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Associations between heavy metal exposure and vascular age: a large cross-sectional study

Yuntao Feng[1,4†], Chengxing Liu[1†], Litang Huang[2†], Jun Qian[1], Na Li[3], Hongwei Tan[1*] and Xuebo Liu[1*]

Abstract

Background Heavy metal exposure is an emerging environmental risk factor linked to cardiovascular disease (CVD) through its effects on vascular ageing. However, the relationship between heavy metal exposure and vascular age have not been fully elucidated.

Methods This cross-sectional study analyzed data from 3,772 participants in the National Health and Nutrition Examination Survey (NHANES) from 2005 to 2016. We measured urinary concentrations of nine heavy metals and assessed their associations with vascular age, estimated pulse wave velocity (ePWV) and heart vascular age (HVA). Additionally, sex-stratified analyses, Weighted Quantile Sum (WQS) regression and Bayesian Kernel Machine Regression were conducted to explore the effects of individual and mixed metal exposures.

Results Exposure to metals such as cadmium (Cd) cesium (Cs), cobalt (Co), and lead (Pb) was significantly associated with increased vascular age, with odds ratios (OR) ranging from 1.05 to 3.48 in full adjusted models. Sex-stratified analyses indicated that individual metal including cobalt (Co) and cadmium (Cd) exposures had a more substantial impact on males. WQS analysis consistently showed combined heavy metals exposure had stronger associations with increased vascular age in men (OR for HVA = 3.89, 95% CI 2.91–5.28).

Conclusions This study highlights a significant association between heavy metal exposure and increased vascular age. Stratified analyses illustrated men might be more susceptible to the combined effects of multiple heavy metal exposure. The findings underscore the importance of considering sex-specific responses and interventions measures in cardiovascular risk assessments and managements. Further research is needed to validate these findings and to develop more precise public health strategies targeting environmental risks.

Keywords Heavy metal exposure, Vascular ageing, Vascular age, Estimated pulse wave velocity

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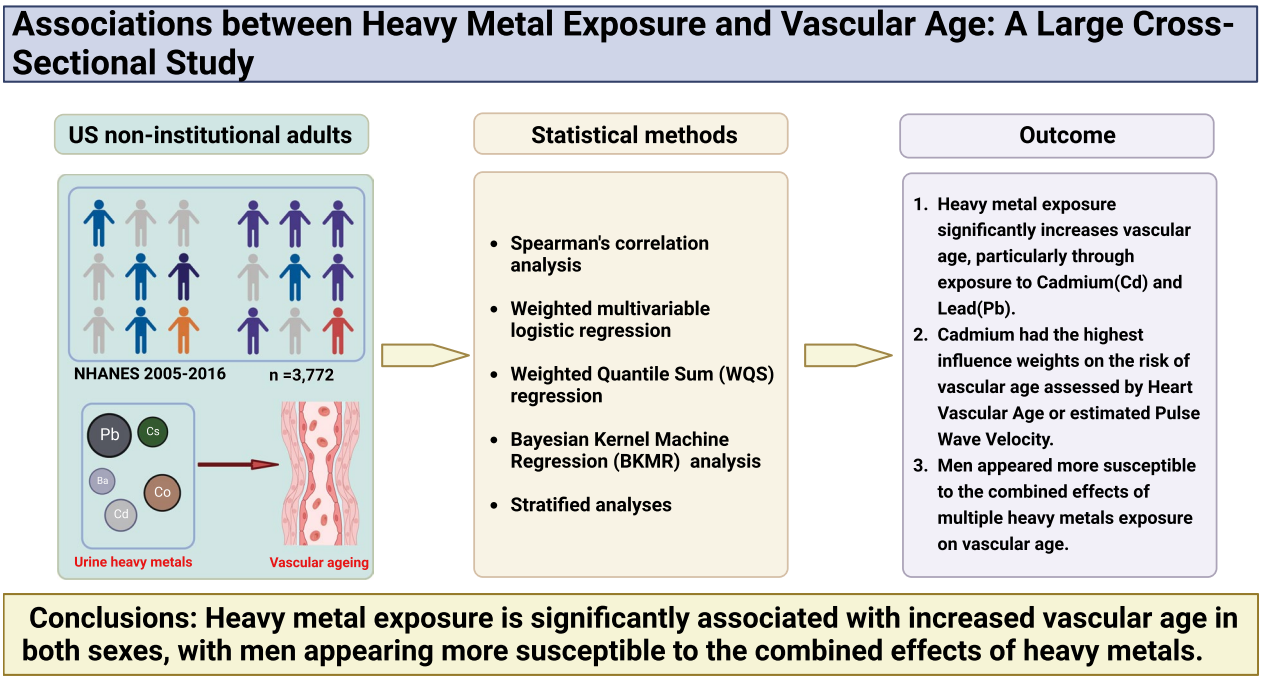
Graphical Abstract



Introduction

Cardiovascular diseases (CVD) remain the leading cause of mortality worldwide, with ageing being a significant risk factor [1–3]. As the vascular system ages, it undergoes structural and functional changes, such as increased arterial stiffness and reduced endothelial function, which elevate the risk of cardiovascular diseases including hypertension, stroke, and heart failure [4, 5]. Understanding the factors that influence vascular ageing is essential for early detection and prevention of cardiovascular events.

Extensive research have highlighted the negative effects of heavy metals, such as cadmium and lead, on vascular ageing [6–9]. These metals are known to induce oxidative stress, inflammation, and endothelial dysfunction, all of which can accelerate the ageing process of the vascular system. It should be emphasized that chronological age alone is too simplistic to predict cardiovascular events. Determining 'vascular age' is essential for cardiovascular risk stratification, considering age-related changes in vessels and cell phenotypes vary with clinical contexts [10, 11]. However, despite abundant evidence linking heavy metals exposure with cardiovascular pathology and arterial ageing, few studies have concurrently measured metal concentrations and vascular age in a representative general population. Moreover, significant gaps remain in our understanding of how environmental exposures contribute to vascular ageing.

