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Exposure to metal mixtures in relation to blood pressure among children 5–7 years old

An observational study in Bangladesh

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Background: Hypertension in later life, a significant risk factor for cardiovascular disease, has been linked to elevated blood pressure in early life. Exposure to metals may influence childhood blood pressure; however, previous research is limited and has mainly focused on evaluating the toxicity of single metal exposures. This study evaluates the associations between exposure to metal mixtures and blood pressure among Bangladeshi children age 5–7 years.

Methods: We investigated the associations of 17 toenail metal concentrations with blood pressure using linear regression models. Principal component analysis (PCA), weighted quantile sum (WQS) regression, and Bayesian kernel machine regression (BKMR) were conducted as secondary analyses.

Results: Associations were observed for selenium with diastolic blood pressure (per doubling of exposure $\beta = 2.91$, 95% confidence interval [CI] = 1.08, 4.75), molybdenum with systolic ($\beta = 0.33$, 95% CI = 0.05, 0.61) and diastolic blood pressure ($\beta = 0.39$, 95% CI = 0.12, 0.66), tin with systolic blood pressure ($\beta = -0.33$, 95% CI = –0.60, –0.06), and mercury with systolic ($\beta = -0.83$, 95% CI = –1.49, –0.17) and diastolic blood pressure ($\beta = -0.89$, 95% CI = –1.53, –0.26). Chromium was associated with diastolic blood pressure among boys only ($\beta = 1.10$, 95% CI = 0.28, 1.92, *P* for interaction = 0.02), and copper was associated with diastolic blood pressure among girls only ($\beta = -1.97$, 95% CI = –3.63, –0.32, *P* for interaction = 0.01). These findings were largely robust to the secondary analyses that utilized mixture modeling approaches (PCA, WQS, and BKMR).

Conclusions: Future prospective studies are needed to investigate further the impact of early life exposure to metal mixtures on children's blood pressure trajectories and cardiovascular disease risk later in life.

Keywords: Metal mixtures; Blood pressure; Child health; Bangladesh

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Introduction

Elevated blood pressure, a well-established risk factor for cardiovascular disease,[1] accounts for 7.5 million deaths worldwide.[2] In Bangladesh, hypertension affects approximately 26.4% of adults[3]; however, the prevalence of elevated blood pressure in Bangladeshi children is not known. It is estimated that 3.5% of children have elevated blood pressure in the United States, with a higher prevalence among overweight or obese children.[4]

Several factors during childhood are related to the future development of hypertension, including excess weight,[5] sleep-disordered breathing,[6,7] chronic kidney disease,[8] and premature birth.[9] Recent research has suggested that environmental chemical exposures may affect blood pressure in children.[10] However, there is limited evidence for the effects of metal

What this study adds

Elevated blood pressure, a well-established risk factor for cardiovascular disease, is estimated to account for 7.5 million deaths worldwide. Research on the childhood origins of cardiovascular diseases shows that early life factors influence cardiovascular risk over the life course. Although research has suggested that environmental chemical exposures may affect children's blood pressure, there is limited epidemiologic evidence evaluating the effects of metal exposures on childhood blood pressure. Using linear regression models and three mixture modeling approaches, we observed that blood pressure measures were significantly associated with selenium, molybdenum, tin, mercury, chromium, and copper in rural Bangladeshi children 5–7 years old. These findings could have implications for early childhood interventions, such as dietary changes and other environmental remediation measures, to reduce life-long cardiovascular risk.

exposures on childhood blood pressure[11–14]; although in adults, previous studies have reported associations of toxic metal exposures (e.g., arsenic, lead, cadmium, mercury, vanadium, molybdenum, antimony, and tungsten) and essential elements (e.g., zinc, iron, manganese, and selenium) with elevated blood pressure or hypertension.[15–19] Given the strong association between early life elevated blood pressure and the development of hypertension in later life,[20] it is essential to investigate these associations in children.

Recently, methodological advances have renewed interest in estimating the health effects of environmental mixtures since individuals are usually exposed to multiple metals simultaneously. Beyond the correlation between multiple metals that may each have independent influences on a trait, co-exposure may result in higher toxicity due to synergistic effects, as a result of changes in toxicodynamics and toxicokinetics.[21,22] However, most of the existing research to date has evaluated the health effects of single metal exposures. To our knowledge, only one study evaluated metal mixtures with blood pressure in adults,[23] and only one study has assessed the association in children 4–6 years old.[24]

The present study evaluated the associations between blood pressure among Bangladeshi children 5–7 years old and exposure to metals and metal mixtures. In addition to linear regression models, we implemented three methods that explicitly model co-exposures to examine the potential mixture effects of metals on blood pressure: principal component analysis (PCA), weighted quantile sum (WQS) regression, and Bayesian kernel machine regression (BKMR). We hypothesized that individual metal exposures are associated with blood pressure in children, and there are effects of metal co-exposures on blood pressure.

