Original Research Article

ENVIRONMENTAL EPIDEMIOLOGY

OPEN

Urinary trace metals in association with fetal ultrasound measures during pregnancy

Stephani S. Kim[a], John D. Meeker[b], Max T. Aung[b], Youfei Yu[c], Bhramar Mukherjee[c], David E. Cantonwine[d], Thomas F. McElrath[d], Kelly K. Ferguson[a,*]

Abstract: Toxic metals have been associated with lower birth weight while essential metals have been associated with higher birth weight. Evidence for other metals is either inconsistent or limited in terms of number of studies. This study analyzed 17 urinary metals, individually and as a mixture, and their association with measures of fetal growth in the LIFECODES birth cohort. Ultrasound was used to measure the abdominal circumference, head circumference, and femur length and measures were used to calculate estimated fetal weight at ~26 and ~35 weeks. We calculated the *z* score based on gestational age at scan, and estimated fetal weight (EFW) was combined with birth weight for longitudinal analyses. Metals were measured in samples collected at ~26 weeks. We used linear mixed-effects models to examine associations between metals and repeated measures of each outcome, controlling for covariates. Principal components analysis reduced the biomarkers to predictors that may share some commonality. We found that an interquartile range increase in selenium was inversely associated with femur length *z* score as well as other growth outcomes. Other essential metals, however, were associated with an increase in growth. Finally, the PCA component comprised of arsenic, mercury, and tin was associated with decreased head circumference *z* score (–0.14 [95% CI, –0.23, –0.05]).

Keywords: metals, prenatal exposure, fetal growth, birth weight, mixtures.

Introduction

Infants with poor fetal growth, defined as low birth weight (<2,500 g), small for gestational age (SGA, <10th percentile), or intrauterine growth restriction (IUGR), are at an increased risk for neonatal morbidity and mortality as well as adverse health outcomes in adulthood, such as cardiovascular disease and impaired neurodevelopment.[1–3] There are several established risk factors for poor fetal growth, including maternal age, maternal health conditions, socioeconomic factors, and smoking or alcohol use during pregnancy.[4–6] Exposure to environmental chemicals has been associated with restricted fetal growth as well.[7–13]

Everyone experiences exposure to metals from the environment. Some are naturally occurring or found in common dietary sources while others are released from anthropogenic activities. In addition, some metals are known toxicants, such as lead (Pb), arsenic (As), cadmium (Cd), and mercury (Hg), whereas others are essential for human health, e.g., copper (Cu), manganese (Mn), selenium (Se), and zinc (Zn).[14–18] Several studies have found that higher exposure to toxic metals is associated with poor fetal growth.[10,19,20] On the other hand, essential metals have been associated with decreased fetal growth when intake is insufficient or when levels are too high (as with Mn).[21–26] However, there have been inconsistent results for these studies, many metals have limited or no data regarding fetal growth, and many studies of toxic metals are done only in communities with very high levels of exposure.[7]

In addition, while most of these studies focus on measurements taken at birth, such as birth weight or SGA, few have assessed associations with ultrasound measures during pregnancy.[12,27,28] Examining the effect of chemicals on fetal growth

What this study adds

Many studies have examined the relationship between maternal exposure to metals and birth weight, but they tend to focus on toxic metals (e.g., lead), populations with high levels of exposure, and investigate one chemical at a time. In our study of a generalizable US population, we examined 17 toxic and essential metals in urine samples from the third trimester of pregnancy and examined associations between individual as well as combined measurements and longitudinal ultrasound measures of fetal growth. Surprisingly, urinary selenium was associated with decreased fetal growth; however, associations between other essential metals were associated with increased fetal growth. Exposure to "seafood-related" metals as a group was associated with decreased fetal growth.

[a]Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina; [b]Department of Environmental Health Sciences, University of Michigan School of Public Health, Ann Arbor, Michigan; [c]Department of Biostatistics, University of Michigan School of Public Health, Ann Arbor, Michigan; and [d]Division of Maternal-Fetal Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts

Sponsorships or competing interests that may be relevant to content are disclosed at the end of the article.

This research was supported by the Intramural Research Program of the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (Z1AES103321). Additional funding was provided by NIEHS (R01ES018872). Analysis was provided by the Children's Health Exposure Analysis Research (CHEAR) Program (U2CES02655).

Researchers can apply for access by contacting the LIFECODES Birth Cohort study team at Brigham and Women's Hospital.

SDC Supplemental digital content is available through direct URL citations in the HTML and PDF versions of this article (www.environepidem.com).

Human Studies Research: This study was deemed exempt by the Institutional Review Boards (IRB) at the University of Michigan and the National Institute of Environmental Health Sciences. The protocol for the LIFECODES birth cohort was approved by the IRB at Brigham and Women's Hospital.

*Corresponding Author. Address: 111 TW Alexander, Mail Drop A-3, Research Triangle Park, NC 27709. E-mail: kelly.ferguson2@nih.gov (K.K. Ferguson).

