# The effects of vitamin D on albuminuria relative to blood lead in patients with type 2 diabetes

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# Abstract

**Background:** Environmental lead exposure has been linked with reduced kidney function. However, evidence about its role in diabetic kidney damage, especially when considering the nutritional status of vitamin D, is sparse.

**Methods:** This observational study comprised 4,033 diabetic patients from seven communities in Shanghai, China. The associations of blood lead with urinary albumin-to-creatinine ratio (UACR) and albuminuria, defined as UACR ≥30 mg/g, according to serum 25-hydroxyvitamin D [25(OH)D] levels were analyzed using linear and Poisson regression models, respectively.

**Results:** A doubling of blood lead level was associated with a 10.7% higher UACR (95% CI, 6.19% to 15.5%) in diabetic patients with 25(OH)D <50 nmol/L, whereas the estimate declined to 2.03% (95% CI, −5.18% to 9.78%) in those with 25(OH)D ≥50 nmol/L. The difference in the association for albuminuria prevalence was also observed between the two groups, with risk ratios of 1.09 (95% CI, 1.03–1.15) and 0.99 (95% CI, 0.86–1.14) per doubling of blood lead level, respectively. Furthermore, the increment of UACR in relation to blood lead appeared to be two times higher in patients with 25(OH)D <50 nmol/L and estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 $m^2$ than those with eGFR ≥60 mL/min/1.73 $m^2$. While in patients with 25(OH)D ≥50 nmol/L, there was no association between blood lead and UACR regardless of eGFR category.

**Conclusions:** Higher blood lead levels were associated with increased urinary albumin excretion in diabetic patients with vitamin D deficiency, which became more pronounced in the presence of reduced eGFR. Further prospective studies are needed to validate our findings and to determine whether vitamin D supplementation yields a benefit.


# 1. Background

Type 2 diabetes and its complications pose a major threat to public health. It is estimated that 463 million adults had diabetes worldwide in 2019, with a projection for a 51% increase to700 million by 2045 [1]. The epidemic of diabetes has led to a substantial burden of diabetic kidney disease (DKD), which is now the leading cause of end-stage renal disease and closely linked to excess mortality and cardiovascular events in patients with diabetes [2]. Globally in 2017, DKD accounted for almost a third of 35.8 million disability-adjusted life-years resulted from chronic kidney disease [3]. In the setting of the rising prevalence of diabetes, efforts to prevent and/or slow progression of DKD by identifying novel risk factors that could be modifiable are imperative.

Lead is a ubiquitous toxicant with well-established adverse health effects [4]. Despite the overall decline in lead pollution in the past few decades, lead exposure still occurs through ingestion of contaminated food and drinking water, as well as inhalation of polluted air in areas with heavy traffic or industrial emissions [5]. Lead nephrotoxicity at high levels of exposure has long been recognized; however, mounting evidence indicates that environmental lead exposure, even at blood lead levels below 5 μg/dL,

can already contribute to impaired kidney function [6]. Importantly, patients with diabetes have been identified to be a susceptible population who are at greater risk for nephrotoxic effects from lead [7]. Two longitudinal studies previously reported a significant association between low-level lead exposure and progressive loss of kidney function among diabetic patients with late stages of nephropathy [8, 9]. Nevertheless, these studies were limited by small sample size ($n < 100$), and the influence of lead on biomarkers for early DKD continues to be elucidated. Although the exact mechanisms whereby lead adversely affect kidney remain uncertain, oxidative stress and inflammatory responses are believed to be two convincing actions underlying lead nephrotoxicity [10].

Development of readily available and effective treatments may help reduce kidney impairment due to lead exposure, especially for high-risk populations including diabetic patients. Vitamin D is a pleiotropic steroid hormone characterized by anti-fibrotic, anti-oxidative, and anti-inflammatory properties, in addition to its classical effects on bone metabolism [11]. A recent *in vivo* study in rats revealed benefits of vitamin D in alleviating lead-induced renal injury via anti-inflammatory and anti-oxidative pathways [12]. Furthermore, vitamin D has a negative effect on the renin-angiotensin system, which has been found to be activated after exposure to lead [10, 13]. Thus, it can be reasonably hypothesized that vitamin D may protect against kidney damage from lead exposure.

China is the epicenter of the world's diabetes epidemic. Meanwhile, with rapid industrialization and urbanization, China has been experiencing serious environmental problems such as air pollution (e.g. haze events). Accordingly, higher lead exposure is more common in Chinese population, as reflected by several times higher blood lead levels than in US population [14, 15]. The present study aimed to examine whether blood lead is associated with albuminuria, an early clinical indicator of the presence of DKD [16], and to assess the potential modification effects of vitamin D in Chinese patients with type 2 diabetes.

