological evidence on the risk of anemia is still limited. Previous studies have shown a controversial conclusion between selenium and risk of anemia. Semba et al. reported that low selenium level is linked to an increased risk of anemia among school children [11] and older adults [12]. However, several studies reported that there were no statistically significant evidence supporting this correlation between selenium and hemoglobin concentration [13,14]. Therefore, the purpose of the study was to evaluate the linear and non-linear relationship between blood selenium level and the risk of anemia using the National Health and Nutrition Examination Survey (NHANES) continuously collected from 2011 to 2016.

MATERIALS AND METHODS

Study population

Our data were collected from NHANES, which was a series of surveys based on general population of the United States to collect data based on demographic survey, nutrition interview, and clinical laboratory examination [15]. The NHANES procedures were designed by the National Center for Health Statistics (NCHS). Written informed consent from all participants were provided. NHANES included a total of 14,758 participants who were not pregnant and aged 20 years or older in 3 continuous cycles 2011 - 2012, 2013 - 2014, and 2015 - 2016. Among them, 5,390 participants were excluded who were both missing blood selenium levels and hemoglobin concentrations data. Thirty-four participants missing information on key covariates were not considered. These missing covariates overlapped partially. Finally, 9,335 data were included in the present study (Figure1).

Measurements of Se

Selenium (Se) concentrations in blood were calculated using inductively coupled plasma mass spectrometry (ICP-MS) (https://wwwn.cdc.gov/Nchs/Nhanes/2017-2018/PBCD_J.htm#LBXBSE). If blood selenium concentrations were below detection limit, selenium levels were calculated with the detection divided by the square root of 2. NHANES quality assurance and quality control (QA/QC) protocols meet the requirements of the 1988 Clinical Laboratory Improvement Act mandates.

Anemia assessment

Suspected anemia cases were identified by hemoglobin concentrations, which is commonly considered as a biomarker for anemia. Anemia status was assumed in the presence of hemoglobin concentrations < 130 g/L for men and < 120 g/L for women in accordance with the WHO standard.

Covariates

Self-administered questionnaires were used to collect information on age, gender, race/ethnicity (Mexican American, Non-Hispanic Black, Non-Hispanic White, Other Hispanic and Other Race), education levels, poverty income ratio (PIR), smoking status, work activity and dietary intake information including fiber, protein, calcium, vitamin D, and magnesium. Education backgrounds are classified as below high school, high school graduate or equivalent, some college or above. Smoking status was viewed as self-reported current smokers (≥ 100 cigarettes and smoking currently), former smokers (≥ 100 cigarettes and no smoking currently), and never smokers (< 100 cigarettes in the entire life)[16]. Height and weight were measured to calculate the body mass index (BMI) as the ratio of weight (kg) and height (meters^2). Work activity was divided into three level activity intensity vigorous, moderate, and light work activities basing on activity intensity per week. Poverty income ratio (PIR) was calculated through a household size specific threshold to assess family income. The dietary intake interview was conducted basing on previous 24 hours dietary recall including total dietary energy, protein and fiber, vitamin D, calcium, and magnesium information.

Statistical analysis

All the analyses were performed with the statistical software packages R (http://www. R-project.org, The R Foundation), Free Statistics software version 1.3, and Empower Stats (http://www.empowerstats.com). The continuous variable is expressed as the mean (standard deviation) or as the median (interquartile range). Comparisons of categorical variables (gender, racial group, education status, poverty income ratio, smoking status, and work activity) were presented as cases (n) and percentage (%). Considering possible non-linear relationships, blood selenium level was divided into five quintiles. The linear trend test was conducted by assigning the blood selenium quintiles as a continuous variable. The generalized additive model (GAM) and a two-piecewise regression model were used to further identify the potential non-linear relationship between blood selenium and anemia. Model 1 adjusted for none. Model 2 adjusted for gender, age, and race/ethnicity (Mexican American, non-Hispanic Black, non-Hispanic White, other Hispanic, and other Race). Model 3 adjusted for all potential confounding factors listed in Table 1 (gender, age, race, education level, PIR, BMI, smoking status, physical activity, and dietary intake, including total energy, fiber, protein, calcium, vitamin D, and magnesium). Finally, we investigated the possible influence of age (20 - 39 years, 40 - 59 years , 60 years and older), gender, race (Mexican American, non-Hispanic Black, non-Hispanic White, other Hispanic, other Race), education levels (college education or above,

Table 1. Characteristics of the study population by anemia status (n = 9,335).

