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The role of coronary artery calcification in metal-related cardiovascular disease

Arce Domingo-Relloso [a,b,c,*], Katlyn E. McGraw [b], Irene Martinez-Morata [b], Yuchen Zhang [a], Kathrin Schilling [b], Ronald A. Glabonjat [b], Ziqing Wang [a], Kiros Berhane [a], Brent A. Coull [d], Marta Galvez-Fernandez [e], Miranda R. Jones [f,g], Wendy S. Post [f,g], Joel Kaufman [h], Tiffany R. Sanchez [b,i], Maria Tellez-Plaza [j], Graham R. Barr [k], Steven Shea [k], Ana Navas-Acien [b], Linda Valeri [a,l]

[a] Department of Biostatistics, Columbia University Mailman School of Public Health, New York, NY, USA
[b] Department of Environmental Health Sciences, Columbia University Mailman School of Public Health, New York, NY, USA
[c] IE University School of Science and Technology, Madrid, Spain
[d] Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA, USA
[e] Astrazeneca, Madrid, Spain
[f] Department of Oncology, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD, USA
[g] Department of Epidemiology, Johns Hopkins University Bloomberg School of Public Health, Baltimore MD, USA
[h] Department of Medicine, University of Washington, Seattle, WA, USA
[i] Department of Epidemiology, University of Colorado, Anschutz Medical Center, CO, USA
[j] Department of Chronic Diseases Epidemiology, National Center for Epidemiology, Instituto de Salud Carlos III, Madrid, Spain
[k] Departments of Medicine and Epidemiology, Columbia University Irving Medical Center, New York, NY, USA
[l] Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA

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ABSTRACT

Metals are associated with cardiovascular disease (CVD), but the underlying pathways remain largely unclear. We evaluated the potential intermediate role of coronary artery calcification (CAC) trajectory on the association between urinary metals and incident CVD, accounting for competing risks by death from other causes. We used data from 6,459 participants of the Multi-Ethnic Study of Atherosclerosis (MESA). CAC was measured longitudinally using the spatially weighted calcium score in five exams, starting in 2000. Participants were followed for CVD events through 2019. Cadmium, cobalt, copper, uranium, tungsten, and zinc were measured in urine at the baseline visit (2000–2002). We used a causal inference algorithm with a path-specific effects approach for longitudinal mediation analysis to evaluate the intermediate role of CAC on the association between metals and incident CVD. The association with incident CVD mediated through the CAC trajectory was statistically significant for cadmium, cobalt, copper, tungsten, and zinc. The number of CVD cases (95% CI) per 100,000 person-years attributable to an interquartile range (IQR) increase in metal levels through the longitudinal trajectory of CAC was 44 (20, 72) for cadmium, 21 (6, 39) for cobalt, 19 (2, 36) for copper, 18 (2, 38) for tungsten, and 43 (26, 62) for zinc. This study supports that part of the association between urinary metals and CVD is attributable to changes in CAC over time. In particular, half of the association between urinary cadmium and CVD might be mediated by longitudinal changes in CAC. This study could inform strategies for early detection and prevention of CVD based on urinary metal levels.

1. Introduction

Physicians are increasingly considering the importance of incorporating measurements of environmental exposures to clinical practice (Rabinowitz and Barry, 2024). Metals are cardiovascular disease (CVD) risk factors, as supported by mounting epidemiologic and

* Corresponding author at: Departments of Biostatistics and Environmental Health Sciences, Columbia University Mailman School of Public Health, 722 West 168th Street, Suite R615, 10032 New York, NY, USA.
E-mail address: ad3531@cumc.columbia.edu (A. Domingo-Relloso).

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mechanistic evidence (Sevim et al., 2020; Chowdhury et al., 2018; Yang et al., 2020; Lamas et al., 2023). Exposure to metals might accelerate the development of atherosclerosis, including the accumulation of variable amounts of calcium in the coronary arteries (i.e., coronary artery calcification (CAC)) (Mori et al., 2018). CAC is traditionally measured non-invasively through cardiac computed tomography (CT), and can be quantified using a novel semi-automated, threshold-free method called spatially weighted calcium score (CAC-SW) (Liang et al., 2012; Shea et al., 2021).

CAC-SW levels are highly predictive of coronary heart disease (CHD) events (Greenland et al., 2018; Nasir and Cainzos-Achirica, 2021). In a previous study, we found that non-essential (cadmium, tungsten, uranium) and essential (cobalt, copper, zinc) metals are longitudinally associated with CAC in the Multi-Ethnic Study of Atherosclerosis (MESA) (McGraw et al., 2024). We also found that the same metals are prospectively associated with clinical cardiovascular events including incident CVD and CHD (Martinez-Morata et al., 2024). The amount of the association between different metals and incident CVD that might be explained by changes in CAC over time, however, is unknown.

Mediation analysis methods do not generally incorporate longitudinal measures of the potential mediator, and thus fail to incorporate changes over time preceding the clinical outcome. In addition, mediation analysis generally ignores competing risks by death or reports disease risk either reconstructing a population in which no one dies or considering death as a censoring event (Young et al., 2020). This leads to biased estimates, as the competing event (i.e., death) makes it impossible for the event of interest (e.g., CVD) to happen, and information on whether participants would have developed the outcome is missing for those who die during the follow-up.

The MESA includes more than 6,000 participants from multiple centers across the US with different ethnicities, and a long follow-up of almost two decades with repeated measures of CAC over time. These characteristics provide a unique opportunity to study the potential intermediate role of the longitudinal changes of CAC on the association between urinary metals and CVD risk. In this work, we used a novel approach to quantify the mediating role of CAC, measured longitudinally at five time points using CAC-SW, on the association between baseline urinary metal biomarkers and clinical CVD events. Our algorithm accounts for competing risks by death using a path-specific effects framework (Avin et al., 2024). This allows us to evaluate the number of CVD cases attributable to each metal through the longitudinal trajectory of CAC, and the number of CVD cases that do not happen due to participants dying during the follow-up. We prioritized nonessential (cadmium, tungsten, uranium) and essential (cobalt, copper, zinc) metals that have been previously associated with CAC and CVD in MESA (McGraw et al., 2024; Martinez-Morata et al., 2024).

