

ORIGINAL RESEARCH ARTICLE

Lead acetate exposure alters renal water reabsorption and gut microbiota in mice: Potential involvement of cerebral cavernous malformations 3 and the gut–kidney axis

Feiyu Zhao[†], Liuxue Yang^{2†}, Dan Liang^{3†}, Xu Liang¹, Hang Zhou¹, Ying Zhang¹, Xinru Liang¹, Jiaomin He¹, Xinru Ma¹, Muyao Chen⁴, and Yi Sun^{1,5*}¹Department of Toxicology, School of Public Health, Guilin Medical University, Guilin, Guangxi, China²Department of Science Experiment Center, School of Public Health, Guilin Medical University, Guilin, Guangxi, China³Department of Endocrinology, The Second Affiliated Hospital of Guilin Medical College, Guilin, Guangxi, China⁴Department of Biology, Tongji University High School (Sci-Tech), Shanghai, China⁵Department of Environmental and Occupational Medicine, School of Public Health, Guilin Medical University, The Guangxi Key Laboratory of Environmental Exposomics and Entire Lifecycle Health, Guilin, Guangxi, China

[†]These authors contributed equally to this work.

***Corresponding author:**Yi Sun
(sunyi@glmu.edu.cn)

Citation: Zhao F, Yang L, Liang D, *et al.* Lead acetate exposure alters renal water reabsorption and gut microbiota in mice: Potential involvement of cerebral cavernous malformations 3 and the gut–kidney axis. *Asian J Water Environ Pollut.* doi: 10.36922/AJWEP026300204

Received: July 25, 2026**Revised:** August 27, 2026**Accepted:** September 10, 2026**Published online:** October 28, 2026

Copyright: © 2026 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.

Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract

Lead is a widespread, cumulative environmental pollutant with confirmed renal and gastrointestinal toxicity, but the mechanism underlying lead-induced alterations in renal water reabsorption and the potential role of the gut–kidney axis remain unclear. This study aims to explore the potential role of cerebral cavernous malformations 3 (CCM3) in lead-induced alteration of renal tubular water reabsorption and its potential association with the gut–kidney axis. C57BL/6J mice were administered drinking water containing 0.05%, 0.25%, and 0.5% lead acetate for 30 days. Urine was collected weekly for quantitative analysis. After exposure, a desmopressin (dAVP) challenge test was performed. Blood lead concentration was detected by inductively coupled plasma mass spectrometry. Kidney tissues were evaluated by hematoxylin and eosin (H&E) staining and Western blot. Fecal samples were collected before and after exposure for 16S rRNA gut microbiota sequencing. Data were analyzed by repeated-measures analysis of variance (ANOVA) and one-way ANOVA using GraphPad Prism 8.0. As exposure dose increased, blood lead concentration increased dose-dependently in all exposure groups. Urine output decreased significantly in medium- and high-dose groups after 24-hour water deprivation and dAVP challenge. H&E staining showed dose-dependent renal tubular necrosis. The composition and diversity of gut microbiota were significantly altered. Renal CCM3 protein expression was significantly increased (most prominent in the medium-dose group), glutathione peroxidase 4 (GPX4) protein was significantly decreased in the high-dose group, and aquaporin (AQP) 2 and AQP4 proteins showed an upward trend without statistical significance. Lead exposure induces renal tubular structural injury and alters renal water reabsorption function. This process may be associated with gut microbiota dysbiosis, which affects renal CCM3 expression via the gut–kidney axis and further regulates AQP2/4 levels through GPX4-related pathways. This study provides preliminary correlational evidence and proposes a testable hypothesis for

future mechanistic research.

Keywords: Lead; Cerebral cavernous malformations 3; Glutathione peroxidase 4; Aquaporin 2/4; Gut–kidney axis

1. Introduction

Despite the continuous implementation of various prevention and control measures and standards worldwide over the past 30 years, such as the World Health Organization's ban on lead-containing paints and the revision of lead limits in China's National Food Safety Standard for Contaminants in Food (GB 2762-2022) in 2022, widespread lead use in the last century has left significant environmental residues. According to the World Health Organization's estimates, approximately 1 million deaths globally are attributable to lead exposure-related diseases each year, with the highest disease burden in low- and middle-income countries in South Asia and Africa. Nearly one-third of the world's population is affected by chronic low-level lead exposure, which causes irreversible damage to multiple organ systems even at low doses. In recent years, rapid growth in lead production and consumption, along with new exposure pathways such as PM_{2.5} inhalation, has kept lead a high-priority cumulative environmental pollutant. Due to multiple exposure routes and insidious toxic effects, the health burden of lead exposure continues to rise. Therefore, investigating the health hazards and toxic mechanisms of lead is of great significance for long-term health risk assessment and prevention.

The kidneys and gastrointestinal tract are well-recognized target organs of lead toxicity. Lead accumulates preferentially in the kidneys, and blood lead concentrations above 500 µg/L can cause measurable renal damage. Studies have shown that even trace levels of lead (29 ng/g in food) can interfere with enzyme activity in renal cells and disrupt cellular metabolic homeostasis. Lead exposure not only directly causes intestinal tissue damage and inflammatory responses, but also directly or indirectly alters the composition and diversity of the gut microbiota.¹ In turn, gut microbiota regulates the toxicokinetics of lead *in vivo*. In mice, antibiotic-induced microbiota disruption increases intestinal permeability, exacerbates inflammation, impairs tight junction integrity, elevates blood lead levels and target-organ lead accumulation, and reduces fecal lead excretion.² However, the correlation between lead-induced renal toxicity and gastrointestinal toxicity requires further investigation.³

Organ crosstalk via endocrine, neural, or paracrine signaling pathways is an emerging frontier in understanding lead toxicity mechanisms. Building on the “intestinal–renal syndrome” theory proposed in 2011⁴, the “gut–kidney axis” concept has been established, revealing the close functional linkage between the intestine and kidney under both physiological and pathological conditions. Some studies suggest microbial signals in the human kidney, with differences between healthy individuals and kidney cancer patients, providing indirect support for gut–kidney interactions, though this conclusion remains controversial.⁵ It remains unclear whether lead exposure disturbs the gut microbiota and further exacerbates renal injury via the gut–kidney axis.

Brain cavernous malformation 3 (CCM3/*PDCD10*) is a well-characterized lead-responsive gene⁶ and a risk factor for vascular developmental lesions. Recent studies have shown that intestinal CCM3 deficiency causes extensive colonic epithelial damage, impairs mucin secretion, alters gut microbiota abundance, and increases lipopolysaccharide production, ultimately exacerbating cerebral cavernous malformations via the gut–brain axis.^{7–9} In the kidney, CCM3 is essential for maintaining normal water reabsorption in the collecting duct and supports renal epithelial homeostasis and function.^{10,11} Therefore, we hypothesize that CCM3 may serve as a key mediator in the gut–kidney axis under lead exposure. However, the changes in renal CCM3 after lead exposure and its role in the gut–kidney axis remain unexplored.

