

Plasma selenium and zinc alter associations between nephrotoxic metals and chronic kidney disease: Results from NHANES database 2011–2018

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ABSTRACT

Introduction: Chronic kidney disease (CKD) is a condition defined as a persistent change in kidney structure or function, or both, that compromises human health. Environmental exposure to heavy metals (e.g. cadmium, lead, arsenic and mercury) is common, and high exposure levels are known to cause nephrotoxicity. Micronutrients such as selenium and zinc are positively associated with better kidney function and renal outcomes. This study determined the associations between CKD and heavy metal exposures measured in blood or urine within a community-dwelling population, and assessed whether and how selenium and zinc modified the associations.

Method: Data were extracted from 4 cycles of the US National Health and Nutrition Examination Survey (NHANES) database (2011–2012, 2013–2014, 2015–2016 and 2017–2018).

Results: Univariate analysis showed that higher quartiles of plasma lead and cadmium concentration were more likely associated with CKD than the lowest quartile, and along with folate, were linked to greater odds of CKD. Conversely, as plasma selenium and serum zinc increased, the odds of CKD decreased. Multivariate analysis had similar results after adjusting for relevant confounders. Higher plasma cadmium quartiles were associated with higher odds of CKD. Associations between higher quartiles of plasma selenium and serum zinc were significantly associated with lower odds of CKD.

Conclusion: Elevated blood levels of heavy metals increase CKD, whereas elevated concentrations of plasma selenium and serum zinc decrease CKD. A high serum zinc concentration appears to interact with low-toxicity heavy metals to reduce CKD risk. This study suggests that increased selenium and zinc in the body along with avoidance of heavy metal exposures could protect against CKD.

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INTRODUCTION

Chronic kidney disease (CKD) is a clinical condition comprising persistent changes in kidney function or structure, or both. It is characterised by irreversible and progressive evolution, increasing the risk of complications and mortality. As the 16th leading cause of mortality worldwide, CKD affects 8–16% of the global population.^{1,2} Clinically, CKD is classified by albuminuria (an indicator of glomerular damage) and the glomerular filtration rate (GFR) (a well-established marker of renal excretory function). Both are considered

reliable predictors of the outcomes of long-term CKD.³ In CKD, GFR <60 mL/min/1.73 m² or proteinuria ≥30 mg/24h for more than 3 months indicates persistent renal structural or functional abnormalities.⁴ CKD is divided into 5 stages according to GFR and 3 steps according to proteinuria. The staging system helps to determine the monitoring method and intensity of CKD. Prognostic factors include age, sex, race, plasma cholesterol concentration and smoking. Adverse outcomes associated with CKD include death and end-stage renal disease (ESRD),⁵ which may

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CLINICAL IMPACT

What is New

- This study highlights the protective effects of micronutrients against chronic kidney disease (CKD) among patients who have high blood levels of heavy metals.
- Higher blood cadmium levels significantly increased the risk of CKD.
- Conversely, the elevation of serum selenium and zinc neutralised toxic heavy metals consisting of lead, cadmium and mercury. The incidence of CKD was decreased.

Clinical Implications

- Increasing the blood concentrations of selenium and zinc as well as avoiding heavy metal exposures might help to prevent the development of CKD.
- The accumulation of selenium and zinc appears to alleviate the damage caused by heavy metals to the kidneys.

be prevented through proper clinical diagnosis and management.⁶

Global industrialisation continues to meet the needs of modern humans, but the associated costs include substantial pollution of the environment with various toxic pollutants, including heavy metals.⁷ The accumulation of toxic heavy metals in the environment may have deleterious effects on human health.⁸ Heavy metals are classified as those that have high atomic mass in the range of 63.5–200.6 daltons.⁹ Environmental heavy metal exposures, including those of cadmium, lead, arsenic and mercury, are common, and high exposure levels in certain populations are recognised as nephrotoxic.^{10–12} Other heavy metals might also harm the body when they exceed specific thresholds, although the toxic levels of heavy metals are affected by many factors, such as exposure route, dose and oxidative status, in addition to human factors, such as age, genetics, sex and dietary status.¹³ Toxic heavy metals are prevalent nephrotoxins that unfortunately, have not been fully studied in humans.

A previous study reported an association between certain heavy metals and kidney disease, which was largely explained by toxic effects on proximal tubular function.¹⁴ A case-controlled study with a population-

based cohort found an association between red blood cell (RBC) lead levels and an increased risk of ESRD.¹⁵ While the toxic effects of heavy metals are deleterious to humans, micronutrients are essential for maintaining normal human physiology. Micronutrients such as selenium, zinc and folic acid are positively associated with better renal outcomes and kidney function.¹⁶

Several protective mechanisms of selenium and zinc against metal toxicity in animals and cells have previously been reported.¹⁷ Even dysregulation of metal or trace-metal homeostasis in biological systems can lead to severe deleterious effects in many diseases.¹⁸ However, the combined effects of environmental heavy metal exposures measured in human bodies and micronutrients such as selenium, zinc and folate on CKD and renal function remain to be assessed. This study aimed to investigate the associations between CKD and heavy metal exposures measured in the urine or blood among the general community-dwelling population, and whether and how selenium and zinc modified the associations.

METHOD

Study design and data source

This cross-sectional retrospective cohort study extracted all patient data from National Health and Nutrition Examination Survey (NHANES) database collected by National Center for Health Statistics (NCHS) of US Centers for Disease Control and Prevention. The survey assesses US children and adults' health and nutritional status, using a complex, multistage design to collect and analyse the data representative of US national non-institutionalised population. Data are released on a biennial cycle for research purposes, and NCHS permits researchers to use the data. Participants in NHANES complete home interviews and are invited to undergo an extensive examination at NHANES Mobile Examination Center, including physical examinations, speciality measurements and laboratory tests. Thus, participants' assessments in NHANES database are robust and multidimensional and can be equated to population-level estimates.¹⁹

Study population

All data were extracted from 4 released 2-year cycles in the NHANES database (2011–2012, 2013–2014, 2015–2016 and 2017–2018), for a total of 8 years. Adults aged 18 years or older, who had complete information of the variables of interest, were eligible for inclusion. Subjects with ESRD, defined as an estimated GFR (eGFR) of <15 mL/min/1.73 m², and

participants without eGFR data were excluded. The included population was divided into participants with or without CKD, defined as having an eGFR <60 mL/min/1.73 m² for further comparisons.