Variables	Total (n = 9,335)	No Anemia (n = 8,276)	Anemia (n = 1,059)	p
Age, mean ± SD	49.5 ± 17.7	48.8 ± 17.4	55.1 ± 18.8	< 0.001
Gender, n (%)				< 0.001
Female	4,701 (50.4)	4,024 (48.6)	677 (63.9)	
Male	4,634 (49.6)	4,252 (51.4)	382 (36.1)	
Race, n (%)				< 0.001
Mexican American	1,185 (12.7)	1,067 (12.9)	118 (11.1)	
Non-Hispanic black	2,121 (22.7)	1,661 (20.1)	460 (43.4)	
Non-Hispanic white	3,657 (39.2)	3,405 (41.1)	252 (23.8)	
Other Hispanic	998 (10.7)	905 (10.9)	93 (8.8)	
Other race	1,374 (14.7)	1,238 (15)	136 (12.8)	
PIR, mean ± SD	2.5 ± 1.6	2.5 ± 1.7	2.2 ± 1.5	< 0.001
Education level, n (%)				< 0.001
College education or above	5,208 (55.8)	4,671 (56.5)	537 (50.8)	
Graduated from high school	2,050 (22.0)	1,824 (22)	226 (21.4)	
< High school	2,073 (22.2)	1,778 (21.5)	295 (27.9)	
Smoking status, n (%)				< 0.001
Never smoker	5,300 (56.8)	4,635 (56)	665 (62.8)	
Former smoker	2,220 (23.8)	1,963 (23.7)	257 (24.3)	
Current smoker	1,815 (19.4)	1,678 (20.3)	137 (12.9)	
Work activity, n (%)				< 0.001
Light work activity	5,759 (61.7)	5,018 (60.6)	741 (70)	
Moderate work activity	1,889 (20.2)	1,683 (20.3)	206 (19.5)	
Vigorous work activity	1,687 (18.1)	1,575 (19)	112 (10.6)	
BMI (kg/m^2), mean ± SD	29.2 ± 7.0	29.1 ± 7.0	29.7 ± 7.7	0.011
Energy (gm), mean ± SD	2,116.0 ± 969.8	2,146.9 ± 980.4	1,866.6 ± 839.8	< 0.001
Protein (gm), mean ± SD	81.3 ± 41.8	82.6 ± 42.3	70.9 ± 35.7	< 0.001
Fiber (gm), mean ± SD	17.2 ± 10.8	17.4 ± 10.9	15.5 ± 10.0	< 0.001
Calcium (mg), mean ± SD	920.2 ± 567.9	933.3 ± 574.2	814.6 ± 502.4	< 0.001
Magnesium (mg), mean ± SD	299.2 ± 151.6	303.6 ± 152.7	263.9 ± 137.1	< 0.001
Vitamin D (mg), mean ± SD	4.5 ± 5.7	4.6 ± 5.8	4.2 ± 4.9	0.047
Hb (g/L), mean ± SD	139.5 ± 15.3	142.8 ± 12.2	113.2 ± 10.7	< 0.001
Se (μg/L), median (IQR)	192.8 (178.3, 208.2)	194.5 (179.8, 209.4)	180.8 (164.3, 195.4)	< 0.001

graduated from high school, < high school), PIR (< 1, ≥ 1), BMI (< 25, 25 - 29.9, ≥ 30), calcium intake (< 800 mg, ≥ 800 mg), magnesium intake (< 260 mg, ≥ 260 mg), work activity (light work activity, moderate work activity, vigorous work activity) and smoking status (never smokers, former smokers, and current smokers) on the relationship between blood selenium and anemia by stratified analysis.

RESULTS

Baseline characteristics of selected participants

Table 1 generalized the characteristics of the study population according to anemia or not. The mean age of study participants was 49.5 (49.5 ± 17.7) years, and 50.4% of them were females. Among the 9,335 participants, 1,059 were confirmed as anemia cases. Participants with anemia were prone to be female, older, non-Hispanic black, with a lower dietary intake (vitamin D, calcium, magnesium, protein, and fiber), had higher BMI and lower selenium level. Median (quartile range,

Table 2. Adjusted odds ratios (ORs) (95% CI) for prevalence of anemia with quintile changes in blood selenium levels multivariable-adjusted logistic regressions.