Accordingly, this study investigated the changes in renal and intestinal CCM3 and gut microbiota composition after lead exposure in mice, to preliminarily explore the role of CCM3 in lead-induced renal injury and its potential association with the gut–kidney axis.

2. Materials and methods

2.1. Materials

2.1.1. Experimental animals

Twenty-eight female C57BL/6J mice (36 weeks old, body weight 28 ± 2 g) were purchased from Hunan Slike Jinda Biotechnology Co., Ltd. (China) and housed in a specific pathogen-free barrier system. Thirty-six-week-old female mice were selected based on pre-experimental results:

mice at this age exhibit stable and high sensitivity to lead-induced renal injury with low individual variation, which facilitates the observation of water reabsorption phenotypes. All animal procedures were approved by the Animal Ethics Committee of Guilin Medical University (GLMC-IACUC-20241040).

2.1.2. Reagents and instruments

Reagents used included desmopressin acetate (dAVP; GLPBIO, United States), high-efficiency radioimmunoprecipitation assay tissue/cell lysis buffer (Solarbio, Catalog No. R0010), PMSF solution (Solarbio, China), bicinchoninic acid protein concentration assay kit (Catalog no. P0010S; Beyotime, China), rainbow 245 broad-spectrum protein marker (Catalog no. PR1920; Solarbio, China), tricolor pre-stained protein marker (YEASEN, China), QuickBlock™ Western blocking buffer (Beyotime, China), Omni-ECL™ ultra-sensitive chemiluminescence detection kit (Catalog no. SQ201; YEASEN, China), anti- β -actin mouse monoclonal antibody (GB12001; Servicebio, China), anti- β -actin rabbit polyclonal antibody (GB11001; Servicebio, China), anti-PDCD10/CCM3 rabbit antibody (ab180706; Abcam, United Kingdom), anti-glutathione peroxidase 4 (GPX4) rabbit antibody (ab125066; Abcam, United Kingdom), anti-cyclooxygenase 2 (COX2) antibody (ab179800, Abcam, United Kingdom), aquaporin (AQP) 2 rabbit polyclonal antibody (A16209; ABclonal, United States), AQP4 rabbit monoclonal antibody (A11210; ABclonal, United States), mouse IgG secondary antibody (GB111739; Servicebio, China), and rabbit IgG secondary antibody (GB111738; Servicebio, China). Instruments used included a Varioskan LUX multifunctional microplate reader (Thermo Fisher Scientific, United States), a FluorChem M multi-color fluorescence gel imaging system (Protein Simple, United States), and a NexION 2000 inductively coupled plasma mass spectrometry instrument (PerkinElmer, United States). The instrumental limit of detection for lead was 0.03 $\mu\text{g/L}$, and the limit of quantification was 0.1 $\mu\text{g/L}$.

2.2. Methods

2.2.1. Animal grouping and treatment

Mice were randomly divided into four groups ($n = 7$ per group): control, low-dose, medium-dose, and high-dose. Exposure groups received drinking water containing 0.05%, 0.25%, and 0.5% lead acetate for 30 days, respectively, while the control group received distilled water. The dose gradients were selected based on established mouse subchronic lead poisoning models^{2,3}, which induce stable target organ damage with acceptable animal tolerance.

Housing conditions were controlled at 20 °C–26 °C,

40%–70% humidity, and a 12 h light/12 h dark cycle, with free access to food. After 30 days of exposure, fluid deprivation-vasopressin tests were performed, followed by euthanasia and sample collection.

2.2.2. Blood lead detection

A standard lead stock solution (1,000 $\mu\text{g/L}$) was prepared and diluted with 1% nitric acid containing 0.05% triethanolamine to generate standard curve solutions at concentrations of 0.5, 2, 5, 10, 50, 100, and 200 $\mu\text{g/L}$. A total volume of 50 μL of peripheral blood was diluted 30-fold with 1% nitric acid containing 0.05% triethanolamine, mixed thoroughly, and centrifuged at 12,000 rpm for 25 min. A total volume of 1 mL of supernatant was analyzed using a PerkinElmer NexION 2000 inductively coupled plasma mass spectrometry instrument.

2.2.3. Urine collection

On days 0, 7, 14, 21, and 28, mice were placed in metabolic cages and fasted for 24 h. After 24 h, mice were returned to home cages, and urine and feces were collected. Fecal samples were snap-frozen in liquid nitrogen and stored at $-80\text{ }^{\circ}\text{C}$ together with urine samples. Body weight and water consumption were measured on the day before each metabolic cage collection.

2.2.4. Fluid deprivation-vasopressin test

After 30 days of exposure, mice were water-deprived for 24 h with free access to food. Subsequently, mice received an intraperitoneal injection of 1 ng/g body weight dAVP (1 $\mu\text{g/kg}$), a dose consistent with established mouse urinary concentration test protocols. Urine was collected in metabolic cages at 6 h and 12 h post-injection, and total urine volume was measured. All urine samples were stored at $-80\text{ }^{\circ}\text{C}$.

2.2.5. Hematoxylin and eosin staining for renal tissue

Kidney tissues were fixed in 10% formalin, dehydrated, and embedded in paraffin. Sections (6 μm thick) were deparaffinized with xylene, rehydrated through a graded alcohol series, stained with hematoxylin, differentiated with 0.1% hydrochloric acid ethanol, counterstained with eosin, dehydrated, cleared with xylene, and mounted with neutral resin. Pathological changes were observed and imaged using a Panthera digital trinocular microscope camera system (Motic Industries Group Co., Ltd., China). Two independent pathologists performed blinded, semi-quantitative tubular injury scoring using a standard scale: 0: no injury; 1: <10% tubules injured; 2: 10%–25% tubules injured; 3: 26%–50% tubules injured; 4: >50% tubular necrosis or cast formation.

2.2.6. Western blot analysis

Kidney tissues were homogenized into a powder in liquid nitrogen on ice, and total protein was extracted with a radioimmunoprecipitation assay lysis buffer. Protein concentration was determined using the bicinchoninic acid assay. Samples were denatured at 95 °C for 5 min with 5× loading buffer, then subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis, membrane transfer, blocking, primary antibody incubation (1:1,000), secondary antibody incubation (1:1,000), and chemiluminescent detection. Band intensity was quantified using ImageJ software (version 1.53a) and normalized to β -actin.

2.2.7. 16S rRNA sequencing analysis of gut microbiota

Fecal samples were collected at day 0 (before exposure) and day 21 of lead exposure, after 24-hour fasting in metabolic cages. Fresh feces were transferred to 1.5 mL Eppendorf tubes, snap-frozen in liquid nitrogen for 15 min, and stored at –80 °C.

Total genomic DNA was extracted from fecal samples. The V3–V4 variable region of the 16S rRNA gene was amplified using primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). Amplicons were purified with magnetic beads and quantified by fluorescence. High-throughput sequencing was performed on the Illumina NovaSeq 6000 platform.