Environmental Epidemiology (2020) 4:e075

Received: 22 May 2019; Accepted 1 October 2019

Published online 11 February 2020

DOI: 10.1097/EE9.0000000000000075

measured longitudinally across pregnancy may improve ability to detect associations and enable detection of critical windows of susceptibility. We analyzed 17 trace metals in urine samples and examined associations with longitudinal ultrasound as well as delivery measurements of fetal growth. This study augments existing data by investigating metals that have not been studied in this context and by examining combinations of metals using principal components analysis (PCA).

Methods

Study participants

The LIFECODES birth cohort is an ongoing prospective cohort study originally designed to identify risk factors for preeclampsia. Pregnant women who are planning to deliver at Brigham and Women's Hospital (BWH) in Boston, MA, are consented and enrolled at <15 weeks gestation and participate in up to four study visits (median: 10, 18, 26, and 35 weeks). Women complete a questionnaire to capture demographic information, along with medical and pregnancy history at the first visit. Biological samples are collected at each of the four study visits and stored at −80°C. This study utilizes data from women who were enrolled in 2006–2008 and were selected for a nested case–control study of preterm birth. The case–control study included nearly all cases of preterm birth (n = 130), defined as birth <37 weeks of gestation, and randomly selected controls (n = 352) in a 3:1 ratio.[29,30] For this analysis, we included women from this nested case–control study who had an available urine sample from the third study visit (n = 390).

Ultrasounds were performed and reviewed by board-certified sonologists following the guidelines set by the American College of Obstetricians and Gynecologists (ACOG). All participants received an ultrasound at approximately 18 weeks of gestation to assess fetal anatomy, as per ACOG guidelines. Ultrasounds at visits 3 and 4 (median 26 and 35 weeks of gestation) were not part of the study or clinical protocol but were performed at the participant's request or if there was a suspected abnormality. Visit 3 ultrasounds were taken at a median of 26 weeks of gestation (range: 20–32 weeks) and visit 4 ultrasounds were taken at a median of 35 weeks of gestation (range: 30–40 weeks). Ultrasound measures of estimated fetal weight, femur length, and abdominal circumference were available from 197 participants at visit 3 and from 211 participants at visit 4 (n = 136 with both). Ultrasound measures of head circumference were available for 197 participants at visit 3 and 209 participants at visit 4 (n = 134 with both). Anthropometric measurements from these ultrasounds included femur length (mm), head circumference (mm), and abdominal circumference (mm). We calculated estimated fetal weight (EFW) using the Hadlock formula.[31] We limited our analysis of ultrasound data to those scans available from visits 3 and 4, as these occurred at or after the time of exposure measurement. In addition, we examined birth weight (BW, g) at delivery (n = 390) and, among a subset, birth length (cm) (n = 369), and placental weight (g) (n = 79). We calculated *z* scores based on gestational age at scan for all ultrasound measures as well as birth weight using all singleton pregnancies delivered at BWH from 2006 to 2012.[32]

Urinary trace metals analysis

In collaboration with the Children's Health Exposure Analysis Resource (CHEAR), urine samples from the third visit were analyzed for As, barium (Ba), beryllium (Be), Cd, chromium (Cr), Cu, Pb, Hg, Mn, molybdenum (Mo), nickel (Ni), Se, tin (Sn), thallium (Tl), tungsten (W), uranium (U), and Zn using Thermo Fisher (Waltham, MA) ICAPRQ inductively coupled plasma mass spectrometer and CETAC ASX-520 autosampler by NSF International (Ann Arbor, MI). The methods for metal analysis are described elsewhere.[30] Concentrations with machine-read values were kept as is, even if they were below the limit of detection (LOD), while those with no machine-read value or a value of zero were replaced with the LOD divided by the square root of two.[33,34] If a metal was below the LOD for >70% of the population, we treated it as a binary variable (detect vs. nondetect) in all analyses.

Statistical analyses

We examined demographic characteristics, medical history, and pregnancy history of the women in the study, including maternal age, race, education, type of health insurance, prepregnancy body mass index (BMI), self-reported tobacco use during pregnancy, self-reported alcohol use during pregnancy, parity, assisted reproductive technology (ART), and sex of neonate. All analyses used inverse probability weights to adjust for the case–control study design and to allow for generalizable results to the base LIFECODES cohort.[8,35]

