1 **Association of single and joint metals with albuminuria and estimated glomerular filtration**

2 **longitudinal change in middle-aged adults from Spain: the Aragon Workers Health Study**

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31 **Running title**: Environmental metals and longitudinal renal markers change

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 changes 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. Exposure reduction and mitigation interventions of metals may improve prevention and decrease the burden of renal disease in the population.

INTRODUCTION

The kidney is a target organ of acute metal toxicity because of its ability to filter, reabsorb and accumulate divalent metals.[1] In general, this toxicity is related to altered glomerular (endothelium and podocytes), and tubular (proximal or distal tubules and the Henle loop structures) damage.[1] There is a solid body of evidence supporting that high exposures to metals such as arsenic (As), cadmium (Cd) and lead (Pb) are nephrotoxic.[2–5] In particular, high exposure to As has been related to proteinuria and albuminuria,[3] and high exposure to Cd and Pb has been related to lower estimated glomerular filtration rate (eGFR) and other renal biomarkers.[5] 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.[6]

Metals are naturally present in the ground water and earth's crust. Increased metal extraction after the industrial revolution resulted in substantial environmental pollution.[7,8] While a recent decline in exposure has been documented for some metals,[9,10] the general population remains exposed to metals through the air, drinking water, diet and smoking.[8] Exposure to non-essential metals has been associated with an extensive list of adverse health conditions, including cancer, cardiovascular disease, cognitive outcomes and mortality.[11,12] Alternatively, while essential metals deficiency has been related to several diseases, including renal damage,[13] essential metals excess has also been related with adverse health effects.[14,15]

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 exposure correlations.[16]

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. As secondary analyses, we also evaluated the cross-sectional association of these metals with renal damage markers.[16]

METHODS

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.[17] 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 to 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 Figure 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.

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.[18] 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).

127   *Serum creatinine-based eGFR.* Serum creatinine was quantified by the modified kinetic Jaffe

128 method to isotope dilution mass spectrometry. The eGFR was calculated based on serum creatinine

129 using the CKD-EPI abbreviated formula and expressed as ml/min/1.73m^2.[19] Since only six

130 participants showed baseline eGFR levels below the standard cut-off of 60 ml/min/1.73m^2 for

131 defining reduced eGFR,[19] we did not include the reduced eGFR categorical endpoint in our cross-

132 sectional analyses. For longitudinal analyses, we calculated the annual change in eGFR as the

133 difference between follow-up and baseline eGFR divided by the years between both visits. As a

134 secondary endpoint, we created a binary endpoint for annual decreases $\geq$5 ml/min/1.73m^2 (yes, no),

135 which is close to 1 SD deviation in our distribution of annual eGFR change.

136 **Urine metals determinations**

137   Urine samples were collected in polypropylene tubes, frozen within 1 to 2 hours of collection

138 and stored at <−70ºC in the Occupational Medicine Service Unit, Opel Factory, Figueruelas (Spain).

139 In the AWHS, the concentrations of total As, Ba, Cd, Co, Cr, Cu, Mo, Sb, Ti, U, V, W, Zn, Pb and

140 selenium (Se) in urine were determined using inductively coupled plasma mass spectrometry (ICP-

141 MS) with dynamic reaction cell at the University of Huelva, Spain. We did not include urine Pb and

142 Se for the analyses because urine is not a well-established biomarker of exposure.[8] Urinary

143 concentrations of As species, including arsenobetaine, were measured using anion exchange high

144 performance liquid chromatography coupled to ICP-MS. The quality control assurance (precision

145 and accuracy) was performed by using a ClinChek® urine lyophilized material for trace elements

146 analysis at two different levels of concentration (Recipe, Munchen, Germany).