The NCHS Research Ethics Review Board approved NHANES.

Data collection

Assessment of CKD

Creatinine measurements were obtained from standardised biochemical profiles collected by NHANES for each participant. The Beckman Synchron LX20 Modular Chemistry Analyzer (Beckman Coulter, Indianapolis, IN, US) used the Jaffe rate method (kinetic alkaline picrate) to determine creatinine concentrations in urine, serum or plasma. GFR was estimated from recalibrated serum creatinine using the 4-variable Modification of Diet in Renal Disease (MDRD) study equation. Isotope dilution mass spectrometry traceable MDRD study equation using standardised creatinine: $GFR = 175 \times (\text{standardised serum creatinine})^{-1.154} \times (\text{age})^{-0.203} \times 0.742$ (for female participants) $\times 1.212$ (for African American) was applied.²⁰ Estimated GFR is reported in mL/min/1.73 m². CKD was defined by an eGFR of less than 60 mL/min/1.73 m².

Assessment of blood lead, cadmium, mercury and urinary arsenic

Blood samples were processed, frozen at -20 °C and sent to the Centers for Disease Control and Prevention's National Center for Environmental Health (NCEH), US for testing. Detailed descriptions of the laboratory methods used by NHANES can be found on the NHANES website. Whole blood was analysed for cadmium, mercury, lead and total urinary arsenic by inductively coupled plasma dynamic cell reaction mass spectrometry (ICP-MS) in the Laboratory Sciences Division of the NCEH.²¹ Urine creatinine concentration was determined as a marker of urine dilution and was selected by Jaffe rate response using a Beckman Synchron Analyzer LX20 Modular Chemistry Analyzer (Beckman Coulter, Indianapolis, IN, US).

Assessment of plasma selenium, serum zinc and serum folate

Briefly, cyclic selenium was tested in the trace element laboratory using ICP-MS.²¹ Serum zinc was measured using the same method as the other trace metals in the same facility.²² Microbiological assays measure whole-blood folate, whereas serum folate is measured by

isotope dilution high-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). RBC folate was then calculated using data from both assays.

Covariates

Demographic data, including age, race, sex, education level and household income-to-poverty ratio, were obtained through face-to-face interviews conducted by trained interviewers using the Household and Sample Demographic Questionnaire and the Computer-Assisted Personal Interviewing system. Collected data were weighted according to the NHANES protocol.

Body mass index (BMI) values were obtained from NHANES examination measurements for anthropomorphic data and calculated as weight in kg divided by height in m². Included participants were split into underweight (<18.5 kg/m²), average (18.5–24.9 kg/m²), overweight (25–29.9 kg/m²) and obese (≥ 30.0 kg/m²) according to their BMI. For smoking status, participants were categorised as never smokers, exposed to ambient tobacco smoke, or active smokers. Active smokers were defined as having smoked >100 cigarettes in their lifetime and answering “yes” to the question: “Do you smoke now?” or having a serum cotinine level >10 µg/L (ng/mL). Exposure to environmental tobacco smoke was defined as detectable serum cotinine of 0.015, but not more than 10 µg/L (ng/mL). Non-smokers smoked fewer than 100 cigarettes in their lifetime or had a serum cotinine level <10 µg/L (ng/mL). Alcohol consumption was categorised based on responses to survey questions defining alcohol consumption, including excessive drinking, characterised by a reply ≥ 4 times per week to the following question: “In the past 12 months, how often did you drink an alcoholic beverage?” Vigorous physical activity was determined based on individuals' self-reported vigorous physical exercise versus no physical activity in the past 30 days.

High blood pressure was defined by those who answered “yes” to the following questions or met the criteria: “Have you been told on 2 or more different visits that you have hypertension, also known as high blood pressure?” or “Because of your hypertension, have you ever been told to take prescription medications?” or have a mean systolic blood pressure of ≥ 140 mmHg over 3 consecutive measurements or have a mean diastolic blood pressure of over 3 straight steps ≥ 90 mmHg. The following questions and criteria define diabetes: “Apart from pregnancy, have you ever been told by a doctor or a health professional that you have

diabetes or sugar diabetes?” or “Are you currently taking insulin?” or “Are you currently taking diabetes medication to lower blood glucose, sometimes called oral antidiabetic medications?” or have HbA1c of $\geq 6.5\%$ (≥ 48 mmol/mol) or laboratory-measured fasting blood glucose >125 mg/dL (6.94 mmol/L). Hyperlipidaemia was defined as a self-reported answer “yes” to the question or met the criteria: “Have you ever been told by a doctor or a health professional to take a prescription drug to lower blood cholesterol?” or have a total cholesterol level of >200 mg/dL (5.20 mmol/L). History of cardiovascular disease, including coronary heart disease, angina, congestive heart failure, myocardial infarction and stroke, was defined by the question: “Has a doctor or a health professional ever told you that you have (disease)?” Chronic obstructive pulmonary disease (COPD) is described as positive responses to the questions: “Has a doctor or a health professional ever told you that you have emphysema?” or “Has a doctor or a health professional ever told you that you have chronic bronchitis?”