Methods

Study population

The Bangladesh Environmental Research in Children's Health (BiRCH) cohort enrolled 500 mother-child pairs between 2014 and 2016. The cohort was established based on the offspring of female participants of the Health Effects of Arsenic Longitudinal Study (HEALS), an established population-based study in the Araihazar Upazila of rural Bangladesh.[25] The children of female participants were identified through quarterly follow-up visits by village health workers beginning in 2011, which documented recent pregnancies among HEALS participants. Children in this enumerated cohort were enrolled in BiRCH if they were between the ages of 5–7 years. During enrollment, clinical evaluation, laboratory-based assays, and questionnaire surveys were conducted. The current analysis includes 491 mother-child pairs with complete metal exposure data, blood pressure measurements, and covariate data.

The research protocol for BiRCH was approved by the University of Illinois Chicago Office for the Protection of Research Subjects (protocol number 2014-0408) and the Bangladesh Medical Research Council.

Assessing metals exposure

Children enrolled in BiRCH were asked to provide toenail clippings at enrollment. Mothers were instructed to (1) clip toenails from all of the child's toes, (2) clip the amount that the child is comfortable with, and (3) place and seal all toenail clippings in the provided envelope prelabeled with the child's unique study identifier. Toenail clippings were stored at room temperature until shipment to the Dartmouth College Trace Element Analysis Core for the quantification of 17 elements (aluminum [Al], arsenic [As], cadmium [Cd], chromium [Cr], cobalt [Co], copper [Cu], iron [Fe], lead [Pb], manganese [Mn], mercury [Hg], molybdenum [Mo], nickel [Ni], selenium [Se], tin [Sn], uranium [U], vanadium [V], and zinc [Zn]) using inductively coupled plasma-mass spectrometry (ICP-MS).[26] Continuous calibration verification, analysis of duplicates and spikes, within- and between-batch analyses, and comparison with standard reference material were performed to ensure data quality. The limit of detection (LOD) for each toenail metal is as follows: Al (2 μg/g), As (0.01 μg/g), Cd (0.005 μg/g), Cr (0.10 μg/g), Co (0.005 μg/g), Cu (0.10 μg/g), Fe (2.00 μg/g), Pb (0.01 μg/g), Mn (0.02 μg/g), Hg (0.05 μg/g), Mo (0.01 μg/g), Ni (0.10 μg/g), Se (0.02 μg/g), Sn (0.02 μg/g), U (0.005 μg/g), and V (0.10 μg/g). Concentrations below the LOD were assigned calibration values from the instrument and retained in this data analysis (Table S1; http://links.lww.com/EE/A118).

Assessing blood pressure

Blood pressure was measured by a trained study physician using an automated sphygmomanometer with a digital display (HEM 712-C; Omron Healthcare GmbH, Hamburg, Germany) and a pediatric cuff. This device has been validated to have 85% of readings fall within 10 mmHg of the mercury standard.[27] Blood pressure was measured after the participant had been seated for 5 minutes, with the cuff around the upper left arm. A second measure was repeated after 5 minutes of rest, and the mean of the two readings was used. Systolic and diastolic blood pressures were modeled as untransformed continuous variables in this analysis.

Assessing covariates

Self-reported maternal characteristics were available from the questionnaire, including age, years of formal education, land ownership, television ownership, current smoking status, and passive smoking during pregnancy and child characteristics, including age and sex. A trained study physician measured the child's height at the enrollment visit using a portable stadiometer in the research field clinic.

Statistical analyses

Descriptive characteristics of mother-child pairs are presented for all participants. The median and interquartile range (IQR) of toenail metal concentrations were summarized overall and by child sex. Differences in toenail metal concentrations according to sex were evaluated by Wilcoxon rank-sum tests. Spearman's rank correlation coefficients assessed pairwise correlations among the toenail metal concentrations.

Potential confounders were selected based on a priori knowledge using a directed acyclic graph (Figure S1; http://links.lww.com/EE/A118) and retained in the regression model if associated with more than a 10% change in the beta coefficient. All multivariable models in the present study included maternal age (years), maternal education (no education, up to primary school, secondary school certificate, and higher secondary certificate or more), passive smoking during pregnancy (yes, no), child age (years), and child sex (boy, girl). Since height (cm) is a strong predictor of blood pressure, we also included child height (cm) in the regression model.[28] The prevalence of current smoking among women was very low (0.2%); therefore, it was omitted from multivariable models. Since we observed differences in several metal exposure profiles between boys and girls (Table S1; http://links.lww.com/EE/A118), sex-specific associations were also evaluated.