147   The limits of detection were 0.006 µg/L for most elements, except 0.007 µg/L for U, 0.008

148 µg/L for W, and 0.001 µg/L for arsenobetaine. The percentages of participants with concentrations

149 below the limit of detection and the corresponding inter batch coefficients of variation are shown in

150 Supplemental Table S2. Urine metal levels below the limit of detection (up to 0.7% of

151 determinations) were imputed as the limit of detection divided by the square root of two following

152 common practice.[20] While inorganic As is considered toxic for humans, arsenobetaine is a form of

153 organic As found in seafood intake that is non-toxic.[21] In populations with substantial seafood intake,

154 such as in the present study,[22] it is recommended to remove arsenobetaine variability from total As to

155 eliminate the contribution of seafood arsenicals to total and methylated As.[23] Thus, to assess

156 inorganic As exposure, we estimated total As levels that are independent of arsenobetaine by using a

157 residual-based method[23] (from now on just As for simplicity).

158 **Other variables**

159 Participants underwent an interview, which included information on age, sex, education,

160 smoking status and medication use, and a physical examination, to measure height, weight and blood

161 pressure by trained staff following standard protocols. Hypertension was defined as a

162 systolic/diastolic blood pressure ≥140/90 mmHg, a self-reported diagnosis or current use of

163 antihypertensive medication. Fasting serum glucose was measured by spectrophotometry. Whole

164 blood glycated hemoglobin was measured by reverse-phase cationic exchange chromatography and

165 quantified by double wave-length colorimetry quantification. Diabetes was defined as a clinical

166 diagnosis of diabetes, fasting serum glucose ≥126 mg/dL, glycated hemoglobin ≥6.5% or current use

167 of glucose-lowering medication.

168 **Statistical methods**

169 *Single metals and renal damage.* Metal concentrations were divided by urine creatinine to

170 account for urine dilution and log-transformed to improve normality. We calculated the median and

171 interquartile range of the study endpoints across participant characteristics and categories of urine

172 metal levels. In addition, the urinary metal levels across participant characteristics, and their pairwise

173 Spearman correlations were reported in the Supplemental Material. For longitudinal analyses,

174 adjusted geometric mean ratios (GMRs) of annual relative changes in ACR and the mean differences

175 (MDs) in annual eGFR changes comparing the 80th to the 20th metal percentiles were estimated from

176 linear regression models. Metals were also modeled as tertile categories to compare the two highest

177 to the lowest tertiles of metals distributions. Adjusted odds ratios (ORs) for annual ACR increase

178 ≥20% and annual eGFR decrease ≥5 ml/min/1.73m^2 were obtained from logistic models. For

179 secondary cross-sectional analyses, the corresponding GMRs of baseline ACR and the MDs in

180 baseline eGFR were estimated from linear regression models. We also assessed non-linear

181 relationships by modeling the metal variables as restricted quadratic splines with knots at the 10th,

182 50th, and 90th percentiles of their distribution. All models were adjusted for age, sex, education

183 (≤high school, >high school), smoking status (never, former, current), BMI (kg/m^2), diabetes

184 (yes/no), and hypertension (yes/no). In addition, models for ACR-related endpoints were further

185 adjusted for baseline eGFR; longitudinal models for ACR endpoints were further adjusted for

186 baseline ACR; and longitudinal models for eGFR endpoints were additionally adjusted for baseline

187 eGFR.

188   *Joint metals and renal damage.* We evaluated the joint association of metal mixtures with the

189 annual change in ACR and eGFR levels (as continuous outcomes) by implementing BKMR with the

190 *bkmr* package in R.[16] Given the elevated number of metals included in our study, to have

191 parsimonious BKMR models that facilitate convergence, we first conducted a principal component

192 (PC) and hierarchical cluster analyses to split the evaluated metals into metal mixtures based on

193 shared similarities. Second, for PCs with more than two relevant metals and for each outcome of

194 interest, we introduced all metals within the mixture in a flexible kernel and kept the same

195 adjustment covariates as in main regression models. For each conducted BKMR model, we estimated

196 the Posterior Inclusion Probabilities (PIPs) to quantify the relative importance of each metal for each

197 outcome, as they are a ranking measure to see how much the data favor the inclusion of a variable in

198 the BKMR model. In addition, we also evaluated the dose-response relationships of each metal and

199 the outcomes of interest when the other metals of the mixture were fixed to a given percentile, which

200 enables the identification of potential interactions within the metals.[16] For both PC and BKMR

201 analyses, metals were treated as z-score variables to standardize their levels. BKMR was fitted using

202   the 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'

203   predictor vectors for different individuals, and r_m the tuning parameter that control the smoothness

204   of the kernel function (specified with a uniform prior distribution with default values 0 and 100 for

205   the lower and upper bound, respectively). The number of iterations was fixed to 20000.

