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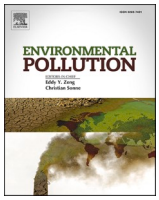

Association of single and joint metals with albuminuria and estimated glomerular filtration longitudinal change in middle-aged adults from Spain: The Aragon workers health study☆

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ABSTRACT

The nephrotoxicity of low-chronic metal exposures is unclear, especially considering several metals simultaneously. We assessed the individual and joint association of metals with longitudinal change in renal endpoints in Aragon Workers Health Study participants with available measures of essential (cobalt [Co], copper [Cu], molybdenum [Mo] and zinc [Zn]) and non-essential (As, barium [Ba], Cd, chromium [Cr], antimony [Sb], titanium [Ti], uranium [U], vanadium [V] and tungsten [W]) urine metals and albumin-to-creatinine ratio (ACR) (N = 707) and estimated glomerular filtration rate (eGFR) (N = 1493) change. Median levels were 0.24, 7.0, 18.6, 295, 3.1, 1.9, 0.28, 1.16, 9.7, 0.66, 0.22 μg/g for Co, Cu, Mo, Zn, As, Ba, Cd, Cr, Sb, Ti, V and W, respectively, and 52.5 and 27.2 ng/g for Sb and U, respectively. In single metal analysis, higher As, Cr and W concentrations were associated with increasing ACR annual change. Higher Zn, As and Cr concentrations were associated with decreasing eGFR annual change. The shape of the longitudinal dose-responses, however, was compatible with a nephrotoxic role for all metals, both in ACR and eGFR models. In joint metal analysis, both higher mixtures of Cu–Zn–As–Ba–Ti–U–V–W and Co–Cd–Cr–Sb–V–W showed associations with increasing ACR and decreasing eGFR annual change. As and Cr were main drivers of the ACR change joint metal association. For the eGFR change joint metal association, while Zn and Cr were main drivers, other metals also contributed substantially. We identified potential interactions for As, Zn and W by other metals with ACR change, but not with eGFR change. Our findings support that Zn, As, Cr and W and suggestively other metals, are nephrotoxic at relatively low exposure levels. Metal exposure reduction and mitigation interventions may improve prevention and decrease the burden of renal disease in the population.

Abbreviations: ACR, albumin to creatinine ratio; AWHS, Aragon Workers Health Study; BKMR, Bayesian kernel machine regression; BMI, body mass index; CI, confidence interval; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; GMR, geometric mean ratio; ICP-MS, inductively coupled plasma mass spectrometry; MD, mean difference; NHANES, National Health and Nutrition Examination Survey; OR, odds ratio; PC, principal component; PIP, posterior inclusion probability; US, United States.

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1. Introduction

The kidney is a target organ of acute metal toxicity because of its ability to filter, reabsorb and accumulate divalent metals (Barbier et al., 2005). In general, this toxicity is related to altered glomerular (endothelium and podocytes), and tubular (proximal or distal tubules and the Henle loop structures) damage (Barbier et al., 2005). There is a solid body of evidence supporting that high exposures to metals such as arsenic (As), cadmium (Cd) and lead (Pb) are nephrotoxic (Orr and Bridges, 2017; Robles-Osorio et al., 2015; Savolainen, 1995; Ekong et al., 2006). In particular, high exposure to As has been related to proteinuria and albuminuria (Robles-Osorio et al., 2015), and high exposure to Cd and Pb has been related to lower estimated glomerular filtration rate (eGFR) and other renal biomarkers (Ekong et al., 2006). However, the role of these metals on renal damage at low-to-moderate exposure levels remains unclear. In addition, epidemiological studies evaluating other metals, including essential and non-essential metals, are limited (Luo and Hendryx, 2020).

Metals are naturally present in the ground water and earth's crust. Increased metal extraction after the industrial revolution resulted in substantial environmental pollution (U.S. Geological Survey (USGS; Nordberg et al., 2015). While a recent decline in exposure has been documented for some metals (Fourth, 2009; Choi et al., 2017), the general population remains exposed to metals through the air, drinking water, diet and tobacco smoke (Nordberg et al., 2015). Exposure to non-essential metals has been associated with an extensive list of adverse health conditions, including cancer, cardiovascular disease, cognitive impairment and mortality (Nigra et al., 2016; Chowdhury et al., 2018). Alternatively, while essential metals deficiency has been related to several diseases, including renal damage (Prasad, 2003), essential metals excess has also been related with adverse health effects (Galan-Chilet, 2017; Domingo-Relloso et al., 2019).

In epidemiological studies, the association of metals beyond As, Cd and Pb, including essential metals, and metal mixtures, with renal disease biomarkers has seldom been investigated. In addition, because metals are naturally found in combination with other metals, and because of potentially common exposure sources and metabolic pathways, the study of metal mixtures and their associations with health outcomes is of increasing interest. However, most studies still focus on single metals or evaluate the co-exposure to metals with simple two-way interaction models. The Bayesian Kernel Machine Regression (BKMR) approach was developed to study multi-pollutant mixtures in a more flexible and informative way. BKMR performs variable selection on the mixtures components and allows to estimate non-linear and non-additive associations between a mixture of correlated exposures and an outcome while accounting for the uncertainty introduced by the exposures correlations (Bobb et al., 2015).

Our aim was to evaluate the longitudinal association of essential (cobalt [Co], copper [Cu], molybdenum [Mo] and zinc [Zn]) and non-essential (As, barium [Ba], Cd, chromium [Cr], antimony [Sb], titanium [Ti], uranium [U], vanadium [V] and tungsten [W]) urine metals with the annual change of renal damage markers (urine albumin to creatinine ratio [ACR] and estimated glomerular filtration rate [eGFR]) in the Aragon Workers Health Study (AWHS), a cohort of middle-aged adults from Spain. We further assessed the joint association of metal mixtures with renal markers change endpoints by applying BKMR methods (Bobb et al., 2015). As secondary analyses, we also evaluated the cross-sectional association of these metals with renal damage markers.

2. Methods

2.1. Study population

The AWHS is a prospective cohort based on the annual health exams in a car assembly plant in Figueruelas (Zaragoza, Spain) that started in 2009–2010 (Casasnovas, 2012). All workers were invited to participate and 5678 decided to enroll (response rate was 95.6%). Workers were excluded from the cohort if they had clinically overt cardiovascular disease, or a major clinical condition limiting survival to <3 years. Subsequently, 2678 participants (out of the 5678) who were 40–55 years old were included in a sub-cohort for subclinical atherosclerosis imaging, which was conducted in the 2011–2014 examination visit (from now on denoted as "baseline"). A total of 1889 participants of the imaging sub-cohort had baseline urine available for metal determinations (AWHS-metal study). Among AWHS-metal participants, 1519 had complete information on urine albumin, serum creatinine and covariables of interest at the baseline (for more details, see flowchart in Supplemental Fig. S1).

For longitudinal analysis, a subset of 707 and 1493 participants had additional repeated measures of urine albumin and serum creatinine, respectively, from subsequent annual occupational exams, which allowed to estimate annual changes in albuminuria and estimated glomerular filtration rate (eGFR) levels. The latest available annual exam is from now on denoted as "follow-up". Albuminuria determinations were discontinued prematurely for logistic reasons unrelated to the study endpoints. Indeed, sociodemographic characteristics comparing the 707 with the 1493 individuals with follow-up endpoints were similar (Supplemental Table S1). The median (interquartile range) time of follow-up was 1.0 (0.9, 1.1) years for albuminuria measures, and 2.2 (1.9, 3.0) years for serum creatinine measures.

The study was approved by the central Institutional Review Board of Aragon. All participants provided written informed consent.