# 2. Methods

## 2.1 Study population

The METAL (Environmental Pollutant Exposure and Metabolic Diseases) study is a population-based study designed to investigate risk factors for vascular complications in individuals with type 2 diabetes [17, 18]. From May to August 2018, Chinese patients with diabetes were enrolled from seven communities in Huangpu and Pudong District, Shanghai. Eligible patients had been identified from the registration system of each community healthcare center according to the diagnosis criteria as fasting plasma glucose ≥ 7.0 mmol/L, glycated hemoglobin ($HbA_{1c}$) ≥ 6.5%, or a previous diagnosis by healthcare professionals. Those who reported taking insulin before age 40 years were excluded. Among the remaining patients, one-half were randomly selected for participation. In total, 4,937 patients with type 2 diabetes were included and received an examination. All the participants had lived in the local area for at least 6 months and reported no occupational exposure to lead. After excluding individuals with missing questionnaire data or samples (n = 124) and those with missing laboratory data (n = 780), 4,033 participants were finally analyzed (**Additional file 1: Figure S1**).The study protocol was approved by the

Ethics Committee of Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine. All study participants provided written informed consent.

### 2.2 Data collection

A standard questionnaire was administered by trained study personnel to collect information on sociodemographic characteristics, lifestyle factors, personal and family medical history, and medications. Diabetes duration was defined as the time from the date of diabetes onset to the date of enrollment. Current smoking was defined as having smoked at least 100 cigarettes in lifetime and currently smoking cigarettes [19]. Anthropometric parameters including body weight, height, and blood pressure were measured according to a standard protocol. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

Venous blood samples were drawn after an overnight fast of at least 8 hours. Upon collection, centrifugation was completed within 1 hour and frozen on site. Morning spot urine samples were obtained under normative retention guidelines. All samples were placed on dry ice and shipped to the central laboratory, which had been certified by the College of American Pathologists Laboratory Accreditation Program. $HbA_{1c}$ was assessed by high-performance liquid chromatography (MQ-2000PT, Medconn, Shanghai, China). Plasma glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, and serum creatinine were performed using a Beckman Coulter AU680 (Brea, Germany). Urinary albumin and creatinine were measured using a turbidimetric immunoassay and an enzymatic method, respectively (Beckman Coulter AU680, Brea, USA). Serum 25-hydroxyvitamin D [25(OH)D] was detected using a chemiluminescence assay (SIEMENS ADVIA Centaur XP, China). In accordance with the guidelines from the Endocrine Society, 25(OH)D levels < 50 nmol/L were deemed to be deficient [20].

Blood lead level was determined by atomic absorption spectrometry (BH2200, China), as described previously [21]. The standard curves were established with good linearity ($r > 0.995$). Outliers were detected by duplicate runs. Quality control samples were tested in all analytical rounds and showed satisfactory results. The limit of detection for blood lead was 0.1 μg/L. None of the samples exhibited values below the detection limit. The inter-assay coefficient of variation for blood lead was less than 10% [21].

### 2.3 Variable definition

The estimated glomerular filtration rate (eGFR) was calculated from age, sex, race/ethnicity, and serum creatinine using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [22]. Subjects with preserved kidney function were considered as having an eGFR ≥ 60 mL/min/1.73 $m^2$. The urinary albumin excretion rate was recorded as the urinary albumin-to-creatinine ratio (UACR). Kidney damage was indicated by an increase of UACR. Albuminuria was defined as UACR ≥ 30 mg/g following the American Diabetes Association statement [23]. Microalbuminuria and macroalbuminuria were defined as UACR of 30–299 and ≥ 300 mg/g, respectively.

### 2.4 Statistical analysis

Data on characteristics of study participants were summarized as frequencies for categorical variables and mean ± standard deviation (SD) or median (interquartile range, [IQR]) for continuous variables. Linear regression model was used to determine the association between blood lead and UACR in the total sample as well as by 25(OH)D status. In the linear analysis, UACR was log transformed because of skewed distribution, and the percent changes in mean UACR associated with tertiles of blood lead level were calculated by exponentiating beta coefficient and subtracting by 1. We also fitted blood lead as a continuous variable after log transformation and estimated the effect for a doubling of lead level. Model 1 was the unadjusted model. Adjustment included known risk factors for kidney disease and variables that affected the coefficient of the linear regression of blood lead level with UACR by > 10% as follows: age, sex, BMI, smoking (model 2), LDL cholesterol, HDL cholesterol, triglycerides, $HbA_{1c}$, duration of diabetes, systolic blood pressure, use of antihypertensive medications, and eGFR (model 3). To evaluate the dose-response relationship of blood lead level with UACR by 25(OH)D status, we created a restricted cubic spline with three knots of log-transformed lead level using the fully adjusted analyses.

Poisson regression model with a robust error variance was used to calculate risk ratios (RRs) and 95% confidence intervals (CIs) for albuminuria in relation to blood lead level. There is no longer any good justification for fitting logistic regression when outcomes are not rare, whereas Poisson regression can yield unbiased estimates of relative risk in this setting [24]. For these analyses, the same adjustment was applied. A parallel model with microalbuminuria as the outcome was conducted.

We examined possible effect modification for age, sex, use of antihypertensive medications, diabetes duration, and BMI in the final model. We also performed sensitivity analyses by excluding participants with pre-existing retinopathy and by excluding participants with blood lead levels ≥ 100 μg/L to minimize the potential impact of extreme outliers of lead measurements. All statistical analyses were performed using SAS (version 9.4; SAS Institute, Cary, NC) and R software (version 3.5.3; R Foundation for Statistical Computing). A two-sided $P$ value < 0.05 was considered statistically significant.