Raw reads were quality-controlled using Trimmomatic, assembled using FLASH, and chimeras were removed. The DADA2 algorithm was used for amplicon sequence variant (ASV) clustering. Taxonomic annotation was performed against the SILVA 138 reference database. Alpha diversity indices (Chao1, Shannon) were calculated using QIIME2. Beta diversity was analyzed by principal component analysis based on Bray–Curtis distance. Functional prediction of Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathways was performed using PICRUSt2.

2.3. Statistical analysis

All data are expressed as mean $\pm$ standard deviation. Sample size was estimated using G*Power 3.1 ($\alpha = 0.05$, power = 0.8, effect size $f = 0.8$); seven mice per group were included to account for a 15% dropout rate.

For longitudinal endpoints (body weight, water intake, urine volume), repeated-measures analysis of variance (ANOVA) was used with time as the within-subject factor and treatment as the between-subject factor, followed by a Bonferroni post-hoc test. One-way ANOVA with Tukey's

post-hoc test was used for single-time-point intergroup comparisons. All data were tested for normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test). For microbiota data, the Kruskal–Wallis test with Dunn's post-hoc test and false-discovery rate correction was applied. Statistical analysis was performed using GraphPad Prism 8.0, and $p < 0.05$ was considered statistically significant. Pathological evaluation and Western blot quantification were performed in a blinded manner.

3. Results

3.1. Effects of lead on physiological and renal toxicity in mice

3.1.1. Body weight changes

Body weight was measured every seven days during exposure. Body weight trends were generally consistent across groups, with control mice maintaining slightly higher body weights than lead-exposed groups, indicating that lead exposure exerted a mild effect on body growth (Figure 1A). Repeated-measures ANOVA showed a significant main effect of time on body weight, but no significant group $\times$ time interaction. Statistically significant body weight differences were only observed between baseline and day 14 in the control and medium-dose groups (Figure 1B).

3.1.2. Blood lead levels

Blood lead concentration increased in a dose-dependent manner with increasing lead acetate dose, and inter-group differences were statistically significant (Figure 1C). Based on weekly water consumption and body weight, estimated actual lead intake was approximately 45 mg/kg/day, 225 mg/kg/day, and 450 mg/kg/day for the low-, medium-, and high-dose groups, respectively.

3.1.3. Water consumption

Average daily water consumption was measured weekly. Water consumption was negatively correlated with lead dose, and statistically significant differences were observed among exposure groups (Figure 2A), consistent with the known taste-aversive property of lead acetate.

3.1.4. Urine volume measurement

Weekly baseline urine volume decreased with increasing lead dose, but inter-group differences were not statistically significant (Figure 2B). After 24-hour water deprivation, urine volume was lower in all exposure groups than in the control group, and approached near-zero levels in the medium- and high-dose groups (Figure 2C). Twelve hours after dAVP injection, urine volume increased in all groups. Urine volume in the low-dose group was comparable to

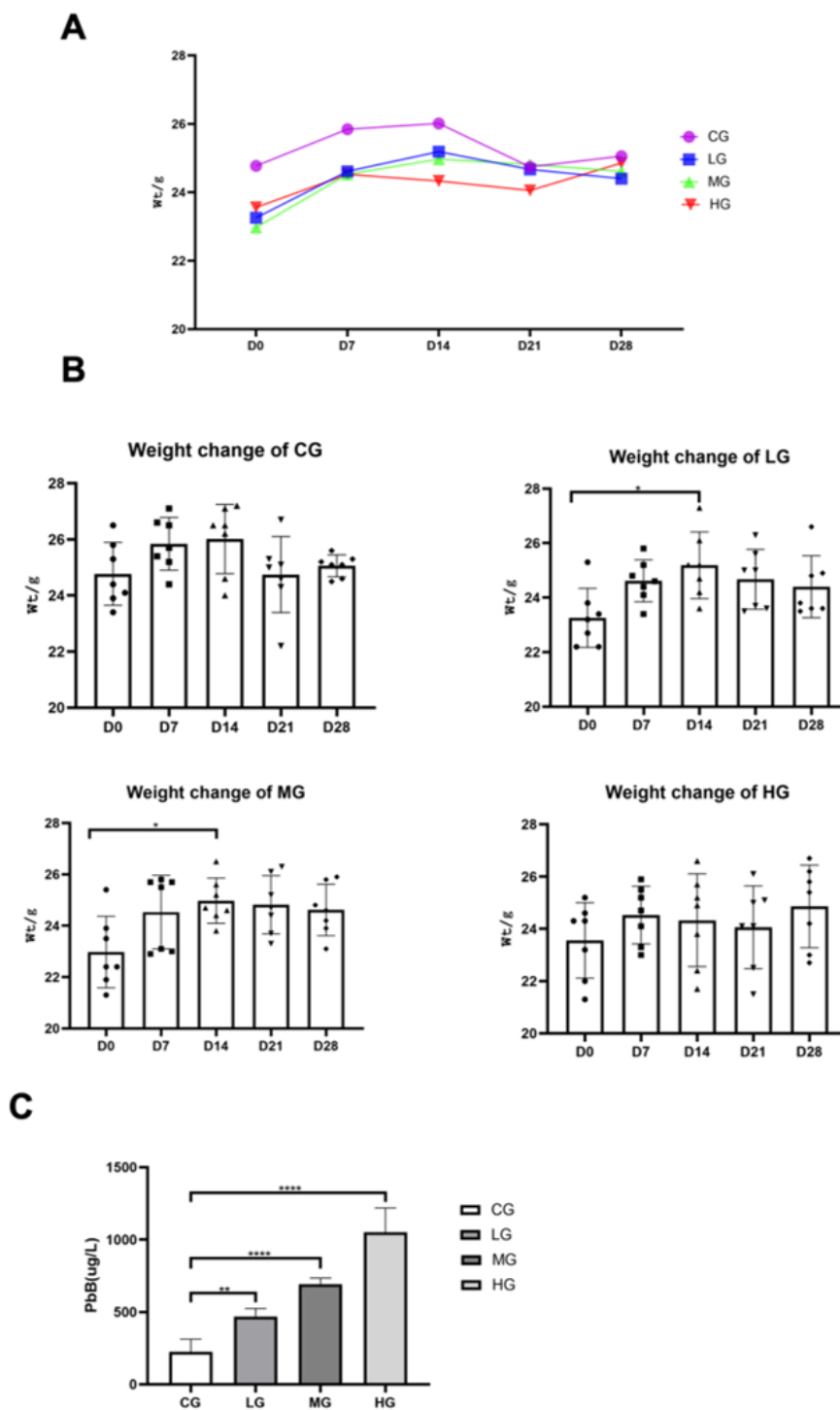


Figure 1. Body weight changes and blood lead levels in each treatment group. (A) Body weight changes of mice during 28-day exposure. (B) Body weight comparison at baseline and day 14. (C) Blood lead concentrations in each group at the end of exposure. Data are presented as mean $\pm$ standard deviation ($n = 7$ per group). * $p < 0.05$ vs. control group. Statistical analysis was performed by repeated-measures analysis of variance (ANOVA) (A) and one-way ANOVA with Tukey's post-hoc test (B, C).