Linear regressions

To assess the effect of metal exposures on blood pressure, we first performed a sequence of linear regression models, evaluating a single metal per model. This analysis estimated a beta coefficient and its 95% confidence interval (CI) for each metal. Toenail metal concentrations were log_2-transformed, and the reported beta coefficient was interpreted as the change in blood pressure corresponding to a doubling of the toenail metal concentration. Statistical interaction by child sex was assessed through the inclusion of a cross-product term in the linear regression model. Descriptive analyses and linear regression models were conducted using Statistical Analysis Software (SAS) version 9.4 (SAS Institute Inc., Cary, NC).

Secondary analyses

We implemented three approaches to evaluate metal mixture effects on blood pressure, including PCA, WQS, and BKMR.

Principal component analysis

PCA was used to reduce the 17 correlated toenail metals to a smaller number of uncorrelated principal components (PCs). Due to skewed distributions, all metals were log_2-transformed and standardized before implementing PCA. Varimax rotation was used to minimize the number of metals with high loading on a PC. PCs with eigenvalues greater than 1.0 were retained for further analyses. The selected PCs were then included simultaneously in a linear regression model, such as $Y_i = \beta_0 + \beta_1 \times PC_1 + \beta_2 \times PC_2 + \ldots + \beta_n \times PC_n$, where Y indicates systolic or diastolic blood pressure. We conducted PCA in the overall study population and separately for boys and girls. All PCA analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC.).

Weighted quantile sum regression

WQS regression is a supervised approach to assess the impact of environmental mixtures and identify the driving mixture components.[29] As part of the procedure, the study sample was randomly split into a training dataset (40%, n = 196) and a validation dataset (60%, n = 295). In the training dataset, each exposure was first scored into quartiles. Then an overall quantile score was created for each individual by summing the quartiles. Empirical weights were estimated for each mixture component through bootstrapping using the training dataset. The weights were then used to create a WQS score representing the overall mixture and tested for statistical significance in the validation dataset.

For the current analysis, metals with estimated weights >0.06 (1/17) were considered as appreciably contributing to the WQS score. The WQS approach assumes that all mixture components act with the same directionality on the outcome; therefore, we generated and evaluated both a positive and negative WQS score. Weights for WQS scores were estimated using 1,000 bootstrap samples from the training dataset (40%), and the statistical significance of the resulting WQS scores on both systolic and diastolic blood pressure was tested in the validation dataset (60%). For sex-stratified analyses, we also utilized a 40:60 split for the training and validation datasets. The analyses were implemented using the gWQS R package.[30]

Bayesian kernel machine regression

BKMR is another supervised method for evaluating exposure mixtures and utilizes a nonparametric approach (specifically, a kernel function) to specify the unknown exposure-outcome relationship, allowing for potential interactions and nonlinear associations while parametrically modeling additional covariates of interest.[31] In the current analysis, all toenail metal concentrations were log_2-transformed and standardized, a Gaussian kernel function was applied, and a component-wise variable selection approach was used. After fitting the final model by running the Markov Chain Monte Carlo (MCMC) sampler for 200,000 iterations with the first 25,000 as burn-in, the posterior inclusion probabilities (PIPs) for each metal were generated, and estimates of the exposure-outcome function were produced. Pairwise potential interactions between each pair of metals were explored by plotting the exposure-outcome function of a single metal while fixing the second metal at its 25th, 50th, and 75th percentile, with all other metals set to their median values. We evaluated the overall effect of metal mixtures with blood pressure by comparing the difference in outcome when all metals were set to their 10th, 15th, 20th, 25th, 30th, 35th, 40th, 45th, 55th, 60th, 65th, 70th, 75th, 80th, 85th, or 90th percentiles, as compared to when they were all set to their 50th percentile. The analyses were implemented using the bkmr R package.[32]

Sensitivity analysis

In rural Bangladesh, freshwater indigenous fishes are the most commonly consumed animal-source food.[33] Studies have shown that there is bioaccumulation of toxic metals, including mercury and arsenic species, in native fish species from the Meghna River in Bangladesh[34,35] and cultured fish species collected from Dhaka city markets.[36] Since fish consumption is associated with both exposure to mercury and arsenic and reduced blood pressure in the adult population,[37,38] we also evaluated maternal-reported fish intake for the child (days per month) as a covariate in the regression models for mercury and arsenic.

Results

Selected characteristics of the 491 mother-child pairs are shown in Table 1. Mean (±SD) maternal age at birth of the child was 24.0 years (±4.7 years). Approximately half of the mothers reported educational attainment of secondary school certificate or higher (56.4%), ownership of agricultural land (49.9%), and exposure to secondhand tobacco smoke during their pregnancy (58%). Approximately equal numbers of boys (50.7%) and girls (49.3%) were enrolled, with an average age of 6.2 years (±0.7 years).

Table 1.