There are various methods to assess vascular age. Structurally, arteriosclerosis results from changes in the architecture and function of the arterial wall, representing vascular ageing [12]. Estimated Pulse Wave Velocity (ePWV) is an indicator that measures the velocity of arterial blood flow, reflecting arterial stiffness and serving as a reliable predictor of vascular age [13, 14]. Additionally, the concept of vascular age prediction based on risk scoring offers another approach to calculating vascular age. Heart vascular age (HVA) is a conceptual measurement that matches an individual's cardiovascular risk with their chronological age, providing an intuitive measure of cardiovascular risk and vascular age [15].

Sex, as a major cardiovascular risk factor, significantly influences various aspects of cardiovascular health and mortality [16]. Although the risk factors and mechanisms underlying vascular ageing are well-established, significant gaps remain in our understanding of sex differences in vascular ageing, particularly in the context of environmental exposures [17]. Recent studies have highlighted the protective role of female sex in vascular diseases, particularly through differences in inflammation and vascular remodeling [18].Moreover, men and women often exhibit differential vascular ageing risk profiles,

which may be influenced by environmental factors such as heavy metal exposure [19–22]. These findings underscore the need for sex-specific approaches in assessing the effects of heavy metals on vascular ageing, in order to optimize prevention and treatment strategies for both men and women.

The primary objective of this study is to investigate the association between heavy metal exposure and vascular ageing using various statistical methods, addressing significant knowledge gaps in the field. Additionally, we aim to explore the potential sex specific differences of the relationship between heavy metal exposure and vascular ageing, providing a more precise preventive strategy for the control of heavy metals exposures in the general populations.

Methods

Data source

The National Health and Nutrition Examination Survey (NHANES) is a large cross-sectional study that assesses the health and nutritional status of non-institutionalized American adults and children by collecting demographic, dietary, examination, and laboratory data on a biennial basis. The study protocol and procedures were approved by the Institutional Review Board of the National Center for Health Statistics (NCHS), and all adult participants provided written informed consent before participation. These data can be found on the website of the Centers for Disease Control and Prevention (CDC) at https://wwwn.cdc.gov/Nchs/Nhanes/.

We retrieved data from six consecutive NHANES survey cycles conducted biennially between 2005 and 2016, encompassing specific cycles (e.g., 2005–2006, 2007–2008, etc.), with follow-up until the end of December 2019. Inclusion criteria encompassed participants aged 20 years and older with complete urinary heavy metal measurements and relevant vascular age data. After excluding participants with missing metal data, uncertain medical histories, and insufficient information for calculating vascular age, a total of 3,772 participants were included in the statistical analysis (Figure S1).

Measurements of urinary heavy metals

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is a multi-element analytical technique used to measure the following elements in urine: barium (Ba), cadmium (Cd), cobalt (Co), cesium (Cs), lead (Pb), antimony (Sb), thallium (Tl), tungsten (Tu) and uranium (Ur). Sample collection was conducted using standardized NHANES protocols, with urine samples preserved at − 20 °C until analysis. Creatinine analysis utilizes the Jaffé rate reaction, where creatinine reacts with picric acid in an alkaline solution to form a red creatinine-picrate complex. ICP-MS was calibrated with certified standards, and quality control (QC) procedures included the use of internal standards and replicate analyses to ensure analytical accuracy and precision. Values below the detection limit (LOD) are replaced with the square root of LOD divided by 2. We adjusted the concentrations of all metals through urinary creatinine (ug/g). Due to the skewed distribution of metals in the data, natural logarithm (Ln) transformation was used for subsequent analysis.

For detailed information on metal urine sampling, storage, measurement, and quality control (QC) procedures, please visit https://wwwn.cdc.gov/Nchs/Nhanes/2015-2016/SSKL_I.htm#Description_of_Laboratory_Methodology.

Vascular age

Vascular age is assessed by ePWV and HVA based on Framingham risk score (FRS)[23]

ePWV is a non-invasive method of measuring pulse wave velocity (PWV) that incorporates mean blood pressure (MBP), calculated from systolic blood pressure (SBP) and diastolic blood pressure (DBP), and age [24]. Blood pressure measurements were obtained using a standardized protocol, where the average of three consecutive readings taken by trained professionals after a five-minute rest period was used for analysis [25].The formula for calculating ePWV is as follows:

$$\begin{aligned} \mathrm{ePWV} = {} & 9.587 - 0.402 \times age + 4.560 \times 10^{-3} \\ & \times age^2 - 2.621 \times 10^{-5} \times age^2 \times MBP \\ & + 3.176 \times 10^{-3} \times MBP \times age - 1.832 \\ & \times 10^{-2} \times MBP. \end{aligned}$$

$$\mathrm{MBP} = DBP + 0.4 \times [SBP - DBP]$$

HVA represents the age of individuals with the same cardiovascular disease risk as assessed by the FRS. The FRS calculation includes factors such as age, total cholesterol, high-density lipoprotein cholesterol, upper arm systolic pressure, duration of hypertension treatment, smoking status, and diabetes, which provides sex-specific results [15]. In the mediation analysis, we explored what factors in the calculations of ePWV and HVA may have contributed to the increased vascular age.

For computational convenience, we used 80 years and 30 years to replace the "> 80" and "< 30" data in HVA, respectively. According to the literature, 60 years is a critical cardiovascular risk threshold, and the normal value for PWV reflecting arteriosclerosis is typically less than

10 m/s [26, 27]. Therefore, we defined an HVA ≥ 60 years or an ePWV ≥ 10 m/s as elevated vascular age. Additionally, participants whose HVA exceeds their chronological age were defined as HVA acceleration.

Confounders and Covariates

A directed acyclic graph (DAG) was employed to identify potential confounding variables according to prior knowledge and literature [28–30]. A single minimal sufficient adjustment set of variables was identified, which, when controlled for, is expected to block biasing pathways in the final analyses (Supplementary Figure S2). This included the following variables: (1) Demographic Factors: Age, sex, and race/ethnicity. (2)Socioeconomic Status (SES): Level of education, family poverty income ratio (PIR). (3) Lifestyle Factors: Body mass index (BMI), smoking status, and drinking status. (4) Social Environment: Marital status.

Statistical analysis

Spearman's correlation analysis was used to evaluate the relationships between metals after Ln transformation. Considering the complex sampling design of NHANES, we utilized sample weights (WTSA2YR) to estimate population-representative results in subsequent analyses, as recommended by the official analysis guide, ensuring the representativeness of the samples in the population. Unfortunately, the algorithmic complexity of the Weighted Quantile Sum (WQS) regression and Bayesian Kernel Machine Regression (BKMR) renders them unsuitable for weighted data.