Abbreviations: CG: Control group; HG: High-dose group; LG: Low-dose group; MG: Medium-dose group.

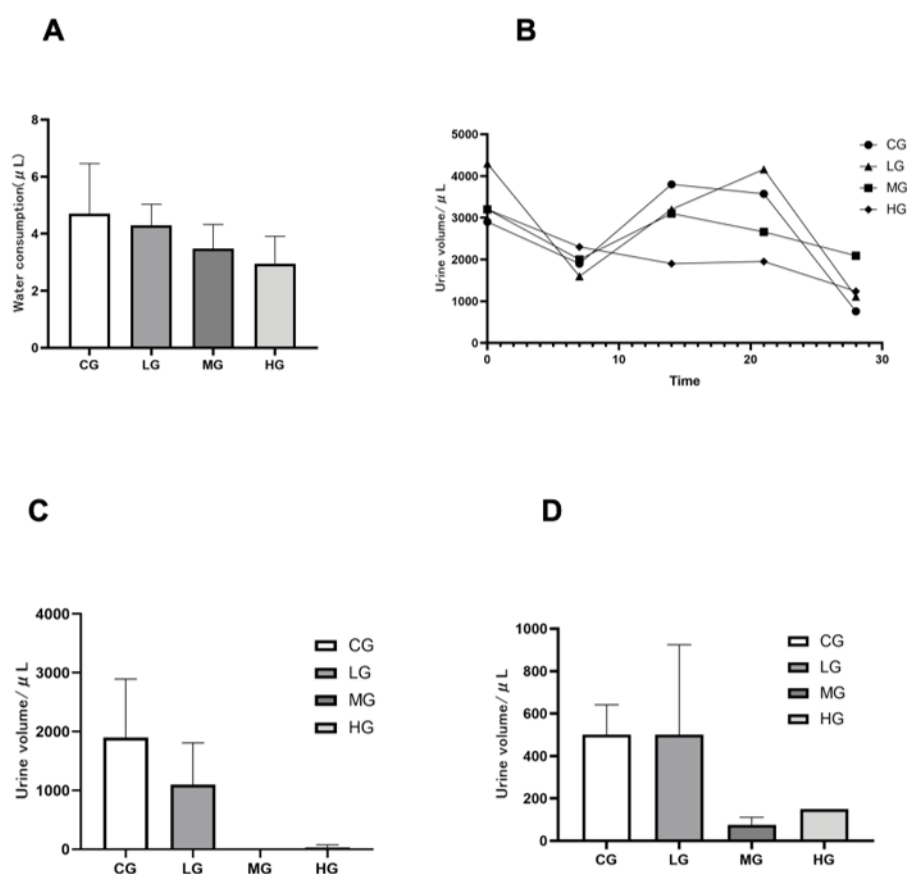


Figure 2. Water consumption and urine volume changes before and after arginine vasopressin injection. (A) Average daily water consumption per mouse in each group. (B) Baseline urine volume changes during exposure. (C) Urine volume after 24-hour water deprivation. (D) Urine volume 12 hours after desmopressin acetate-arginine vasopressin injection. Data are presented as mean $\pm$ standard deviation ($n = 7$ per group). * $p < 0.05$ vs. control group. Statistical analysis was performed by repeated-measures analysis of variance (ANOVA) (A, B) and one-way ANOVA (C, D). Abbreviations: CG: Control group; HG: High-dose group; LG: Low-dose group; MG: Medium-dose group.

the control group, while urine volume in the medium- and high-dose groups remained significantly lower than controls (Figure 2D).

3.1.5. Renal organ coefficient

The renal coefficient was calculated as (renal weight/body weight) $\times$ 100. The renal coefficient was increased in lead-exposed groups, with a statistically significant difference between the control and medium-dose groups (Figure 3A).

3.1.6. Pathological changes in renal tissue

Hematoxylin and eosin staining showed that the low-dose group had largely intact renal tissue architecture with no obvious pathological changes. The medium-dose group showed localized tubular epithelial degeneration and necrosis, cellular swelling, nuclear pyknosis/fragmentation/dissolution, cytoplasmic vacuolization, and protein casts

in some tubules. The high-dose group exhibited extensive tubular epithelial degeneration and necrosis, disorganized tubular arrangement, vacuolar degeneration, nuclear condensation, and mild interstitial inflammatory cell infiltration (predominantly lymphocytes) (Figure 3B). Semi-quantitative tubular injury scores confirmed a dose-dependent increase in renal injury in lead-exposed groups.

3.1.7. Western blot results

Compared with the control group, CCM3 protein expression increased significantly in all lead-exposed groups, with the greatest elevation in the medium-dose group (Figure 4A, $p < 0.05$). GPX4 protein expression was significantly decreased in the high-dose group (Figure 4B, $p < 0.05$). AQP2 and AQP4 protein levels were higher in all exposure groups than in controls, but the differences did not reach statistical significance (Figure 4C).