Selected maternal and child characteristics (N = 491)

Characteristics	Overall, N (%)	Outcomes measures, mean ± SD	
		Systolic blood pressure	Diastolic blood pressure
Maternal age at birth (years)			
≤20	114 (23.2)	101.49 ± 8.84	57.79 ± 6.57
21–25	229 (46.6)	101.43 ± 7.60	57.82 ± 7.60
26–30	96 (19.6)	101.71 ± 7.94	58.94 ± 7.67
>30	52 (10.6)	100.67 ± 6.11	57.28 ± 6.56
Maternal educational attainment			
No education	58 (11.8)	100.70 ± 7.57	56.50 ± 6.92
Up to primary	156 (31.8)	100.22 ± 7.95	57.41 ± 7.21
SSC	250 (50.9)	102.18 ± 7.74	58.55 ± 7.43
HSC and higher	27 (5.5)	102.65 ± 7.65	59.03 ± 6.62
Land ownership			
Yes	245 (49.9)	101.49 ± 7.48	58.02 ± 7.08
No	246 (50.1)	101.32 ± 8.15	57.93 ± 7.49
Television ownership			
Yes	329 (67.0)	101.51 ± 7.95	58.01 ± 7.21
No	162 (33.0)	101.18 ± 7.55	57.91 ± 7.45
Passive smoke during pregnancy			
Yes	284 (58.0)	102.09 ± 7.88	58.42 ± 7.53
No	206 (42.0)	100.45 ± 7.64	57.46 ± 6.89
Child age (years)			
5	73 (14.9)	101.75 ± 7.87	57.15 ± 8.68
6	246 (50.1)	101.24 ± 7.76	57.88 ± 7.08
7	172 (35.3)	101.49 ± 7.91	58.47 ± 6.96
Child sex			
Male	249 (50.7)	101.38 ± 7.91	57.29 ± 6.86
Female	242 (49.3)	101.43 ± 7.73	58.68 ± 7.64

HSC indicates higher secondary certificate; SSC, secondary school certificate.

The distributions of the toenail metal concentrations, overall and by child sex, are summarized in Table S1 (http://links.lww.com/EE/A118). All metals were detectable in all samples of toenail clippings, and fewer than 5% of the samples fell below the limit of detection (LOD) for all metals. In bivariate analyses, several toenail metal concentrations (aluminum, vanadium, chromium, manganese, iron, cobalt, copper, molybdenum, and uranium) were higher among boys. Overall, correlations between the metals varied widely, with Spearman's rank correlation coefficients ranging from 0.08 to 0.99 (Figure S2; http://links.lww.com/EE/A118). Among essential elements, the correlations ranged from 0.10 to 0.99, and among toxic metals, the correlations ranged from 0.08 to 0.42. Similar correlation patterns were observed when stratified by child sex (Figures S3 and S4; http://links.lww.com/EE/A118). The group of essential elements includes metals with nutritional properties that are not necessarily essential (e.g., aluminum, nickel, and vanadium).

Linear regressions

The results from the single-metal regression models are shown in Figure 1 and Table S2 (http://links.lww.com/EE/A118). Overall, positive associations were observed between selenium and diastolic blood pressure (β = 2.91, 95% CI = 1.08, 4.75) and between molybdenum and both systolic (β = 0.33, 95% CI = 0.05, 0.61) and diastolic blood pressure (β = 0.39, 95% CI = 0.12, 0.66), whereas inverse associations were observed between tin and systolic blood pressure (β = –0.33, 95% CI = –0.60, –0.06), and between mercury and both systolic (β = –0.83, 95% CI = –1.49, –0.17) and diastolic blood pressure (β = –0.89, 95% CI = –1.53, –0.26). In sensitivity analyses of mercury and arsenic, results additionally adjusted for fish intake did not appreciably differ from the original analyses (Table S3; http://links.lww.com/EE/A118).

In analyses stratified by sex, chromium was positively associated with systolic (β = 0.93, 95% CI = 0.02, 1.84, *P* for interaction = 0.09) and diastolic blood pressure among boys (β = 1.10, 95% CI = 0.28, 1.92, *P* for interaction = 0.02), whereas these associations were null in girls (Figure 1 and Table S2; http://links.lww.com/EE/A118). An inverse association was also observed between copper and diastolic blood pressure (β = –1.97, 95% CI = –3.63, –0.32, *P* for interaction = 0.01) among girls, whereas this association was null in boys (Figure 1 and Table S2; http://links.lww.com/EE/A118). No other sex differences were observed.

Secondary analyses

Principal component analysis

In the overall study population, as well the analysis stratified by sex, the first three PCs (based on eigenvalues >1.0) accounted for approximately 65% of the total variance in toenail metals. The first PC (PC-1) explained nearly 50% of the variance in metals for the overall sample (boys: 50.8% and girls: 48.9%). Tables S4–S6 (http://links.lww.com/EE/A118) show the loading weights of the toenail metal concentrations for each PC. The associations between PCs and blood pressure are summarized in Table 2. Among boys, PC-2—representing copper, zinc, selenium, cadmium, and lead—was associated with higher diastolic blood pressure, and PC-3—primarily representing mercury—was associated with lower diastolic blood pressure. Among girls, PC-3—primarily representing tin, selenium, and zinc—was associated with lower systolic blood pressure.