For descriptive statistical characteristics, weighted means (standard errors) and sample sizes (weighted percentages) were used to represent continuous and categorical variables, respectively. Weighted variance tests and weighted chi-square tests were used to compare intergroup differences. The relationships between different metal exposures and various vascular age indices were analyzed using weighted multivariable logistic regression. We used the Benjamini & Hochberg method (FDR), a more flexible refinement of the Bonferroni method, to correct for multiple comparisons in single-exposure associations, in order to minimize the false discovery rate of Type I errors [31]. Stratified analyses explored the relationships between different metal exposures and various vascular age indices across sexes.

Given that heavy metals are a mixture of multiple, intercorrelated constituents, we employed Weighted Quantile Sum (WQS) regression to examine the combined effects of these constituents [32]. WQS regression is particularly suited to analyzing complex environmental mixtures with high collinearity among constituents, as it generates a composite index that reflects the mixture's cumulative effect. To create this index, we categorized each constituent into quantiles and employed a two-step process for weight estimation. First, we split the dataset into a training set (40%) and a validation set (60%), using the training data to derive weights for each constituent through bootstrap sampling. For each bootstrap sample, weights were estimated through an optimization function that constrained them to sum to one, ensuring that all weights remained between 0 and 1. The final WQS index, representing the combined exposure effect, was derived by averaging weights across bootstraps and was then tested in the validation set to assess its association with the outcome. This approach helps balance the contribution of each constituent while reducing the potential confounding effects of highly correlated exposures. We used the 'gWQS' R package to conduct WQS analysis, which has showed good performance in characterizing environmental mixtures and assessing the contributions of individual components within the mixture [32–35].

Additionally, we employed a Bayesian Kernel Machine Regression (BKMR) model to assess the combined impact of heavy metals on vascular age. This approach allows for the identification of nonlinear and non-additive relationships within heavy metals mixtures. We calculated posterior inclusion probabilities (PIPs) to estimate the relative contributions of each component in the metal mixture to the outcomes with a threshold of 0.5 indicating significance and analyzed the dose–response relationships between single metals and various vascular age indices while fixing other metal concentrations. Besides, we analyzed the dose–response relationship between single metals and various vascular age indicators while holding the concentrations of other metals constant, considering the 25th, 50th, and 75th percentiles of the remaining heavy metals. Finally, after adjusting for all covariates, the Markov Chain Monte Carlo algorithm was used for 10,000 iterations to verify model convergence.

All statistical analyses were conducted using R software (Version 4.2.1, The R Foundation; http://www.R-project.org) and EmpowerStats software (Version 5.0, X&Y Solutions, Inc., Boston, MA; http://www.empowerstats.com). A p-value of less than 0.05 was deemed statistically significant.

Results

Characteristics of participants and metals distribution

Table 1 presents the baseline information of 3772 study participants, representing over 170 million non-institutionalized residents of the United States. The average age of participants was 47.61 ± 0.40 years, with 50.11% being female, and most subjects being non-Hispanic whites (70.89%). Participants who have never married, with high levels of total cholesterol (TC), triglycerides (TG),

Table 1 Baseline characteristics of study population

Characteristics	Overall	Heart/vascular age		Arterial stiffness	
		≤ 60	> 60	Low	High
Age, (years)	47.61 (0.40)	39.18 (0.36)	64.38 (0.38)	42.41 (0.36)	71.80 (0.34)
Sex (%)					
Female	1873 (50.11)	1124 (48.88)	749 (52.55)	1459 (49.65)	414 (52.24)
Male	1899 (49.89)	1117 (51.12)	782 (47.45)	1420 (50.35)	479 (47.76)
Race/ethnicity, n (%)					
White	1755 (70.89)	975 (67.80)	780 (77.05)	1244 (68.82)	511 (80.55)
Black	716 (9.91)	413 (10.00)	303 (9.73)	544 (10.24)	172 (8.36)
Mexican	598 (8.25)	400 (9.82)	198 (5.11)	513 (9.28)	85 (3.41)
Other	703 (10.95)	453 (12.39)	250 (8.11)	578 (11.66)	125 (7.68)
FamilyPIR, n (%)					
< 1.3	1149 (20.02)	671 (19.64)	478 (20.78)	880 (19.95)	269 (20.37)
1.3–3.5	1468 (37.09)	816 (34.67)	652 (41.92)	1059 (35.45)	409 (44.76)
> 3.5	1155 (42.88)	754 (45.69)	401 (37.30)	940 (44.60)	215 (34.87)
Marital status, n (%)					
Divorced/separated/widowed	811 (18.33)	276 (11.92)	535 (31.09)	463 (14.48)	348 (36.27)
Married/living with a partner	2298 (64.52)	1406 (65.43)	892 (62.71)	1792 (65.33)	506 (60.74)
Never married	663 (17.15)	559 (22.65)	104 (6.19)	624 (20.19)	39 (2.99)
Education, n (%)					
College graduate or above	904 (30.58)	630 (34.59)	274 (22.60)	731 (32.05)	173 (23.70)
Some college	1089 (31.04)	699 (32.49)	390 (28.15)	875 (31.80)	214 (27.51)
High school or less	1779 (38.38)	912 (32.92)	867 (49.25)	1273 (36.15)	506 (48.79)
Smoke, n (%)					
Never	2040 (53.47)	1381 (59.66)	659 (41.16)	1617 (54.74)	423 (47.56)
Former	965 (26.43)	431 (22.00)	534 (35.25)	593 (22.81)	372 (43.33)
Now	767 (20.10)	429 (18.35)	338 (23.59)	669 (22.46)	98 (9.11)
Alcohol use, n (%)					
Never	539 (10.77)	285 (9.55)	254 (13.19)	378 (9.55)	161 (16.41)
Former	627 (14.18)	246 (10.87)	381 (20.77)	391 (12.49)	236 (22.04)
Mild	1279 (36.71)	703 (34.28)	576 (41.55)	922 (35.05)	357 (44.45)
Moderate	548 (17.50)	404 (20.15)	144 (12.22)	482 (19.01)	66 (10.44)
Heavy	779 (20.85)	603 (25.16)	176 (12.28)	706 (23.89)	73 (6.65)
Diastolic blood pressure	69.31 (0.29)	69.37 (0.33)	69.19 (0.45)	69.29 (0.31)	69.40 (0.61)
Systolic blood pressure	121.07 (0.38)	115.90 (0.36)	131.34 (0.72)	117.41 (0.31)	138.09 (1.07)
BMI (kg/cm2)	28.82 (0.14)	28.24 (0.17)	29.99 (0.24)	28.90 (0.17)	28.46 (0.21)
HbA1c (%)	5.58 (0.02)	5.35 (0.01)	6.02 (0.03)	5.51 (0.02)	5.91 (0.03)
Total cholesterol (mg/dl)	194.75 (0.88)	190.91 (1.09)	202.40 (1.50)	194.37 (1.03)	196.51 (1.86)
Total Triglyceride (mg/dL)	120.75 (1.40)	110.00 (1.73)	142.15 (2.22)	119.65 (1.66)	125.90 (2.49)
Gamma-glutamyltransferase (U/L)	28.01 (1.04)	26.92 (1.50)	30.17 (0.99)	28.15 (1.24)	27.32 (1.26)
CVD, n (%)					
No	3367 (91.76)	2183 (97.37)	1184 (80.58)	2715 (95.41)	652 (74.74)
Yes	405 (8.24)	58 (2.63)	347 (19.42)	164 (4.59)	241 (25.26)
Hypertension, n (%)					
No	2251 (63.90)	1778 (79.34)	473 (33.18)	2031 (71.84)	220 (26.91)
Yes	1521 (36.10)	463 (20.66)	1058 (66.82)	848 (28.16)	673 (73.09)
DM, n (%)					
No	1449 (42.36)	1211 (54.70)	238 (17.81)	1301 (47.63)	148 (17.83)
preDM	1573 (41.95)	914 (40.34)	659 (45.16)	1158 (40.58)	415 (48.35)
Diabetes	750 (15.68)	116 (4.96)	634 (37.02)	420 (11.79)	330 (33.83)