Statistical analysis

Statistical analysis was performed using SAS statistical software, version 9.4 (SAS Inc, Cary, NC, US). NHANES draws samples from a complex sampling scheme that combines variables from different study periods. Using sample weights provided by NHANES, it is possible to estimate the equivalent total number of individuals in the whole country from the study sample. Continuous variables are presented as means and standard errors (SE), and categorical variables are presented as unweighted numbers and weighted proportions. Differences in means between groups were compared using the SURVEYREG procedure for continuous variables. Rao-Scott chi-square tests were also performed on categorical variables using the SURVEYFREQ programme to examine differences in proportions between groups. Logistic regression was performed using the SURVEYLOGISTIC procedure to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for associations between prevalent CKD and study variables. Variables that reached statistical significance in univariate analysis were entered into multivariate models for adjustment. A 2-sided P value <0.05 was determined to be statistically significant. Three types of associations were assessed. First, roughly linear associations between $\log_{10}$ GFR and blood lead, cadmium, mercury, urinary arsenic, plasma selenium, serum zinc, serum and erythrocyte folate levels. Second, presence of CKD with blood metals, crude and adjusted associations between urinary arsenic, plasma selenium, serum zinc, serum and erythrocyte folate quartiles.

Third, quartile cut-off values were determined by the distribution of data across all subjects for combined effects of blood metals, urinary arsenic and plasma selenium or serum zinc on CKD. In a combined effects analysis, “high” levels represent the highest quartile (Q4), whereas “low” levels represent the lowest quartile (Q1).

RESULTS

Study sample selection

The flow diagram of study participants' inclusion and exclusion is presented in Fig. 1. The sample data were extracted from the 4 released cycles of the NHANES database. A total of 39,156 participants identified as eligible, and 23,825 patients aged >18 years were selected. Individuals with ESRD or those with no eGFR results were excluded, leaving 21,251 participants. Finally, 9557 subjects with laboratory measures of heavy metals and micronutrients of interest were included as the primary cohort for the subsequent analysis. Sample weights were assigned to each sampled individual by NHANES for calculating the equivalent population size in the entire US. After weighting, the cohort size ($n=9557$) was equivalent to 236,263,413 community-dwelling adults of the US (Fig. 1).

Study population characteristics

Blood and urine measures, demographic characteristics, lifestyle and comorbid conditions of the study population

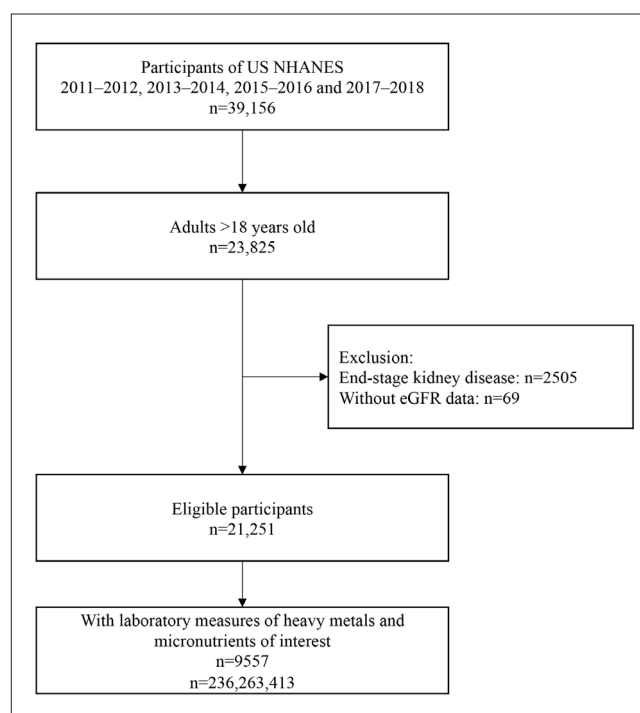


Fig. 1. Flow diagram of study sample selection.

with or without CKD are summarised in Table 1. The mean age of the study cohort was 46.7 ± 0.03 years, and 51.5% were females. Most participants were non-Hispanic whites (64.4%) and non-smokers (57.0%). The most common comorbid conditions of the study cohort were hyperlipidaemia (52.0%) and hypertension (34.7%). Regardless of being continuous variables or categorised in quartiles, blood lead, plasma selenium, serum folate and RBC folate showed significantly different distributions between participants with or without CKD. Significant differences in distribution were noted in blood cadmium levels in quartiles between subjects with or without CKD. Blood mercury, as a continuous variable, also showed differences between CKD and non-CKD participants. Significant differences were found in all other variables, except for education level and excessive alcohol consumption between individuals with and without CKD (Table 1).

Associations between selenium, zinc, folate, lead, cadmium, mercury, arsenic levels and prevalent CKD

Crude associations between log transformed eGFR and levels of plasma selenium, zinc, serum folate and RBC folate are shown in Fig. 2. Serum zinc was positively associated with $\log_{10}$ eGFR with β (SE) 2.1 (1.4) $\mu\text{g}/\text{dL}$, $P=0.160$. However, blood selenium with β (SE) -1.1 (1.9) $\mu\text{g}/\text{L}$, $P=0.578$, serum total folate with β (SE) -6.9 (1.2) $\mu\text{g}/\text{L}$ (ng/mL), $P<0.001$, and RBC folate with β (SE), -136.3 (20.7) ng/mL, $P<0.001$ were inversely associated with $\log_{10}$ eGFR (Fig. 2).

Crude associations between log-transformed eGFR and levels of blood lead, cadmium, mercury and urine arsenic are illustrated in Fig. 3. Urine arsenic was positively associated with $\log_{10}$ eGFR with β (SE) 1.2 (2.7) $\mu\text{g}/\text{L}$, $P=0.649$. Blood lead with β (SE) -0.5 (0.1) $\mu\text{g}/\text{dL}$, $P<0.001$, cadmium with β (SE) -0.04 (0.04) $\mu\text{g}/\text{L}$, $P=0.332$, and total mercury in blood with β (SE) -0.005 (0.098) $\mu\text{g}/\text{L}$, $P=0.961$ were inversely associated with $\log_{10}$ eGFR (Fig. 3).