2.2. Renal damage assessment

Albuminuria. All included participants provided urine samples collected at the first voiding urine in the morning, at both baseline and follow-up visits. Urine albumin was measured by automated nephelometric immunochemistry (Behring Institute). Urinary creatinine was quantified to assess urine dilution by the modified kinetic Jaffe method by isotope dilution mass spectrometry. For cross-sectional analyses, we determined albumin-to-creatinine ratio (ACR), expressed as mg/g of creatinine (Levey et al., 2011). Elevated ACR defined as having a ACR above the standard of 30 mg/g was not assessed in our cross-sectional analyses given that only 30 participants had baseline ACR ≥30 mg/g. For longitudinal analyses, we calculated the annual relative change in ACR as the ratio of follow-up to baseline ACR raised to the inverse of the time in years from baseline to follow-up visit. As a secondary endpoint, we further categorized ACR change using the arbitrary cut-off of 1.20 to identify annual increases in ACR ≥20% (no, yes).

Serum creatinine-based eGFR. Serum creatinine was quantified by the modified kinetic Jaffe method to isotope dilution mass spectrometry. The eGFR was calculated based on serum creatinine using the CKD-EPI abbreviated formula and expressed as ml/min/1.73m^2 (Levey et al., 2009). Since only six participants showed baseline eGFR levels below the standard cut-off of 60 ml/min/1.73m^2 for defining reduced eGFR (Levey et al., 2009), we did not include the reduced eGFR categorical endpoint in our cross-sectional analyses. For longitudinal analyses, we calculated the annual change in eGFR as the difference between follow-up and baseline eGFR divided by the years between both visits. As a secondary endpoint, we created a binary endpoint for annual decreases ≥5 ml/min/1.73m^2 (yes, no), which is close to 1 SD deviation in our distribution of annual eGFR change.

2.3. Urine metals determinations

Urine samples were collected in polypropylene tubes, frozen within 1–2 h of collection and stored at <−70 °C in the Occupational Medicine Service Unit, Opel Factory, Figueruelas (Spain). In the AWHS, the concentrations of total As, Ba, Cd, Co, Cr, Cu, Mo, Sb, Ti, U, V, W, Zn, Pb and selenium (Se) in urine were determined using inductively coupled

plasma mass spectrometry (ICP-MS) with dynamic reaction cell at the University of Huelva, Spain. We did not include urine Pb and Se for the analyses because urine is not a well-established biomarker of exposure (Nordberg et al., 2015). Urinary concentrations of As species, including arsenobetaine, were measured using anion exchange high performance liquid chromatography coupled to ICP-MS. The quality control assurance (precision and accuracy) was performed by using a ClinCheck® urine lyophilized material for trace elements analysis at two different levels of concentration (Recipe, Munchen, Germany).

The limits of detection were 0.006 μg/L for most elements, except 0.007 μg/L for U, 0.008 μg/L for W, and 0.001 μg/L for arsenobetaine. The percentages of participants with concentrations below the limit of detection and the corresponding inter batch coefficients of variation are shown in Supplemental Table S2. Urine metal levels below the limit of detection (up to 0.7% of determinations) were imputed as the limit of detection divided by the square root of two following common practice (Hornung and Reed, 1990). While inorganic As is considered toxic for humans, arsenobetaine is a form of organic As found in seafood intake that is non-toxic (Navas-Acien et al., 2011). In populations with substantial seafood intake, such as in the present study (Sotos-Prieto et al., 2015), it is recommended to remove arsenobetaine variability from total As to eliminate the contribution of seafood arsenicals to total and methylated As (Jones et al., 2016). Thus, to assess inorganic As exposure, we estimated total As levels that are independent of arsenobetaine by using a residual-based method (Jones et al., 2016) (from now on just As for simplicity).

2.4. Other variables

Participants underwent an interview, which collected information on age, sex, education, smoking status and medication use, and a physical examination, to measure height, weight and blood pressure by trained staff following standard protocols. Hypertension was defined as a systolic/diastolic blood pressure ≥140/90 mmHg, a self-reported diagnosis or current use of antihypertensive medication. Fasting serum glucose was measured by spectrophotometry. Whole blood glycated hemoglobin was measured by reverse-phase cationic exchange chromatography and quantified by double wave-length colorimetry quantification. Diabetes was defined as a clinical diagnosis of diabetes, fasting serum glucose ≥126 mg/dL, glycated hemoglobin ≥6.5% or current use of glucose-lowering medication.

2.5. Statistical methods

Single metals and renal damage. Metal concentrations were divided by urine creatinine to account for urine dilution and log-transformed to improve normality. We calculated the median and interquartile range of the study endpoints across participant characteristics and categories of urine metal levels. The urinary metal levels across participant characteristics, and their pairwise Spearman correlations are reported in the Supplemental Material. For longitudinal analyses, adjusted geometric mean ratios (GMRs) of annual relative changes in ACR and the mean differences (MDs) in annual eGFR changes comparing the 80th to the 20th percentiles of the metals distribution were estimated from linear regression models. Metals were also modeled as tertile categories to compare the two highest to the lowest tertiles of metals distributions. Adjusted odds ratios (ORs) for annual ACR increase ≥20% and annual eGFR decrease ≥5 ml/min/1.73m^2 were obtained from logistic models. For secondary cross-sectional analyses, the corresponding GMRs of baseline ACR and the MDs in baseline eGFR were estimated from linear regression models. We also assessed non-linear relationships by modelling the metal variables as restricted quadratic splines with knots at the 10th, 50th, and 90th percentiles of their distribution. All models were adjusted for age, sex, education (≤high school, >high school), smoking status (never, former, current), BMI (kg/m^2), diabetes (yes/no), and hypertension (yes/no). In addition, models for ACR-related endpoints were further adjusted for baseline eGFR; longitudinal models for ACR endpoints were further adjusted for baseline ACR; and longitudinal models for eGFR endpoints were additionally adjusted for baseline eGFR.

Joint metals and renal damage. We evaluated the joint association of metal mixtures with the annual change in ACR and eGFR levels (as continuous outcomes) by implementing BKMR with the *bkmr* package in R (Bobb et al., 2015). Given the elevated number of metals included in our study, to have parsimonious BKMR models that facilitate convergence, we first conducted a principal component (PC) and hierarchical cluster analyses to split the evaluated metals into metal mixtures based on shared similarities. Second, for PCs with more than two relevant metals and for each outcome of interest, we introduced all metals within the mixture in a flexible kernel and kept the same adjustment covariates as in main regression models. For each conducted BKMR model, we estimated the Posterior Inclusion Probabilities (PIPs) to quantify the relative importance of each metal for each outcome, as they are a ranking measure to see how much the data favor the inclusion of a variable in the BKMR model. In addition, we also evaluated the dose-response relationships of each metal and the outcomes of interest when the other metals of the mixture were fixed to a given percentile, which enables the identification of potential interactions within the metals mixture (Bobb et al., 2015). For both PC and BKMR analyses, metals were treated as z-score variables to standardize their levels. BKMR was fitted using a Gaussian kernel, which is calculated as $K(z,z') = \exp\{-\sum_{m=1}^{M} r_m(z_m - z'_m)^2\}$, being z and z' predictor vectors for different individuals, and r_m the tuning parameter that control the smoothness of the kernel function (specified with a uniform prior distribution with default values 0 and 100 for the lower and upper bound, respectively). The number of iterations was fixed to 20000.

Sensitivity analyses. First, given the fact that our eGFR change definition does not reflect between-visits fluctuations, we repeated the analysis for annual eGFR change calculated as the slope of all available eGFR measures for each participant. Second, diabetes can increase zinc urinary excretion (Cunningham et al., 1994). We, thus, repeated the association analyses of Zn among non-diabetic participants. Third, we also repeated the analyses modelling the metals in ug/L (i.e, non-creatinine standardized) with separate adjustment for urine creatinine. Fourth, we repeated the analyses adjusting all models by physical activity and by family history of diabetes and hypertension. Moreover, the length of follow-up time was different for each participant. Consequently, we repeated the analyses additionally adjusting for follow-up time. In addition, Zn status in the body can influence Cd absorption and toxicity (Santonen et al., 2015). To address potential residual confounding by zinc status, we evaluated the Cd results in models additionally adjusted for Zn. To compare the BKMR results with results from the traditional linear regression, for statistically significant metals from single-metal linear regression models, we additionally conducted a fully adjusted linear regression model, in which we further adjusted for all other significant metals (i.e. multiple-metal model). Finally, smoking is a source of exposure for some metals (Nordberg et al., 2015) and a well-established cardiovascular risk factor. Thus, for most relevant metals, we assessed potential differential associations in subgroups defined by smoking.