Table 1 (continued)

Continuous variables are presented as the weighted mean and 95% confidence interval (CI), category variables are presented as the proportion and 95% confidence interval

Family PIR: family poverty income ratio

HbA1c, and a history of cardiovascular disease and diabetes are more likely to be classified into the high vascular age group as defined by HVA or ePWV.

Table S1 describes the distribution of metal concentrations, with a detection rate of over 70% for all metals in participants' urine. Except for antimony (Sb), the detection rates of other metals in urine of the participants exceeded 85%, indicating a widespread distribution of heavy metals exposure. Spearman coefficients for ln-transformed metals showed the strongest correlation between Cs and Tl (r = 0.59). Other metals exhibited moderate to weak correlations (Supplementary Figure S2).

The relationship between single heavy metal exposure and vascular age

Weighted logistic regression analysis found an association between metal exposure and vascular age (Tables 2 and 3). In the fully adjusted model, a unit increase in ln-transformed Cd, Cs, Pb, and Ur was associated with a 248% (OR = 3.48, 95% CI 2.72–4.46), 104% (OR = 2.04, 95% CI 1.56–2.67), 101% (OR = 2.01, 95% CI 1.70–2.38), and 30% (OR = 1.30, 95% CI 1.11–1.51) increased risk of higher HVA calculated vascular age, respectively. When metal concentrations were categorized into quartiles with the lowest quartile as the reference, a similar association between metals and increased vascular age was observed (Table 2).

The association between metal exposure and increased vascular age calculated by ePWV was also evaluated (Table 3). In the fully adjusted model, a unit increase in ln-transformed Cs, Pb, Cd, and Co was associated with a 128% (OR = 2.28, 95% CI 1.60–3.26), 89% (OR = 1.89, 95% CI 1.49–2.40), 88% (OR = 1.88, 95% CI 1.48–2.40) and 78% (OR = 1.78, 95% CI 1.37–2.31), increased risk of higher vascular age, respectively. Consistent results were

Table 2 Associations of single urinary metals with Heart/Vascular age

Metals	Model	Continuous OR(95%CI)	Quartile 1	Quartile 2 OR(95%CI)	Quartile 3 OR(95%CI)	Quartile 4 OR(95%CI)	*p* for trend
Ba	Model1	1.05 (0.95,1.16)	Reference	0.63 (0.50,0.79)**	0.69 (0.54,0.88)**	0.95 (0.77,1.18)	0.082
	Model2	1.05 (0.91, 1.20)	Reference	0.66 (0.46, 0.94)*	0.63 (0.43, 0.90)*	0.92 (0.66, 1.29)	0.338
Cd	Model1	3.30 (2.83,3.85)**	Reference	2.70 (1.94,3.75)**	5.29 (3.84,7.27)**	13.01 (9.44,17.93)**	< 0.0001
	Model2	3.48 (2.72, 4.46)**	Reference	2.62 (1.78, 3.86)**	4.65 (3.11, 6.96)**	12.21 (7.77,19.20)**	< 0.0001
Cs	Model1	2.01 (1.67,2.41)**	Reference	1.56 (1.18,2.05)**	2.46 (1.86,3.25)**	2.63 (2.09,3.30)**	< 0.0001
	Model2	2.04 (1.56, 2.67)**	Reference	1.58 (1.15, 2.17)*	2.52 (1.71, 3.71)**	2.62 (1.90, 3.63)**	< 0.0001
Co	Model1	1.43 (1.25,1.64)**	Reference	1.25 (0.98,1.59)	1.74 (1.36,2.24)**	1.81 (1.41,2.32)**	< 0.0001
	Model2	1.23 (0.98, 1.54)	Reference	1.11 (0.82, 1.49)	1.53 (1.09, 2.14)*	1.49 (1.01, 2.20)*	0.047
Pb	Model1	2.42 (2.11,2.78)**	Reference	2.40 (1.82,3.16)**	3.64 (2.82,4.70)**	5.57 (4.23,7.35)**	< 0.0001
	Model2	2.01 (1.70, 2.38)**	Reference	1.92 (1.37, 2.70)**	2.93 (2.16, 3.98)**	3.43 (2.43, 4.84)**	< 0.0001
Sb	Model1	1.03 (0.89,1.19)	Reference	1.26 (0.97,1.63)	1.22 (0.94,1.59)	1.11 (0.82,1.51)	0.795
	Model2	1.08 (0.88, 1.33)	Reference	1.35 (0.91, 2.01)	1.35 (0.93, 1.96)	1.21 (0.78, 1.88)	0.633
Tl	Model1	0.96 (0.81,1.13)	Reference	0.92 (0.74,1.14)	0.77 (0.63,0.95)*	0.94 (0.73,1.21)	0.719
	Model2	1.24 (0.98, 1.56)	Reference	1.17 (0.87, 1.56)	0.92 (0.68, 1.23)	1.39 (0.99, 1.96)	0.103
Tu	Model1	1.07 (0.96,1.19)	Reference	1.12 (0.88,1.43)	1.18 (0.91,1.52)	1.24 (0.97,1.58)	0.126
	Model2	1.07 (0.90, 1.26)	Reference	1.28 (0.88, 1.87)	1.27 (0.88, 1.83)	1.26 (0.87, 1.82)	0.418
Ur	Model1	1.27 (1.14,1.42)**	Reference	1.14 (0.89,1.47)	1.41 (1.08,1.85)*	1.78 (1.35,2.36)**	< 0.0001
	Model2	1.30 (1.11, 1.51)**	Reference	1.10 (0.79, 1.54)	1.51 (1.03, 2.22)*	1.77 (1.18, 2.66)*	0.002