2. Methods

2.1. Study population

The MESA is a multi-center, prospective cohort study of subclinical and clinical CVD (Bild et al., 2002). Between 2000 and 2002, 6,814 participants were recruited at six study sites in Baltimore MD, Chicago IL, Los Angeles CA, New York NY, St. Paul MN, and Winston Salem NC. Participants were 45–84 years old, from four race and ethnic groups (White, Black, Hispanic, and Chinese) and free of clinical CVD at baseline. They completed up to five clinic visits (2000–2002, 2002–2004, 2004–2006, 2005–2007, and 2010–2012). Informed consent was given by participants who met enrollment criteria. The Institutional Review Board at each study site approved the study.

The inclusion criteria for this study, including a flow diagram, have been described elsewhere (McGraw et al., 2024), and are detailed in the Supplementary Methods and in Supplementary Fig. 1. The final sample size included 6,527 participants with repeated measures of CAC.

2.2. Urinary metals

The analytical protocols to measure metals in MESA have been published (Schilling et al., 2023; Glabonjat et al., 2024) and are described in the Supplementary Material. Briefly, urine samples were taken at MESA exam 1 (2000–2002) and metals were analyzed using Inductively Coupled Plasma Mass Spectrometry after dilution (Julshamn et al., 2013). Metal concentrations were divided by urine creatinine concentrations, measured using the Jaffe reaction method (Jaffe, 1886), to correct for urine dilution, and are expressed as μg metal/g creatinine. For a subset of participants with metals analyzed at MESA exams 1 and 5 (N = 594), the intraclass correlation coefficient ranged from 0.50 to 0.72 for cobalt and uranium, respectively, supporting that a single baseline metal measure is a moderate to good reflection of long-term metal levels (McGraw et al., 2024). We did not include metal measurements at exam 5 as, in order to conduct a mediation analysis, the exposure needs to precede the mediator, and in our study, CAC (our mediator of interest) was measured in MESA exams 1–5 (i.e, before metal measurements at exam 5) (see Supplementary Fig. 2). We included six metals with prior evidence of being associated with CAC progression and incident CVD in MESA: cadmium, cobalt, copper, uranium, tungsten, and zinc (McGraw et al., 2024; Martinez-Morata et al., 2024). While other metals such as arsenic and lead have shown associations with cardiovascular disease, they were not associated with CAC and incident CVD in previous work in MESA (McGraw et al., 2024; Martinez-Morata et al., 2024). In order to investigate mediated effects, a statistically significant association between the exposure and the outcome and between the exposure and the mediator is required. Thus, we did not include those other metals. Natural log-transformation was applied to all metals for analysis. Correlations between metals were low to moderate, and are shown in Supplementary Fig. 3.

2.3. Cardiac computed tomography (CT) scanning and coronary artery calcification

Procedures of cardiac CT scans to measure CAC in MESA have been described previously (Carr et al., 2005). Arterial trajectories across the surface of the heart were determined within 8 mm. Then, a phantom-based adjustment was applied, and candidate calcified plaques were identified. The criteria for calcification were for each plaque to be composed of at least 4 contiguous voxels with an attenuation level of 130 Hounsfield units or greater (Carr et al., 2005). The selection criteria for undergoing cardiac CT scans are described in the Supplementary Material.

Several methods exist to quantify CAC. The traditional Agatston score (CAC-AS) uses a specific but conservative algorithm for lesion detection, which does not take into account available information in the CT scan. CAC-AS has the limitation that it ignores the presence of calcification below a certain threshold, classifying levels early in the calcification stages as CAC-AS = 0 (Liang et al., 2012; Shea et al., 2021). CAC-AS is a continuous measure that is dichotomized as 0 and 1, where 0 refers to no measured calcification, and 1 refers to any measured calcification per the Agatston scoring criteria. The spatially weighted calcium score (CAC-SW) is a semi-automated threshold-free CAC scoring method. This method assigns weights to each image voxel according to the phantom and neighboring voxels, thus maximizing the CT scan information. The detailed algorithm for calculation, providing the continuous CAC score, has been published (Shea et al., 2021). CAC-SW has been shown to predict incident CHD events even among participants with CAC-AS = 0 (Shea et al., 2021). We used longitudinal measurements of CAC-SW across MESA exams 1–5 for the analyses. We used Multiple Imputation in Chained Equations (MICE) (Azur et al., 2011) to impute missing values in CAC in exams 1–5, based on the values of the other visits and other covariates. Details of the MICE procedure can be found in the Supplementary Methods, Supplementary Table 1, and Supplementary Fig. 4.

2.4. Cardiovascular disease ascertainment

Participants were followed for incident cardiovascular events and mortality through December 2019; the median (interquartile range, IQR) follow up was 17.5 (11.2, 18.4) years. CVD diagnosis procedures in MESA have been published elsewhere (Bild et al., 2002) and are detailed in the Supplementary Methods. Incident CVD events were defined as myocardial infarction, resuscitated cardiac arrest, definite and probable angina, stroke, atherosclerotic deaths including CHD and other CVD deaths. Information about death events was systematically collected from the National Death Index database (Access and National Death, 2024). Participants were followed until the first CVD record of any type, death, loss to follow-up, or the administrative censoring date (December 31, 2019), whichever happened first.

2.5. Covariates

Self reported age (years), sex (male, female), race and ethnicity (White, Black, Hispanic, or Chinese), education, smoking status (never, former, current smoker), cigarette pack-years (calculated by multiplying the intensity in packs per day by duration in years) and use of lipid-lowering and hypertension medications (yes, no) were collected using questionnaires at MESA exam 1. Height and weight were measured to calculate body mass index (BMI, kg/m^2). Resting systolic and diastolic blood pressure were measured 3 times in the seated position using a Dinamap model Pro 100 automated oscillometric sphygmomanometer with the last 2 measurements averaged for analysis. Low- and high-density lipoprotein cholesterol (LDL, HDL, mg/dL blood), and calibrated fasting plasma glucose (FPG, mg/dL plasma), were assessed using standard laboratory techniques. Diabetes mellitus (DM) was defined by the 2003 American Diabetes Association fasting criteria and categorized by normal (<100 mg/dL plasma FPG) or impaired fasting glucose (100–125 mg/dL plasma FPG), untreated or treated diabetes (≥126 mg/dL plasma FPG or taking diabetes medications). Estimated glomerular filtration rate (eGFR, mL/min/1.73 m^2) is a measure of kidney function and can influence both metal excretion in urine and CVD risk. It was calculated using the new creatinine and cystatin-C based Chronic Kidney Disease Epidemiology Collaboration equation, without accounting for race and ethnicity (Inker et al., 2021).