The associations between levels of blood lead, cadmium, mercury, plasma selenium, serum folate, RBC folate, serum zinc in quartiles and prevalent CKD are summarised in Table 2. Univariate analysis showed greater odds for CKD compared to the lowest quartile (Q1) and higher quartiles (Q2–Q4) of blood lead and cadmium. In addition, serum and RBC folate in the highest quartile (Q4) were associated with greater odds for CKD. Decreased odds for CKD were associated with increasing plasma selenium and serum zinc (Q2–Q4). Multivariable analysis maintained these results after adjusting for relevant confounders. Compared to Q1,

higher blood cadmium quartiles were associated with greater odds for CKD (adjusted OR [AOR] 1.93, 95% CI 1.25–3.00 for Q3; AOR 2.79, 95% CI 1.58–4.92 for Q4). For plasma selenium and serum zinc, statistically significant associations were noted between higher quartiles (vs Q1) and lower odds for CKD (Table 2).

Combined effects of selenium and zinc with blood lead, cadmium, mercury and urine arsenic on CKD risk

After adjusting for relevant confounders in multivariable analysis, high selenium and low cadmium was associated with significantly reduced odds for CKD (AOR 0.23, 95% CI 0.07–0.72) compared to low selenium and high cadmium. High zinc with either high lead or low lead was associated with significantly lower odds for CKD (AOR 0.10, 95% CI 0.03–0.33 for high zinc and high lead; AOR 0.08, 95% CI 0.01–0.65 for high zinc and low lead) compared to low zinc and high lead. Similarly, compared to low zinc and high cadmium, high zinc with either high or low cadmium was associated with significantly lower odds for CKD (AOR 0.10, 95% CI 0.02–0.43 for high zinc and high cadmium; AOR 0.11, 95% CI 0.02–0.76 for high zinc and low cadmium). In addition, compared to low zinc and high mercury, high zinc with either high or low mercury was associated with significantly lower odds for CKD (AOR 0.15, 95% CI 0.02–0.97 for high zinc and high mercury; AOR 0.09, 95% CI 0.02–0.58 for high zinc and low mercury) (Table 3).

DISCUSSION

This study investigated associations between CKD and heavy metal exposure measured in the blood or urine of a general community-based population. It also determined whether and how selenium and zinc might alter the associations, using the comprehensive data of 4 cycles of NHANES.

Findings showed that elevated plasma selenium and serum zinc interact with low-toxicity heavy metals to reduce odds of CKD, suggesting that increasing selenium or zinc levels and avoiding heavy metal exposures may help protect against developing CKD. Subjects with higher blood cadmium levels had significantly increased likelihood of CKD, whereas those with higher level of plasma selenium or serum zinc had significantly reduced CKD risk. Regarding combined effects, high serum zinc strongly reduced the likelihood of CKD among those who had high blood lead, high cadmium and high mercury levels, suggesting that zinc can modify the harm posed by heavy metals on the kidneys.

Table 1. Blood and urine parameters, demographic characteristics, lifestyle and comorbid conditions of the study population with or without CKD.

Variables	Overall ^a (n=9557)	CKD		P value
		No (n=8845)	Yes (n=712)	
Blood lead, mean ± SD, µg/dL	1.3 ± 0.04	1.2 ± 0.04	1.5 ± 0.1	<0.001
Q1 (≤0.6)	1712 (26.6)	1665 (27.6)	47 (11.4)	<0.001
Q2 (>0.6–1.0)	1861 (27.9)	1759 (28.3)	102 (21.5)	
Q3 (>1.0–1.6)	1803 (24.4)	1642 (23.8)	161 (31.9)	
Q4 (>1.6)	1728 (21.2)	1519 (20.2)	209 (35.2)	
Missing	2453	2260	193	
Blood cadmium, mean ± SD, µg/L	0.5 ± 0.01	0.5 ± 0.01	0.5 ± 0.04	0.323
Q1 (≤0.2)	2107 (34.6)	2040 (36.0)	67 (13.9)	<0.001
Q2 (>0.2–0.3)	1397 (20.5)	1301 (20.6)	96 (19.6)	
Q3 (>0.3–0.6)	1969 (24.8)	1761 (23.6)	208 (41.9)	
Q4 (>0.6)	1631 (20.1)	1483 (19.7)	148 (24.6)	
Missing	2453	2260	193	
Blood mercury, mean ± SD, µg/L	1.3 ± 0.06	1.4 ± 0.1	1.2 ± 0.1	0.021
Q1 (≤0.4)	1820 (27.0)	1694 (27.2)	126 (23.8)	0.219
Q2 (>0.4–0.8)	1875 (26.1)	1734 (26.2)	141 (25.1)	
Q3 (>0.8–1.6)	1663 (24.2)	1504 (23.9)	129 (28.8)	
Q4 (>1.6)	1776 (22.7)	1653 (22.8)	123 (22.3)	
Missing	2453	2260	193	
Urinary arsenic, mean ± SD, µg/g creatinine	16.6 ± 1.5	16.6 ± 1.6	17.7 ± 3.3	0.757
Q1 (≤3.7)	842 (29.3)	791 (29.6)	51 (24.5)	0.596
Q2 (>3.7–6.5)	790 (24.6)	736 (24.6)	54 (24.8)	
Q3 (>6.5–14.3)	820 (24.3)	769 (24.3)	51 (25.1)	
Q4 (>14.3)	826 (21.8)	759 (21.5)	67 (25.6)	
Missing	6279	5790	489	
Plasma selenium, µg/L	195.3 ± 0.9	195.7 ± 0.9	189.6 ± 2.0	0.002
Q1 (≤176.8)	1723 (22.1)	1551 (21.4)	172 (32.5)	<0.001
Q2 (>176.8–191.4)	1826 (25.5)	1714 (25.7)	112 (22.6)	
Q3 (>191.4–207.1)	1756 (25.7)	1642 (26.0)	114 (20.4)	
Q4 (>207.1)	1799 (26.7)	1678 (26.9)	121 (24.5)	
Missing	2453	2260	193	
Serum folate, ng/mL	19.3 ± 0.3	18.8 ± 0.3	26.4 ± 1.1	<0.001
Q1 (≤11.4)	2284 (25.2)	2144 (25.5)	140 (19.8)	<0.001
Q2 (>11.4–16.3)	2170 (23.7)	2058 (24.2)	112 (17.3)	
Q3 (>16.3–23.9)	2096 (25.1)	1967 (25.4)	129 (20.5)	
Q4 (>23.9)	2092 (26.0)	1844 (24.9)	248 (42.4)	
Missing	915	832	83	