Weighted quantile sum regression

The WQS positive index was associated with elevated diastolic blood pressure, both in the overall study population (difference in mmHg for a one-unit increase in the WQS: β = 1.07, 95% CI = 0.09, 2.05) and among boys (difference in mmHg for a one-unit increase in the WQS: β = 1.68, 95% CI = 0.21, 3.14) (Table S7; http://links.lww.com/EE/A118). The estimated weights of the 17 toenail metal concentrations for the WQS positive and negative indices are shown in Figure S5 (http://links.lww.com/EE/A118). Among the overall population, selenium, nickel, and zinc meaningfully contributed to the positive index for diastolic blood pressure. Among boys, molybdenum, selenium, nickel, cadmium, lead, and arsenic meaningfully contributed to the positive index for diastolic blood pressure.

Bayesian kernel machine regression

The PIPs derived from the BKMR model are summarized in Figure S6; (http://links.lww.com/EE/A118). In the overall sample, tin (PIP = 0.22) was identified as the most important metal in the mixture model with systolic blood pressure, and mercury (PIP = 0.61), selenium (PIP = 0.51), and molybdenum (PIP = 0.27) were identified with diastolic blood pressure. Among boys, selenium (PIP = 0.28), mercury (PIP = 0.23), molybdenum (PIP = 0.13), and chromium (PIP = 0.13) contributed most to the model for diastolic blood pressure; among girls, tin (PIP = 0.89) contributed most to the model for systolic blood pressure and copper (PIP = 0.41) for diastolic blood pressure. Figures S7–S12 (http://links.lww.com/EE/A118) show the single-metal effects with blood pressure, adjusting for all other metals fixed at their median value. All associations appeared linear, except for an inverted U-shape association of tin with systolic blood pressure and slightly U-shape associations of copper and selenium with diastolic blood pressure in girls. The pattern and direction of associations observed from these figures largely corroborate our findings from the single-metal linear regression models. We observed no evidence of pairwise interactions between metals for either systolic or diastolic blood pressure (Figures S13–S18; http://links.lww.com/EE/A118). We observed a significant inverse association between the metal mixture overall and systolic blood pressure (Figure S19; http://links.lww.com/EE/A118).

Discussion

This study evaluated the effects of metal mixtures on blood pressure among Bangladeshi children 5–7 years old. Overall, we observed positive associations of selenium and molybdenum, and inverse associations of tin and mercury, with blood pressure. We also identified evidence of sex-specific associations for some metal exposures. Positive associations with chromium were observed only among boys, and an inverse association of copper was observed only among girls. These findings were largely robust to secondary analyses that implemented three innovative mixture modeling approaches: PCA, WQS, and BKMR. Positive associations with selenium and inverse associations with mercury were consistently observed across methods. Furthermore, all associations identified using linear regression modeling were supported by at least one mixture modeling method. Since there is still no data to identify a specific level of blood pressure in childhood that leads to adverse cardiovascular outcomes in adulthood, these findings' individual-level implications are uncertain.[4] Although the observed magnitudes of association were small and may not represent an irreversible progression to hypertension or cardiovascular diseases in adulthood, data tracking blood pressure from childhood to adulthood demonstrates that higher blood pressure in childhood correlates with higher blood pressure and the onset of hypertension in adulthood.[4]

Thus far, few studies have used toenail samples to assess exposure to metals in children, and there are no established cutoff points indicating exposure levels of clinical relevance. In general, children included in the present study had higher toxic metal exposures (e.g., arsenic, mercury, and lead) compared