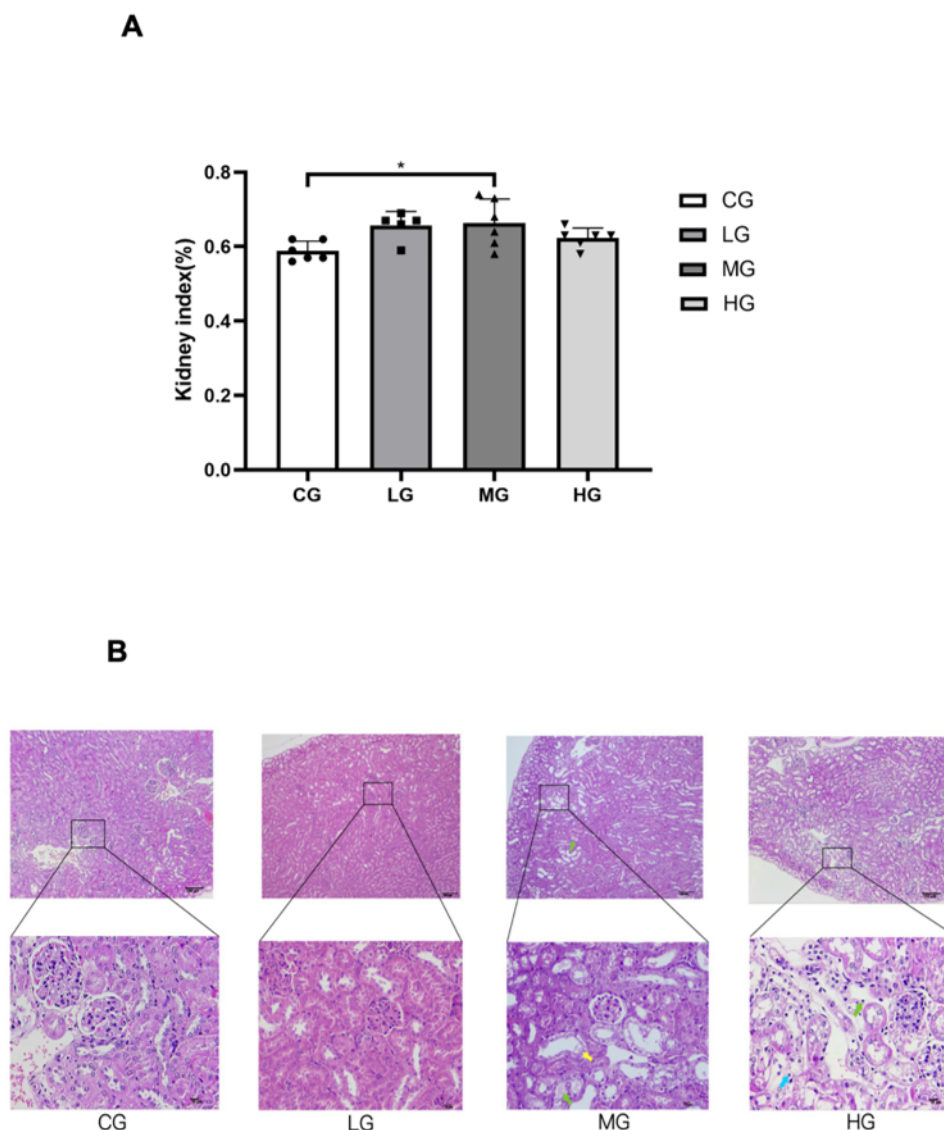


Figure 3. Renal coefficients and hematoxylin and eosin staining results of kidney tissue. (A) Renal organ coefficients in each group. (B) Representative hematoxylin and eosin staining images of renal tissue (scale bar: 50 μ m; magnification: 50 $\times$) and semi-quantitative tubular injury scores. Data are presented as mean $\pm$ standard deviation ($n = 7$ per group). * $p < 0.05$ vs. control group. Statistical analysis was performed by one-way analysis of variance with Tukey's post-hoc test.

Abbreviations: CG: Control group; HG: High-dose group; LG: Low-dose group; MG: Medium-dose group.

3.2. Analysis of gut microbiota

3.2.1. Gut microbiota diversity and amplicon sequence variant distribution

Venn diagram analysis identified 97 shared ASVs across all eight groups. Total ASV counts were 1,184 and 1,126 in the control group; 941 and 401 in the low-dose group; 1,465 and 1,762 in the medium-dose group; and 643 and 735 in the high-dose group before and after exposure, respectively. Richness decreased markedly in the low-dose group (from

941 to 401) and slightly in the control group, while it increased in the medium- and high-dose groups following 21 days of exposure (Figure 5B). Consistently, alpha diversity (Chao1 and Shannon indices) showed no significant differences among groups at baseline, but both indices were markedly reduced after 21 days of low-dose lead exposure. Furthermore, principal component analysis of beta diversity showed clear segregation of microbial communities before and after lead exposure, along with distinct clustering patterns among the different exposure dosages.

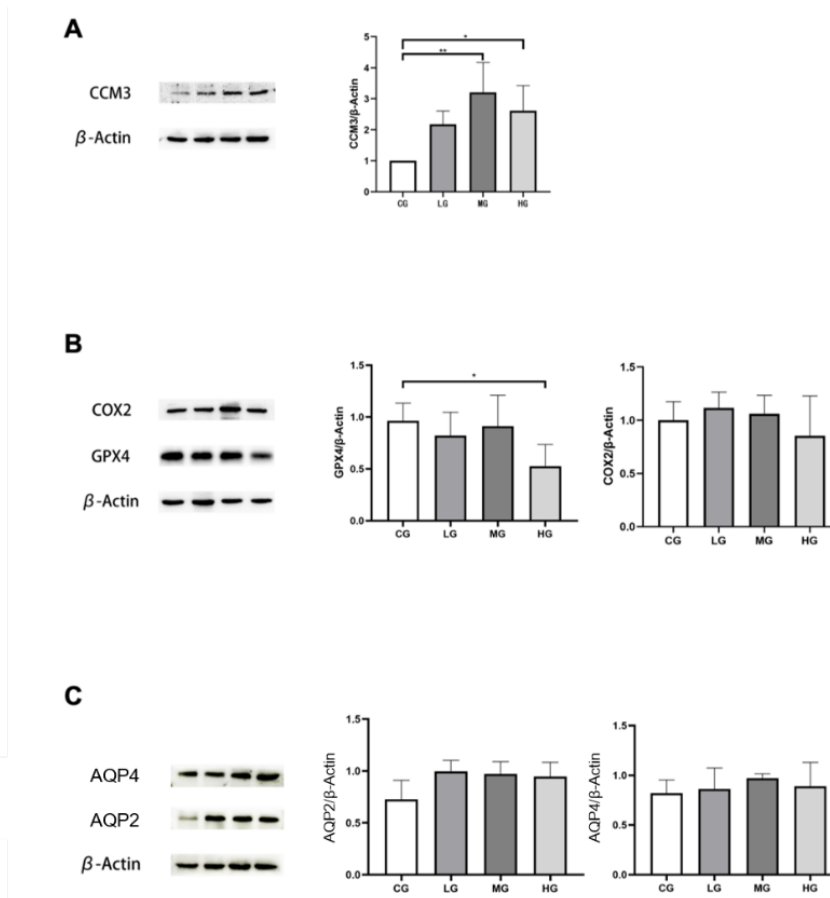


Figure 4. Western blot results of renal tissues in each group. (A) Cerebral cavernous malformations 3 (CCM3) protein expression and quantification. (B) Glutathione peroxidase 4 (GPX4) protein expression and quantification. (C) Aquaporin (AQP) 2 and AQP4 protein expression and quantification. β -actin was used as a loading control. Representative blots are shown from independent biological replicates. Data are presented as mean $\pm$ standard deviation ($n = 7$ per group). * $p < 0.05$ vs. control group. Statistical analysis was performed by one-way analysis of variance with Tukey's post-hoc test. Abbreviations: CG: Control group; HG: High-dose group; LG: Low-dose group; MG: Medium-dose group.

3.2.2. Compositional alterations in gut microbiota

Taxonomic profiling revealed substantial alterations in community structure after 21 days of lead treatment (Figure 5A). Compared with baseline, the control group showed elevated abundances of the mucin-degrading bacteria *Akkermansia* and *Dubosiella*. In the low-dose group, *Staphylococcus*, *Bifidobacterium*, *Allobaculum*, *Turicibacter*, and *Lactobacillus* were significantly enriched, whereas *Lachnospiraceae* decreased. The medium-dose group showed increased proportions of *Alistipes* and *Helicobacter*, accompanied by depleted *Lachnospiraceae*. In the high-dose group, *Turicibacter*, *Muribaculum*, *Helicobacter*, and *Alistipes* were prominently enriched, while *Lachnospiraceae* remained persistently low (Figure 5A). Hierarchical cluster heatmap analysis further confirmed distinct group-specific taxonomic profiles and separation between treatment regimens (Figure 6A),

consistent with lead-induced gut microbiota dysbiosis.