Exposure	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Blood selenium, µg/L	0.97 (0.97, 0.98) < 0.001	0.98 (0.97, 0.98) < 0.001	0.98 (0.97, 0.98) < 0.001
Quintiles of blood selenium levels, µg/L			
Q1 (105.38 - 174.89)	Ref	Ref	Ref
Q2 (174.90 - 187.28)	0.51 (0.43, 0.60) < 0.001	0.51 (0.43, 0.62) < 0.001	0.52 (0.43, 0.64) < 0.001
Q3(187.29 - 198.59)	0.34 (0.28, 0.42) < 0.001	0.38 (0.31, 0.46) < 0.001	0.38 (0.31, 0.48) < 0.001
Q4 (198.60 - 212.71)	0.25 (0.21, 0.31) < 0.001	0.28 (0.23, 0.35) < 0.001	0.29 (0.23, 0.37) < 0.001
Q5 (212.72 - 734.80)	0.22 (0.17, 0.27) < 0.001	0.24 (0.20, 0.31) < 0.001	0.24 (0.19, 0.32) < 0.001
p for trend	< 0.001	< 0.001	< 0.001

Model 1 adjust for: none.
Model 2 adjust for: gender, age, and race.
Model 3 adjust for all covariates listed in Table 1.

Table 3. Adjusted percentage changes (95% CI) of hemoglobin concentration with quintile changes in blood selenium by multivariable-adjusted linear regressions (n = 9,335).

Exposure	Model 1 ß (95% CI)	Mode 2 ß (95% CI)	Model 3 ß (95% CI)
Blood selenium, µg/L	0.13 (0.12, 0.14) < 0.001	0.1 (0.09, 0.11) < 0.001	0.09 (0.08, 0.1) < 0.001
Quintiles of blood selenium			
Q1 (105.38 - 174.89)	Ref	Ref	Ref
Q2 (174.90 - 187.28)	4.59 (3.64, 5.54) < 0.001	3.81 (3.04, 4.58) < 0.001	3.77 (2.95, 4.58) < 0.001
Q3(187.29 - 198.59)	6.92 (5.97, 7.87) < 0.001	5.12 (4.35, 5.90) < 0.001	5.01 (4.20, 5.83) < 0.001
Q4 (198.60 - 212.71)	9.28 (8.34, 10.24) < 0.001	6.87 (6.10, 7.64) < 0.001	7.06 (6.24, 7.88) < 0.001
Q5 (212.72 - 734.80)	11.12 (10.17, 12.07) < 0.001	8.25 (7.48, 9.03) < 0.001	8.22 (7.40, 9.04) < 0.001
p for trend	< 0.001	< 0.001	< 0.001

Model 1 adjust for: none.
Model 2 adjust for: gender, age, and race.
Model 3 adjust for all covariates listed in Table 1.

IQR) blood selenium levels were 192.8 (178.3, 208.2) µg/L, 194.5 (179.8, 209.4) µg/L, and 180.8 (164.3, 195.4) µg/L in total population, non-anemia group, and anemia group, respectively.

Multiple regression model and analysis of non-linear relationship

The relationship between blood selenium level and anemia was shown in Table 2 by using multiple regression analysis. Univariate analysis shows that blood Se concentration was statistically associated with anemia ($p < 0.001$). After full adjustment, a statistically positive correlation between blood selenium level and anemia was observed (Q5 vs. Q1 = 11.12 (95% CI: 10.17 - 12.07), p for trend < 0.001), and p-trend indicated non-equidistant changes. A non-linear association between selenium level and anemia was observed by using a generalized additive model (GAM) after adjusting for all potential confounders (Figure 2). According to the piecewise linear regression analysis (Table 4), a positive association was observed when blood selenium below 205.89 µg/L was detected. No statistically significant correlation was found at blood selenium > 205.89 µg/L.

Table 3 showed the relationship between blood selenium levels and hemoglobin concentration. After adjusting for all covariates, comparing to the lowest quintiles, the OR was 8.22 (95% CI: 7.40 - 9.04) (p - trend < 0.001). A similar non-linear link between selenium and hemoglobin concentration was identified (Figure 2).

Table 4. Threshold effect analysis of blood selenium on anemia and hemoglobin concentration with the use of segmented linear regression model.