2.6. Statistical methods

Fig. 1 depicts the directed acyclic graph for our setting. We performed a longitudinal mediation analysis using the CAC data collected at baseline and follow-up (exams 1–5). Our exposure variables were the six creatinine-corrected urinary metal concentrations evaluated at exam 1, in separate models, and our outcome was time to first CVD event in years. We used an adaptation of the mediational g-formula that we developed for survival mediation analysis accounting for competing risks. We used a path-specific effects framework for a continuous exposure and a time-to-event outcome to conduct longitudinal mediation analysis accounting for competing risks (Domingo-Relloso et al., 2026) (see Supplementary Methods). To do so, we consider death as another mediator which has a deterministic relationship with both the mediator of interest (CAC) and the outcome (in the sense that, if an individual dies, no further values of the mediator can be observed, and the outcome cannot happen).

We used a semi-parametric additive hazards model with CVD incidence as the outcome and the metal of interest as the exposure, adjusted for CAC (in all visits) and baseline age, sex, race/ethnicity, study site, education, smoking status, cigarette pack-years, BMI, and eGFR (model 1). We further adjusted for clinical CVD risk factors (LDL and HDL cholesterol, diabetes mellitus, sBP, lipid lowering and hypertension medication, model 2), which could also be mediators on the association between metals and CVD. The code of the algorithm is publicly available and can be found in the following Github repository: https://github.com/arcedr/CM_CR_gFormula.

Our model reports the effects in differences in hazards (number of cases attributable to an IQR increase in the exposure per number of person-years) (Lange and Hansen, 2011), which constitutes an absolute measure of risk that is relevant for public health. While differences in hazards are popular in epidemiological studies and represent a measure of impact for public health, recent work has demonstrated that they do not have a causal interpretation (Hernán, 2010). Thus, in addition to differences in hazards, we provide results in differences in survival probability scale, i.e., being free of CVD after 20 years. Confidence intervals were calculated using quantile bootstrap with 1,000 iterations. Empirical p-values were calculated inverting the CI, as the α such that the confidence interval with coverage probability $1-\alpha$ has the value of the parameter hypothesized under the null hypothesis (i.e., 0) exactly on top of one of the endpoints of the interval (Bradley Efron, 2025). P-values were corrected by multiple comparisons using the Benjamini-Hochberg approach to account for the fact that we are analyzing six metals in different models (Benjamini et al., 2001). The statistical significance threshold for p-values was set to 0.05. For bootstrap confidence intervals, results were considered statistically significant when the confidence intervals did not include the null value of 0.

We ran the mediational g-formula algorithm comparing the metals'

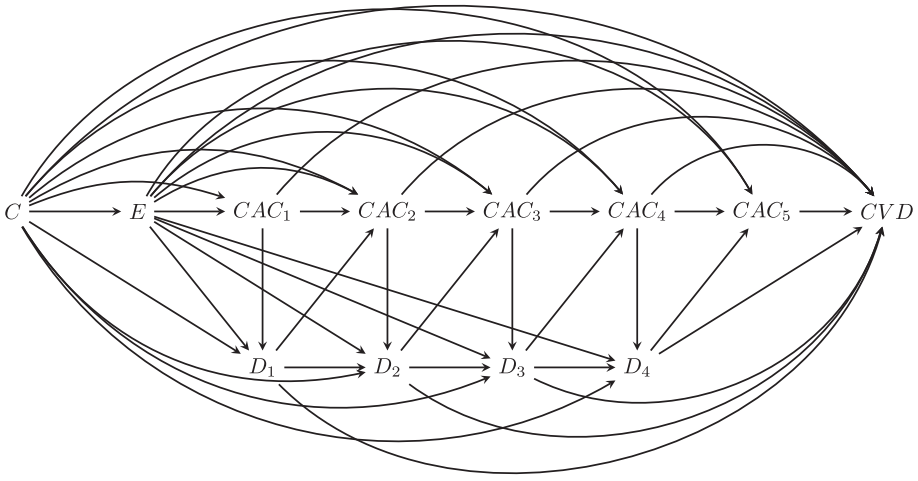

Fig. 1. Directed Acyclic Graph of the association between each urinary metal (E) and time to cardiovascular disease (CVD) potentially mediated through the longitudinal trajectory of coronary artery calcification (CAC). Abbreviations: C, confounders; E, exposures (urinary metals); CAC_i, coronary artery calcification measures at exam i; D_i, death indicator at exam i; CVD, cardiovascular disease.

75th to 25th percentiles. We thus have four quantities of interest:

- *Total effect*: number of CVD cases per 100,000 person-years attributable to an IQR increase in each metal concentration.
- *Direct effect*: association of the metal with CVD through pathways not involving CAC or death. Number of CVD cases per 100,000 person-years attributable to an IQR increase in each metal concentration that do not happen through CAC or death pathways.
- *Natural indirect effect through CAC* (the mediator of interest): association between the metal and CVD through the CAC trajectory. Number of CVD cases per 100,000 person-years attributable to an IQR increase in each metal concentration that happen through the CAC trajectory.
- *Natural indirect effect through death* (the competing event): association between the metal and CVD through death (i.e., preventing CVD from happening). Number of CVD cases per 100,000 person-years attributable to an IQR increase in each metal concentration avoided by the fact that the individual died before the CVD event happened.

The identifiability assumptions that need to be fulfilled for the effects to be identifiable, as well as the mathematical formulation of the effects, are described in the Supplementary Methods.

We ran several sensitivity analyses. First, to evaluate the impact of MICE imputation in our results, we ran a sensitivity analysis for metals that showed statistically significant indirect effects, including only the first time point of CAC-SW (i.e., without taking into account the longitudinal trajectory), and removing missing values of CAC-SW (i.e., without imputation). We did not do sensitivity analyses excluding missing values for all CAC-SW time points due to the high number of missing values in subsequent visits (Supplementary Table 2). Second, given previous evidence of differential associations between urinary cadmium and CAC for men and women (McGraw et al., 2024), we ran a mediation analysis for cadmium separately for men and women. We also conducted a sensitivity analysis stratifying our mediation models by CAC-AS status for those metals that showed statistically significant indirect effects through CAC-SW in primary analyses. Last, we ran Model 1 for all metals introducing a quadratic term to assess potential non-linear associations between metals and CAC and metals and CVD.