3.2.3. Functional pathway prediction

PICRUSt2 functional profiling based on KEGG pathways indicated that the altered gut microbiota were predominantly involved in metabolic pathways (Figure 6B). Among these, carbohydrate metabolism, cofactor and vitamin metabolism, amino acid metabolism, and terpenoid and polyketide metabolism were the most highly represented categories, followed by pathways related to genetic information processing, cellular processes, environmental information processing, and organismal systems. These functional shifts align with established effects of lead exposure on intestinal metabolic disruption and oxidative stress.

In summary, 21 days of lead exposure induced profound gut microbiota dysbiosis across all exposure groups, with

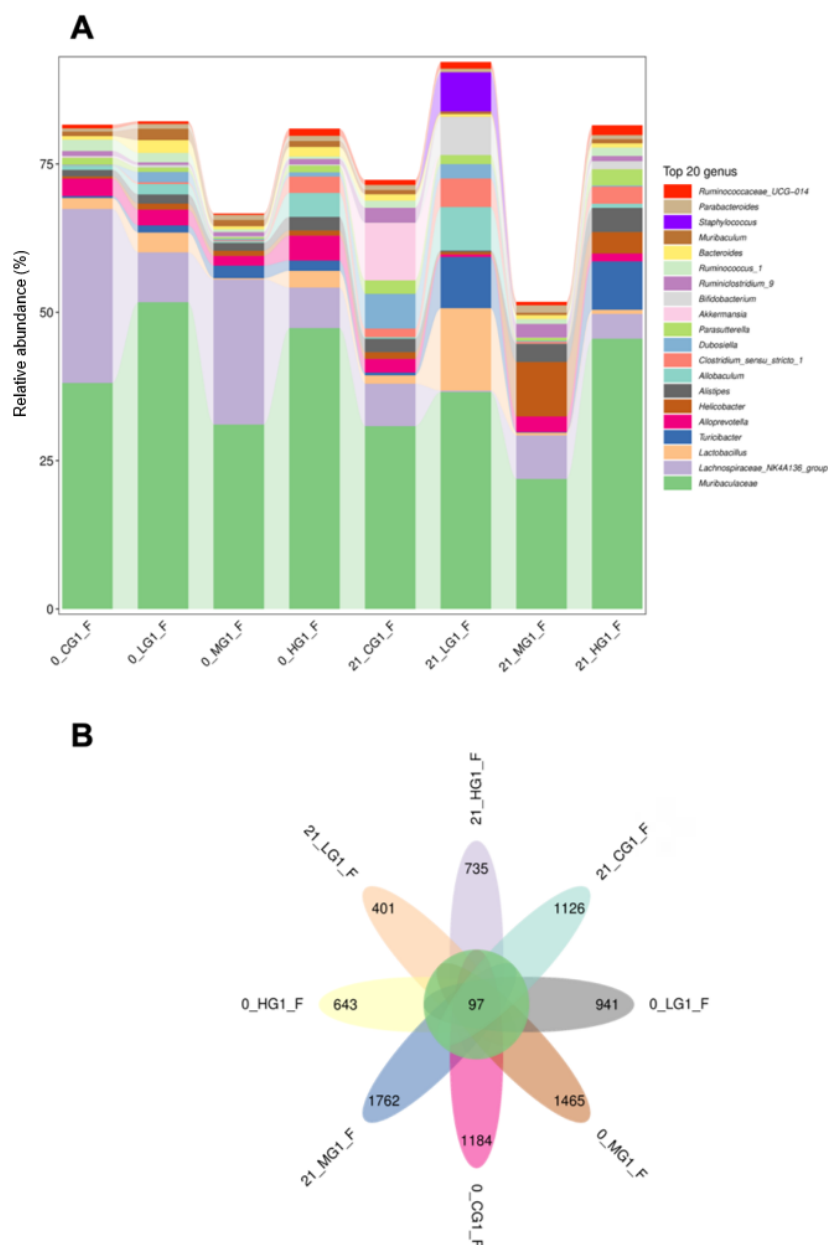


Figure 5. Gut microbiota diversity and differential taxa analysis. (A) Differential taxa abundance among groups after 21-day exposure. (B) Alpha diversity indices (Chao1 and Shannon) at day 0 and day 21. Data are presented as mean $\pm$ standard deviation ($n = 7$ per group). * $p < 0.05$ vs. control group. Statistical analysis was performed using the Kruskal–Wallis test with Dunn's post-hoc test.

the low-dose cohort showing the most severe reduction in richness and alpha diversity.

4. Discussion

This study showed that lead exposure induced dose-dependent increases in blood lead, renal tubular structural injury, and enhanced renal water reabsorption in mice. Renal CCM3 protein was significantly upregulated, GPX4

was downregulated at high doses, and AQP2/AQP4 showed non-significant increases. In parallel, lead exposure caused significant gut microbiota dysbiosis and altered predicted metabolic pathways.

Epidemiological studies have consistently linked lead exposure to chronic kidney dysfunction and accelerated CKD progression.¹² Muntner *et al.*¹³ reported that higher blood lead levels were associated with increased chronic

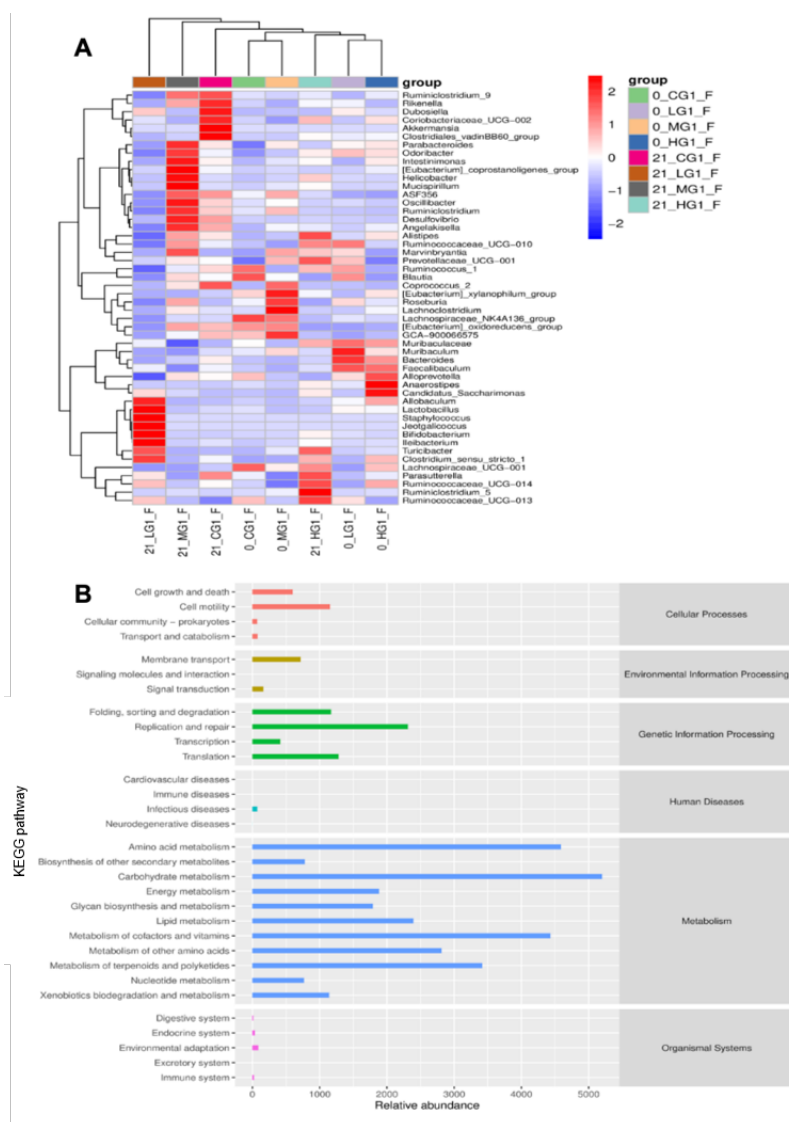


Figure 6. Gut microbiota composition and functional prediction. (A) Cluster heatmap of differential taxa and Venn diagram of amplicon sequence variant abundance. (B) Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathway prediction of gut microbiota.