Outcome	β/OR (95% CI)	p for value
Anemia		
Inflection point	**205.89**	
< 205.89	**0.97 (0.96, 0.97)**	**< 0.0001**
> 205.89	**1.00 (0.99, 1.01)**	**0.8431**
Log likelihood ratio test	**< 0.001**	
Hemoglobin		
Inflection point	**207.55**	
< 207.55	**0.018 (0.017, 0.020)**	**< 0.0001**
> 207.55	**0.001 (-0.001, 0.002)**	**0.5336**
Log likelihood ratio test	**< 0.001**	

Log likelihood ratio test results comparing linear regression model with two piecewise linear regression model adjusted for all covariates listed in Table 1.

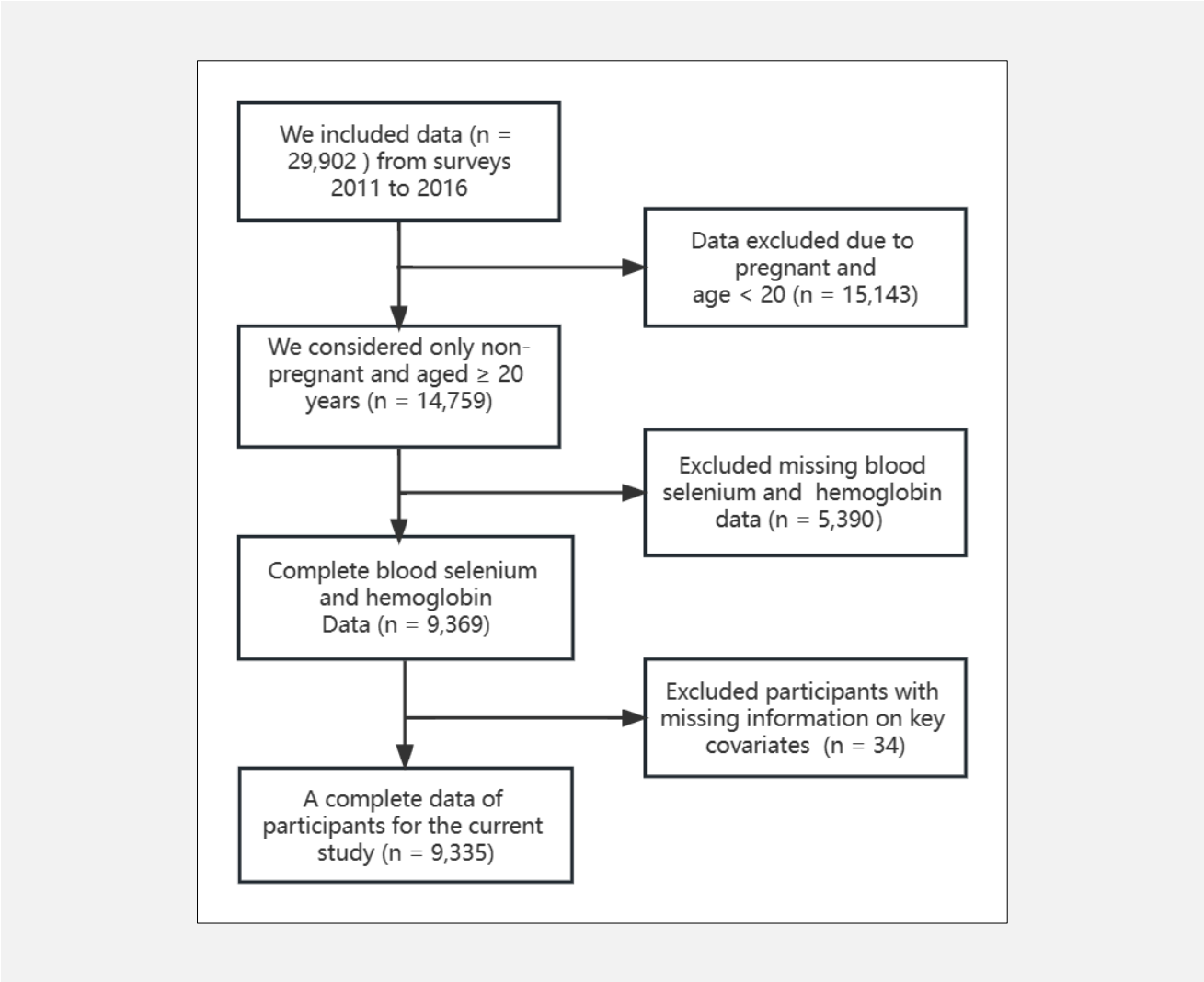

Figure 1. The flow chart of the study.