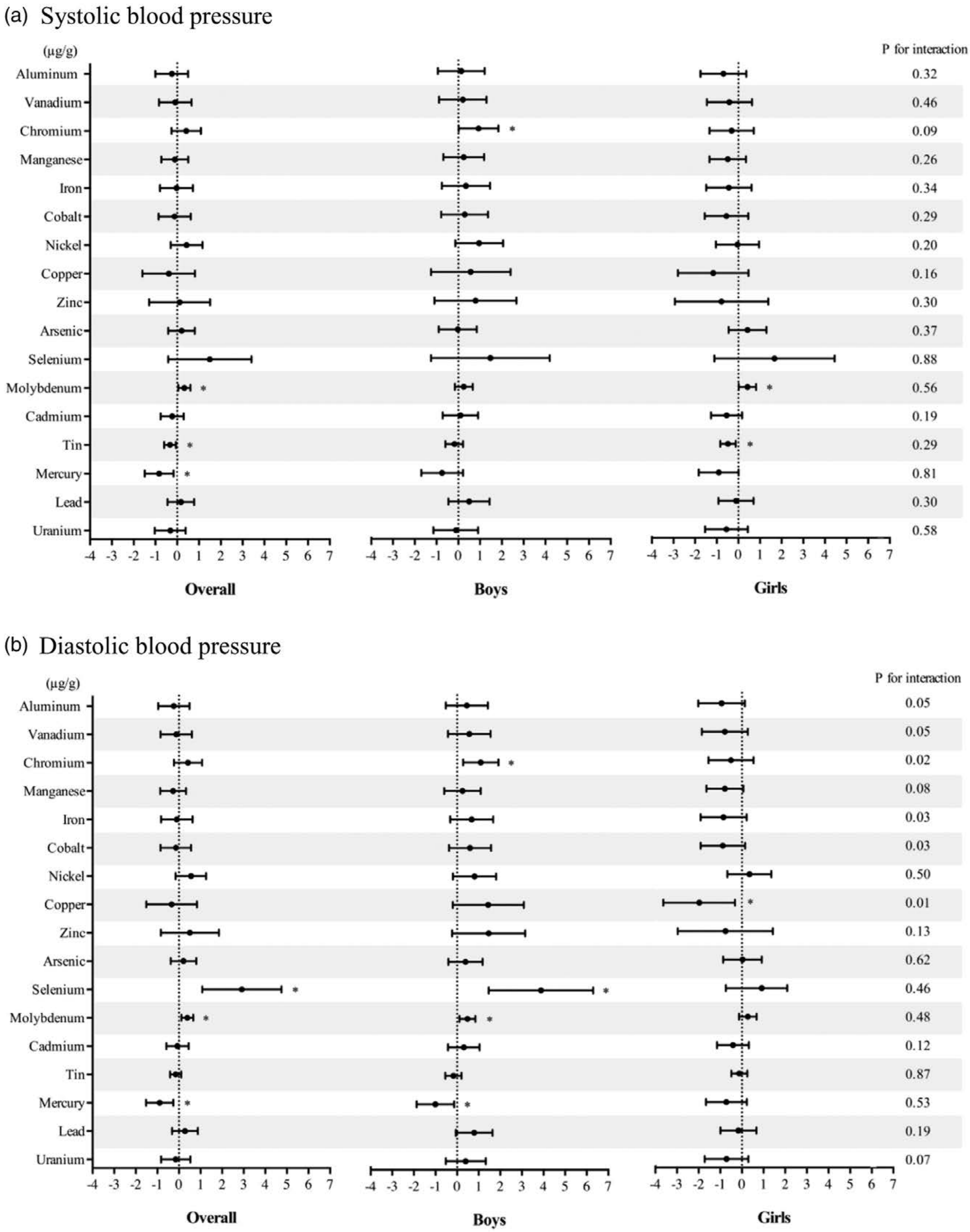

Figure 1. Associations between toenail metal concentrations and blood pressure. Mean difference (95% CI) in systolic (A) and diastolic (B) blood pressures per doubling of toenail metal concentrations from single-metal models, shown for overall population and stratified by sex. All models were adjusted for maternal age, maternal education, passive tobacco smoke exposure during pregnancy, child age, child sex (except sex-specific analyses), and height. Asterisks indicated statistical significance at $P < 0.05$.

Table 2.

Mean difference in systolic and diastolic blood pressures in relation to one-unit increase in principal component scores

	β (95% CI)[a]		
Association	**Overall (N = 491)**	**Boys (N = 249)**	**Girls (N = 242)**
PC-1	Al, V, Cr, Mn, Fe, Co, Ni, Cu, As, U	Al, V, Cr, Mn, Fe, Co, Ni, Cu, As, Pb, U	Al, V, Cr, Mn, Fe, Co, Ni, As, U
SBP	–0.12 (–0.82, 0.57)	0.31 (–0.66, 1.28)	–0.25 (–1.22, 0.72)
DBP	–0.31 (–0.98, 0.36)	0.47 (–0.38, 1.33)	–0.69 (–1.69, 0.31)
PC-2	Cu, Zn, Se, Cd, Pb	Cu, Zn, Se, Cd, Pb	Cu, Zn, Se, Cd, Hg, Pb
SBP	0.21 (–0.46, 0.88)	0.70 (–0.27, 1.67)	–0.87 (–1.81, 0.07)
DBP	0.59 (–0.06, 1.24)	1.45 (0.60, 2.31)	–0.45 (-1.42, 0.52)
PC-3	Sn	Hg	Zn, Se, Sn
SBP	–0.62 (–1.28, 0.05)	–0.89 (–1.86, 0.09)	–1.22 (–2.15, –0.28)
DBP	–0.26 (–0.90, 0.38)	–1.27 (–2.13, –0.42)	–0.65 (–1.61, 0.32)

Listed metals had PC loadings ≥|0.40|.

[a]All models were adjusted for maternal age, maternal education, passive tobacco smoke exposure during pregnancy, child age, child sex (except sex-specific analyses), and height.