Model 1 was crude model; Model 2 was adjusted for age, sex, race/ethnicity, level of education, family poverty income ratio (PIR), body mass index,marital status, smoking status, drinking status. Continuous, ln-transformed concentration of metal

Q: quartile; Ba:barium; Cd:cadmium; Co: cobalt; Cs: cesium; Pb: lead; Sb: antimony; Tu: tungsten; Tl: thallium; Ur: uranium

* Test for trend (p for trend) was tested by incorporating the variables of the median of each quartile into the regression model; *: $p < 0.05$;**: $p < 0.01$

observed in the regression analysis of categorized metals (Table 3).

To further clarify the impact of metal exposure on vascular age risk, we explored the relationship between metal exposure and vascular age exceeding chronological age (vascular age acceleration). However, weighted logistic regression analysis did not reveal significant statistical association between most metal exposures and vascular age acceleration (Table S2). After adjusting for multiple comparisons, exposures to different metals such as Cd, Cs, and Pb remained significantly associated with various vascular age indicators (adjusted $p < 0.05$, Table S3).

The relationship between multi metal exposures and vascular age

To elucidate the impact of multiple heavy metal exposures on vascular age, we conducted Weighted Quantile Sum (WQS) analysis, which were proved to be effective in environmental research [32, 36]. This analysis provides a comprehensive understanding of the effects of mixed metal exposures on vascular age. In the fully adjusted model, an increase in the quartiles of the WQS index was significantly positively associated with increased HVA (OR = 2.99, 95% CI 2.52–3.57) and ePWV (OR = 2.20, 95% CI 1.67–2.92) calculated vascular age (Table 4). Furthermore, after adjusting for all covariates, the metals with the highest influence weights on the risk of vascular age assessed by HVA or ePWV were Cd (0.65 and 0.36) and Pb (0.23 and 0.27) (Fig. 1A and B).

We also used Bayesian Kernel Machine Regression (BKMR) to explore nonlinear relationships between metal exposures and vascular age, as well as to examine interactive effects between different metals [37]. Consistent with the WQS results, BKMR identified Cd and Pb as the most important factors influencing increased vascular age calculated by HVA (PIP 1.00 and 0.96) or ePWV (PIP 1.00 and 0.99)(Table S4). Besides, the BKMR model also identified the dose–response relationship between metal exposure and different vascular age indicators. When all other metals were at their median levels, Cd and Pb were positively associated with an increased risk of vascular age (Figure S4 A-B). And the combined effect of urinary metals was significantly associated with increased vascular age (measured by HVA and ePWV) when the concentrations of all metal mixtures were at or above the 55th percentile (Figure S4 C-D).

Table 3 Associations of single urinary metals with Estimated Pulse Wave Velocity

Metals	Model	Continuous OR(95%CI)	Quartile 1	Quartile 2 OR(95%CI)	Quartile 3 OR(95%CI)	Quartile 4 OR(95%CI)	*p* for trend
Ba	Model1	1.10 (0.98,1.23)	Reference	0.81 (0.60,1.09)	0.95 (0.72,1.25)	1.16 (0.87,1.54)	0.058
	Model2	1.29 (1.10, 1.53)**	Reference	1.13 (0.73, 1.77)	1.45 (0.77, 2.72)	1.85 (1.20, 2.86)*	0.012
Cd	Model1	2.23 (1.94,2.55)**	Reference	2.81 (1.96, 4.02)**	5.27 (3.52, 7.89)**	7.33 (5.01,10.71)**	< 0.0001
	Model2	1.88 (1.48, 2.40)**	Reference	2.40 (1.36, 4.26)**	3.42 (1.71, 6.82)**	4.07 (2.16, 7.66)**	< 0.001
Cs	Model1	2.02 (1.63,2.50)**	Reference	1.86 (1.33,2.61)**	2.76 (1.96,3.90)**	3.11 (2.20,4.40)**	< 0.0001
	Model2	2.28 (1.60, 3.26)**	Reference	1.91 (1.23, 2.97)**	2.51 (1.52, 4.12)**	3.27 (1.88, 5.68)**	< 0.001
Co	Model1	1.69 (1.47,1.95)**	Reference	1.11 (0.84,1.47)	1.78 (1.34,2.36)**	2.34 (1.77,3.08)**	< 0.0001
	Model2	1.78 (1.37, 2.31)**	Reference	0.62 (0.38, 1.00)	1.49 (0.95, 2.34)	2.71 (1.72, 4.28)**	< 0.0001
Pb	Model1	2.37 (2.03,2.76)**	Reference	2.84 (1.83, 4.40)**	4.92 (3.40, 7.12)**	7.25 (4.86,10.81)**	< 0.0001
	Model2	1.89 (1.49, 2.40)**	Reference	2.19 (1.23, 3.90)*	3.82 (2.39, 6.10)**	3.66 (2.02, 6.64)**	< 0.001
Sb	Model1	0.96 (0.82,1.11)	Reference	1.18 (0.87,1.60)	1.14 (0.84,1.55)	1.02 (0.74,1.40)	0.808
	Model2	1.11 (0.88, 1.39)	Reference	1.43 (0.80, 2.54)	1.41 (0.81, 2.45)	1.52 (0.96, 2.40)	0.153
Tl	Model1	0.85 (0.71,1.02)	Reference	0.85 (0.66,1.09)	0.74 (0.57,0.95)	0.84 (0.63,1.11)	0.308
	Model2	1.12 (0.82, 1.53)	Reference	1.31 (0.77, 2.24)	0.87 (0.55, 1.37)	1.46 (0.84, 2.52)	0.323
Tu	Model1	1.15 (1.04,1.29)*	Reference	1.12 (0.85,1.47)	1.37 (1.04,1.80)*	1.47 (1.09,1.97)*	0.011
	Model2	1.33 (1.09, 1.62)**	Reference	1.33 (0.86, 2.04)	2.26 (1.39, 3.69)**	2.05 (1.21, 3.47)*	0.012
Ur	Model1	1.22 (1.11,1.35)**	Reference	1.09 (0.80,1.49)	1.32 (0.92,1.89)	1.68 (1.24,2.26)**	< 0.0001
	Model2	1.33 (1.06, 1.67)*	Reference	1.29 (0.74, 2.26)	2.11 (1.04, 4.28)*	2.08 (1.09, 3.95)*	0.015