Table 1. Blood and urine parameters, demographic characteristics, lifestyle and comorbid conditions of the study population with or without CKD. (Cont'd)

Variables	Overall ^a (n=9557)	CKD		P value
		No (n=8845)	Yes (n=712)	
RBC folate, ng/mL	409.0 ± 9.8	401.1 ± 9.7	530.0 ± 25.1	<0.001
Q1 (≤41.1)	2231 (24.9)	2101 (25.2)	130 (20.2)	<0.001
Q2 (>41.1–396.9)	2101 (22.3)	1991 (22.6)	110 (17.2)	
Q3 (>396.9–556.3)	2140 (25.0)	2004 (25.3)	136 (21.0)	
Q4 (>556.3)	2125 (27.8)	1879 (26.9)	246 (41.6)	
Missing	960	870	90	
Serum zinc, µg/dL	88.5 ± 0.5	88.7 ± 0.5	84.5 ± 1.8	0.013
Q1 (≤71.0)	229 (8.8)	195 (8.2)	34 (20.2)	0.003
Q2 (>71.0–80.4)	484 (19.0)	445 (18.9)	39 (20.8)	
Q3 (>80.4–90.3)	725 (32.0)	684 (32.3)	41 (26.6)	
Q4 (>90.3)	903 (40.2)	863 (40.6)	40 (32.4)	
Missing	7216	6658	558	
Age, years	46.7 ± 0.3	45.2 ± 0.3	68.5 ± 0.5	<0.001
18–29	1895 (21.0)	1890 (22.4)	5 (0.7)	
30–39	1516 (17.4)	1497 (18.3)	19 (3.8)	
40–59	3078 (35.1)	2995 (36.7)	83 (13.1)	
60–79	2533 (22.6)	2157 (20.3)	376 (55.4)	
80+	535 (3.9)	306 (2.3)	229 (27.0)	
Sex				
Male	4627 (48.5)	4316 (49.4)	311 (36.0)	<0.001
Female	4930 (51.5)	4529 (50.6)	401 (64.0)	
Race				
Non-Hispanic whites	3578 (64.4)	3170 (63.4)	408 (78.9)	<0.001
Non-Hispanic blacks	2007 (11.3)	1887 (11.6)	120 (8.1)	
Other Hispanic	1350 (8.9)	1297 (9.3)	53 (3.3)	
Others + Mexican American	2622 (15.3)	2491 (15.7)	131 (9.7)	
Poverty income ratio				
Not poor ≥1	6649 (84.1)	6118 (83.8)	531 (87.9)	0.029
Poor <1	2004 (15.9)	1886 (16.2)	118 (12.1)	
Missing	904	841	63	
Education level				
≤ High school	4021 (37.5)	3656 (37.1)	365 (43.5)	0.029
College or above	5086 (62.5)	4741 (62.9)	345 (56.5)	
Missing	450	448	2	
Vigorous physical activity	1909 (22.6)	1834 (23.4)	75 (11.9)	<0.001

Table 1. Blood and urine parameters, demographic characteristics, lifestyle and comorbid conditions of the study population with or without CKD. (Cont'd)

Variables	Overall ^a (n=9557)	CKD		P value
		No (n=8845)	Yes (n=712)	
BMI, category				
Underweight	284 (2.5)	262 (2.5)	22 (2.5)	0.019
Normal	2670 (27.8)	2518 (28.2)	152 (21.5)	
Overweight	3020 (32.0)	2782 (32.0)	238 (33.2)	
Obese	3583 (37.7)	3283 (37.3)	300 (42.7)	
Smoking status				
Never	5491 (57.0)	5137 (57.3)	354 (53.1)	0.001
Former	2196 (24.7)	1921 (23.8)	275 (37.2)	
Current smoker	1750 (18.3)	1667 (18.9)	83 (9.6)	
Missing	120	120	0	
Excessive alcohol consumption	426 (5.5)	396 (5.5)	30 (5.6)	0.952
Hypertension	3678 (34.7)	3130 (32.0)	548 (73.6)	<0.001
DM	1620 (12.5)	1355 (11.1)	265 (32.1)	<0.001
Hyperlipidaemia	5042 (52.0)	4515 (50.4)	527 (75.2)	<0.001
CVD history	1006 (9.0)	738 (7.2)	268 (34.8)	<0.001
COPD	659 (6.9)	566 (6.5)	93 (13.2)	<0.001

BMI: body mass index; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease; CVD: cardiovascular disease; DM: diabetes mellitus; Q: quartile

^a The cohort size (n=9557) was equivalent to 236,263,413 weighted population in the US after applying the sample weights provided by NHANES.

Values in bold are statistically significant.

Unless indicated otherwise, all values in brackets are expressed in no. (%) of participants.