All statistical analyses were performed using the R software (version 3.6.2). The statistical code can be made available upon reasonable request to the corresponding author.

3. Results

Descriptive analysis. Older participants, as well as female participants showed higher levels of ACR and lower eGFR at baseline visit (Table 1). In addition, participants who were current smokers, participants with diabetes or hypertension, and participants with higher urinary levels of most metals, showed higher ACR levels at baseline. Median metal levels,

Table 1
Median (interquartile range) of ACR and eGFR at baseline visit and annual change by participants characteristics and urine metal levels.

	ACR (N = 707)			eGFR (N = 1493)		
	N	Baseline ACR	Annual ACR change	N	Baseline eGFR	Annual eGFR change
Overall	707	3.09 (2.32, 4.48)	0.99 (0.78, 1.24)	1493	86.2 (84.1, 90.1)	0.89 (−1.56, 3.46)
<50 years	232	3.02 (2.25, 4.47)	0.95 (0.77, 1.19)	487	88.7 (86.7, 91.8)	1.04 (−1.33, 3.71)
50–55 years	380	3.05 (2.32, 4.45)	1.01 (0.80, 1.25)	784	86.0 (84.4, 86.6)	0.73 (−1.66, 3.37)
≥55 years	95	3.25 (2.48, 4.68)	0.90 (0.72, 1.25)	222	84.2 (81.4, 86.1)	0.58 (−1.46, 3.65)
Female	39	3.79 (2.81, 4.42)	0.92 (0.67, 1.13)	61	66.5 (64.8, 81.6)	5.74 (0.87, 10.05)
Male	668	3.04 (2.28, 4.47)	0.99 (0.79, 1.24)	1432	86.3 (84.2, 90.1)	0.74 (−1.61, 3.34)
≤High School	431	3.15 (2.32, 4.87)	0.98 (0.78, 1.23)	938	86.3 (84.2, 90.4)	1.11 (−1.41, 3.75)
>High School	276	2.95 (2.32, 4.33)	1.00 (0.78, 1.24)	555	86.1 (83.4, 89.8)	0.50 (−1.81, 3.13)
Never smoking	187	3.03 (2.35, 4.53)	1.01 (0.77, 1.23)	359	86.1 (82.3, 89.5)	0.83 (−1.38, 3.47)
Former smoking	290	2.89 (2.16, 4.31)	0.98 (0.76, 1.24)	648	86.1 (83.9, 90.0)	0.84 (−1.83, 3.37)
Current smoking	230	3.26 (2.47, 4.81)	0.97 (0.80, 1.22)	486	86.5 (84.4, 91.8)	1.04 (−1.45, 3.72)
No Obesity	544	3.02 (2.27, 4.25)	0.99 (0.79, 1.22)	1146	86.3 (84.2, 90.5)	0.86 (−1.57, 3.39)
Obesity	163	3.50 (2.52, 6.01)	0.99 (0.75, 1.28)	347	85.9 (83.3, 88.3)	0.97 (−1.56, 3.91)
No diabetes	656	3.02 (2.25, 4.37)	0.99 (0.78, 1.24)	1371	86.2 (84.0, 90.0)	0.86 (−1.59, 3.41)
Diabetes	51	4.44 (3.07, 7.60)	0.99 (0.77, 1.16)	122	86.2 (84.3, 93.2)	1.22 (−1.26, 3.77)
No HTA	445	2.86 (2.15, 4.08)	1.00 (0.80, 1.24)	903	86.3 (84.2, 90.2)	0.63 (−1.63, 3.40)
HTA	262	3.50 (2.57, 5.65)	0.97 (0.74, 1.22)	590	86.1 (83.9, 89.9)	1.11 (−1.31, 3.59)
Metals[a]						
Co ≤ 0.24 μg/g	318	3.05 (2.29, 4.36)	1.00 (0.79, 1.24)	745	86.2 (83.2, 90.0)	0.89 (−1.47, 3.39)
Co > 0.24 μg/g	389	3.12 (2.33, 4.84)	0.97 (0.77, 1.22)	748	86.3 (84.3, 90.2)	0.88 (−1.61, 3.55)
Cu ≤ 7.0 μg/g	366	3.04 (2.34, 4.32)	0.97 (0.78, 1.18)	752	86.2 (84.1, 90.0)	0.92 (−1.66, 3.39)
Cu > 7.0 μg/g	341	3.15 (2.30, 4.82)	1.00 (0.78, 1.29)	741	86.3 (84.1, 90.5)	0.83 (−1.48, 3.66)
Mo ≤ 18.6 μg/g	324	3.03 (2.23, 4.33)	1.00 (0.81, 1.25)	749	86.1 (82.5, 89.9)	0.97 (−1.52, 3.46)
Mo > 18.6 μg/g	383	3.17 (2.37, 4.66)	0.97 (0.74, 1.22)	744	86.3 (84.2, 90.4)	0.83 (−1.65, 3.46)
Zn ≤ 295 μg/g	368	2.96 (2.27, 4.31)	1.01 (0.79, 1.26)	745	86.1 (83.6, 89.7)	1.16 (−1.33, 3.58)
Zn > 295 μg/g	339	3.20 (2.36, 5.01)	0.97 (0.76, 1.21)	748	86.3 (84.2, 91.3)	0.49 (−1.78, 3.36)
As ≤ 3.1 μg/g	370	3.12 (2.42, 4.51)	0.97 (0.76, 1.20)	748	86.3 (84.2, 89.9)	0.96 (−1.57, 3.39)
As > 3.1 μg/g	337	2.97 (2.16, 4.34)	1.00 (0.81, 1.29)	745	86.2 (83.9, 90.5)	0.70 (−1.56, 3.60)
Ba ≤1.9 μg/g	383	3.03 (2.34, 4.50)	0.99 (0.78, 1.21)	750	86.2 (83.9, 89.7)	0.70 (−1.93, 3.30)
Ba >1.9 μg/g	324	3.11 (2.27, 4.45)	0.98 (0.78, 1.29)	743	86.3 (84.2, 91.5)	1.05 (−1.30, 3.72)
Cd ≤ 0.28 μg/g	338	2.97 (2.17, 4.20)	1.01 (0.80, 1.24)	750	86.2 (83.5, 90.0)	1.03 (−1.41, 3.40)
Cd > 0.28 μg/g	369	3.17 (2.42, 5.06)	0.97 (0.75, 1.22)	743	86.2 (84.2, 90.2)	0.59 (−1.65, 3.57)
Cr ≤ 1.16 μg/g	319	3.04 (2.24, 4.43)	0.98 (0.78, 1.21)	746	86.2 (83.3, 90.0)	0.90 (−1.41, 3.38)
Cr > 1.16 μg/g	388	3.11 (2.34, 4.63)	0.99 (0.78, 1.25)	747	86.2 (84.2, 90.2)	0.89 (−1.76, 3.68)
Sb ≤ 52.5 ng/g	336	3.09 (2.35, 4.39)	0.97 (0.79, 1.19)	747	86.1 (82.1, 89.9)	0.81 (−1.38, 3.54)
Sb > 52.5 ng/g	371	3.06 (2.26, 4.56)	1.00 (0.76, 1.29)	746	86.3 (84.4, 90.5)	0.92 (−1.69, 3.42)
Ti ≤ 9.7 μg/g	371	2.87 (2.16, 4.27)	1.00 (0.78, 1.25)	747	86.2 (83.4, 89.7)	0.70 (−1.86, 3.30)

(*continued on next page*)

Table 1 (*continued*)