206   *Sensitivity analyses.* First, given the fact that our eGFR change definition does not reflect

207   between-visits fluctuations, we repeated the analysis for annual eGFR change calculated as the slope

208   of all available eGFR measures for each participant. Second, because diabetes can increase zinc

209   urinary excretion,[24] we repeated the association analyses of Zn among non-diabetic participants.

210   Third, we also repeated the analyses modelling the metals in ug/L (i.e, non-creatinine standardized)

211   with separate adjustment for urine creatinine. Fourth, we repeated the analyses adjusting all models

212   by physical activity and by family history of diabetes and hypertension. Moreover, because the

213   length of follow-up time is different for each participant, we repeated the analyses adjusting for

214   follow-up time. In addition, because Zn status in the body can influence Cd absorption and toxicity,[25]

215   we evaluated the Cd results in models additionally adjusted for Zn, and vice versa. To compare the

216   BKMR results with results from the traditional linear regression, for statistically significant metals

217   from single-metal linear regression models, we additionally conducted a fully adjusted linear

218   regression model, in which we further adjusted for all other significant metals (i.e. multiple-metal

219   model). Finally, smoking is a source of exposure for some metals[8] and a well-established

220   cardiovascular risk factor. Thus, we assessed potential differential associations by smoking by

221   conducting subgroup analysis of most relevant metals.

222   All statistical analyses were performed using the R software (version 3.6.2). The statistical

223   code can be made available upon reasonable request to the corresponding author.

224

225   **RESULTS**

226   *Descriptive analysis.* Older participants, as well as female participants showed higher levels

227   of ACR and lower eGFR at baseline visit (Table 1). In addition, participants who were current

228   smokers, participants with diabetes or hypertension, and participants with higher urinary levels of

229   most metals, showed higher ACR levels at baseline. Median metal levels, in μg/g unless otherwise

230   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

231   Co, Cu, Mo, Zn, As, Ba, Cd, Cr, Sb, Ti, U, V and W, respectively (Supplemental Table S3).

232   Participants older than 55 years had higher levels of Cu, Zn, As, Ba, Cd, Ti, U, V and W compared

233   to participants younger than 50 years. Ever smokers had higher levels of Zn, As, Ba and Cd. Obese

234   participants had higher levels of Zn and Ti but lower Ba and U. Co and Cd showed the strongest

235   positive correlation (r spearman=0.53) (Supplemental Table S4), while Cd and U showed the

236   strongest negative correlation (r spearman=−0.19).

237   *Single metals and longitudinal renal endpoints.* The GMR (95% CI) of ACR change

238   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

239   1.07 (1.01, 1.13) for W (Table 2) (i.e., a 15%, 7% and 7%, respectively, higher annual increase in

240   ACR). For eGFR, the estimated MD (95% CI) of eGFR annual change (ml/min/1.73m^2) comparing

241   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

242   (0.09, 0.67) for Ba (Table 3). Figure 1 graphically showed strongly supportive associations specially

243   for As, Cr and W with increasing ACR annual change, and for Zn, As, and Cr with decreasing eGFR

244   change at the higher metal exposure range (i.e. the confidence intervals mostly did not include the

245   null value). The dose-response shape for all of the evaluated metals, however, was generally

246   compatible with a nephrotoxic role of metals both in ACR and eGFR annual change models. The

247   associations of Zn, As, and Cr with increased ACR and decreased eGFR were also confirmed in

248   complementary analysis with the categorized endpoints for annual ACR increase ≥20% and eGFR

249   decrease ≥5 ml/min/1.73m^2 (Supplemental Table S5). In secondary cross-sectional analysis,

250   increased urinary Co, Cu, Zn, Cd, Cr and W levels were associated with higher baseline ACR

251   (Supplemental Table S6).