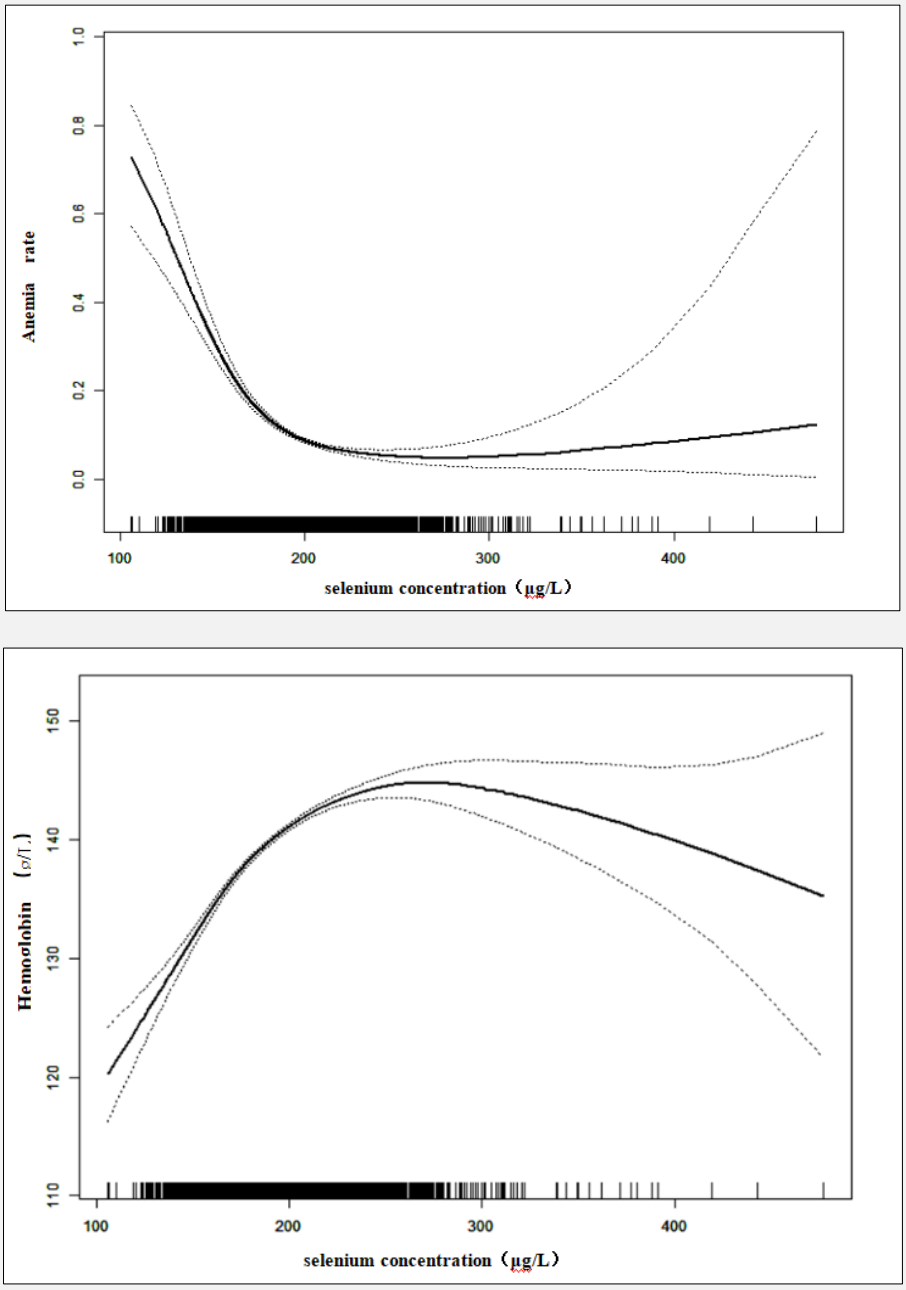

Figure 2. The correlation between blood selenium and anemia and hemoglobin concentration based on generalized additive model (GAM).

The GAM model adjusted for all covariates listed in Table 1.

Sensitivity analysis

For the purpose of ensuring the stability of the results, subgroup analysis was conducted to reveal the potential interaction between blood selenium and anemia (Supplemental Table 1). We also found the interactions between blood selenium with age on anemia, race on anemia, and smoking status on anemia.

DISCUSSION

In our study, we examined a non-linear relationship between blood selenium levels and risk of anemia in US adults, based on the NHANES program from 2011 to 2016. After adjusting for all potential confounders, we revealed that blood selenium inversely associated with risk of anemia when blood selenium was lower than 205.89 μg/L, whereas the curve was flattened at > 205.89 μg/L blood selenium. At the same time, a similar non-linear link between blood selenium and hemoglobin concentration was detected.