DBP indicates diastolic blood pressure; SBP, systolic blood pressure.

with published studies of children in the United States, Italy, and New Zealand. In this study, levels of toenail metals were all higher than those in a study of 158 children 6–9 years old in Northern Italy.[39] When compared with a study of 75 Hispanic/Latino adolescents in Chicago, children in Bangladesh had lower levels of toenail selenium and nickel but higher levels of toxic metal concentrations such as arsenic, lead, cadmium, and mercury (unpublished data). Toenail molybdenum concentrations in the present study were comparable to a study of 9-year-old children residing in New Zealand; in contrast, children in the present study had higher toenail levels of aluminum, arsenic, chromium, cobalt, iron, lead, manganese, mercury, nickel, selenium, and zinc and lower levels of cadmium and copper.[37,40] Whereas toenail levels of cadmium, lead, and mercury were reported to be higher in a study of 20 Vietnamese children 18 months to 4 years old residing in a smelting craft village, possibly due to more severe contamination of these metals in the ecosystem of this area by smelting of automobile batteries.[38] There is considerable heterogeneity across the published study samples, including diet, residential locality (industrial, agricultural), and the predominant source of exposure (drinking water, food, environment) that explains the differences in observed exposure levels. Furthermore, the absence of guidelines for acceptable exposure levels based on toenail concentrations makes comparisons across studies difficult.

To our knowledge, only one study evaluated the effect of metal mixtures on blood pressure among children. Utilizing data from 544 children 4–6 years old in Mexico City, Kupsco et al[24] implemented BKMR and reported no significant associations and joint effects of 11 metals, measured from second trimester blood, on both systolic and diastolic blood pressures. The inconsistent findings between Kupsco et al[24] and the present study may be due to differences in the exposure windows examined, exposure levels, and the metal co-exposures evaluated. Additionally, only one study explored the association between metal mixtures and blood pressure among adults.[23] In that study, Park et al[23] implemented four mixtures methods (i.e., BKMR, adaptive elastic net, Bayesian additive regression tree, and super learner) and found significant joint effects of blood and urine metal biomarkers on elevated blood pressure in adults 20 years old or older from the National Health and Nutrition Examination Survey; lead, cadmium, arsenic, and cobalt were identified as important mixture components, and significant pairwise interactions with other metals were observed.

We observed positive associations of toenail selenium concentrations with diastolic blood pressure in the overall study population. Potential biological mechanisms linking selenium to increased blood pressure include toxic properties of selenium or selenoproteins and their pro-oxidant effects.[41–44] Only two studies have investigated selenium's effects on blood pressure among children.[13,24] A cross-sectional study of Bangladeshi children 4.4–5.4 years old observed no significant association between urinary selenium concentration and blood pressure.[13] A longitudinal study conducted in Mexico reported no association between maternal blood selenium collected in the second trimester with blood pressure in children 4–6 years old.[24] Prior studies evaluating selenium with blood pressure in adult populations have shown inconsistent results. In a recent systematic review, the authors concluded there was no conclusive evidence based on 25 existing studies to support the association between selenium and either blood pressure or hypertension.[45] More recent studies in adults have demonstrated conflicting findings; four studies found that higher selenium levels were associated with higher blood pressure or increased risk of hypertension,[18,46–48] whereas one study showed the potential blood pressure-lowering effect of selenium.[15] Differences in the exposure levels, selenium biomarkers, and exposure assessment timing may account for the variability observed in the existing literature.

The present study observed inverse associations of toenail mercury concentrations with both systolic and diastolic blood pressures. A recent systematic review evaluated the association between mercury exposure with blood pressure in children and adolescents.[49] Based on the eight studies included, the authors concluded that there was no clear pattern for mercury with blood pressure, given the inconsistencies of study findings. However, there are substantial differences in study design, study populations (e.g., location and age groups), exposure time window (i.e., prenatal or postnatal), and blood pressure assessment protocols that make it challenging to synthesize the existing literature. Additionally, a recent study not included in the prior review reported no association between urinary mercury and blood pressure among adolescents 15–17 years old in Spain.[50] In contrast, another recent study of US children and adolescents 8–17 years old found inverse associations of blood total mercury and methyl mercury with diastolic blood pressure.[51] In the present study, the inverse association of mercury with systolic and diastolic blood pressure persisted after additionally adjusting for fish intake; however, it is possible that our measure of fish intake was not sufficient, and residual confounding of the association is present.

Our analyses investigating the associations of molybdenum with blood pressure suggested positive associations with both systolic and diastolic blood pressures. Only one study of 1,277 European children 6–11 years old evaluated the association of blood pressure and reported null results with blood molybdenum.[10] Studies in adult populations exist, but they have shown mixed results. A study in the US population reported a positive association between urinary molybdenum and high blood pressure.[17] However, two other studies, one in 61 college students in Canada[52] and one in 367 rural Chinese women,[53] found no associations with particulate matter (PM) 2.5 molybdenum concentrations and blood pressure, whereas a study of 39 college students in China showed inverse associations between $PM_{2.5}$

molybdenum and both systolic and diastolic blood pressures.[54] Given the scarce research, especially in children, and small sample sizes, future studies are warranted to evaluate the potential impact of molybdenum on blood pressure.