	ACR (N = 707)			eGFR (N = 1493)		
	N	Baseline ACR	Annual ACR change	N	Baseline eGFR	Annual eGFR change
Ti > 9.7 μg/g	336	3.31 (2.44, 4.74)	0.97 (0.79, 1.20)	746	86.3 (84.3, 91.6)	1.06 (−1.39, 3.75)
U ≤ 27.2 ng/g	347	3.04 (2.22, 4.43)	1.00 (0.78, 1.24)	747	86.2 (83.4, 90.0)	0.83 (−1.80, 3.39)
U > 27.2 ng/g	360	3.12 (2.36, 4.55)	0.98 (0.79, 1.21)	746	86.2 (84.2, 90.2)	0.96 (−1.49, 3.58)
V ≤ 0.66 μg/g	374	2.94 (2.23, 4.42)	0.99 (0.79, 1.22)	752	86.3 (84.2, 90.1)	0.62 (−1.69, 3.39)
V > 0.66 μg/g	333	3.18 (2.38, 4.71)	0.97 (0.77, 1.25)	741	86.1 (83.9, 90.1)	1.04 (−1.37, 3.64)
W ≤ 0.22 μg/g	349	2.96 (2.27, 4.32)	0.99 (0.79, 1.22)	746	86.2 (83.9, 90.0)	0.86 (−1.56, 3.39)
W > 0.22 μg/g	358	3.19 (2.34, 4.83)	0.99 (0.77, 1.25)	747	86.2 (84.1, 90.2)	0.90 (−1.57, 3.60)

Abbreviations: BMI, body mass index; HTA, hypertension; eGFR, estimated glomerular filtration rate.
[a] Metals categorized below and above the median levels from the sample of 1519 participants with complete baseline information.

Table 2
Geometric mean ratio (95% confidence interval) of annual relative ACR change by urinary metal levels in adult participants from the Aragon Workers Health Study (N = 707).

	Tertile 1	Tertile 2	Tertile 3	$p80^{th}$ vs $p20^{th}$	p-value
Essential metals					
Co	1.00 (reference)	1.06 (0.97, 1.17)	1.04 (0.95, 1.14)	1.05 (0.99, 1.12)	0.08
Cu	1.00 (reference)	0.95 (0.87, 1.05)	1.06 (0.96, 1.16)	1.03 (0.97, 1.09)	0.30
Mo	1.00 (reference)	0.91 (0.83, 1.00)	0.94 (0.86, 1.03)	0.96 (0.90, 1.02)	0.17
Zn	1.00 (reference)	0.91 (0.83, 0.99)	0.95 (0.87, 1.05)	1.00 (0.95, 1.05)	0.97
Non-essential metals					
As	1.00 (reference)	1.06 (0.97, 1.16)	1.13 (1.03, 1.25)	1.15 (1.04, 1.28)[a]	0.008
Ba	1.00 (reference)	0.99 (0.91, 1.09)	1.06 (0.97, 1.16)	1.02 (0.97, 1.08)	0.43
Cd	1.00 (reference)	1.02 (0.92, 1.11)	0.99 (0.90, 1.09)	1.01 (0.96, 1.06)	0.69
Cr	1.00 (reference)	0.98 (0.90, 1.08)	1.08 (0.99, 1.18)	1.07 (1.01, 1.13)	0.02
Sb	1.00 (reference)	1.08 (0.98, 1.18)	1.02 (0.93, 1.12)	1.02 (0.96, 1.09)	0.48
Ti	1.00 (reference)	1.00 (0.91, 1.09)	1.01 (0.92, 1.11)	1.00 (0.94, 1.06)	0.92
U	1.00 (reference)	0.95 (0.87, 1.04)	1.04 (0.95, 1.14)	1.03 (0.97, 1.09)	0.29
V	1.00 (reference)	0.96 (0.88, 1.05)	1.00 (0.92, 1.10)	0.99 (0.94, 1.05)	0.83
W	1.00 (reference)	0.99 (0.91, 1.09)	1.07 (0.98, 1.17)	1.07 (1.01, 1.13)	0.02

Abbreviations: CI, confidence interval; ACR, albumin-to-creatinine ratio.Models were adjusted for age (years), sex (male, female), education (≤high school, >high school), smoking status (never, former, current), body mass index (kg/m^2), diabetes status (no, yes), hypertension status (no, yes) and baseline eGFR levels.
The 80th and 20th percentiles for essential and non-essential metals (μg/g, except for Sb and U that are ng/g) were 0.43 and 0.15 for Co; 11.25 and 4.5 for Cu; 34.9 and 9.04 for Mo; 473 and 186 for Zn; 4.81 and 2.20 for As; 3.65 and 0.98 for Ba; 0.46 and 0.17 for Cd; 2.02 and 0.77 for Cr; 118.7 and 29.1 for Sb; 15.34 and 5.82 for Ti; 46.0 and 16.3 for U; 1.05 and 0.42 for V; and 0.42 and 0.12 for W.
The tertile cut-off for essential and non-essential metals (μg/g, except for Sb and U that are ng/g) were: 0.18 and 0.31 for Co; 5.7 and 8.9 for Cu; 12.8 and 26.0 for Mo; 256 and 374 for Zn; 2.57 and 3.80 for As; 1.39 and 2.71 for Ba; 0.22 and 0.35 for Cd; 0.93 and 1.52 for Cr; 37.2 and 77.3 for Sb; 7.7 and 12.4 for Ti; 20.9 and 35.5 for U; 0.52 and 0.83 for V; and 0.16 and 0.30 for W.
[a] Metals introduced as restricted quadratic splines with knots at percentiles 10th, 50th, and 90th because of nonlinear relationships.

in μg/g unless otherwise stated, were 0.24, 7.0, 18.6, 295, 3.1, 1.9, 0.28, 1.16, 52.5 (ng/g), 9.7, 27.2 (ng/g), 0.66 and 0.22, for Co, Cu, Mo, Zn, As, Ba, Cd, Cr, Sb, Ti, U, V and W, respectively (Supplemental Table S3). Participants older than 55 years had higher levels of Cu, Zn, As, Ba, Cd, Ti, U, V and W compared to participants younger than 50 years. Ever smokers had higher levels of Zn, As, Ba and Cd. Obese participants had higher levels of Zn and Ti but lower Ba and U. Co and Cd showed the strongest positive correlation (r spearman = 0.53) (Supplemental Table S4), while Cd and U showed the strongest negative correlation (r spearman = −0.19).

Single metals and longitudinal renal endpoints. The GMR (95% CI) of ACR change comparing the 80th to the 20th percentile was 1.15 (1.04, 1.28) for As, 1.07 (1.01, 1.13) for Cr and 1.07 (1.01, 1.13) for W (Table 2) (i.e., a 15%, 7% and 7%, respectively, higher annual increase in ACR). For eGFR, the estimated MD (95% CI) of eGFR annual change ($ml/min/1.73m^2$) comparing the 80th to the 20th percentile was −0.31 (−0.61, −0.01) for Zn, −0.35 (−0.70, 0.00) for As and 0.38 (0.09, 0.67) for Ba (Table 3). Fig. 1 graphically showed strongly supportive

Table 3
Mean difference (95% confidence interval) of annual absolute eGFR change (ml/min/1.73m^2) by urinary metal levels in adult participants from the Aragon Workers Health Study (N = 1493).