kidney failure risk in a large hypertensive cohort. Lin *et al.*¹⁴ further demonstrated that elevated body lead burden accelerated renal function decline in patients with chronic glomerulonephritis. While previous studies have focused on lead-induced interstitial inflammation and tubulointerstitial injury, the impact of lead on renal tubular water reabsorption remains understudied.

Our results showed that under free-drinking conditions, both water intake and urine volume decreased gradually with lead exposure. After standardized water deprivation and dAVP challenge, medium- and high-dose groups exhibited significantly lower urine output than controls. This phenotype cannot be fully explained by reduced water

intake alone, suggesting that lead exposure alters renal water reabsorption capacity. Mechanistically, CCM3 is a critical regulator of renal tubular epithelial homeostasis; CCM3 knockout causes polyuria and polydipsia in mice due to impaired water reabsorption.¹⁰ Accordingly, the increased renal CCM3 expression observed in our study is consistent with the enhanced water reabsorption phenotype.

Renal water reabsorption and urine concentration occur across the proximal tubule, descending limb of the loop of Henle, and collecting duct.¹⁵ Aquaporins (AQP1, 2, 3, 4) constitute the molecular basis of renal water handling.¹⁶ AQP2, the only hormone-regulated water

channel, localizes to the apical membrane and intracellular vesicles of collecting duct principal cells.¹⁷ AQP4 is expressed on the basolateral membrane and contributes to transepithelial water transport.^{18,19} In our study, total AQP2 and AQP4 protein levels showed upward trends, consistent with the pattern that CCM3 deficiency reduces AQP2/AQP4 expression and impairs reabsorption. However, these changes were not statistically significant, and whole-tissue lysate blotting cannot distinguish total protein synthesis from apical membrane insertion. Notably, Wang *et al.*¹⁰ demonstrated that CCM3 regulates AQP2 function primarily by modulating its membrane trafficking rather than total expression.¹⁰ Therefore, AQP2 membrane localization under lead exposure requires further validation.

Arginine vasopressin is the key hormone that regulates renal water reabsorption by promoting AQP2 membrane insertion.²⁰⁻²² The differential urine output after dAVP challenge suggests that lead exposure may alter renal responsiveness to vasopressin, but this interpretation is limited by the lack of endogenous AVP and urine osmolality measurements. Previous studies have linked AVP signaling to ferroptosis in heart failure models.^{23,24} We observed downregulation of GPX4, a central negative regulator of ferroptosis, in high-dose lead-exposed kidneys. Based on these parallel changes, we hypothesize that CCM3 may participate in AVP-associated oxidative stress pathways and indirectly regulate AQP2/4 abundance via GPX4-mediated iron-dependent oxidative stress. This hypothesis remains to be tested by functional validation experiments.

The gut and kidney are both major target organs of lead toxicity, and the gut–kidney axis provides a conceptual framework for understanding their interconnection. Our 16S rRNA sequencing results confirmed that lead exposure significantly altered gut microbiota composition, diversity, and predicted metabolic pathways. The consistent depletion of *Lachnospiraceae*, a major short-chain fatty acid-producing taxon, may impair intestinal barrier integrity and promote systemic low-grade inflammation, which could, in turn, affect renal function.

Based on our findings and existing evidence, we propose a preliminary hypothesis: lead exposure may alter CCM3 expression in the intestinal epithelium, disrupt intestinal barrier function, and induce gut microbiota dysbiosis. Microbial metabolites or inflammatory mediators may then enter the systemic circulation and modulate renal CCM3 expression and tubular function via the gut–kidney axis. This hypothesis requires rigorous experimental verification in future studies.

This study is the first to investigate CCM3's role in lead-induced alterations in renal water reabsorption and to

explore a potential association between lead-induced gut microbiota dysbiosis and renal injury via the gut–kidney axis, providing novel insights into lead nephrotoxicity. However, several important limitations should be acknowledged.

First, this is an observational correlational study. The proposed CCM3/GPX4/AQP regulatory association and gut–kidney axis hypothesis are based on parallel changes, and no causal verification experiments (gene manipulation, inhibitor intervention, fecal microbiota transplantation) were performed. Second, only urine volume was measured; gold-standard renal concentration indicators (urine osmolality, specific gravity, creatinine, electrolytes) were absent. Plasma osmolality, serum sodium, and endogenous AVP were not detected, and no pair-watered control group was included. Third, only 36-week-old female mice were used. Sex hormones modulate AVP–AQP2 signaling and affect toxicokinetics, and age influences renal function and the gut microbiota. The generalizability of the results is limited.

Fourth, exposure doses exceed environmental levels, making them suitable for mechanistic exploration but not directly representative of low-dose environmental exposure. Renal and fecal lead content and positive control groups were not included. Fifth, all samples were collected after 24-hour fasting, which may confound gut microbiota composition and renal water-electrolyte handling. Sixth, whole-tissue lysate Western blot cannot distinguish cytoplasmic vs. membrane-localized AQP2/AQP4, and the membrane targeting status remains undetermined.

Seventh, the ferroptosis phenotype was not comprehensively validated; only GPX4 was measured, and lipid peroxidation, labile iron, and reduced glutathione/oxidized glutathione ratio were not assessed. Eighth, renal function biomarkers (blood urea nitrogen, serum creatinine) and injury markers (kidney injury molecule-1, neutrophil gelatinase-associated lipocalin) were not measured, and the causal link between tubular necrosis and altered water reabsorption remains unconfirmed. Lastly, KEGG pathway results are functional predictions based on 16S rRNA sequencing, not actual metabolomic data, which limits mechanistic interpretation.

5. Conclusion

In conclusion, lead exposure induces renal tubular structural injury and alters renal water reabsorption in mice. The potential mechanism may involve CCM3, an environmentally responsive gene, regulating GPX4 expression and further affecting AQP2/4 abundance. Lead exposure also disrupts gut microbiota homeostasis and metabolic pathways. As a lead-responsive gene expressed

in both renal and intestinal epithelium, CCM3 may play an important role in lead-induced renal injury via the gut–kidney axis.