## 3. Results

### 3.1 Population characteristics

Of the 4,033 patients with type 2 diabetes, 1,862 (46.2%) were male, and the mean age was 67.1 (SD, 8.7) years. The median blood lead level was 26 (IQR, 18–36) μg/L. The median blood 25(OH)D level was 41 (IQR, 32–50) nmol/L. Characteristics of participants by tertiles of blood lead level are shown in Table 1. Overall, participants in the higher lead tertiles were older, had higher proportions of men and current smokers, were more likely to have a history of hypertension, and had longer duration of diabetes and poorer metabolic profiles including higher blood pressure, triglycerides, and UACR and lower eGFR. The levels of 25(OH)D did not differ significantly across lead tertiles.

Table 1
Characteristics of study participants by tertiles of blood lead level.

| | Tertile of blood lead (μg/L) | | | | |
|---|---|---|---|---|---|
| Characteristics | Total | T1 (≤ 20) | T2 (20–32) | T3 (≥ 32) | *P* |
| Number of participants | 4,033 | 1,374 | 1,285 | 1,374 | |
| Age, years | 67.1 ± 8.7 | 66.4 ± 8.5 | 66.5 ± 8.7 | 68.3 ± 8.7 | < 0.001 |
| Female, *n* (%) | 2,171 (53.8) | 773 (56.3) | 713 (55.5) | 685 (49.9) | 0.001 |
| Current smoking, *n* (%) | 727 (18.0) | 181 (13.2) | 246 (19.1) | 300 (21.8) | < 0.001 |
| History of hypertension, *n* (%) | 2,628 (65.2) | 874 (63.6) | 797 (62.0) | 957 (69.7) | < 0.001 |
| Use of medications | | | | | |
| Antidiabetic drugs, *n* (%) | 2,832 (70.2) | 967 (70.4) | 901 (70.1) | 964 (70.2) | 0.98 |
| Lipid lowering drugs, *n* (%) | 1,523 (37.8) | 507 (36.9) | 485 (37.7) | 531 (38.6) | 0.64 |
| Antihypertensive drugs, *n* (%) | 1,746 (43.3) | 590 (42.9) | 526 (40.9) | 630 (45.9) | 0.04 |
| ACEI or ARB drugs, *n* (%) | 882 (21.9) | 307 (22.3) | 255 (19.8) | 320 (23.3) | 0.09 |
| Duration of diabetes, years | 8 (3–15) | 8 (3–15) | 8 (3–15) | 10 (4–16) | < 0.001 |
| BMI, $kg/m^2$ | 25.0 ± 3.6 | 24.8 ± 3.6 | 25.0 ± 3.8 | 25.1 ± 3.5 | 0.21 |
| Systolic blood pressure, mmHg | 144.6 ± 19.7 | 143.1 ± 18.7 | 143.5 ± 19.7 | 147.1 ± 20.4 | < 0.001 |
| Diastolic blood pressure, mmHg | 79.3 ± 10.8 | 78.8 ± 10.4 | 79.0 ± 10.4 | 80.0 ± 11.5 | 0.008 |
| Total cholesterol, mmol/L | 5.10 ± 1.21 | 5.08 ± 1.16 | 5.15 ± 1.19 | 5.08 ± 1.26 | 0.23 |
| LDL cholesterol, mmol/L | 3.14 ± 0.85 | 3.12 ± 0.82 | 3.18 ± 0.84 | 3.13 ± 0.88 | 0.17 |
| HDL cholesterol, mmol/L | 1.21 ± 0.29 | 1.22 ± 0.29 | 1.21 ± 0.31 | 1.19 ± 0.29 | 0.003 |
| Triglyceride, mmol/L | 1.92 ± 1.64 | 1.82 ± 1.32 | 1.94 ± 1.63 | 2.01 ± 1.92 | 0.01 |
| $HbA_{1c}$, % | 7.48 ± 1.37 | 7.49 ± 1.43 | 7.44 ± 1.36 | 7.51 ± 1.31 | 0.40 |
| 25(OH)D, nmol/L | 40.6 (32.4–49.9) | 40.3 (32.2–49.5) | 40.9 (32.9–50.5) | 40.5 (32.2–49.8) | 0.36 |

| | Tertile of blood lead (μg/L) | | | | |
|---|---|---|---|---|---|
| eGFR, mL/min/1.73 $m^2$ | 90.9 (80.0–97.6) | 92.3 (83.5–98.6) | 91.4 (81.3–97.9) | 89.1 (75.3–96.0) | < 0.001 |
| UACR, mg/g | 13.0 (7.4–29.3) | 11.7 (7.0–23.8) | 12.5 (7.2–28.1) | 15.2 (8.0–36.8) | < 0.001 |
| ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; UACR, urinary albumin-to-creatinine ratio; 25(OH)D, 25-hydroxyvitamin D. | | | | | |
| Data are presented as mean ± standard deviation or median (interquartile range) or number (percentage). | | | | | |

Among the participants, 989 (24.5%) had albuminuria (microalbuminuria, 20.6%; macroalbuminuria, 3.9%). In patients with albuminuria, hypertension, a longer duration of diabetes, and glucose-lowering and antihypertensive medications were more prevalent (Table 2). Moreover, these patients were older and had higher BMI, blood pressure, triglycerides, $HbA_{1c}$, blood lead, and had lower 25(OH)D and eGFR.