All analyses were run using the R software (version 4.3.1). The R package mice (version 3.16.0) was used to conduct MICE imputation. Bootstrap confidence intervals were calculated using parallel computing with the R package foreach (version 1.5.2). Survival models were fitted using the aalen() function of the timereg R package (version 2.0.6). Linear and logistic models for mediators were fitted using the lm() and glm() functions from base R, respectively. The variable names in the MESA dataset, together with their description, can be found in Supplementary Table 3.

3. Results

There were 1,125 incident CVD events, with mean follow-up time of 9 years for cases, and of 16 years for non-cases. Participants that developed CVD were older, more likely to be male and to have treated diabetes mellitus, had lower education and eGFR levels, and had higher sBP levels (Table 1). CAC-SW showed an overall increase over time, although not strictly monotonic, and was higher among those who developed CVD. Median CAC-SW at baseline was 4.2 for those who did not have CVD, and 56.6 for those who had CVD. At exam 5, median CAC-SW was 59.8 for those who did not have CVD, and 253.0 for those who had CVD. Baseline urine levels of cadmium, cobalt, copper and zinc were higher among those who developed CVD.

The total effects (attributable CVD cases/100,000 person-years (95 % CI) per one IQR increase in metal levels) in the difference in hazards scale ranged from 84.9 (−103.5, 276.5) for uranium to 311.6 (161.8, 477.9) for cobalt (Table 2, Fig. 2). The indirect effect for the association between metals and CVD through the CAC trajectory was statistically significant for all metals except uranium in models not adjusted for clinical risk factors (model 1). The number of CVD cases/100,000 person-years (95 % CI) attributable to an IQR increase in metal levels through the longitudinal trajectory of CAC (mediated effect) ranged from 21.1 (5.7, 39.1) for cobalt to 43.8 (19.9, 71.7) for cadmium (Table 2, Fig. 2). The indirect effect through death (avoided CVD cases/100,000 person-years (95 % CI) due to death from causes other than CVD, i.e., CVD cases that did not occur due to death for other causes related to exposure to the metal) was −10.1 (−16.9, −3.5) for cadmium, and not statistically significant for the other metals. When evaluating the difference in probability of being free of CVD after 20 years, the indirect effect through CAC was also statistically significant for all metals except

Table 1
Participant characteristics by cardiovascular disease (CVD) status.

Variables	No CVD (N = 5,334)	Incident CVD (N = 1,125)	p-value
Age (years)	61 (53, 69)	68 (60, 75)	<0.001
Sex %			<0.001
Female	55.1	42.2	
Male	44.9	57.8	
Education level %			<0.001
≤ High School	34.5	42.0	
Some College	23.2	23.5	
> College	42.3	34.5	
BMI (kg/m^2)	27.4 (24.4, 31.1)	27.9 (25.1, 31.4)	0.004
eGFR (mL/min/1.73 m^2)	78.4 (67.9, 89.4)	73.5 (62.4, 85.7)	<0.001
Smoking %			0.004
Never	51.9	46.7	
Former	35.5	40.4	
Current	12.6	12.9	
Race / ethnicity %			0.28
White	38.5	39.9	
Chinese	12.4	10.4	
Black	27.6	27.3	
Hispanic	21.5	22.4	
LDL cholesterol mg/dL	116 (96, 136)	115 (96, 137)	0.67
HDL cholesterol mg/dL	49 (41, 60)	46 (39, 56)	<0.001
Diabetes %			<0.001
Normal	76.4	63.6	
IFG	13.2	15.0	
Untreated DM	2.4	3.1	
Treated DM	7.9	18.3	
sBP mmHg	122 (110, 138)	133 (118, 149)	<0.001
Lipid medication %			<0.001
No	84.9	78.1	
Yes	15.1	21.9	
Hypertension medication %			<0.001
No	65.7	49.2	
Yes	34.3	50.8	
CAC-SW exam 1	4.2 (0.5, 35.4)	56.6 (6.1, 237.8)	<0.001
CAC-SW exam 2	7.0 (0.8, 48.7)	69.2 (9.1, 290.2)	<0.001
CAC-SW exam 3	54.6 (7.9, 256.4)	289.1 (51.1, 1092.0)	<0.001
CAC-SW exam 4	34.1 (6.4, 193.6)	218.1 (35.6, 817.3)	<0.001
CAC-SW exam 5	59.8 (10.8, 236.6)	253.0 (55.4, 683.5)	<0.001
Cadmium μg/g	0.53 (0.35, 0.80)	0.55 (0.36, 0.81)	0.32
Cobalt μg/g	0.39 (0.28, 0.57)	0.40 (0.29, 0.56)	0.18
Copper μg/g	12.3 (10.0, 15.6)	13.0 (10.4, 16.8)	<0.001
Uranium μg/g	0.005 (0.003, 0.011)	0.005 (0.003, 0.011)	0.41
Tungsten μg/g	0.06 (0.04, 0.10)	0.06 (0.04, 0.11)	0.22
Zinc μg/g	527 (356, 788)	614 (413, 935)	<0.001
Follow-up time (years)	17.8 (14.3, 18.5)	9.2 (4.4, 13.3)	<0.001

Abbreviations: CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; LDL, low density lipoprotein; HDL, high density lipoprotein; IFG, impaired fasting glucose; DM, diabetes mellitus; sBP, systolic blood pressure; CAC-SW, spatially weighted calcification score.
The table shows median (interquartile range) for continuous variables, percentages for categorical variables.
P-values were obtained from χ^2 tests for categorical variables, t-tests for normally distributed variables, and Mann-Whitney *U* tests for non-normally distributed variables.

Table 2
Direct, indirect and total effects (number of cardiovascular disease cases per 100,000 person-years) of one interquartile-range increase in urinary metals (μg/g) on cardiovascular disease incidence through longitudinal changes in spatially weighted calcium scores accounting for competing risks by death in the differences in hazards scale.