To date, the optimal intake of selenium worldwide has not been conclusively determined [17]. In the USA, based on the Food and Nutrition Board of the Institute of Medicine, the recommended intake of selenium is 55 μg/day. The recommended intake of selenium in the UK for adults is 60 μg/day and 75 μg/day for female and male, respectively. The tolerable upper intake is 400 μg/day [18]. A peer-reviewed paper showed that the recommended safe intake of Se was around 800 μg/day with no adverse effects observed. Up to 1,600 μg/day for selenium intake showed that mild adverse effects were observed [19]. A toxic level was 5,000 μg/day, where serious adverse effects happened, such as hair, toes, and nails fall out, breath smells of garlic, acute respiratory distress syndrome, myocardial infarction, and kidney failure. In past decades, more and more studies indicated that the physiological and toxic dose ranges are narrow. Despite nutritional value, a growing number of studies suggested that both too low and too high selenium levels were significantly associated with many chronic diseases, such as type 2 diabetes, advanced prostate cancer [9,20], hypertension [21], nervous system diseases [22], and nonalcoholic fatty liver disease [10]. Considering the narrow physiological range of selenium, participants are vulnerable to be exposed in geographical deficiency and toxic environments, which might help us better understanding why high blood selenium exposure is linked to increased anemia risk. In our study, we indicated a non-linear association between blood selenium and anemia. Especially, a negative association with blood selenium and anemia risk was reported when the blood selenium concentration was less than 205.89 μg/L. In contrast, there were no significant associations between blood selenium and anemia at a blood selenium concentration higher than 205.89 μg/L.

Observational studies suggest that biochemical selenium deficiency was related to the etiology of anemia [12, 23]. Laboratory studies report that selenium, as part of glutathione peroxidase, protects biomacromolecules and biofilm structures from peroxides and keeps cell dissociation rate low, thus protecting normal physiological functions of cells [24]. Glutathione peroxidase prevents hemolysis of red blood cells and therefore may be relevant to hemoglobin level [24]. The regulation of oxidative stress is a necessary condition for red blood cells to carry oxygen, and the deficiency of antioxidant enzymes, as well as obstacles to mature and longevity of red blood cells [25]. The underlying mechanism of this association is still unclear, and the antioxidant activity of selenium may play an important role in these mechanisms. Conceivably, high blood selenium may influence the pathogenesis of anemia through the toxicity of selenomethionine [26]. The morphology of selenium in blood warrants further study.

A few epidemiological reports have demonstrated these links between selenium and anemia. Consistent with our studies, a similar non-linear dose-response link was noticed in a cross-sectional survey among 2,902 adults in the USA [27], where the inflecting point of serum selenium level in the study was 143 ng/ml, which was lower in our study. In our study, we measured the concentration of selenium in whole blood, which is generally higher than serum. An adverse linear association between selenium intake level and anemia has also been reported among 2,092 aged 65 or older adults [28]. Another study observed that low circulating selenium was linked to increased anemia risk in older community-dwelling adults [29]. In general, a negative independent association between blood selenium and anemia was explored in our study. Meanwhile, a threshold effect was noted, which could guide optimum blood selenium level to reducing the risk of anemia.

Our study has certain advantages. First, as of now, we have the largest number of participants compared with previous studies. Second, in order to analyze the potential non-linear relationship, a GAM model and a smooth curve fitting were used. Third, sensitivity analysis was conducted to ensure the stability of the analysis results. Fourth, we first measured selenium in whole blood in contrast to previous studies. However, the study has several limitations. We acknowledge that a cross-sectional study of NHANES data cannot prove causality from the results. In addition, due to the cross-sectional analysis nature, we could not eliminate unknown or unmeasured factors, although we controlled many known confounding factors. We did not consider the types of anemia. Additionally, there may be other nutrients that share a common source with selenium, such as the diet, which may also affect the relationship between selenium levels and anemia. Another limitation was that only a single blood selenium was measured; other specimens, such as serum/plasma and urine were not available. In future research, comparing different selenium morphology and biomarkers will be useful for better understanding the relationship between selenium and anemia. Finally, the study participants were limited to USA residents, this conclusion may not be generalizable to other residents.

Declaration of Interest:
There are no conflicts of interest to declare.

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