	Tertile 1	Tertile 2	Tertile 3	p80th vs p20th	p-value
Essential metals					
Co	1.00 (reference)	0.22 (−0.27, 0.72)	−0.11 (−0.61, 0.40)	−0.22 (−0.53, 0.10)	0.17
Cu	1.00 (reference)	−0.00 (−0.50, 0.50)	0.14 (−0.36, 0.65)	0.03 (−0.27, 0.34)	0.82
Mo	1.00 (reference)	−0.12 (−0.62, 0.37)	−0.10 (−0.60, 0.40)	0.06 (−0.22, 0.34)	0.66
Zn	1.00 (reference)	−0.23 (−0.73, 0.27)	−0.62 (−1.12, −0.12)	−0.31 (−0.61, −0.01)	0.05
Non-essential metals					
As	1.00 (reference)	−0.62 (−1.12, −0.12)	−0.59 (−1.10, −0.08)	−0.35 (−0.70, 0.00)	0.05
Ba	1.00 (reference)	0.44 (−0.05, 0.94)	0.72 (0.23, 1.22)	0.38 (0.09, 0.67)	0.01
Cd	1.00 (reference)	0.28 (−0.22, 0.78)	0.09 (−0.43, 0.60)	−0.02 (−0.27, 0.24)	0.83
Cr	1.00 (reference)	0.16 (−0.34, 0.66)	−0.26 (−0.76, 0.24)	−0.19 (−0.48, 0.10)	0.19
Sb	1.00 (reference)	0.26 (−0.23, 0.76)	−0.21 (−0.71, 0.29)	−0.17 (−0.51, 0.17)	0.34
Ti	1.00 (reference)	0.16 (−0.34, 0.66)	0.47 (−0.04, 0.97)	0.19 (−0.15, 0.52)	0.27
U	1.00 (reference)	−0.28 (−0.78, 0.22)	−0.09 (−0.60, 0.42)	−0.13 (−0.43, 0.18)	0.56
V	1.00 (reference)	0.10 (−0.40, 0.60)	0.23 (−0.27, 0.73)	0.03 (−0.28, 0.34)	0.84
W	1.00 (reference)	0.24 (−0.26, 0.74)	0.06 (−0.44, 0.56)	−0.02 (−0.32, 0.29)	0.88

Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate.
Models were adjusted for age (years), sex (male, female), education (≤high school, >high school), smoking status (never, former, current), body mass index (kg/m^2), diabetes status (no, yes), and hypertension status (no, yes).
The 80th and 20th percentiles for essential and non-essential metals (μg/g, except for Sb and U that are ng/g) were 0.43 and 0.15 for Co; 11.25 and 4.5 for Cu; 34.9 and 9.04 for Mo; 473 and 186 for Zn; 4.81 and 2.20 for As; 3.65 and 0.98 for Ba; 0.46 and 0.17 for Cd; 2.02 and 0.77 for Cr; 118.7 and 29.1 for Sb; 15.34 and 5.82 for Ti; 46.0 and 16.3 for U; 1.05 and 0.42 for V; and 0.42 and 0.12 for W.
The tertile cut-off for essential and non-essential metals (μg/g, except for Sb and U that are ng/g) were: 0.18 and 0.31 for Co; 5.7 and 8.9 for Cu; 12.8 and 26.0 for Mo; 256 and 374 for Zn; 2.57 and 3.80 for As; 1.39 and 2.71 for Ba; 0.22 and 0.35 for Cd; 0.93 and 1.52 for Cr; 37.2 and 77.3 for Sb; 7.7 and 12.4 for Ti; 20.9 and 35.5 for U; 0.52 and 0.83 for V; and 0.16 and 0.30 for W.

associations specially for As, Cr and W with increasing ACR annual change, and for Zn, As, and Cr with decreasing eGFR change at the higher metal exposure range (i.e. the confidence intervals mostly did not include the null value). The dose-response shape for all of the evaluated metals, however, was generally compatible with a nephrotoxic role of metals both in ACR and eGFR annual change models. The associations of Zn, As, and Cr with increased ACR and decreased eGFR were also confirmed in complementary analysis with the categorized endpoints for annual ACR increase ≥20% and eGFR decrease ≥5 ml/min/1.73m^2 () (Supplemental Table S5). In secondary cross-sectional analysis, increased urinary Co, Cu, Zn, Cd, Cr and W levels were associated with higher baseline ACR (Supplemental Table S6).

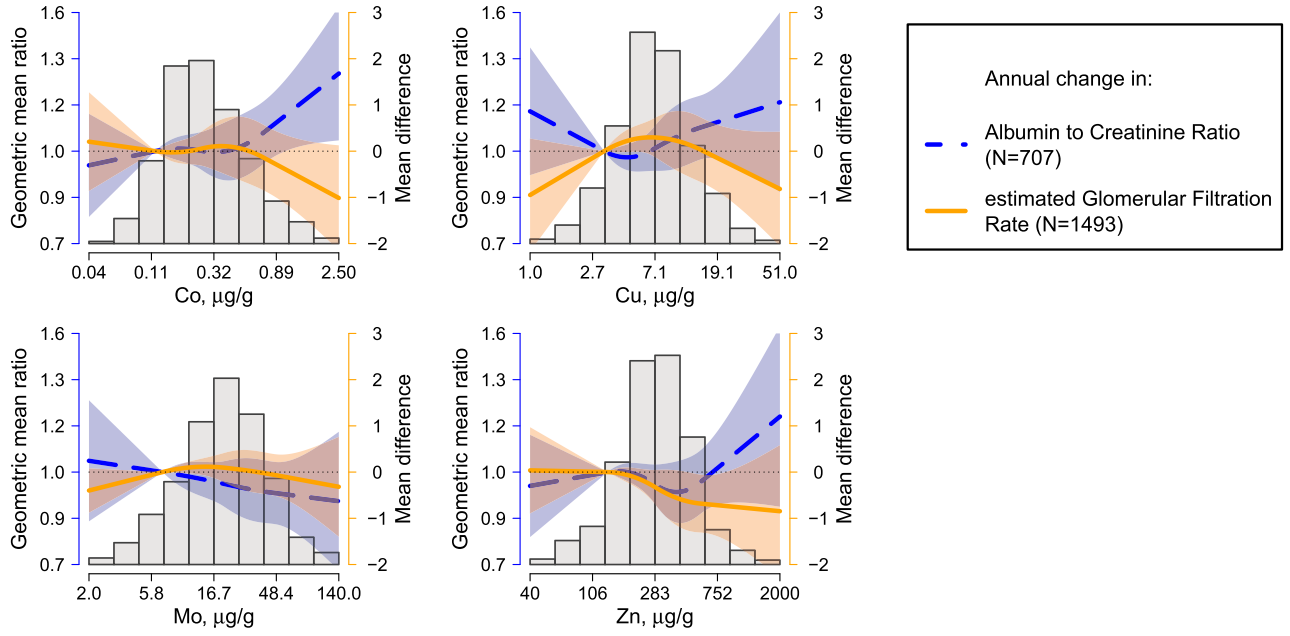
Joint metals and longitudinal renal endpoints. We first grouped the metals into mixtures by implementing PC and hierarchical cluster analysis. The first mixture included Cu, Zn, As, Ba, Ti, U, V and W (named PC1 from now on), the second mixture included Co, Cd, Cr, Sb, V and W (named PC2 from now on) (Supplemental Fig. S2). Fig. 2 shows a positive dose-response shape for both PC1 and PC2 mixtures as a whole with the change in ACR excretion, while the dose-response shape with eGFR change was inverse, suggesting that higher levels of PC1 and PC2 mixtures are related with higher ACR excretion and with decreased eGFR. For ACR change models, the highest metal-specific posterior inclusion probabilities (PIPs), which help to identify the most important metals within each mixture in relation to each outcome, were observed for As (PIP = 0.55) within PC1 and for Cr (PIP = 0.61) within PC2 (Table 4). For eGFR change, while the highest PIPs were observed for Zn (PIP = 0.71) within PC1 and for Cr (PIP = 0.74) within PC2, the PIPs for the other PC2 metals were also substantial. Finally, for the ACR change associations we visually identified potential interactions between As–V, Zn–Ba and Zn–Cu in BKMR models with PC1 metals (Supplemental Fig. S3), and between Cd–Sb, W–Sb, W–Cd and W–Co in BKMR models with PC2 metals (Supplemental Fig. S4). We did not identify interactions between metals in BKMR models for eGFR change (Supplemental Figs. S5 and S6).