Acknowledgments

None.

Funding

This work was supported in part by the National Natural Science Foundation of China (8266120278, 82060586), the Natural Science Foundation of Guangxi Province (2025GXNSFHA069136, 2023GXNSFAA026122), the China Scholarship Council (CSC, 202208455012), and the National Guangxi College Students Innovation and National Guangxi College Students Innovation and Entrepreneurship Training Program (S202510601199, X202410601232).

Conflict of interest disclosure

The authors declare that they have no competing interests for this research.

Author contributions

Conceptualization: Feiyu Zhao

Data curation: Dan Liang, Xinru Ma

Formal analysis: Liuxue Yang

Funding acquisition: Yi Sun

Investigation: Hang Zhou, Jiaomin He, Muyao Chen

Methodology: Feiyu Zhao

Project administration: Yi Sun

Resources: Liuxue Yang

Supervision: Ying Zhang

Validation: Xu Liang, Xinru Liang

Visualization: Dan Liang

Writing – original draft: Feiyu Zhao, Yi Sun

Writing – review & editing: Yi Sun

Ethics approval and consent to participate

The animal experiments of the project met the requirements of animal welfare ethics and passed the animal welfare ethics investigation by the IACUC of Guilin Medical University (investigation number: GLMC-IACUC-20241040).

Consent for publication

Not applicable.

Availability of data

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Brila I, Lavrinienko A, Tkalenko E, *et al.* Low-level environmental metal pollution is associated with altered gut microbiota of a wild rodent, the bank vole (*Myodes glareolus*). *Sci Total Environ.* 2021;790:148224. doi: 10.1016/j.scitotenv.2021.148224
2. Zhai Q, Qu D, Feng S, *et al.* Oral supplementation of lead-intolerant intestinal microbes protects against lead (Pb) toxicity in mice. *Front Microbiol.* 2020;10:3161. doi: 10.3389/fmicb.2019.03161
3. Rehman K, Fatima F, Waheed I, *et al.* Prevalence of exposure of heavy metals and their impact on health consequences. *J Cell Biochem.* 2018;119(1):157–184. doi: 10.1002/jcb.26234
4. Pironi L. Definitions of intestinal failure and the short bowel syndrome. *Best Pract Res Clin Gastroenterol.* 2016;30(2):173–185. doi: 10.1016/j.bpg.2016.02.011
5. Huang Y, Xin W, Xiong J, *et al.* The intestinal microbiota and metabolites in the gut-kidney-heart axis of chronic kidney disease. *Front Pharmacol.* 2022;13:837500. doi: 10.3389/fphar.2022.837500
6. Sun Y, Zhao Z, Zhang H, *et al.* The interaction of lead exposure and CCM3 defect plays an important role in regulating angiogenesis through eNOS/NO pathway. *Environ Toxicol Pharmacol.* 2020;79:103407. doi: 10.1016/j.etap.2020.103407
7. Dettler MR, Shenkar R, Benavides CR, *et al.* Novel murine models of cerebral cavernous malformations. *Angiogenesis.* 2020;23(4):651–666. doi: 10.1007/s10456-020-09736-8
8. Mayer EA, Nance K, Chen S. The gut-brain axis. *Annu Rev Med.* 2022;73(1):439–453. doi: 10.1146/annurev-med-042320-014032
9. Tang AT, Sullivan KR, Hong CC, *et al.* Distinct cellular roles for PDCD10 define a gut-brain axis in cerebral cavernous malformation. *Sci Transl Med.* 2019;11(520):eaaw3521. doi: 10.1126/scitranslmed.aaw3521
10. Wang R, Wu ST, Yang X, *et al.* Pcd10-Stk24/25 complex controls kidney water reabsorption by regulating Aqp2 membrane targeting. *JCI Insight.* 2021;6(12):e142838. doi: 10.1172/jci.insight.142838
11. Wang Y, Ma B, Jian Y, *et al.* Deficiency of Pcd10 causes urothelium hypertrophy and vesicle trafficking defects in ureter. *FEBS J.* 2024;291(5):1008–1026.

- doi: 10.1111/febs.17022
12. Marsden PA. Increased body lead burden—cause or consequence of chronic renal insufficiency. *N Engl J Med.* 2003;348(4):345-347.
doi: 10.1056/NEJMe020164
 13. Muntner P, Menke A, DeSalvo KB, *et al.* Blood lead and chronic kidney disease in the general United States population: results from NHANES III. *Kidney Int.* 2003;63(3):1044-1050.
doi: 10.1046/j.1523-1755.2003.00812.x
 14. Lin JL, Lin-Tan DT, Hsu KH, Yu CC. Environmental lead exposure and progression of chronic renal diseases in patients without diabetes. *N Engl J Med.* 2003;348(4):277-286.
doi: 10.1056/NEJMoa021672
 15. Feraille E, Sassi A, Olivier V, *et al.* Renal water transport in health and disease. *Pflugers Arch.* 2022;474(8):841-852.
doi: 10.1007/s00424-022-02712-9
 16. Gade W, Robinson B. A brief survey of aquaporins and their implications for renal physiology. *Clin Lab Sci.* 2006;19(2):70-79.
 17. Centrone M, Ranieri M, Di Mise A, *et al.* AQP2 trafficking in health and diseases: an updated overview. *Int J Biochem Cell Biol.* 2022;149:106261.
doi: 10.1016/j.biocel.2022.106261
 18. Zhu B, Zhou W, Chen C, *et al.* AQP4 is an emerging regulator of pathological pain: a narrative review. *Cell Mol Neurobiol.* 2023;43(8):3997-4005.
doi: 10.1007/s10571-023-01422-9
 19. He J, Yang B. Aquaporins in renal diseases. *Int J Mol Sci.* 2019;20(2):366.
doi: 10.3390/ijms20020366
 20. Warren AM, Grossmann M, Christ-Crain M, *et al.* Syndrome of inappropriate antidiuresis: from pathophysiology to management. *Endocr Rev.* 2023;44(5):819-861.
doi: 10.1210/endrev/bnad010
 21. Bonilla-Felix M. Development of water transport in the collecting duct. *Am J Physiol Renal Physiol.* 2004;287(6):F1093-F1101.
doi: 10.1152/ajprenal.00119.2004
 22. Hui C, Khan M, Suheb MZK, *et al.* Arginine vasopressin disorder (diabetes insipidus). In: *StatPearls*. Treasure Island, FL: StatPearls Publishing; 2024.
 23. Bichet DG, Bockenhauer D. Genetic forms of nephrogenic diabetes insipidus (NDI): vasopressin receptor defect (X-linked) and aquaporin defect (autosomal recessive and dominant). *Best Pract Res Clin Endocrinol Metab.* 2016;30(2):263-276.
doi: 10.1016/j.beem.2016.02.010
 24. Sholokh A, Klusmann E. Local cyclic adenosine monophosphate signalling cascades—roles and targets in chronic kidney disease. *Acta Physiol (Oxf).* 2021;232(1):e13641.
doi: 10.1111/apha.13641