	Cadmium	Copper	Cobalt	Uranium	Tungsten	Zinc
Model 1						
Direct effect	59.0 (−95.1, 238.8)	294.3 (166.5, 458.7)	294.6 (144.8, 459.7)	75.0 (−115.1, 253.9)	134.7 (4.2, 272.7)	156.1 (22.8, 278.8)
Indirect effect through CAC	43.8 (19.9, 71.7)	18.7 (2.0, 35.7)	21.1 (5.7, 39.1)	14.7 (−8.8, 37.6)	18.4 (1.6, 37.6)	43.0 (25.6, 62.1)
Indirect effect through death	−10.1 (−16.9, −3.5)	−4.4 (−9.2, 0.4)	−4.3 (−9.5, 0.7)	−2.5 (−8.8, 3.8)	−0.3 (−5.9, 5.3)	−2.6 (−8.3, 2.8)
Total effect	93.6 (−63.3, 279.5)	307.9 (179.2, 474.4)	311.6 (161.8, 477.9)	84.9 (−103.5, 276.5)	153.2 (19.8, 293.5)	195.6 (56.6, 328.4)
Model 2						
Direct effect	78.1 (−85.7, 253.6)	236.3 (83.7, 385.8)	140.2 (6.3, 290.7)	78.6 (−93.8, 254.9)	118.7 (−5.9, 252.3)	69.6 (−62.6, 202.1)
Indirect effect through CAC	38.4 (19.1, 61.1)	3.4 (−10.7, 17.1)	13.5 (−1.6, 29.5)	7.3 (−10.9, 25.9)	9.9 (−4.7, 24.8)	22.8 (8.5, 40.7)
Indirect effect through death	−9.8 (−16.9, −3.3)	−4.2 (−9.5, 1.5)	−5.1 (−11.0, 0.6)	−2.9 (−9.7, 3.9)	−0.4 (−6.2, 5.9)	−2.1 (−7.8, 3.2)
Total effect	106.7 (−60.9, 288.8)	233.5 (80.9, 387.3)	148.9 (12.7, 296.8)	81.9 (−83.1, 261.5)	129.6 (1.9, 264.6)	91.2 (−43.9, 220.6)

Abbreviations: CAC, coronary artery calcification.
Model 1 was adjusted for age, sex, race/ethnicity, study site, education level, body mass index, estimated glomerular filtration rate, smoking status, and cigarette pack-years.
Model 2 was further adjusted for LDL cholesterol, HDL cholesterol, sBP, diabetes status, lipid lowering medication and hypertension medication.
Metals were divided by creatinine and log-transformed to correct for urine dilution.
Direct effects represent the number of cardiovascular disease (CVD) cases per 100,000 person-years attributable to an interquartile range increase in each individual metal not happening through changes in the CAC trajectory.
Indirect effects through CAC represent the number of CVD cases per 100,000 person-years attributable to longitudinal changes in the CAC trajectory following an interquartile range increase in each individual metal.
Indirect effects through death represent the number of avoided CVD cases per 100,000 person-years due to death following an interquartile range increase in each individual metal.
Total effects represent the sum of all three path-specific effects.
FDR corrected p-values for the direct effect (Model 1): for 0.484 cadmium, <0.001 for copper, 0.06 for cobalt, 0.48 for uranium, for 0.07 tungsten, 0.06 for zinc.
FDR corrected p-values for the direct effect (Model 2): for 0.38 cadmium, 0.01 for copper, 0.12 for cobalt, 0.38 for uranium, 0.12 for tungsten, 0.38 for zinc.
FDR corrected p-values for the indirect effect through CAC (Model 1): 0.006 for cadmium, 0.02 for copper, 0.07 for cobalt, 0.22 for uranium, 0.05 for tungsten, <0.001 for zinc.
FDR corrected p-values for the indirect effect through CAC (Model 2): <0.001 for cadmium, 0.63 for copper, 0.19 for cobalt, 0.49 for uranium, 0.29 for tungsten, <0.001 for zinc.
FDR corrected p-values for the indirect effect through death (Model 1): 0.01 for cadmium, 0.19 for copper, 0.17 for cobalt, 0.48 for uranium, 0.90 for tungsten, 0.48 for zinc.
FDR corrected p-values for the indirect effect through death (Model 2): 0.02 for cadmium, 0.22 for copper, 0.19 for cobalt, 0.54 for uranium, 0.90 for tungsten, 0.54 for zinc.
FDR corrected p-values for the total effect (Model 1): 0.33 for cadmium, <0.001 for copper, 0.04 for cobalt, 0.34 for uranium, 0.04 for tungsten, 0.01 for zinc.
FDR corrected p-values for the total effect (Model 2): 0.30 for cadmium, 0.01 for copper, 0.096 for cobalt, 0.38 for uranium, 0.09 for tungsten, 0.28 for zinc.

uranium (Table 3, Fig. 2). The indirect effect through death was statistically significant for cadmium and cobalt.

The associations were slightly attenuated when adjusting the models for clinical CVD risk factors, which could be related to clinical risk factors other than CAC serving as mediators on the association between metals and CVD. However, the indirect effect through CAC was still statistically significant for cadmium and zinc in the difference in hazards scale (Table 2, model 2, Fig. 2), and for cadmium, cobalt, and zinc in the survival probability difference scale (Table 3, model 2, Fig. 2), supporting an effect of metals on atherosclerosis progression independent of clinical risk factors.

In sensitivity analyses, the results were consistent when running a mediation analysis including only the first CAC-SW time point and excluding missing values in CAC-SW (Supplementary Table 4). In models stratified by sex, while the magnitude of the effects among men was higher compared to women, they were not statistically significant and showed wide confidence intervals, consistent with a lack of power. The indirect effect through CAC-SW remained statistically significant for women, but not for men (Supplementary Table 5). There were N = 3,104 participants with CAC-AS = 1, and N = 3,142 participants with CAC-AS = 0. As expected, the direct, indirect, and total effects were stronger for the group already showing CAC at baseline, showing that the intermediate effect of the CAC trajectory on the association between urinary metals and CVD is more pronounced for those already showing calcification at baseline (Supplementary Table 6). Nevertheless, the trends for those with CAC = 0 are similar although attenuated, which shows that the trajectory, in addition to the baseline status, also plays a role in the mediated effect. When including a quadratic term for the metals, the confidence intervals became much wider, but the indirect effect through CAC and through death was statistically significant for cadmium and copper (Supplementary Table 7).

4. Discussion

This large, multi-ethnic, longitudinal study of adults supports that part of the association between increasing levels of essential and non-essential metals measured in urine and CVD is attributable to changes in CAC, a subclinical atherosclerosis marker, over time. Our novel statistical method allowed us to disentangle different pathways that contribute to the association between metals and CVD, incorporating a continuous mediator for repeated CAC-SW over time. For urinary cadmium, half of the association with incident CVD could be mediated by longitudinal changes in CAC. For copper and uranium, other pathways beyond CAC might be more relevant. Further, our approach enabled us to account for competing risks due to death when analyzing time to CVD events, which was particularly important for metals that are strongly associated with all-cause mortality, such as cadmium.