252   *Joint metals and longitudinal renal endpoints.* We first grouped the metals into mixtures by

253   implementing PC and hierarchical cluster analysis. The first mixture included Cu, Zn, As, Ba, Ti, U,

254   V and W (named PC1 from now on), the second mixture included Co, Cd, Cr, Sb, V and W (named

255   PC2 from now on) (Supplemental Figure S2). Figure 2 shows a positive dose-response shape for

256   both PC1 and PC2 mixtures as a whole with the change in ACR excretion, while the dose-response

257   shape with eGFR change was inverse, suggesting that higher levels of PC1 and PC2 mixtures are

258   related with higher ACR excretion and with decreased eGFR. For ACR change models, the highest

259   metal-specific posterior inclusion probabilities (PIPs), which help to identify the most important

260   metals within each mixture in relation to each outcome, were observed for As (PIP=0.55) within PC1

261   and for Cr (PIP=0.61) within PC2 (Table 4). For eGFR change, while the highest PIPs were

262   observed for Zn (PIP=0.71) within PC1 and for Cr (PIP=0.74) within PC2, the PIPs for the other

263   PC2 metals were also substantial. Finally, for the ACR change associations we visually identified

264   potential interactions between As-V, Zn-Ba and Zn-Cu in BKMR models with PC1 metals

265   (Supplemental Figure S3), and between Cd-Sb, W-Sb, W-Cd and W-Co in BKMR models with PC2

266   metals (Supplemental Figure S4). We did not identify interactions between metals in BKMR models

267   for eGFR change (Supplemental Figures S5 and S6).

268   *Sensitivity analyses.* The findings for annual eGFR change calculated as the slope of all

269   available eGFR measures for each individual were generally consistent with the main results, but

270   with somewhat attenuated associations for some metals (Supplemental Table S7). The findings for

271   Zn were essentially identical in analysis restricted to participants without diabetes (data not shown).

272   We also observed consistent results when modelling the metals in ug/L with separate adjustment for

273   urine creatinine, and in models further adjusting for length of follow-up, physical activity, or family

274   history of diabetes and hypertension (data not shown). In multiple-metal models for ACR change

275 (GMR [95% CI]), the association of As became slightly stronger (1.19 [1.05, 1.34]), while for Cr and

276 W became slightly attenuated (1.06 [0.99, 1.14] and 1.05 [0.99, 1.12], respectively). In multiple-

277 metal models for eGFR change (MD [95% CI] ml/min/1.73m^2), the association of Zn and As became

278 slightly stronger (-0.39 [-0.74, -0.05)] and -0.58 [-1.04, -0.12], respectively). These results are

279 consistent with BKMR results. In subgroup analysis we did not observe statistically significant

280 interactions by smoking (Supplemental Table S8).

281

282 **DISCUSSION**

283   Our longitudinal results showed that increasing exposures to Zn, As, Cr, and W are

284 associated with increased ACR and decreased eGFR changes over time. The shape of the

285 longitudinal dose-responses, however, was compatible with a nephrotoxic role for all of the

286 evaluated metals, both in ACR and eGFR models. In joint metal exposure analysis, the associations

287 with ACR were mostly driven by As and Cr. For eGFR, while the associations were mostly driven

288 by Zn and Cr, the contribution of other metals was also relevant. We identified some potential

289 interactions for As, Zn and W by other metals for ACR change, but not for eGFR change.