Industrial development, which intends to meet the demands of a growing and modern population, has contributed to extremely dangerous chemicals in the environment that broadly affect human health—including heavy metals. Several previous studies investigated whether metal exposure is associated with adverse effects on kidney injury biomarkers, kidney function or changes in blood pressure in children or adolescents.^{23,24} Several studies have found significant associations between heavy metals and reductions in GFR, and provided meaningful underlying data on renal function and heavy metals.^{11,12,25} Another study has pointed out that excessive cadmium excretion may indicate renal tubular damage, showing that heavy metals and their combinations may affect renal parameters.¹² The effects of low-level exposure to multiple metals on renal function in early life may have profound implications on developing hypertension, renal disease and renal insufficiency later in life.¹¹ Oxidative stress plays a

crucial role in many diseases, including CKD. In vitro studies have identified arsenic- or cadmium-induced oxidative stress in rat kidney or renal tubular epithelial cells.²³ Mercury, arsenic, lead, cadmium and chromium are the most common heavy metals that can cause chronic or acute poisoning when inhaled or ingested via air, water and food. Different body tissues have different toxic effects on the bioaccumulation of heavy metals.²⁶ Heavy metals can damage cellular processes, including proliferation, growth, apoptosis, damage repair and differentiation. The pathways through which these metals induce toxicity are similar to damage manifestation, including reactive oxygen species production, weakened antioxidant defences, enzymatic inactivation and oxidative stress.

In this study, univariate analysis indicated that higher quartiles of blood lead and cadmium were associated with greater odds of CKD compared with the lowest quartile. However, as plasma selenium and serum zinc

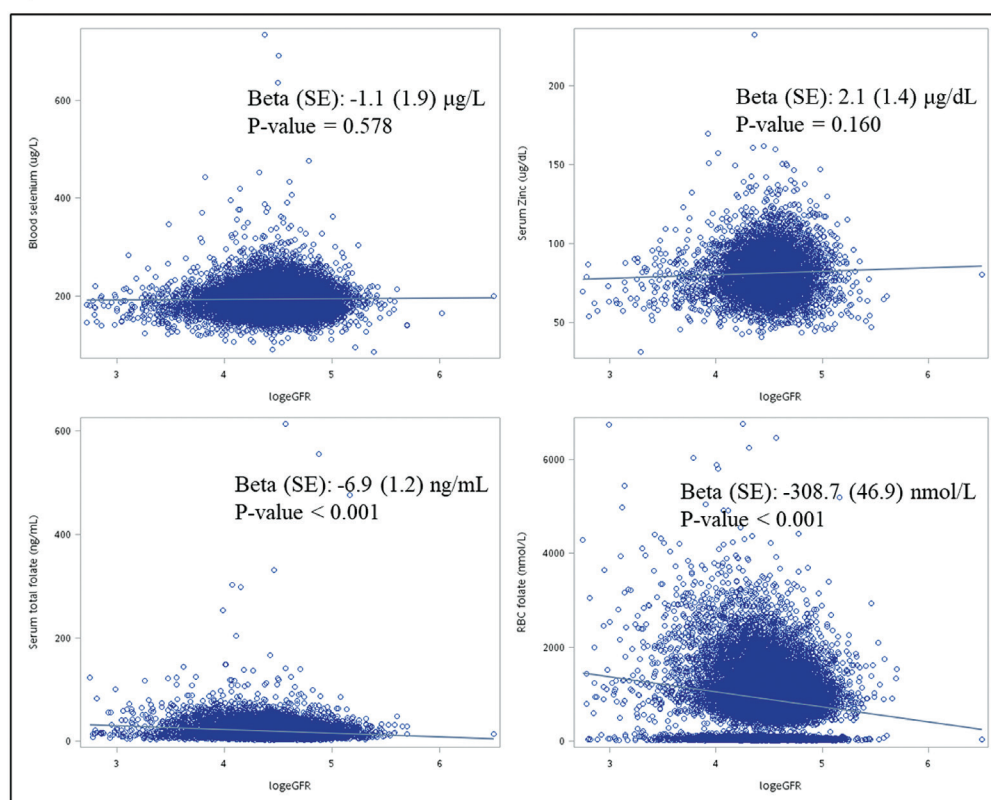


Fig. 2. Associations between $\log_{10}$ GFR, plasma selenium, zinc, serum folate and RBC folate.