Model 1 was crude model; Model 2 was adjusted for age, sex, race/ethnicity, level of education, family poverty income ratio (PIR), body mass index, marital status, smoking status, drinking status. Continuous, ln-transformed concentration of metal

Q: quartile; Ba: barium; Cd: cadmium; Co: cobalt; Cs: cesium; Pb: lead; Sb: antimony; Tu: tungsten; Tl: thallium; Ur: uranium

* Test for trend (p for trend) was tested by incorporating the variables of the median of each quartile into the regression model; *: $p < 0.05$;**: $p < 0.01$

Table 4 Associations between weighted quantile sum regression index and vascular age by WQS

Outcomes	OR	95% CI of OR	*P* value
Heart/vascular age			
Model 1	2.83	(2.54,3.17)	< 0.0001
Model 2	2.99	(2.52, 3.57)	< 0.0001
Estimated pulse wave velocity			
Model 1	2.46	(2.16,2.82)	< 0.0001
Model 2	2.20	(1.67, 2.92)	< 0.0001

Model 1 was crude model; Model 2 was adjusted for age, sex, race/ethnicity, level of education, family poverty income ratio (PIR), body mass index, marital status, smoking status, drinking status

The sex-stratified associations between heavy metal exposure and vascular age

Supplementary Tables S5 and S6 illustrate the associations between individual metal exposure and vascular age calculated by HVA or ePWV across different sex subgroups. In the fully adjusted model, there were no significant sex differences in the associations between heavy metal exposure and ePWV estimated vascular age (interaction $p > 0.05$) (Table S5). Notably, Cd and Co exposure were significantly associated with higher HVA-calculated vascular age in men (Table S6). After adjusting for multiple comparisons, exposure to different single metals remained significantly associated with various vascular age indicators in both genders (adjusted $p < 0.05$, Table S7).

To elucidate the mixed effects of heavy metal exposure on vascular age, we next employed WQS analysis to explore the association between WQS index and vascular age across different sexes (Table S8). In the fully adjusted model, an increase in the quartiles of the WQS index in women was significantly associated with increased vascular age, with ORs of 2.55 (95% CI 1.98–3.31) for HVA and 2.08(95% CI 1.33–3.28) for ePWV. In men, higher WQS index quartiles were also significantly associated with increased vascular age (HVA OR = 3.89, 95% CI 2.91–5.28; ePWV OR = 3.27, 95% CI 2.16–5.03), with the association being slightly stronger than in women (despite no interaction p-value). These findings suggest

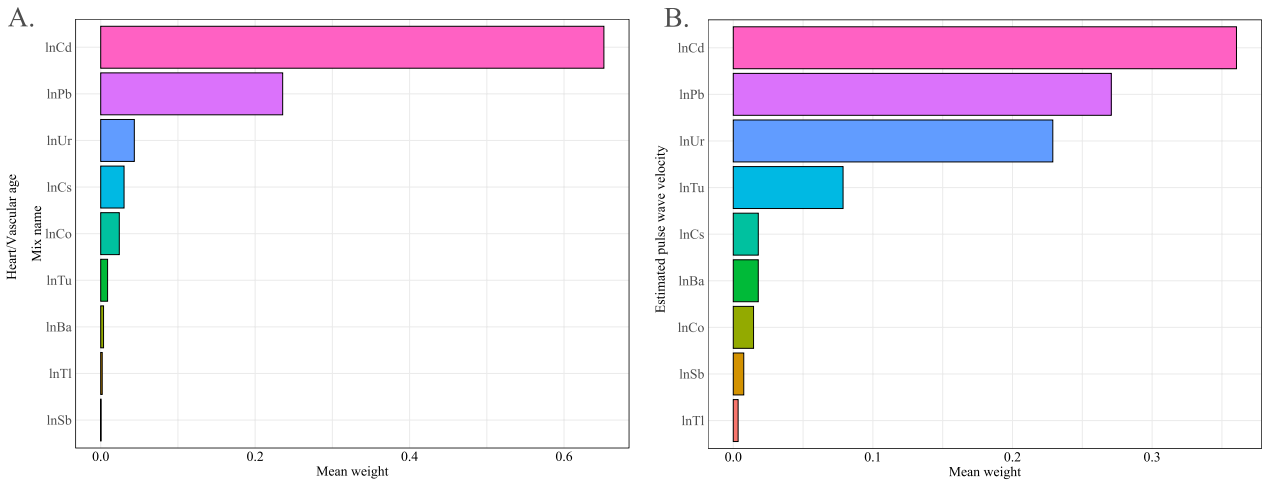


Fig. 1 Weighted values of urinary metals for vascular age in the WQS model. **A** Vascular age calculated by HVA; **B** Vascular age calculated by ePWV; Model was adjusted for age, sex, race/ethnicity, level of education, family poverty income ratio (PIR), body mass index, marital status, smoking status, drinking status. WQS, weighted quantile sum

that men may be more susceptible to the effects of exposure to single or multiple metals on vascular age.