Table 2
Characteristics of study participants by the presence of albuminuria.

| Characteristics | No albuminuria | Albuminuria | *P* |
|---|---|---|---|
| Number of participants | 3,044 | 989 | |
| Age, years | 66.7 ± 8.5 | 68.3 ± 9.0 | < 0.001 |
| Female, *n* (%) | 1,643 (54.0) | 528 (53.4) | 0.75 |
| Current smoking, *n* (%) | 520 (17.1) | 207 (20.9) | 0.02 |
| History of hypertension, *n* (%) | 1,859 (61.1) | 769 (77.8) | < 0.001 |
| Use of medications | | | |
| Antidiabetic drugs, *n* (%) | 2,080 (68.3) | 752 (76.0) | < 0.001 |
| Lipid lowering drugs, *n* (%) | 1,124 (36.9) | 399 (40.3) | 0.054 |
| Antihypertensive drugs, *n* (%) | 1,249 (41.0) | 497 (50.3) | < 0.001 |
| ACEI or ARB drugs, *n* (%) | 644 (21.2) | 238 (24.1) | 0.06 |
| Duration of diabetes, years | 8 (3–15) | 10 (5–18) | < 0.001 |
| BMI, $kg/m^2$ | 24.7 ± 3.6 | 25.8 ± 3.6 | < 0.001 |
| Systolic blood pressure, mmHg | 142.2 ± 19.0 | 151.9 ± 19.9 | < 0.001 |
| Diastolic blood pressure, mmHg | 78.8 ± 10.6 | 80.8 ± 11.2 | < 0.001 |
| Total cholesterol, mmol/L | 5.09 ± 1.17 | 5.12 ± 1.32 | 0.52 |
| LDL cholesterol, mmol/L | 3.14 ± 0.83 | 3.15 ± 0.90 | 0.96 |
| HDL cholesterol, mmol/L | 1.22 ± 0.30 | 1.16 ± 0.27 | < 0.001 |
| Triglycerides, mmol/L | 1.82 ± 1.41 | 2.25 ± 2.17 | < 0.001 |
| $HbA_{1c}$, % | 7.33 ± 1.26 | 7.95 ± 1.57 | < 0.001 |
| 25(OH)D, nmol/L | 41.2 (32.8–50.6) | 38.8 (31.3–48.0) | < 0.001 |
| eGFR, mL/min/1.73 $m^2$ | 91.4 (82.2–97.8) | 89.0 (72.0–97.1) | < 0.001 |
| Blood lead, μg/L | 25 (17–35) | 27 (19–39) | < 0.001 |

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; 25(OH)D, 25-hydroxyvitamin D.

Data are presented as mean ± standard deviation or median (interquartile range) or number (percentage).

### 3.2 Blood lead in relation to UACR and albuminuria and the effect of 25(OH)D

The median UACR in the total population was 13.0 (IQR, 7.4–29.3) mg/g. Blood lead levels were positively associated with UACR in linear regression analyses adjusting for age, sex, BMI, and smoking (Table 3). The association remained unchanged after additional adjustment for potential confounders including serum lipids, $HbA_{1c}$, duration of diabetes, systolic blood pressure, antihypertensive medication use, and eGFR. For each doubling of blood lead level, a significant increase by 8.73% in UACR (95% CI, 4.85–12.8%) was observed. Patients in the highest tertile of blood lead level had a 21.1% higher UACR (95% CI, 11.2–31.7%) than those in the lowest tertile. When stratified by 25(OH)D status, the association of blood lead and UACR became stronger among patients with 25(OH)D < 50 nmol/L (change per doubling of lead level,10.7%; 95% CI, 6.19–15.5%), but was remarkably attenuated and statistically nonsignificant among those with 25(OH)D ≥ 50 nmol/L (change, 2.03%; 95% CI, − 5.18–9.78%) (*P* for interaction = 0.06). Restricted cubic spline analyses showed a sharper increase in UACR with higher blood lead levels, with no departures from linearity and no clear threshold, among patients who had 25(OH)D < 50 nmol/L versus 25(OH)D ≥ 50 nmol/L (Fig. 1).

Table 3
Percent changes (95% CI) of UACR (mg/g) in relation to blood lead level in diabetic patients according to 25(OH)D status.