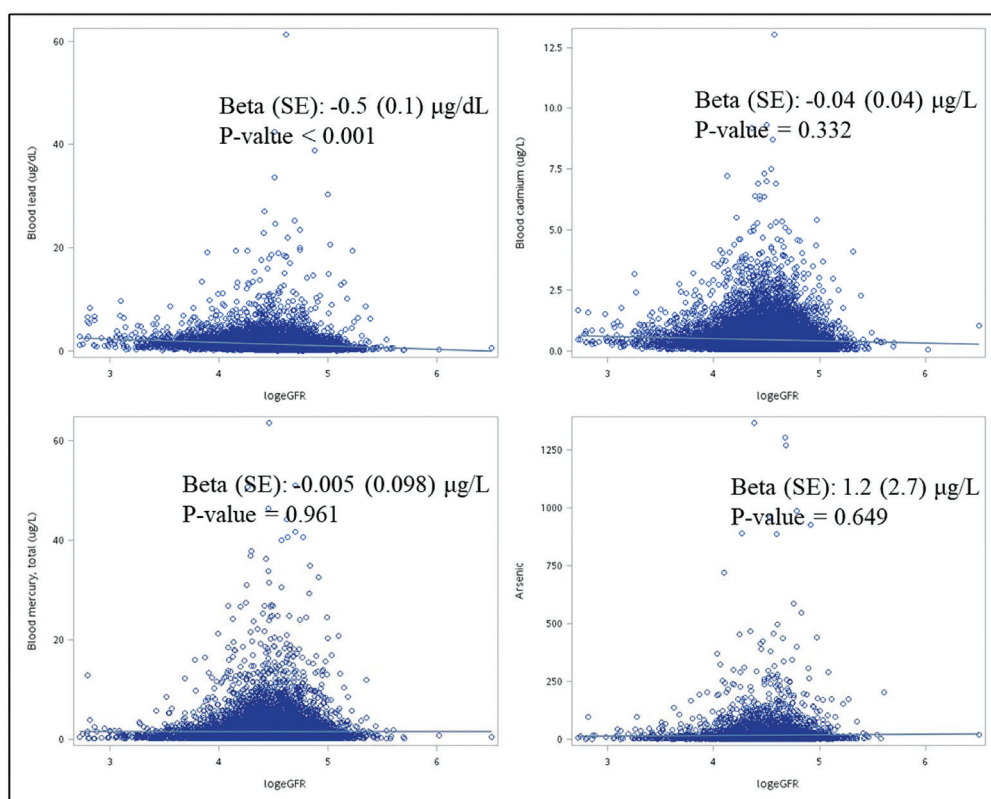


Fig. 3. Associations between $\log_{10}$ GFR, blood lead, cadmium, mercury and urine arsenic.

Table 2. Associations between study variables and prevalent CKD.

Variables	CKD		
	Number	Univariate (OR, 95% CI)	Multivariable (AOR, 95% CI)
Blood lead, µg/dL	7104		
Q1 (≤0.6)		ref	ref
Q2 (>0.6–1.0)		1.84 (1.22–2.76)	0.73 (0.43–1.24)
Q3 (>1.0–1.6)		3.24 (2.14–4.89)	1.01 (0.57–1.78)
Q4 (>1.6)		4.20 (2.76–6.40)	1.01 (0.58–1.77)
Blood cadmium, µg/L	7104		
Q1 (≤0.2)		ref	ref
Q2 (>0.2–0.3)		2.47 (1.70–3.59)	1.49 (0.94–2.35)
Q3 (>0.3–0.6)		4.60 (3.34–6.32)	1.93 (1.25–3.00)
Q4 (>0.6)		3.23 (1.98–5.27)	2.79 (1.58–4.92)
Blood mercury, µg/L	7104		
Q1 (≤0.4)		ref	ref
Q2 (>0.4–0.8)		1.09 (0.81–1.48)	0.84 (0.58–1.22)
Q3 (>0.8–1.6)		1.38 (1.00–1.90)	1.11 (0.75–1.63)
Q4 (>1.6)		1.12 (0.79–1.59)	0.77 (0.49–1.20)
Urine arsenic, µg/g creatinine	3278		
Q1 (≤3.7)		ref	ref
Q2 (>3.7–6.5)		1.22 (0.73–2.03)	1.13 (0.64–2.01)
Q3 (>6.5–14.3)		1.24 (0.71–2.17)	0.93 (0.47–1.87)
Q4 (>14.3)		1.43 (0.80–2.55)	0.74 (0.38–1.46)
Plasma selenium, µg/L	7104		
Q1 (≤176.8)		ref	ref
Q2 (>176.8–191.4)		0.58 (0.40–0.84)	0.57 (0.36–0.90)
Q3 (>191.4–207.1)		0.51 (0.35–0.75)	0.58 (0.36–0.93)
Q4 (>207.1)		0.60 (0.43–0.83)	0.61 (0.40–0.92)
Serum folate, ng/mL	8642		
Q1 (≤11.4)		ref	ref
Q2 (>11.4–16.3)		0.92 (0.63–1.34)	0.93 (0.60–1.44)
Q3 (>16.3–23.9)		1.04 (0.72–1.51)	0.81 (0.50–1.30)
Q4 (>23.9)		2.20 (1.62–2.98)	0.94 (0.64–1.38)
RBC folate, nmol/L	8597		
Q1 (≤41.1)		ref	ref
Q2 (>41.1–396.9)		0.95 (0.69–1.32)	1.11 (0.73–1.70)
Q3 (>396.9–556.3)		1.04 (0.75–1.44)	1.11 (0.70–1.74)
Q4 (>556.3)		1.94 (1.49–2.52)	1.27 (0.86–1.87)
Serum zinc, µg/dL	2341		
Q1 (≤71.0)		ref	ref
Q2 (>71.0–80.4)		0.44 (0.23–0.86)	0.39 (0.18–0.87)
Q3 (>80.4–90.3)		0.33 (0.19–0.60)	0.32 (0.15–0.71)
Q4 (>90.3)		0.32 (0.16–0.66)	0.30 (0.13–0.69)

Multivariable models were adjusted for age (continuous), gender, race, poverty-to-income ratio, education level, vigorous physical activity, body mass index (BMI), smoking status, hypertension, diabetes mellitus (DM), hyperlipidaemia, cardiovascular disease (CVD) and chronic obstructive pulmonary disease (COPD).

Table 3. Combined effects of selenium or zinc with blood lead, cadmium, mercury, urine arsenic on CKD.