Sensitivity analyses. The findings for annual eGFR change calculated as the slope of all available eGFR measures for each individual were generally consistent with the main results, but with somewhat attenuated associations for some metals (Supplemental Table S7). The findings for Zn were essentially identical in analysis restricted to participants without diabetes (data not shown). We also observed consistent results when modelling the metals in ug/L with separate adjustment for urine creatinine, and in models further adjusting for length of follow-up, physical activity, or family history of diabetes and hypertension (data not shown). In multiple-metal models for ACR change (GMR [95% CI]), the association of As became slightly stronger (1.19 [1.05, 1.34]), while for Cr and W became slightly attenuated (1.06 [0.99, 1.14] and 1.05 [0.99, 1.12], respectively). In multiple-metal models for eGFR change (MD [95% CI] ml/min/1.73m^2), the association of Zn and As became slightly stronger (−0.39 [−0.74, −0.05)] and −0.58 [−1.04, −0.12], respectively). These results are consistent with BKMR results. In subgroup analysis we did not observe statistically significant interactions by smoking (Supplemental Table S8).

4. Discussion

Our longitudinal results showed that increasing exposures to Zn, As,

Essential Metals



Non−essential Metals

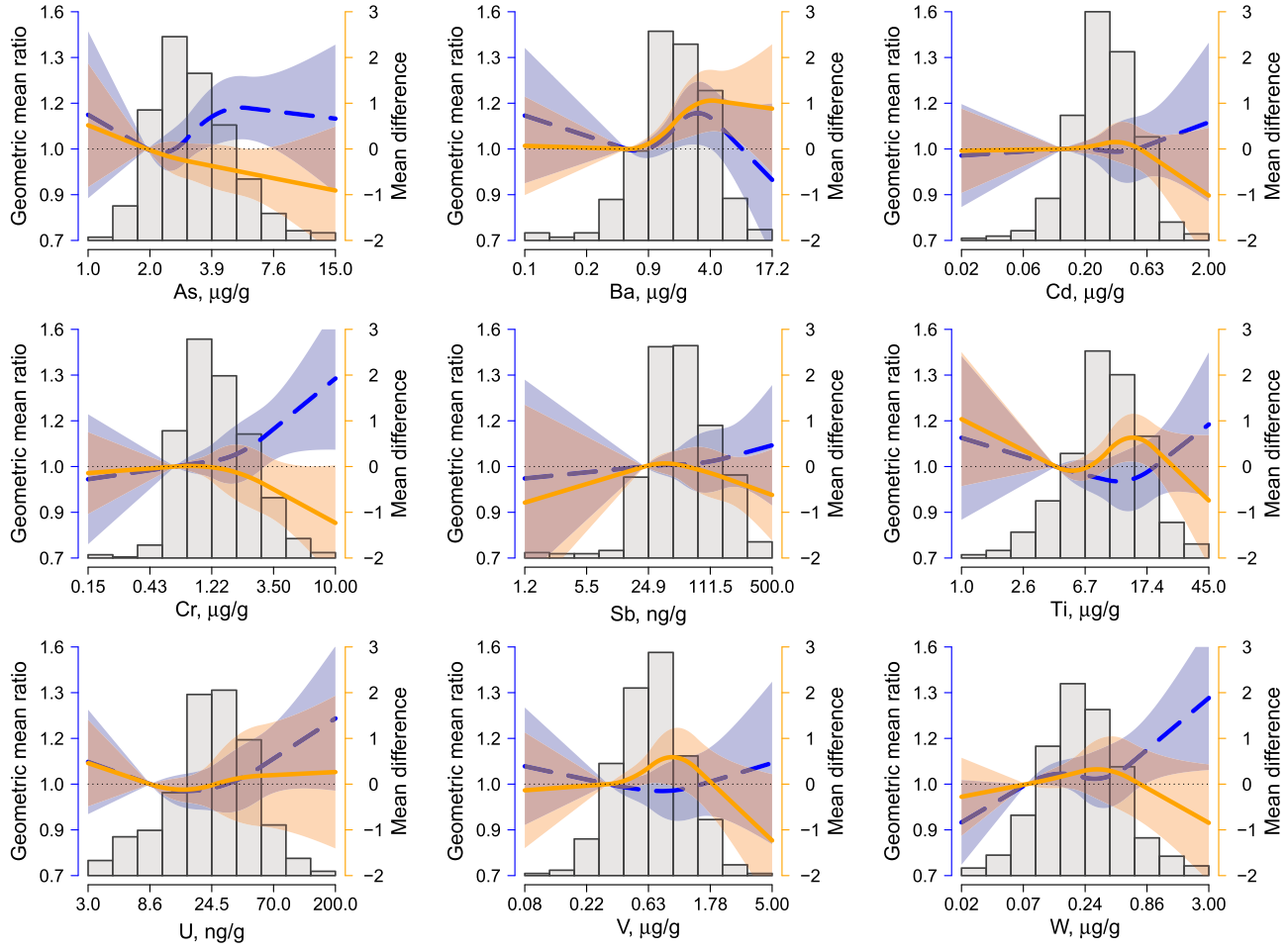


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Fig. 1. Flexible dose-response of urine metals with longitudinal changes in ACR (N = 707) and eGFR (N = 1493) levels in adult participants from the Aragon Workers Health Study.
Lines represent the adjusted geometric mean ratio of annual relative changes in ACR (dashed blue) and the mean difference of annual absolute changes in eGFR (solid orange) based on restricted quadratic splines for log-transformed metals distribution with knots at 10th, 50th and 90th percentiles. The shaded areas represent the corresponding 95% confidence intervals. The reference value was set at 10th percentile of each metal distribution. Models were adjusted for age (years), sex (male, female), education (≤high school, >high school), smoking status (never, former, current), body mass index (kg/m^2), diabetes status (no, yes), hypertension status (no, yes) and baseline eGFR (ml/min/1.73 m^2). Models for annual change in ACR levels were further adjusted for baseline ACR. Histograms in the background represent the distribution of each metal. The 10th, 50th and 90th percentiles (μg/g, except for Sb and U that are ng/g) for metals were 0.12, 0.24 and 0.64 for Co; 3.46, 6.99 and 15.3 for Cu; 5.87, 18.6 and 48.6 for Mo; 145, 295 and 596 for Zn; 1.90, 3.08 and 5.96 for As; 0.67, 1.93 and 4.81 for Ba; 0.12, 0.28 and 0.59 for Cd; 0.63, 1.16 and 2.81 for Cr; 21.8, 52.5 and 173.3 for Sb; 4.21, 9.74 and 19.2 for Ti; 11.7, 27.2 and 62.2 for U; 0.34, 0.66 and 1.36 for V; and 0.09, 0.22 and 0.64 for W. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