290   *Urine metal biomarkers.* Urine is a well-established biosample to evaluate metal exposure,

291 which integrates exposure sources including air, water and food.[25] The metals evaluated in this study

292 have relatively short half-lives in urine, except for Cd, which reflects Cd accumulation in the

293 kidney.[8,26] Under chronically maintained exposure, urine biomarkers could also be a proxy of long-

294 term exposure.[27] In secondary cross-sectional analyses, higher exposure to Zn, As, Cr, Cd and W

295 were associated with higher ACR at baseline, but not with lower eGFR. However, the use of urine

296 metal determinations to assess cross-sectional associations with eGFR is controversial.[28,29] For

297 instance, despite the known nephrotoxic effects of As, Cd and Pb, in several cross-sectional studies

298 urinary levels of these metals have been associated with higher eGFR.[29–31] This could be compatible

299 with reverse causation bias, where decreased renal function might impair metal and creatinine

300   excretion through the kidney, partly resulting in lower urine metal concentrations unrelated to

301   exposure under prevalent renal damage.[32] Therefore, cross-sectional associations of urine metal

302   levels with serum creatinine-based eGFR should be taken cautiously.[32]

303   *Zinc*. Mechanistic research supports that both extremely low and high Zn exposure levels are

304   associated with renal injury.[33–35] Zn deficiency might induce renal disease by increasing oxidative

305   stress and apoptosis, and by decreasing nephron quantity and glomerular filtration surface,[36] while

306   excessive Zn exposure might result in functional changes in the kidney by inducing Cu

307   deficiency.[35,37] In epidemiologic, mostly cross-sectional, studies, both Zn deficiency[13] and excess [38]

308   has been related with adverse renal health conditions. In our longitudinal analysis, higher Zn was

309   associated with lower eGFR but not with higher ACR. Overall, the evidence does not support supra-

310   optimal Zn exposure nor long-term Zn supplementation in Zn-repleted populations for renal disease

311   prevention.

312   *Arsenic.* Arsenic toxicity in the proximal tubule is related to increased ROS production,

313   inflammation and apoptosis, potentially leading to direct podocyte injury.[3] Other studies in humans

314   suggested a role of miRNAs dysregulation in arsenic-related urine albumin excretion.[39] A systematic

315   review of epidemiologic studies concluded that increasing urinary As was cross-sectionally

316   associated with increased ACR and proteinuria.[40] Most recent studies reported null associations for

317   urinary As with abnormal ACR in Chinese adults (mean=69.5 µg/g, N=336)[41], and in NHANES

318   1999-2016 (N=46748).[31] The evidence for eGFR-based endpoints, however, was less clear.[40] Urine

319   As (assessed as the sum of inorganic and methylated arsenic species) was prospectively associated

320   with increased risk of incident CKD among American Indian adults from the Strong Heart Study

321   (median=9.7 µg/g, N=3119).[32] The longitudinal association of As with eGFR decline in our data is

322   novel, since it has not been reported before.

323   *Chromium.* While little is known about the nephrotoxicity of Cr in humans, studies in rats

324   showed that acute and chronic Cr exposure might lead to apoptosis, tubular necrosis and proximal

325 tubule damage.[42,43] Increased urinary Cr was associated with lower eGFR in the National Nutrition

326 and Health Survey in Taiwan (mean urine Cr=0.83 µg/L, N=360)[44] and in the Changhua county

327 (urine Cr levels not reported, N=1328),[45] but not in Southern Taiwan (median urine Cr=0.1 µg/L,

328 N=2447). While no studies have evaluated the prospective association of urine Cr with renal

329 outcomes before, our longitudinal results showed a strongly suggestive relationship between higher

330 urine Cr and eGFR decline. In addition, we observed a novel association of Cr with increased ACR.

331 *Tungsten.* We also observed and association of W with increased ACR change. For the

332 general population, exposure to W comes from air, food ingestion and drinking water and it is

333 expected to be very low.[46] Higher exposure can occur for workers involved in manufacturing

334 processes.[47] In the US, increased urinary W was related with higher eGFR in NHANES cross-

335 sectionally,[28] but with decreased time-to-CKD development in rural Colorado prospectively.[48]

336 *Other metals.* In our study, increased urine Cu (median=10.3 µg/L) was associated with

337 higher ACR in cross-sectional analysis, and with higher odds of ACR increase ≥20%. Cu is an

338 essential nutrient needed for proper renal function.[49] Indeed, studies suggest that Cu-Zn imbalance