Variables	CKD		
	Number	Univariate (OR, 95% CI)	Multivariable (AOR, 95% CI)
Selenium x Lead	1774		
Low Selenium x high Lead		ref	ref
Low Selenium x low Lead		0.13 (0.06–0.29)	0.73 (0.24–2.24)
High Selenium x high Lead		0.73 (0.44–1.21)	0.96 (0.44–2.11)
High Selenium x low Lead		0.14 (0.06–0.35)	0.48 (0.15–1.53)
Selenium x Cadmium	1864		
Low Selenium x high Cadmium		ref	ref
Low Selenium x low Cadmium		0.31 (0.14–0.68)	0.62 (0.27–1.41)
High Selenium x high Cadmium		0.14 (0.05–0.40)	0.92 (0.50–1.69)
High Selenium x low Cadmium		0.87 (0.51–1.49)	0.23 (0.07–0.72)
Selenium x Mercury	1790		
Low Selenium x high Mercury		ref	ref
Low Selenium x low Mercury		1.03 (0.51–2.08)	1.04 (0.46–2.35)
High Selenium x high Mercury		0.80 (0.40–1.60)	0.69 (0.23–2.69)
High Selenium x low Mercury		0.68 (0.32–1.45)	1.02 (0.39–2.69)
Selenium x Arsenic	835		
Low Selenium x high Arsenic		ref	ref
Low Selenium x low Arsenic		0.87 (0.31–2.42)	1.22 (0.41–3.58)
High Selenium x high Arsenic		0.59 (0.21–1.62)	0.32 (0.06–1.66)
High Selenium x low Arsenic		0.20 (0.07–0.56)	0.38 (0.09–1.66)
Zinc x Lead	517		
Low Zinc x high Lead		ref	ref
Low Zinc x low Lead		0.11 (0.02–0.72)	0.50 (0.05–5.02)
High Zinc x high Lead		0.18 (0.06–0.54)	0.10 (0.03–0.33)
High Zinc x low Lead		0.04 (0.01–0.31)	0.08 (0.01–0.65)
Zinc x Cadmium	617		
Low Zinc x high Cadmium		ref	ref
Low Zinc x low Cadmium		0.26 (0.04–1.63)	0.75 (0.08–7.15)
High Zinc x high Cadmium		0.19 (0.04–0.80)	0.10 (0.02–0.43)
High Zinc x low Cadmium		0.08 (0.01–0.45)	0.11 (0.02–0.76)
Zinc x Mercury	560		
Low Zinc x high Mercury		ref	ref
Low Zinc x low Mercury		0.33 (0.10–1.13)	0.36 (0.05–2.37)
High Zinc x high Mercury		0.15 (0.04–0.51)	0.15 (0.02–0.97)
High Zinc x low Mercury		0.16 (0.05–0.59)	0.09 (0.02–0.58)
Zinc x Arsenic	535		
Low Zinc x high Arsenic		ref	ref
Low Zinc x low Arsenic		0.35 (0.10–1.29)	0.21 (0.02–2.76)
High Zinc x high Arsenic		0.10 (0.03–0.35)	0.01 (<0.001–0.13)
High Zinc x low Arsenic		0.13 (0.04–0.43)	0.07 (0.01–0.65)

Multivariable models were adjusted for age (continuous), gender, race, poverty-to-income ratio, education level, vigorous physical activity, body mass index (BMI), smoking status, hypertension, diabetes mellitus (DM), hyperlipidaemia, cardiovascular disease (CVD) and chronic obstructive pulmonary disease (COPD).

increased, the odds of CKD decreased. These results were upheld in multivariate analysis after adjusting for relevant confounders. Statistically significant associations were found between higher quartiles of plasma selenium and serum zinc and lower odds of CKD, similar to results found in previous studies. One previous study indicated that adequate selenium consumption may have a positive effect on the treatment and prevention of CKD.²⁷ Another study reported an association between blood cadmium and blood lead levels and adverse renal outcomes.²⁸ Decreased glomerular filtration results in increasing the accumulation of cadmium and lead in the blood, and decreased urinary excretion of cadmium and lead. Patients with lower eGFR may retain these heavy metals, exacerbating their toxic effects on the other organs and kidneys. Another study investigating CKD using a large-scale health programme in Taiwan found that drinking water containing high arsenic concentrations is a risk factor for the rapid progression of CKD.²⁹

Selenium is an antioxidant nutrient that plays a vital role in biological defence against oxidative damage.²⁴ A previous study suggested that selenium can alter eGFR in environmental toxicant-induced CKD, and highlighted the need for nutrition and environmental modifications to improve kidney health.³⁰ Another study revealed that selenium functions by forming selenoproteins that contribute to antioxidant defence, immune response and thyroid hormone production in various biological processes.³¹ Selenium plays a crucial role in reducing the toxicity of arsenic, lead, mercury and cadmium.

Zinc is an essential metal in many physiological functions, including immune maturation, reproduction and cell growth.¹⁷ It is also an essential component of copper and zinc superoxide dismutase, acts as a protector for thiols and other chemical groups, and provides protection against free radical damage by maintaining sufficient metallothionein levels.³² In many important enzymatic reactions, zinc acts as a vital cofactor, such as for liver alcohol dehydrogenase, carbonic anhydrase and carboxypeptidase. A previous study suggested that low dietary zinc consumption may increase the risk of CKD in individuals with normal renal function.³³ Zinc has similar physical and chemical properties to cadmium and can compete for binding sites in metal-absorbing proteins and enzyme proteins.

Limitations

Our study was cross-sectional and retrospective, and inferences of causality could not be made confidently regarding the observed associations. Reverse causality

might have operated, e.g. patients with milder CKD had higher intakes of selenium and zinc, or their proximal convoluted tubules retained more selenium and zinc, or both, than the patients with severe CKD. Results also might not be generalisable to other populations aside from those within NHANES. Measurements were collected at only one time point, so the cross-sectional nature of the study did not allow for a longitudinal assessment of GFR over at least 3 months. Moreover, the single-point measurements of nephrotoxic metals do not fully reflect each participant's real cumulative exposure levels. In NHANES, urine and blood sample collection times were not standardised, which does not support the complete accuracy of metal and micronutrient levels that may fluctuate over time.³⁴ Despite controlling for covariates, residual and unmeasured confounders and errors in measurement might still have biased the results.

CONCLUSION

The likelihood of CKD is increased in individuals with elevated levels of toxic heavy metals in the blood but is decreased with elevated levels of plasma selenium and serum zinc. High serum zinc appears to interact with low-toxicity heavy metals to reduce the risk of developing CKD. The results suggest that increased blood concentrations of selenium or zinc, or both, along with avoidance of heavy metal exposures, could protect against CKD.

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