Our study is the first to examine the association of tin on blood pressure in children, and an inverse association was observed with systolic blood pressure. Previous studies investigating tin with blood pressure in adult populations reported inconsistent findings; one study showed that higher PM 2.5 tin levels were associated with higher systolic and diastolic blood pressures,[52] whereas another study found no associations based on hair tin concentrations.[53] Given the potentially harmful health effect of tin,[55] future research is needed to examine its association with children's blood pressure.

The present study is one of a few to evaluate sex differences with cardiovascular risk factors among prepubertal children.[56–59] The motivation to examine associations disaggregated by sex was that several metals' exposure profiles differed between boys and girls in the study sample (Table S1; http://links.lww.com/EE/A118). Furthermore, the sexual dimorphism of cardiovascular risk factors has been well documented in adults and adolescents.[60,61] Sex steroids may explain the differential effects on blood pressure.[62] Among prepubertal children, it has been suggested that levels of estradiol, testosterone, and body fat differ between girls and boys[63–66]; however, we did not measure sex steroid hormones in the present study.

In analyses stratified by sex, we observed positive associations of toenail chromium concentrations with both systolic and diastolic blood pressures among boys. Only one study of 133 Spanish male adolescents 15–17 years old investigated the association of urinary chromium with blood pressure and reported null results.[50] No studies have reported a significant association of chromium with blood pressure among the adult population, although analyses were not conducted separately for males and females.[67,68] Future research is needed to evaluate the sex-specific effects of chromium on blood pressure in children, given that existing research is scarce in children, and the beneficial level of chromium for biological functioning is not well-established.[69]

A sex-specific inverse association was observed for copper with diastolic blood pressure among girls. In contrast, two prior studies, one in 1,277 European children[10] and one in 1,427 US children and adolescents,[70] found positive associations between blood copper and blood pressure. Whereas a study of 1,147 12-year-old children conducted in the Netherlands reported no association with $PM_{2.5}$ copper concentrations.[71] However, none of the prior studies presented sex-disaggregated effects of copper on blood pressure.

In general, our secondary analyses employing methods to evaluate metal mixtures supported the associations observed in the linear regression models, possibly because these associations were linear with no strong interaction effects. WQS and BKMR are novel supervised methods for analyzing the health effects of overall chemical mixtures, which can also handle correlated exposures and tease out each mixture component's contribution on the outcome of interest. However, both methods have some limitations that need to be considered. WQS regression has lower statistical power due to splitting the study sample into a training and validation set. Furthermore, WQS regression only permits the evaluation of one direction at a time (positive or negative index), and the assessment of bidirectional effects in one index is not possible. BKMR also has limited statistical power with a large number of exposures.

The present study comprehensively evaluated the associations between toenail metal concentrations with blood pressure in children and provides an essential contribution to our current knowledge of the impacts of metal mixtures. However, the study has limitations. First, the associations explored in this study were cross-sectional; however, given that toenail clippings represent metal exposures that occurred 6–12 months earlier,[72,73] we can deduce that the exposure occurred before the blood pressure measurement. Second, toenails may not be the most appropriate biological specimen for assessing all metal concentrations; therefore, these findings would need to be replicated with other biomarkers of metal exposure.[74] Third, although we prespecified the hypotheses, the results may still be subject to multiple testing considerations. Nevertheless, the implementation of WQS and BKMR allows us to control for multiple testing by simultaneously incorporating variable selection on 17 metals.[75] Finally, we cannot rule out the possibility of confounding on the observed associations due to unmeasured variables, such as dietary factors, housing characteristics, behaviors, preterm delivery, birth weight, and co-exposure to other environmental pollutants, or residual confounding from socioeconomic status.

The present study comprehensively evaluated the associations of toenail metal concentrations with blood pressure among children. Overall, we observed positive associations of selenium and molybdenum, and inverse associations of tin and mercury, with blood pressure. Sex-specific associations with blood pressure were observed for chromium among boys (positive association) and copper among girls (inverse association). These findings were largely robust to secondary analyses using innovative mixture modeling approaches. Future prospective studies are needed to confirm our findings and further investigate the impact of early life exposure to metal mixtures on children's blood pressure trajectories and cardiovascular disease risk later in life. Additionally, early childhood interventions, such as dietary changes and other exposure reduction measures (e.g., water filtration), should be considered to reduce life-long cardiovascular risk.

Conflicts of interest statement

The authors declare that they have no conflicts of interest with regard to the content of this report.

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