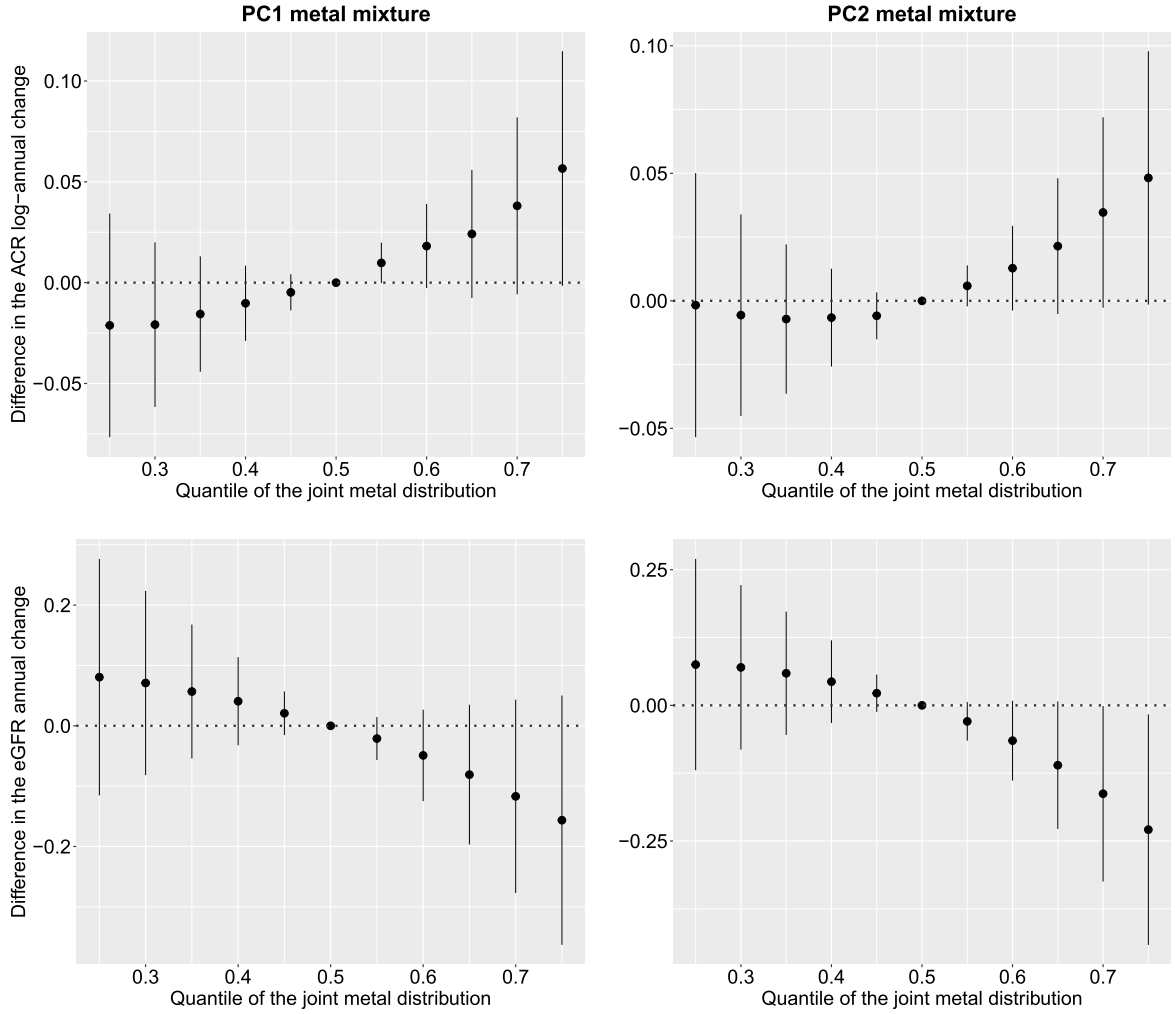

Fig. 2. Estimates and 95% credibility intervals of the PC1 (left panels) and PC2 (right panel) metal mixtures with annual change in ACR (N = 707) and annual change in eGFR levels (N = 1493) when all metals are set at a given percentile compared to all metals set at their 50th percentile.
The dots are the difference in the in the log-annual change for ACR models (upper panels), and the difference in the annual-change for eGFR models (lower panels). Segments represent the 95% credibility intervals. BKMR models were adjusted for age (years), sex (male, female), education (≤high school, >high school), smoking status (never, former, current), body mass index (kg/m^2), diabetes status (no, yes), hypertension status (no, yes) and baseline eGFR (ml/min/1.73m^2). BKMR models for annual relative change in ACR were also adjusted for baseline ACR levels (mg/g).

Cr, and W are associated with increased ACR and decreased eGFR changes over time. The shape of the longitudinal dose-responses, however, was compatible with a nephrotoxic role for all of the evaluated metals, both in ACR and eGFR models. In joint metal exposure analysis, the associations with ACR were mostly driven by As and Cr. For eGFR, while the associations were mostly driven by Zn and Cr, the contribution of other metals was also relevant. We identified some potential interactions for As, Zn and W by other metals for ACR change, but not for eGFR change.

Urine metal biomarkers. Urine is a well-established biosample to evaluate metal exposure, which integrates exposure sources including air, water and food (Santonen et al., 2015). The metals evaluated in this study have relatively short half-lives in urine, except for Cd, which reflects Cd accumulation in the kidney (Nordberg et al., 2015; Roels et al., 1999). Under chronically maintained exposure, urine biomarkers could also be a proxy of long-term exposure (Navas-Acien et al., 2009a). In secondary cross-sectional analyses, higher exposure to Zn, As, Cr, Cd and W were associated with higher ACR at baseline, but not with lower

Table 4
Posterior Inclusion Probabilities in the BKMR models.

	Annual relative change in ACR (N = 707)	Annual absolute change in eGFR (N = 1493)
PC1 metals		
Cu	0.21	0.57
Zn	0.19	**0.71**
As	**0.55**	0.62
Ba	0.16	0.61
Ti	0.21	0.51
U	0.21	0.55
V	0.23	0.58
W	0.32	0.58
PC2 metals		
Co	0.51	0.67
Cd	0.40	0.54
Cr	**0.61**	**0.74**
Sb	0.38	0.56
V	0.42	0.61
W	0.59	0.60

Models adjusted for age, sex, education (≤high school, >high school), smoking status (never, former, current), body mass index, diabetes status (no, yes), hypertension status (no, yes) and baseline eGFR levels (ml/min/1.73m^2). The posterior inclusion probabilities (PIP) obtained from the BKMR quantify the relative importance of each exposure in the model, as they are a ranking measure to see how much the data favor the inclusion of a variable in the model. The highest PIPs within each mixture are shown in bold.

eGFR. However, the use of urine metal determinations to assess cross-sectional associations with eGFR is controversial (Jin et al., 2018; Weidemann et al., 2015). For instance, despite the known nephrotoxic effects of As, Cd and Pb, in several cross-sectional studies urinary levels of these metals have been associated with higher eGFR (Weidemann et al., 2015; Buser et al., 2016; Lee et al., 2020). This could be compatible with reverse causation bias, where decreased renal function might impair metal and creatinine excretion through the kidney, partly resulting in lower urine metal concentrations unrelated to exposure under prevalent renal damage (Zheng et al., 2015). Therefore, cross-sectional associations of urine metal levels with serum creatinine-based eGFR should be taken cautiously (Zheng et al., 2015).

Zinc. Mechanistic research supports that both extremely low and high Zn exposure levels are associated with renal injury (Yanagisawa et al., 1998; Kurihara et al., 2002; Paun et al., 2012). Zn deficiency might induce renal disease by increasing oxidative stress and apoptosis, and by decreasing nephron quantity and glomerular filtration surface (Tomat et al., 2011), while excessive Zn exposure might result in functional changes in the kidney by inducing Cu deficiency (Paun et al., 2012; Saari et al., 1990). In epidemiologic, mostly cross-sectional, studies, both Zn deficiency (Prasad, 2003) and excess (Damianaki et al., 2020) has been related with adverse renal health conditions. In our longitudinal analysis, higher Zn was associated with lower eGFR but not with higher ACR. Overall, the evidence does not support supra-optimal Zn exposure nor long-term Zn supplementation in Zn-repleted populations for renal disease prevention.

Arsenic. Arsenic toxicity in the proximal tubule is related to increased ROS production, inflammation and apoptosis, potentially leading to direct podocyte injury (Robles-Osorio et al., 2015). Other studies in humans suggested a role of miRNAs dysregulation in arsenic-related urine albumin excretion (Kong et al., 2012). A systematic review of epidemiologic studies concluded that increasing urinary As was cross-sectionally associated with increased ACR and proteinuria (Zheng, 2014). Most recent studies reported null associations for urinary As with abnormal ACR in Chinese adults (mean = 69.5 μg/g, N = 336) (Jin et al., 2020), and in NHANES 1999–2016 (N = 46748) (Lee et al., 2020). The evidence for eGFR-based endpoints, however, was less clear (Zheng, 2014). Urine As (assessed as the sum of inorganic and methylated arsenic species) was prospectively associated with increased risk of incident CKD among American Indian adults from the Strong Heart Study (median = 9.7 μg/g, N = 3119) (Zheng et al., 2015). The longitudinal association of As with eGFR decline in our data is novel, since it has not been reported before.