The importance of exposure to metals for CVD development has been extensively documented and recently acknowledged by the American Heart Association (Lamas et al., 2023). Given the burden of CVD in the general US population, strategies for early detection and prevention are needed. The finding that part of the association of metals with CVD can be explained through changes in CAC—which occur years before the onset of clinical events—supports the upstream role of metal exposure and/or urinary excretion in CVD pathogenesis, and presents novel opportunities for early screening and prevention, as exposure to metals is,

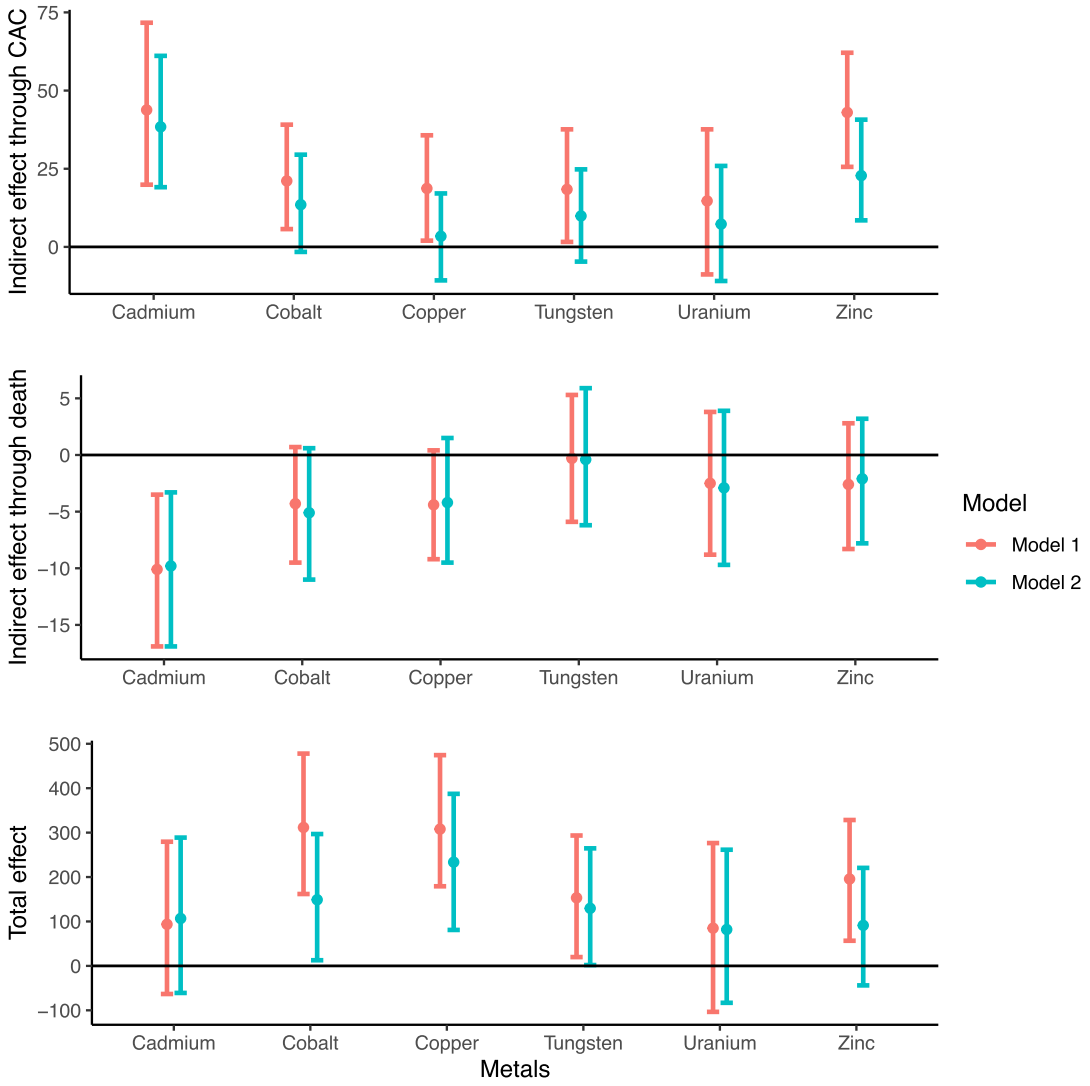

Fig. 2. The y axis represents number of cardiovascular disease (CVD) cases attributable to an interquartile range increase in each metal per 100,000 person-years. For the indirect effect through death, the numbers represent number of CVD cases attributable to an interquartile range increase in each metal avoided due to death per 100,000 person-years. Model 1: adjusted for age, sex, race/ethnicity, study site, education level, body mass index, estimated glomerular filtration rate smoking status, and cigarette pack-years. Model 2: additionally adjusted for LDL cholesterol, HDL cholesterol, sBP, diabetes status, lipid lowering medication and hypertension medication.

at least partially, modifiable.

Sources of exposure to metals might include individual-level sources and lifestyle factors such as smoking, e-cigarettes, dietary preferences, and consumer products, but also community level sources such as air, drinking water, or soil pollution (Martinez-Morata et al., 2023). Previous evidence has documented declines in CVD risk after declines in exposure to toxic metals such as lead and cadmium through regulatory changes (Ruiz-Hernandez et al., 2017; Lieberman-Cribbin et al., 2024), revealing the potential of interventions to reduce exposure and improve related health effects. While interventions to remove metals accumulated in the body have not yet been successful in reducing clinical CVD events, the non-significant inverse association between disodium edetate, an active chelating agent that removes divalent cations (e.g., lead) from the body, compared to placebo (HR 0.93 [0.76, 1.16]) in the Trial to Assess Chelation Therapy 2 (TACT2) (Lamas et al., 2013), suggests a public health benefit for interventions that prevent metal exposures. Yet, assessment of metal removal treatment on subclinical CVD has not been studied.