| | Model 1[a] | | Model 2[b] | | Model 3[c] | |
|---|---|---|---|---|---|---|
| Blood lead (μg/L) | % change (95% CI) | *P* | % change (95% CI) | *P* | % change (95% CI) | *P* |
| Total (*n* = 4,033) | | | | | | |
| Per doubling increment | 16.4 (11.9 to 21.1) | < 0.001 | 13.3 (8.95 to 17.9) | < 0.001 | 8.73 (4.85 to 12.8) | < 0.001 |
| Tertile | | | | | | |
| T1 | 0 (ref) | | 0 (ref) | | 0 (ref) | |
| T2 | 11.9 (1.91 to 22.8) | 0.02 | 9.38 (− 0.24 to 19.9) | 0.06 | 7.53 (− 1.20 to 17.0) | 0.09 |
| T3 | 42.1 (29.6 to 55.8) | < 0.001 | 34.8 (23.0 to 47.6) | < 0.001 | 21.1 (11.2 to 31.7) | < 0.001 |
| 25(OH)D < 50 nmol/L (*n* = 3,038) | | | | | | |
| Per doubling increment | 18.4 (13.2 to 23.9) | < 0.001 | 15.3 (10.2 to 20.7) | < 0.001 | 10.7 (6.19 to 15.5) | < 0.001 |
| Tertile | | | | | | |
| T1 | 0 (ref) | | 0 (ref) | | 0 (ref) | |
| T2 | 14.3 (2.44 to 27.5) | 0.02 | 11.1 (− 0.34 to 23.8) | 0.06 | 8.34 (− 1.91 to 19.7) | 0.11 |
| T3 | 49.0 (33.9 to 65.9) | < 0.001 | 40.9 (26.6 to 56.8) | < 0.001 | 26.1 (14.2 to 39.2) | < 0.001 |
| 25(OH)D ≥ 50 nmol/L (*n* = 995) | | | | | | |
| Per doubling increment | 9.11 (0.86 to 18.0) | 0.03 | 6.96 (− 0.97 to 15.5) | 0.09 | 2.03 (− 5.18 to 9.78) | 0.59 |
| Tertile | | | | | | |
| T1 | 0 (ref) | | 0 (ref) | | 0 (ref) | |
| T2 | 6.70 (− 10.5 to 27.1) | 0.47 | 6.56 (− 10.2 to 26.5) | 0.47 | 6.55 (− 9.32 to 25.2) | 0.44 |

| | Model 1[a] | | Model 2[b] | | Model 3[c] | |
|---|---|---|---|---|---|---|
| T3 | 23.4 (3.54 to 47.0) | 0.02 | 18.4 (− 0.31 to 40.5) | 0.054 | 7.43 (− 8.71 to 26.4) | 0.39 |
| CI, confidence interval; UACR, urinary albumin-to-creatinine ratio; 25(OH)D, 25-hydroxyvitamin D. | | | | | | |
| [a]Model 1: unadjusted. | | | | | | |
| [b]Model 2: adjusted for age, sex, BMI, and smoking. | | | | | | |
| [c]Model 3: model 2 plus LDL cholesterol, HDL cholesterol, triglycerides, $HbA_{1c}$, duration of diabetes, systolic blood pressure, use of antihypertensive medications, and eGFR. | | | | | | |

Assessment of albuminuria yielded consistent results (Fig. 2). In the total population, a doubling of blood lead level was associated with an adjusted RR of 1.07 (95% CI, 1.02–1.13). After stratification, however, the association between blood lead and albuminuria predominantly existed in patients with 25(OH)D < 50 nmol/L (RR per doubling of lead level, 1.09; 95% CI, 1.03–1.15). The associations for microalbuminuria were similar (**Additional file 1: Table S1**). When considered lead exposure and 25(OH)D status jointly, compared to patients with 25(OH)D < 50 nmol/L and lead level in the lowest tertile, the RR for albuminuria was 0.96 (95% CI, 0.76–1.20) for those with 25(OH)D ≥ 50 nmol/L and lead level in the highest tertile (**Additional file 1: Table S2**).

We further analyzed the association between blood lead and UACR according to eGFR category (Table 4). Compared to patients with 25(OH)D < 50 nmol/L and eGFR ≥ 60 mL/min/1.73 $m^2$, blood lead level was more strongly associated with increased UACR among those with eGFR < 60 mL/min/1.73 $m^2$. The increments of UACR per doubling of lead level were 9.11% (95% CI, 4.70–13.7%) and 23.6% (95% CI, 1.41–50.5%), respectively. No significant associations were observed among patients with 25(OH)D ≥ 50 nmol/L regardless of eGFR category.

Table 4
Percent changes (95% CI) of UACR (mg/g) per doubling increment in blood lead level in diabetic patients according to eGFR and 25(OH)D status.

| | *n* | % change (95% CI)[a] | *P* |
|---|---|---|---|
| eGFR ≥ 60 mL/min/1.73 $m^2$ | | | |
| Total | 3,761 | 7.66 (3.90 to 11.6) | < 0.001 |
| 25(OH)D < 50 nmol/L | 2,831 | 9.11 (4.70 to 13.7) | < 0.001 |
| 25(OH)D ≥ 50 nmol/L | 930 | 3.12 (− 3.87 to 10.6) | 0.39 |
| eGFR < 60 mL/min/1.73 $m^2$ | | | |
| Total | 272 | 16.0 (− 2.90 to 38.6) | 0.10 |
| 25(OH)D < 50 nmol/L | 207 | 23.6 (1.41 to 50.5) | 0.04 |
| 25(OH)D ≥ 50 nmol/L | 65 | −26.9 (− 54.2 to 16.6) | 0.18 |

CI, confidence interval; eGFR, estimated glomerular filtration rate; UACR, urinary albumin-to-creatinine ratio; 25(OH)D, 25-hydroxyvitamin D.