339 induces tubular damage.[49, 50,51] Alternatively, the potential toxicity of Cu excess is receiving

340 increasing attention.[52] For instance, higher urine Cu levels have been cross-sectionally associated

341 with proteinuria and low eGFR in Taiwan (median=1.5 µg/L, N=2447),[53] and with low eGFR in

342 China (for Cu levels >20.96 µg/L, N=3553).[54] Lastly, experimental and epidemiological evidence

343 supports that kidney injury is a major effect of high Cd exposure.[55] Consistently, increased urine Cd

344 levels were associated with higher ACR in our cross-sectional analysis, and also in other studies

345 from the US,[30,56] China,[57] and Spain.[58] While we did not find a statistically significant association for

346 Cd with longitudinal ACR or eGFR change, the longitudinal dose-response in our data was

347 compatible a nephrotoxic role of cadmium for the evaluated renal endpoints. Overall, more

348 longitudinal studies that evaluate the change in metal-related renal endpoints are needed, especially

349 in populations exposed to low exposure levels.[59]

350   *Metal mixtures*. Only one cross-sectional study evaluated the association of metal co-

351   exposures with renal disease applying BKMR methods.[6] In that study, the Co-Cd-Hg-Pb mixture was

352   associated with both higher ACR and lower eGFR, and Pb, Co and Cd drove the association for

353   ACR, while Pb drove the association for eGFR.[6] In other cross-sectional studies applying less

354   flexible methodologies found significant associations for Cd-Pb co-exposure with higher ACR and

355   decreased eGFR,[60] for Cd-Cr-Pb mixture with decreased eGFR,[44] and for As-Cd-Hg-Pb with higher

356   ACR but not with decreased eGFR.[61] These results are in line with our finding that PC2 mixture,

357   which includes Cd, Cr and Co, was associated with increased ACR and decreased eGFR longitudinal

358   changes. However, previous metal mixture findings are not completely comparable with our results,

359   given their cross-sectional nature, and the fact that other available metals and metal biomarkers were

360   measured in those studies. Nevertheless, our findings add novel evidence about metal co-exposure

361   and renal marker changes over time and identify some potential interactions between metals, which

362   are supported by mechanistic studies, especially for Zn-Cu,[62] Zn-Ba,[63] and W-Co.[46]

363   *Strengths and limitations.* Our study has several limitations. Because of the small number of

364   women in our sample, we could not evaluate potential interactions by sex. Given the paucity of

365   studies with available metals and repeated measurements of renal damage biomarkers, additional

366   longitudinal studies, including men and women with low metal exposure, are needed to confirm our

367   findings. We used a single urine sample for assessing metal exposure, which might be subject to

368   non-differential physiological fluctuation in individuals and could have attenuated the associations.

369   Also, since we did not have available biomarkers of tubular damage in the AWHS imaging sub-

370   cohort, we cannot discard that our ACR results may partly reflect a dysfunction of albumin

371   reabsorption in the proximal tubules in addition to a disorder in the glomerular filtration barrier.

372   While we adjusted for many relevant factors known to influence renal function, we cannot

373   completely discard the presence of residual confounding by other unmeasured factors, such as

374   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,[64] 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,[15] metabolomics,[65,66] genetic variation in specific genes,[14,58,65,67] epigenetics,[68] and miRNAs and transcription factors [69–73] 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 with substantial implications for renal disease prevention and control.

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

399   reduce environmental exposure of metals may improve renal disease prevention and control at

400   exposure levels that are relevant for general populations.

401

415   **Supplemental material table of contents**

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596

FIGURE LEGENDS

Figure 1. Flexible dose-response relationship 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 (blue) and the mean difference of annual absolute changes in eGFR (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/1.73m^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.

Figure 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).

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*						
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)

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.
* 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).

	Annual ACR change				
	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)*	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.

* Metals introduced as restricted quadratic splines with knots at percentiles 10th, 50th, and 90th because of nonlinear relationships.

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).

	Annual eGFR change (ml/min/1.73m^2)				
	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.

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.