Evidence has consistently shown that exposure to cadmium is a risk factor for CVD (Chowdhury et al., 2018; Tellez-Plaza et al., 2013). Smoking is a major source of cadmium (Tellez-Plaza et al., 2012), and previous studies have proposed cadmium as an important mediator on the association between smoking and both CVD and mortality risk (Fagerberg and Barregard, 2021; Kim et al., 2023). In addition, cadmium has been reported to inhibit vascular repair and impair phosphate metabolism (Keli et al., 2013; Lin et al., 2021). Prior work in MESA showed that urinary cadmium has a strong association with CAC progression (McGraw et al., 2024). Other cross-sectional studies in populations from Sweden (Barregard et al., 2021) and Spain (Grau-Perez et al., 2022) have shown consistent findings. Our findings indicate that a large portion of the effect of cadmium on CVD is mediated through changes in CAC. Cadmium also showed the highest indirect effect through death, which is an indicator of strong competing risks by death, and thus, of the strong association of cadmium with death for causes

Table 3
Direct, indirect, and total effects (difference in 20-year CVD-free probability [percentage points]) of one interquartile-range increase in urinary metals (μg/g) on cardiovascular disease incidence through longitudinal changes in spatially weighted calcium scores accounting for competing risks by death.

	Cadmium	Copper	Cobalt	Uranium	Tungsten	Zinc
Model 1						
Direct effect (%)	−0.89 (−2.70, 0.98)	−4.08 (−5.75, −2.73)	−2.01 (−3.40, −0.65)	−1.02 (−2.62, 0.66)	−1.90 (−3.14, −0.64)	2.18 (−3.54, −0.87)
Indirect effect through CAC (%)	−0.61 (−0.89, −0.35)	−0.26 (−0.44, −0.09)	−0.23 (−0.43, −0.04)	−0.19 (−0.44, 0.04)	−0.23 (−0.42, −0.06)	−0.60 (−0.81, −0.40)
Indirect effect through death (%)	0.14 (0.05, 0.23)	0.06 (−0.004, 0.12)	0.08 (0.005, 0.15)	0.04 (−0.04, 0.13)	0.01 (−0.07, 0.08)	0.04 (−0.04, 0.11)
Total effect (%)	−1.433 (−3.19, 0.84)	−4.28 (−5.98, −2.91)	−2.163 (−3.62, −0.75)	−1.17 (−2.84, 0.56)	−2.13 (−3.37, −0.80)	−2.72 (−4.16, −1.39)
Model 2						
Direct effect (%)	−1.08 (−2.82, 0.55)	−3.04 (−4.49, −1.72)	−1.87 (−3.19, −0.60)	−1.572 (−2.78, −0.33)	−1.57 (−2.78, −0.33)	−0.97 (−2.20, 0.33)
Indirect effect through CAC (%)	−0.51 (−0.76, −0.30)	−0.04 (−0.20, 0.10)	−0.18 (−0.35, −0.03)	−0.13 (−0.29, 0.015)	−0.13 (−0.29, 0.02)	−0.31 (−0.48, −0.15)
Indirect effect through death (%)	0.13 (0.05, 0.22)	0.06 (−0.01, 0.13)	0.07 (0.0, 0.14)	0 (−0.07, 0.08)	0.0 (−0.07, 0.08)	0.03 (−0.05, 0.11)
Total effect (%)	−1.48 (−3.26, 0.17)	−3.03 (−4.48, −1.73)	−1.98 (−3.33, −0.67)	−1.71 (−2.97, −0.46)	−1.71 (−2.97, −0.46)	−1.26 (−2.53, 0.07)

Abbreviations: CAC, coronary artery calcification.
Model 1: adjusted for age, sex, race/ethnicity, study site, education level, body mass index, estimated glomerular filtration rate, smoking status, and cigarette pack-years.
Model 2: additionally adjusted for LDL cholesterol, HDL cholesterol, sBP, diabetes status, lipid lowering medication and hypertension medication.
Metals were log-transformed and divided by creatinine to correct for urine dilution.
Direct effects represent the difference in probability of being free of cardiovascular disease (CVD) after 20 years attributable to an interquartile range increase in each individual metal not happening through changes in the CAC trajectory.
Indirect effects through CAC represent the difference in probability of being free of CVD after 20 years attributable to longitudinal changes in the CAC trajectory following an interquartile range increase in each individual metal.
Indirect effects through death represent the difference in probability of being free of CVD after 20 years attributable to death following an interquartile range increase in each individual metal.
Total effects represent the sum of all three path-specific effects.
FDR corrected p-values for the direct effect (Model 1): for 0.35 cadmium, <0.001 for copper, 0.008 for cobalt, 0.29 for uranium, for 0.009 tungsten, 0.006 for zinc.
FDR corrected p-values for the direct effect (Model 2): for 0.25 cadmium, <0.001 for copper, <0.001 for cobalt, 0.25 for uranium, 0.02 for tungsten, 0.22 for zinc.
FDR corrected p-values for the indirect effect through CAC (Model 1): <0.001 for cadmium, 0.02 for copper, 0.02 for cobalt, 0.09 for uranium, 0.02 for tungsten, <0.001 for zinc.
FDR corrected p-values for the indirect effect through CAC (Model 2): <0.001 for cadmium, 0.55 for copper, 0.06 for cobalt, 0.37 for uranium, 0.13 for tungsten, <0.001 for zinc.
FDR corrected p-values for the indirect effect through death (Model 1): <0.001 for cadmium, 0.14 for copper, 0.09 for cobalt, 0.45 for uranium, 0.82 for tungsten, 0.45 for zinc.
FDR corrected p-values for the indirect effect through death (Model 2): 0.02 for cadmium, 0.18 for copper, 0.15 for cobalt, 0.52 for uranium, 0.99 for tungsten, 0.52 for zinc.
FDR corrected p-values for the total effect (Model 1): 0.17 for cadmium, <0.001 for copper, 0.003 for cobalt, 0.19 for uranium, for 0.003 tungsten, <0.001 for zinc.
FDR corrected p-values for the total effect (Model 2): 0.09 for cadmium, <0.001 for copper, <0.001 for cobalt, 0.21 for uranium, 0.08 for tungsten, 0.09 for zinc.

other than CVD. This is consistent with previous studies reporting strong associations between cadmium and all-cause mortality in MESA and other cohorts (Martinez-Morata et al., 2024; Tellez-Plaza et al., 2012; Menke et al., 2009; Larsson and Wolk, 2016), and highlights the importance of accounting for mortality due to other diseases associated with cadmium, such and cancer (Hartwig, 2013) or lung disease (Sun et al., 2024).

While we did not find statistically significant mediated effects for incident CVD via CAC for urinary uranium, previous studies have shown associations between uranium and both CAC and incident CVD (McGraw et al., 2024; Martinez-Morata et al., 2024). Studies of the health effects of uranium in US populations exposed to high and moderate levels have identified an association with increased oxidative stress and systemic inflammation, supporting the role of this non-essential metal in CVD (Dashner-Titus et al., 2018; Bayesian, 2025). Thus, further mediation studies with increased power should be conducted to investigate the potential intermediate role of CAC on the association between uranium and CVD.