[a]Adjusted for age, sex, BMI, smoking, LDL cholesterol, HDL cholesterol, triglycerides, $HbA_{1c}$, duration of diabetes, systolic blood pressure, use of antihypertensive medications, and eGFR.

### 3.3 Subgroup analyses and sensitivity analyses

The results of UACR relative to blood lead did not substantially change in all population subgroups examined (**Additional file 1: Figure S2**). Within each subgroup, there were generally weaker associations among those with 25(OH)D ≥ 50 nmol/L than those with 25(OH)D < 50 nmol/L, except for the comparable estimates in men (**Additional file 1: Figure S3**). The between-group difference seemed to be more prominent among patients with longer diabetes duration, higher BMI, and antihypertensive medications. Sensitivity analyses after exclusion of individuals with pre-existing retinopathy or individuals with blood lead levels ≥ 100 μg/L showed essentially the same results (**Additional file 1: Table S3**).

## 4. Discussion

In this large sample of Chinese patients with type 2 diabetes, higher blood lead levels were linearly, independently associated with higher UACR and risk of albuminuria. When stratified by 25(OH)D status, the associations maintained only in patients with blood 25(OH)D < 50 nmol/L. Moreover, the increments of UACR in relation to blood lead were greater in the presence of reduced eGFR. These results indicate that environmental lead exposure, even at low levels, may be a risk factor for elevated urinary albumin excretion in diabetic patients who had vitamin D deficiency.

Exposure to lead has decreased substantially in the last couple of decades owing to public health measures phasing out of lead in gasoline and paint, but exposure continues to occur through contaminated food and drinking water, lead paint, smoking, and industrial and combustion emissions [4]. Rapid urbanization in China over the past 30 years has led to numerous environmental issues including lead pollution [25]. In this study, the blood lead level (median, 26 μg/L) was similar to that reported in developed countries a few decades ago [26–28], and was markedly higher than that reported by the 2013–2014 national survey of US population (8.4 μg/L) [15]. Although lead poisoning or high-dose lead exposure is a known cause of kidney injury, a growing body of evidence implicates kidney insufficiency with low-level environmental lead exposure [6]. In addition, suffering from established risk factors for chronic kidney disease such as hypertension and diabetes can increase susceptibility to lead-related nephrotoxicity [7].

Our results are consistent with the limited data available on low-level lead exposure and kidney function measures in diabetic patients. The Normative Aging Study found that increasing baseline blood lead levels from the midpoints of the lowest to the highest quartiles (30–112.5 μg/L) was associated with a 12.8-fold greater increase in the annual rate of rise of serum creatinine in diabetic patients than in nondiabetic patients, but this study was limited to men [7]. In a prospective study of Chinese diabetic patients with chronic kidney disease, each increase of 100 μg in normal body lead burden decreased eGFR by 7.2 mL/min/1.73 $m^2$, 24 times a reduction in nondiabetic patients [8, 29]. Relationship of lead exposure and progressive diabetic nephropathy was subsequently confirmed in a Chinese population with a longer follow-up [9]. However, both studies involved relatively small numbers of patients at late stages of DKD. The current study extends the existing knowledge by demonstrating an association between blood lead and albuminuria in a large sample of diabetic patients, and the effect of lead occurs when eGFR is still preserved, which suggests that exposure to lead may cause kidney damage at an early stage of DKD. Given the remarkable burden of DKD worldwide, our findings emphasize the importance of early intervention to protect kidney health against lead exposure in individuals with diabetes, particularly in areas where prevalence of diabetes is increasing more rapidly than lead exposure is declining.

A novel finding of this study is that the association between blood lead and albuminuria was predominantly present in diabetic patients with deficient blood 25(OH)D. Previous studies have indicated inverse associations of vitamin D levels with the development and progression of chronic kidney disease [30–32]. In this study, we provided first-hand epidemiological data that the influence of lead exposure on albuminuria was dependent of body vitamin D status. The adverse effect of lead on kidney damage indicator might be counteracted or alleviated by high vitamin D levels. Our results, if confirmed in follow-up studies and randomized clinical trials, would have important public health implications for vitamin D supplementation serving as a readily available, safe therapeutic option for diabetic patients who are subjected to lead nephrotoxicity.

Notably, the increment of UACR associated with blood lead was two times higher in diabetic patients with deficient 25(OH)D and reduced eGFR as compared to those with preserved eGFR, showing evidence for greater lead-related albuminuric damage when eGFR loss already exists. Nevertheless, no significant

associations between blood lead and UACR were observed in patients without deficient 25(OH)D regardless of eGFR category. These data demonstrate increased sensitivity to adverse lead effects on urinary albumin excretion in diabetic patients with declined kidney function as well as a special need for vitamin D improvement to prevent albuminuria for these people.