Discussion

This study underscores a significant association between heavy metal exposure and elevated vascular age within a broad U.S. cohort, revealing that higher concentrations of metals such as cadmium (Cd), lead (Pb) and Uranium (Ur) correlate with increased vascular age. These findings align with literature indicating that environmental exposures, including heavy metals, can accelerate biological ageing, particularly in the cardiovascular system [38, 39]. Overall, these findings underscore the potential of heavy metal exposure as a modifiable risk factor influencing vascular health and longevity.

HVA indicates the age of individuals with a comparable cardiovascular disease risk as determined by the FRS [15]. The FRS assesses cardiovascular risk based on factors including age, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, duration of hypertension treatment, smoking status, and diabetes, providing results specific to each sex. Moreover, it will be more reflective of an individual's increased cardiovascular risk when the HVA exceeds the chronological age (HVA acceleration) [23]. As a matter of fact, the cardiovascular age, particularly when calculated using the FRS derived HVA, offers valuable insights into the interplay between cardiovascular health and metabolic factors such as lipid and glucose metabolism [40]. Previous research revealed that single metal exposure or mixed metal exposure were related to increased vascular ageing as calculated by FRS derived risk score [41, 42]. Our results also found that several heavy metals including Cd(cadmium), Cs (cesium), and Pb(lead) were associated with increased risk of higher HVA. Vascular age was calculated using the FRS-derived HVA, which reflects heightened vascular senescence rather than chronological age. Previous research by Hamczyk et al. has suggested that vascular age may serve as a more sensitive metric for identifying individuals at high risk for cardiovascular events [43]. Our findings further support this notion, demonstrating that heavy metal exposure is associated with accelerated vascular ageing, as indicated by an increase in HVA. This suggests that the FRS-derived HVA provides critical value in assessing cardiovascular risk that extends beyond chronological age. To sum up, our findings suggest that exposure to heavy metals is associated with an increase in HVA, indicating a potential pathway through which metals influence cardiovascular health. This relationship underscores the importance of monitoring metal exposure and HVA beyond traditional risk factors in the assessment of cardiovascular risks [44].

Arterial stiffness is a critical factor in the process of vascular ageing [45], reflecting changes in the structure and function of blood vessels that increase with age. This stiffening of the arteries is closely linked to the progression of age-related vascular diseases, making it a key area of study for understanding and potentially mitigating cardiovascular risk [46]. Pulse wave velocity (PWV) is a clinically established measure of arterial stiffness and is often used as an indicator of vascular ageing. Despite its relevance, the widespread clinical application of PWV measurement is hindered by the need for specialized equipment and trained personnel. This study utilized ePWV derived from FRS, presenting a viable alternative for assessing arterial stiffness in environments lacking

the necessary resources for direct PWV measurement. In our study, association analysis revealed that Cadmium (Cd), Cesium (Cs) and Lead (Pb) exposure corresponded to a higher risk of elevated ePWV. Notably, we observed a consistent association between metal exposure and increased vascular age, regardless of whether it was determined by HVA or ePWV. However, due to the distinct methodologies underlying these calculations, the magnitude of vascular age elevation varied across specific metal exposures. HVA incorporates a broader range of cardiovascular risk factors, including chronological age, lipid and glucose levels, hypertension, and smoking status, providing a more comprehensive assessment of vascular health. In contrast, ePWV primarily focuses on chronological age and arterial stiffness indices. Consequently, the inclusion of these factors in the HVA calculation may amplify the observed association between heavy metal exposure and increased vascular age compared to ePWV, which predominantly reflects arterial stiffness. Overall, our results support the association between heavy metal exposure and increased ePWV, reinforcing the utility of ePWV as a surrogate marker in evaluating vascular health [47, 48].

Heavy metals are increasingly recognized for their role in accelerating biological ageing, contributing to the development of age-related diseases [39, 49]. Studies have demonstrated that exposure to metals such as cadmium and lead can significantly hasten the biological ageing process, as measured by markers of cellular senescence [38, 50]. Experiments in mice have demonstrated that cadmium exposure leads to damage in both the intimal and medial layers of the aorta [51]. Mechanistically, cadmium exposure may increase the expression of von Willebrand factor (vWF), a key mediator of endothelial dysfunction, which in turn accelerates vascular ageing [9, 52].In addition, chronic low-level lead exposure causes arterial stiffness and vascular ageing by promoting endothelial dysfunction, lipid disturbance and arteriosclerosis, with studies elucidating its cellular and molecular mechanisms [53–55]. Besides, single heavy metal and mixed heavy metals exposure including cesium, cobalt, and thallium were significantly associated arterial stiffness, lipid disorders and increased cardiovascular risks [56]. Moreover, Pamphlett et al. demonstrated selective metal accumulation in neurons, particularly in the locus ceruleus neurons, which could contribute to accelerated neurodegeneration and vascular ageing through neuroinflammation and blood–brain barrier impairment, adding another dimension to our understanding of metal-induced vascular ageing [57].This neurovascular mechanism underscores the potential systemic effects of heavy metal exposure, highlighting the need for translational studies to elucidate the underlying pathways through which these toxins affect vascular health. In general, chronic exposure to these heavy metals can lead to endothelial dysfunction, increased oxidative stress, and inflammation, thereby exacerbating the process of vascular ageing. Our study aligns with existing literatures by highlighting the adverse impact of heavy metals on vascular ageing [7, 58]. Further research is necessary to investigate how heavy metals might adversely affect vascular ageing, which would possibly offer control measures or drugs targeting the adverse effects of heavy metals exposure on vascular health.