Essential metals are needed in the body for cellular growth and development (Jomova et al., 2022). However, increasing levels of essential metals in extracellular compartments such as serum or urine may not reflect excess intake, but early cardiometabolic dysregulation and release from the intracellular compartments (Malekahmadi et al., 2020; Chen et al., 2023). The fact that elevated essential metals such as copper, zinc, and cobalt showed the strongest association with CVD is remarkable, and while it has been reported in several populations (Domingo-Relloso et al., 2019), it remains a largely overlooked concept. Our mediation analysis shows that most of the association between urinary copper and CVD might involve other alternative pathways beyond CAC. Other pathways such as diabetes, which has been associated with elevated urinary zinc and copper levels (Galvez-Fernandez et al., 2022; Gembillo et al., 2023), might be playing a key role in these associations. Future work in the statistical methods development field should include methods for the evaluation of multiple longitudinal mediating pathways simultaneously, in order to shed light on the different pathways that are involved in metal-related CVD.

Our novel statistical method allowed us to disentangle the different pathways that contribute to the association between metals and CVD, incorporating a continuous mediator that leverages the CAC-SW for measuring CAC status, and to evaluate the role of competing risks, specifically, deaths from non-CVD causes. Traditional Cox proportional hazards models censor people that die as if they had dropped out of the study, and therefore consider they could develop the disease at the same rate as those who remain in the study, leading to a potential over-estimation or underestimation of the association. Other approaches for competing risks in mediation analysis include the survivor average

causal effect (Tchetgen Tchetgen, 2014) and separable effects (Stensrud et al., 2021). These methods, however, are focused on a population that is either unrealistic (all survivors), or not observed (those who would survive no matter the exposure level). Our method decomposes the contribution of each pathway to the association between an exposure and a health outcome while accounting for people that die during follow-up. This provides a better opportunity to investigate different pathways involved in the adverse health effects of elevated urinary metal levels. Other strengths include the large sample size and long follow-up, the inclusion of ethnically diverse participants from different geographic locations, the high-quality surveillance of CVD outcomes, the use of the CAC-SW, a more sensible and accurate method than the traditional Agatston score, to measure CAC, and the ability to account for a wide range of confounders. For example, LDL cholesterol is an important risk factor for CAC and for subsequent CVD, thus, we adjusted Model 2 by LDL cholesterol levels and lipid-lowering medication (92 % statin use). Lipid-lowering medication can strongly modify both LDL cholesterol levels and CAC trajectories. In observational studies, statin use has been associated with increased calcification due to conversion of noncalcified plaque to calcified plaque (Shahraki et al., 2023). In our previous work evaluating the association between metals and CAC in MESA, we conducted a stratified analysis by lipid-lowering medication use and found no evidence of differential association in the two subgroups (McGraw et al., 2025). Future research should evaluate how lipid-lowering medication use might contribute to higher CAC levels.

Our study has several limitations. First, we rely on a single time point for urinary metals measurement. Previous work in MESA using a subset of participants with metals measured in exam 1 and 5 (8–11 years apart) have shown mostly consistent results (McGraw et al., 2024), supporting that single urinary metal measures might reflect long-term exposure and internal dose. Nevertheless, this group of participants represents a very small subset of the population, and thus, potential exposure misclassification cannot be ruled out. Our g-formula algorithm considers discrete time points for death, rather than modeling death as time-to-event, which leads to a loss of granularity and potential measurement error. Future work should include the extension of this algorithm to multi-state models (Valeri et al., 2023), in which the time to death and the time to the event of interest are considered jointly. In addition, we used MICE to impute missing CAC-SW values as our model does not currently support incomplete longitudinal data. Future work should focus on an extension of this method to mixed effects models, in order to compare imputed results to those fitted in incomplete data. Given the importance of including time-varying exposures in mediation designs (Gao et al., 2021; Hao et al., 2022), this method should be extended to mixture models that are able to accommodate longitudinal exposures, such as Bayesian Kernel Machine Regression (Bobb et al., 2015), which accommodates multiple environmental exposures in the same model and evaluates non-linearities, as well as interactions. Overall, caution is needed when interpreting these results because as in any observational study, unmeasured confounding cannot be ruled out.

In conclusion, our method allowed us to identify that the association of urinary cadmium, copper, cobalt, tungsten, and zinc with CVD might be partially mediated by longitudinal changes in CAC. This pathway might be particularly important for cadmium, zinc, and tungsten, while other biological pathways might be more relevant for copper and cobalt. Additionally, we were able to simultaneously characterize, for the first time, the pathway through mortality, which explains to what extent increases in metal concentrations are associated with a reduction in CVD cases due to an increase in competing events that might be more lethal than CVD. Our results could inform strategies for early detection and prevention of CVD based on CAC measurements, and the implementation of preventive strategies to address CVD risk by reducing exposure to metals, especially for cadmium.

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Disclosures

None.

CRediT authorship contribution statement

Arce Domingo-Relloso: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Katlyn E. McGraw:** Supervision, Data curation, Conceptualization. **Irene Martinez-Morata:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Yuchen Zhang:** Methodology. **Kathrin Schilling:** Writing – review & editing, Methodology, Data curation. **Ronald A. Glabonjat:** Writing – review & editing, Methodology, Data curation. **Ziqing Wang:** Software, Methodology. **Kiros Berhane:** Writing – review & editing, Methodology. **Brent A. Coull:** Writing – review & editing, Methodology. **Marta Galvez-Fernandez:** Writing – review & editing, Data curation. **Miranda R. Jones:** Writing – review & editing, Conceptualization. **Wendy S. Post:** Writing – review & editing, Investigation. **Joel Kaufman:** Writing – review & editing, Supervision, Conceptualization. **Tiffany R. Sanchez:** Writing – review & editing, Investigation. **Maria Tellez-Plaza:** Writing – review & editing, Investigation. **Graham R. Barr:** Writing – review & editing, Supervision, Conceptualization. **Steven Shea:** Writing – review & editing, Supervision, Investigation. **Ana Navas-Acien:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Linda Valeri:** Supervision, Methodology, Funding acquisition, Conceptualization.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2026.110095.

Data availability

Data can be made available upon formal request through the MESA website.

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