The possible mechanisms underlying lead nephrotoxicity and the protective effect of vitamin D have been proposed. In experimental studies, low-dose lead exposure could promote oxidative stress that cause increased reactive oxygen species and depletion of nitric oxide [33, 34]. Activation of the transcription nuclear factor-κB (NF-κB) and the intrarenal renin-angiotensin system, infiltration of macrophages, and tubulointerstitial inflammation may also be involved [10, 35]. On the contrary, vitamin D has been shown to reduce levels of reactive oxygen species, suppress renin-angiotensin system and decrease circulating angiotensin II levels, inhibit inflammatory responses, and prevent podocyte loss and glomerulosclerosis [13, 36, 37], thereby potentially protect kidney from damage induced by lead exposure. A recent study in rats revealed that vitamin D alleviated lead induced kidney injury (i.e. interstitial necrosis, glomerular and intertubular fibrosis) through anti-inflammatory and antioxidant pathways [12]. The exact mechanisms by which vitamin D may alter the effect of lead on albuminuria deserve to be further clarified.

About three quarters of our study participants had vitamin D deficiency, as shown by serum 25(OH)D levels < 50 nmol/L. In a large-scale multinational trial of patients with diabetes, 52% had vitamin D deficiency [38]. It has been extensively reported that vitamin D deficiency is prevalent in elderly individuals worldwide, and the situation becomes worse in diabetic patients given the suggestive relationship between low vitamin D levels and insulin resistance [39, 40]. Consequently, global actions to improve vitamin D status are essential and should be taken more aggressively in patients with diabetes.

To our knowledge, the current study is the first to specifically investigate the impact of vitamin D on kidney damage in relation to environmental lead exposure among individuals with diabetes. The strengths of our study include a large sample size, rigorous quality control, well-validated laboratory procedures, and comprehensive adjustment for multiple confounding factors. Moreover, our results are robust in a series of stratified analyses and sensitivity analyses.

Several limitations of this study merit discussion. First, considering the cross-sectional study design, the ability to make inferences regarding causality of the associations detected is limited. However, consistent results in the analysis among participants with preserved eGFR make reverse causation that lead level increase as a result of decreased kidney excretion unlikely. Second, because blood lead level reflects recent and chronic exposure, a single blood measure may not perfectly capture chronic lead exposure. Similarly, a single measurement of UACR may introduce bias by misclassifying individuals, but this type of misclassification is likely to be nondifferential with respect to blood lead level, and such a bias tends to attenuate the results toward the null. Third, the diabetic patients in our study were from community healthcare center, so selection bias probably exists. Fourth, although we adjusted for an array of covariates, several factors (i.e. nutritional status) potentially affecting both lead exposure and kidney

health were not included, and therefore, the possibility of residual or unmeasured confounding cannot be ruled out. Finally, the influence of other correlated environmental nephrotoxicants from the similar sources such as cadmium remains to be determined.

# 5. Conclusion

Our study suggested that the association between blood lead and diabetic kidney damage largely depended on body vitamin D status. Higher blood lead levels were associated with increased urinary albumin excretion in diabetic patients with vitamin D deficiency, which became more pronounced in the presence of reduced eGFR. Further prospective studies and randomized clinical trials are needed to validate our findings and to determine whether vitamin D supplementation yields a benefit.

# Abbreviations

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI: body mass index; CI: confidence interval; DKD: diabetic kidney disease; eGFR: estimated glomerular filtration rate; $HbA_{1c}$: glycated hemoglobin; HDL: high-density lipoprotein; IQR: interquartile range; LDL: low-density lipoprotein; RR; risk ratio; SD: standard deviation; UACR: urinary albumin-to-creatinine ratio; 25(OH)D: 25-hydroxyvitamin D.

# Declarations

### Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine. All study participants provided written informed consent.

### Consent for publication

Not applicable.

### Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Competing interests

The authors declare that they have no competing interests.

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### Authors' contributions

BW and HW conceived and designed the study, analyzed the data, and drafted the manuscript. JC, YC, YW, YC, CC, WZ and FX collected data, contributed to data interpretation, and commented on drafts. NW and YL designed the study and reviewed/edited the manuscript. All authors approved the final version of the manuscript.

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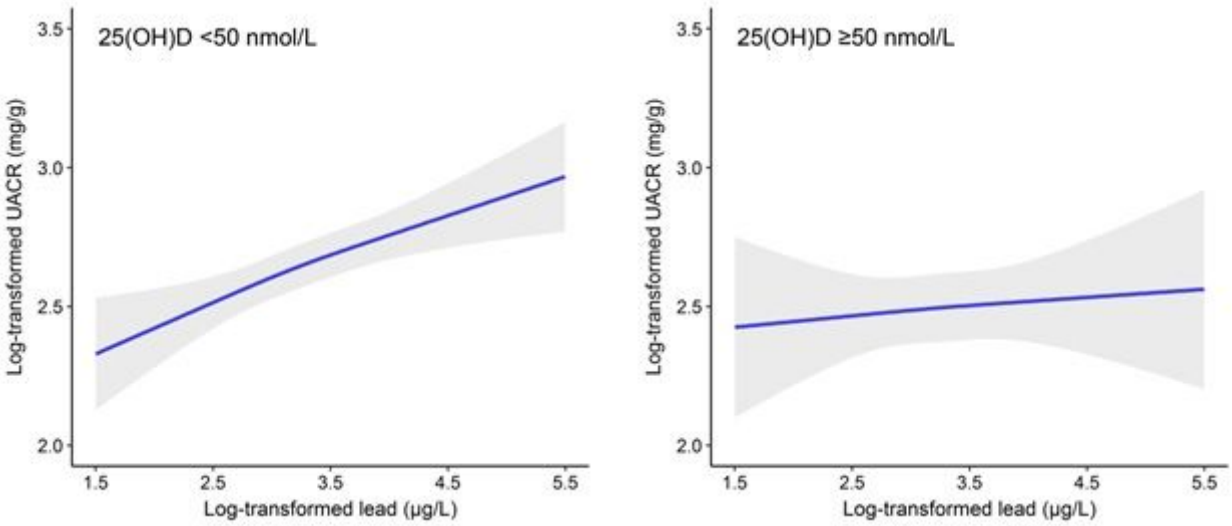
# Figures