Our findings, using both HVA and ePWV markers, consistently identified cadmium and lead as a major contributor to vascular ageing. This aligns with evidence linking low-level chronic cadmium and lead exposure to endothelial dysfunction, oxidative stress, and arteriosclerosis [39, 49, 51, 54, 55, 59, 60]. These results underscore the critical need for environmental control measures aimed at reducing cadmium and lead accumulation, which could help mitigate their detrimental effects on human health. Notably, a study by Wang et al. on humic substances suggests that soil humic acid can reduce the bioavailability of heavy metals, offering a promising environmental strategy to minimize their impact by stabilizing metals in the soil [61]**.** These insights highlight the potential for targeted public health policies focused on controlling heavy metal exposure, which might serve as effective measures for mitigating vascular ageing.

Our study also revealed gender differences in the response to metal exposure, with males showing a stronger association both with single-metal exposure and with the combined effects of multiple metals. Previous studies have highlighted sex-specific neurotoxic effects of heavy metal exposure, which may be attributed to varying levels of metal accumulation between men and women [22]. Epidemiological and laboratory research indicates that males often carry a higher burden of heavy metals, increasing their susceptibility to adverse effects, including impaired cognitive development [62]. Trace element deficiencies, such as those in iron, further influence the absorption and processing of heavy metals, exacerbating their impact on vascular health [63, 64]. Nutritional status also plays a significant role, as men and women often exhibit different nutrient profiles, which can affect how heavy metals are absorbed and metabolized [65, 66]. Moreover, variations in endocrine, genetic, biochemical, and environmental factors contribute to the unequal vulnerability between sexes, potentially explaining why men may experience more pronounced vascular aging when exposed to environmental toxins [21]. To comprehensively understand this phenomenon, it is essential to

pursue large-scale, multicenter, multi-population observational studies, as randomized controlled trials on heavy metal exposure would be ethically unfeasible.

Strengths and limitations

Our study offers multiple strengths but also acknowledges inherent limitations.. Firstly, it employs a sophisticated statistical framework, including weighted logistic regression, WQS regression, and BKMR, to evaluate the relationship between heavy metal exposure and vascular age. These methods enhance the reliability of our findings, particularly in handling complex interactions and mixed exposures, though they may introduce variability due to model assumptions. Secondly, the use of HVA and ePWV as surrogate markers for vascular age represents a major strength. Derived from the Framingham Risk Score, these measures offer a clinically relevant evaluation of cardiovascular health and its deterioration due to environmental factors. However, the reliance on surrogate markers, derived from the Framingham Risk Score and not direct clinical measurements, could lead to imprecision. This imprecision could affect our ability to fully capture vascular stiffness changes related to heavy metal exposure. Thirdly, our study's approach to examining both individual and combined metal exposures addresses the complexities of real-world environmental exposure, which often involves multiple toxins.

While this study provides evidence linking heavy metal exposure to increased vascular age, it is important to acknowledge several limitations. Firstly, the cross-sectional design of the study limits our ability to establish causality between heavy metal exposure and vascular age. Longitudinal studies are required to confirm the temporal sequence and causative effects of metal exposure on vascular health. Our reliance on cross-sectional data implies that observed associations may also be influenced by reverse causation or unaccounted temporal factors, adding caution to any causal interpretation. Moreover, despite adjustments for numerous confounders, the potential for residual confounding due to unmeasured or inadequately measured variables persists. Factors such as dietary habits, genetic predispositions, and other environmental exposures could simultaneously influence metal levels and vascular health. For example, nutritional status influences heavy metal absorption and processing [63, 65, 66], potentially amplifying the detrimental effects on vascular health. The direction and magnitude of such confounding are difficult to precisely estimate, but it is plausible that the observed associations between metal exposure and vascular aging could be more pronounced in populations with poorer nutritional status, leading to a bias toward overestimating the effects of heavy metals on vascular aging in these groups. Additionally, another limitation lies in the age specificity of the HVA model, which was validated primarily for individuals aged 40–65. The applicability and accuracy of this model in estimating vascular age outside this age range require further investigation, as using it for older or younger populations could introduce measurement biases. Moreover, the use of ePWV, derived from risk scores rather than direct clinical measurements, may introduce variability in accurately estimating vascular stiffness. The absence of direct physical measurement tools could compromise the precision of the study's findings. Although the sample is representative of the general U.S. population, the results may not fully apply to populations from other regions or countries with different environmental, socioeconomic, and health profiles. The study also suggests potential sex differences in the impact of heavy metals on vascular health; however, these findings were not statistically significant. Therefore, the observed trends should be interpreted cautiously, and future studies with larger sample sizes are warranted to further elucidate these differences.

Conclusions

In conclusion, our research provides evidence supporting an association between heavy metal exposure and accelerated vascular ageing, as measured by HVA and ePWV. Additionally, our findings indicate a potentially greater impact of metal exposure on vascular age in males compared to females, though these results require cautious interpretation. Overall, our study highlights the importance of public health initiatives aimed at monitoring and reducing environmental metal exposure to mitigate cardiovascular risk and promote health, especially among vulnerable populations. However, due to the study's observational design and limitations, the clinical significance of these findings remains exploratory, and further longitudinal studies are needed to substantiate these associations. If confirmed, these findings could guide risk assessment and preventive strategies for vascular health, especially in metal-exposed populations.

Abbreviations

FRS	Framingham risk score
HVA	Heart vascular age
ePWV	Estimated pulse wave velocity
LDL-C	Low-density lipoprotein cholesterol
TC	Total cholesterol
TG	Triglyceride
WQS	Weighted quantile sum
BKMR	Bayesian Kernel Machine Regression

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Author contributions
YF: Conceptualization; Methodology; Data curation; Formal analysis; Software; Project administration; Writing—original draft. CL: Conceptualization; Supervision; Validation; Writing—original draft. LH: Methodology; Data curation; Supervision. JQ: Investigation; Methodology; Resources; Supervision; Validation; Funding acquisition; Writing—review, editing. NL: Data curation; Resources; Writing—review, editing. HT: Investigation; Methodology; Validation; Funding acquisition; Project administration; Resources; Supervision. XL Conceptualization; Methodology; Funding acquisition; Resources; Supervision; Validation; Writing— review, editing.

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Data availability
The data can be found in the National Health and Nutrition Examination Survey: https://www.cdc.gov/nchs/nhanes/index.htm.

Declarations

Competing interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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