Chromium. While little is known about the nephrotoxicity of Cr in humans, studies in rats showed that acute and chronic Cr exposure might lead to apoptosis, tubular necrosis and proximal tubule damage (Zheng et al., 2020; Wedeen and Qian, 1991). Increased urinary Cr was associated with lower eGFR in the National Nutrition and Health Survey in Taiwan (mean urine Cr = 0.83 μg/L, N = 360) (Tsai et al., 2017) and in the Changhua county (urine Cr levels not reported, N = 1328) (Yuan, 2021), but not in Southern Taiwan (median urine Cr = 0.1 μg/L, N = 2447). While no studies have evaluated the prospective association of urine Cr with renal outcomes before, our longitudinal results showed a strongly suggestive relationship between higher urine Cr and eGFR decline. In addition, we observed a novel association of Cr with increased ACR.

Tungsten. We also observed and association of W with increased ACR change. For the general population, exposure to W comes from air, food ingestion and drinking water and it is expected to be very low (van der Voet et al., 2007). Higher exposure can occur for workers involved in manufacturing processes (Agency for Toxic Substances and Disease Registry (ATSDR), 2005). In the US, increased urinary W was related with higher eGFR in NHANES cross-sectionally (Jin et al., 2018), but with decreased time-to-CKD development in rural Colorado prospectively (Fox et al., 2021).

Other metals. In our study, increased urine Cu (median = 10.3 μg/L) was associated with higher ACR in cross-sectional analysis, and with higher odds of ACR increase ≥20%. Cu is an essential nutrient needed for proper renal function (Palvelková, M., et al., 2018). Indeed, studies suggest that Cu–Zn imbalance induces tubular damage (Palvelková, M., et al., 2018; Nogawa et al., 1984; Eom et al., 2020). Alternatively, the potential toxicity of Cu excess is receiving increasing attention (Stern, 2010). For instance, higher urine Cu levels have been cross-sectionally associated with proteinuria and low eGFR in Taiwan (median = 1.5 μg/L, N = 2447) (Tsai, 2021), and with low eGFR in China (for Cu levels >20.96 μg/L, N = 3553) (Yang et al., 2019). Lastly, experimental and epidemiological evidence supports that kidney injury is a major effect of high Cd exposure (Agency for Toxic Substances and Disease Registry (ATSDR), 2012). Consistently, increased urine Cd levels were associated with higher ACR in our cross-sectional analysis, and also in other studies from the US (Buser et al., 2016; Ferraro et al., 2010), China (Zhang et al., 2015), and Spain (Grau-Perez, 2017). While we did not find a statistically significant association for Cd with longitudinal ACR or eGFR change, the longitudinal dose-response in our data was compatible a nephrotoxic role of cadmium for the evaluated renal endpoints. Overall, more longitudinal studies that evaluate the change in metal-related renal endpoints are needed, especially in populations exposed to low exposure levels (Byber et al., 2016).

Metal mixtures. Only one cross-sectional study evaluated the association of metal co-exposures with renal disease applying BKMR methods (Luo and Hendryx, 2020). In that study, the Co–Cd–Hg–Pb mixture was associated with both higher ACR and lower eGFR, and Pb, Co and Cd drove the association for ACR, while Pb drove the association for eGFR (Luo and Hendryx, 2020). In other cross-sectional studies applying less flexible methodologies found significant associations for Cd–Pb co-exposure with higher ACR and decreased eGFR (Navas-Acien et al., 2009b), for Cd–Cr–Pb mixture with decreased eGFR (Tsai et al., 2017), and for As–Cd–Hg–Pb with higher ACR but not with decreased eGFR (Sanders et al., 2019). These results are in line with our finding that PC2 mixture, which includes Cd, Cr and Co, was associated with increased ACR and decreased eGFR longitudinal changes. However, previous metal mixture findings are not completely comparable with our results, given their cross-sectional nature, and the fact that other available metals and metal biomarkers were measured in those studies. Nevertheless, our findings add novel evidence about metal co-exposure and renal marker changes over time and identify some potential interactions

between metals, which are supported by mechanistic studies, especially for Zn–Cu (Harris, 2001), Zn–Ba (Awatef et al., 2017), and W–Co (van der Voet et al., 2007).

Strengths and limitations. Our study has several limitations. Because of the small number of women in our sample, we could not evaluate potential interactions by sex. Given the paucity of studies with available metals and repeated measurements of renal damage biomarkers, additional longitudinal studies, including men and women with low metal exposure, are needed to confirm our findings. We used a single urine sample for assessing metal exposure, which might be subject to non-differential physiological fluctuation in individuals and could have attenuated the associations. Also, since we did not have available biomarkers of tubular damage in the AWHS imaging sub-cohort, we cannot discard that our ACR results may partly reflect a dysfunction of albumin reabsorption in the proximal tubules in addition to a disorder in the glomerular filtration barrier. While we adjusted for many relevant factors known to influence renal function, we cannot completely discard the presence of residual confounding by other unmeasured factors, such as specific drugs use and comorbidities. Also, the sample size in this study was moderate, which may compromise power, especially in the setting of multiple-comparison correction of statistical significance threshold. Nonetheless, the associations were widely consistent in BKMR analysis, which assessed all metals at a time and is less susceptible to the multiple testing problem, thus providing robustness to our main results. While BKMR is considered as one of the most advanced methods for evaluating correlated environmental chemicals on health (Gibson et al., 2019), it also has its own limitations. For instance, BKMR is computationally intensive. Other methods not implemented in our study, such as weighted quantile sum regression or quantile g-computation, may offer less computationally intensive solutions to estimate mixture effects. Finally, human studies suggested the role of oxidative stress (Domingo-Relloso et al., 2019), metabolomics (Galvez-Fernandez et al., 2022a; Galvez-Fernandez et al., 2022b), genetic variation in specific genes (Galan-Chilet, 2017; Grau-Perez, 2017; Galvez-Fernandez et al., 2022a; Grau-Perez et al., 2018), epigenetics (Domingo-Relloso et al., 2022), and miRNAs and transcription factors (Nguyen and Kim, 2022a; Nguyen and Kim, 2022b; Nguyen and Kim, 2022c; Nguyen and Kim, 2022d; Nguyen, 2023) as potential mechanisms for metal-related health endpoints. While we could not evaluate molecular mechanisms potentially explaining our findings because the required data were not currently available in our study population, future mechanistic evaluation of key molecular pathways for renal disease based on omics data are guaranteed. Our study has also other strengths in addition to the longitudinal design, the standardized protocols and quality control of the AWHS data collection methods and the use of state-of-the-art statistical methods to comprehensively evaluate mixtures. For instance, the relatively healthy mid-age study population -up to 57 years old-, which makes our results relevant and, potentially, with substantial public health implications.

5. Conclusions

In conclusion, we identified Zn, As, Cr, W, and suggestively other metals, as potential risk factors of renal disease at relatively low exposure levels. While additional longitudinal studies, including men and women, and mechanistic studies evaluating the potential molecular pathways involved in metal-related renal disease are needed, our results support that intensified policies to reduce environmental exposure to metals may improve renal disease prevention and control at exposure levels that are relevant for general populations.

Author statement

M. Tellez-Plaza, M. Grau-Perez, R. Pastor-Barriuso and J. Redon conceptualized the study; M. Leon-Latre, J.A. Casasnovas, B. Moreno-Franco, M. Laclaustra-Gimeno, E. Guallar and M. Tellez-Plaza were actively involved in the data generation. T. Garcia-Barrera and JL Gomez-Ariza conducted the laboratory metal analyses; M. Grau-Perez performed the data curation; M. Grau-Perez and A. Domingo-Relloso conducted the statistical analyses; All authors contributed to the interpretation of the results; M. Grau-Perez, R. Pastor-Barriuso, J. Redon and M. Tellez-Plaza wrote the original draft; M. Grau-Perez, A. Domingo-Relloso, A. Navas-Acien, R. Pastor-Barriuso, J. Redon and M. Tellez-Plaza reviewed and edited the initial draft manuscript. All authors reviewed, provided feedback and approved the final version of the manuscript.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Unrestricted data sharing is not allowed. The data that support the findings of this study are, however, available from the corresponding author upon reasonable request by qualified researchers trained in human subject confidentiality protocols.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.120851.

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