## Figure 1

Dose-response relationship between blood lead level and UACR in diabetic patients according to 25(OH)D status. Data were fitted using a restricted cubic spline for log-transformed blood lead level with three knots in linear regression models adjusting for age, sex, BMI, smoking, LDL cholesterol, HDL cholesterol, triglycerides, HbA1c, duration of diabetes, systolic blood pressure, use of antihypertensive medications, and eGFR. Solid lines indicate smoothed fitted relationships and shaded areas indicate 95% CIs. CI, confidence interval; UACR, urinary albumin-to-creatinine ratio; 25(OH)D, 25-hydroxyvitamin D.

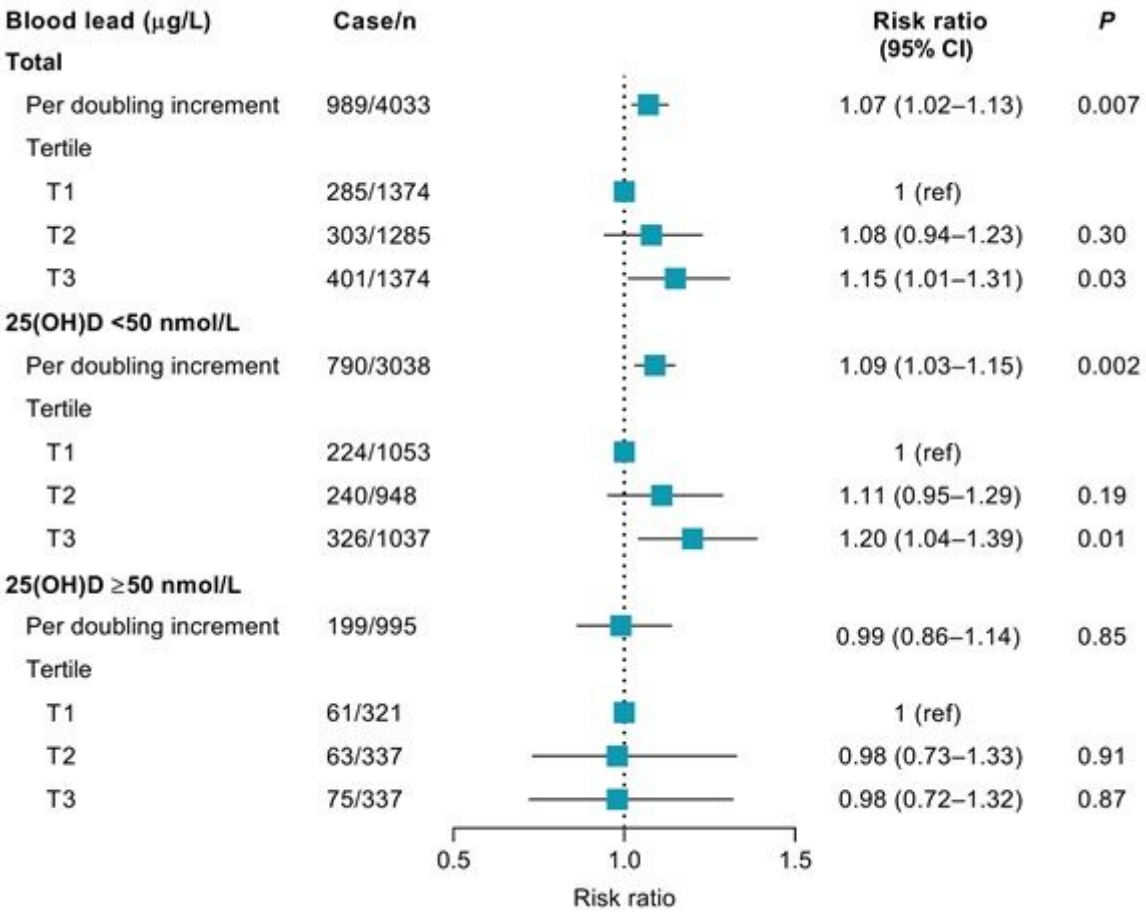


**Figure 2**

Risk ratios (95% CI) of albuminuria in relation to blood lead level in diabetic patients according to 25(OH)D status. The models were adjusted for age, sex, BMI, smoking, LDL cholesterol, HDL cholesterol, triglycerides, HbA1c, duration of diabetes, systolic blood pressure, use of antihypertensive medications, and eGFR. Albuminuria was defined as UACR ≥30 mg/g. CI, confidence interval; UACR, urinary albumin-to-creatinine ratio; 25(OH)D, 25-hydroxyvitamin D.

# Supplementary Files

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