We calculated the 25th, 50th, and 75th percentiles for each metal and reported the number and percentage below LOD. We used linear mixed-effects (LME) models to calculate the unadjusted and adjusted differences and 95% confidence intervals (CI) for repeated growth measurements in association with an interquartile range (IQR) increase in each exposure biomarker, or with detection of the exposure biomarker for those metals with <30% detection. LME models included a random intercept for each participant and a random slope for gestational age at growth measurement. Outcome measurements for these models included: repeated *z* score for femur length, head circumference, and abdominal circumference from visits 3 and 4; and a combination of EFW and BW *z* scores from visits 3, 4, and delivery. We additionally used linear regression models to calculate unadjusted and adjusted associations with BW (g) for comparison to other studies, and birth length (cm) and placental weight (g) which were available among a subset of participants. All adjusted models included specific gravity (continuous), maternal age (continuous), race/ethnicity (white, black, and other), education (≤high school graduate, technical school, junior college/some college, ≥college graduate), prepregnancy BMI (continuous), and gestational age at time of ultrasound (continuous) for the fetal growth measurements. We additionally adjusted for gestational age at delivery (continuous) for models that included BW *z* score at delivery. Type of insurance (public vs. private), self-reported use of alcohol and tobacco, and use of ART were considered as covariates, but did not cause a >10% change in the forward selection procedure. Finally, all primary models were mutually adjusted for coexposures because the urinary trace metals demonstrated low to moderate correlations with Pearson coefficients ranging from −0.22 to 0.41[30] and this model had better fit compared with the models with each metal individually.

We additionally utilized PCA as a mixtures method to reduce the number of chemicals into predictors that may share some commonality, such as exposure source. PCA is a variable reduction method that mathematically creates principal components (PCs) using linear combinations of the variables that have maximal variance and are mutually uncorrelated. For our analysis, we calculated PCs independent of the outcome.[36] Additional description of the PC calculations for this set of metals is provided elsewhere.[30] We fit PCs into the LME and linear regression models adjusting for the same covariates used in our primary model, except for specific gravity since urinary metals were corrected for specific gravity before calculation of PCs.

Sensitivity analyses

We performed several sensitivity analyses to test the robustness of our findings. First, because the ultrasounds were not part of the original study design, we had missing data from visits 3 and 4. Therefore, we used imputed datasets that were created using

the multiple imputation by chained equation (MICE) method. The details of this methodology are described elsewhere.[37] Briefly, we created 50 imputed datasets, ran the LME model across all datasets, and pooled the estimates. Second, we created single pollutant models for comparison with models that were not mutually adjusted. Third, for essential metals only, we created tertiles of urinary concentrations to examine potential nonlinear associations between exposures and estimated fetal weight and BW *z* scores, setting the reference as the second tertile. Finally, we ran an additional sensitivity analysis in which we excluded preterm births and removed the inverse probability weights. We used SAS 9.4 (Cary, NC) for all statistical analyses, except for the multiple imputation analysis where we used R version 3.4.4 and the package mice.[38]

Results

Table 1 describes the demographic and health characteristics of the study population. Our study population was predominantly ≥30 years (69%), white (60%), and had some postsecondary education (72%). Approximately half of the women in our population were normal weight or <25 kg/m^2 (57%). The majority self-reported not drinking alcohol (95%) or using tobacco (94%) during pregnancy. For slightly less than half of the population, this pregnancy was their first (44%).

Table 2 outlines the weighted distributions of urinary trace metals. As, Mo, and Zn were detected in 100% of the samples analyzed. Ba, Cu, Hg, Mn, Se, and Sn were detected in over 90% of the samples. However, detection of Be, Cr, U, and W was less than 70%; therefore, we analyzed these as detect versus nondetect and denoted this difference by shading on subsequent tables. Urinary concentrations of As were markedly higher in our study population compared with women in the National Health and Nutrition Examination Survey (NHANES).[30] Urinary concentrations of Cu, Mn, Ni, and Zn were slightly higher than those to those from NHANES or the Canadian Health Measures Survey, and levels of other trace metals were comparable.[30] We also compared metal concentrations between individuals who had an ultrasound at visit 3 and at visit 4 and those who did not (eTable 1; http://links.lww.com/EE/A74). We observed minimal differences between the groups, with the exception of urinary Mn which was higher among individuals who received an ultrasound at visit 3 compared with those who did not.

We observed several notable associations between fetal growth measures and urinary trace metals in the unadjusted (eTable 2; http://links.lww.com/EE/A74) and adjusted (Figure 1, eTable 3; http://links.lww.com/EE/A74) multipollutant models. We observed a pattern of decreases in fetal growth *z*-scores for Se across all outcome measures. For example, an IQR increase in urinary Se was associated with a 0.43 SD decrease in estimated fetal weight and BW *z* scores (95% CI = −0.76, −0.09). Detection of Cr was also associated with a 0.23 SD (95% CI = −0.52, 0.09) decrease in femur length *z* score. On the other hand, Zn was associated with higher fetal growth *z* scores for all measures except for head circumference. Additionally, we observed a 0.30 SD increase (95% CI = 0.08, 0.52) in femur length *z* score in association with an IQR difference in urinary Mo. Associations between Mo and other fetal growth measures were also positive but more imprecise.

We also analyzed associations between the metals and raw birth weight (g) as well as birth length (cm), and placental weight (g) (eTable 4; http://links.lww.com/EE/A74). Although we have birth weights for all infants, the data for birth length (n = 374) and placenta weight (n = 74) were only available among a subset. In these analyses, we observed an inverse association with Se with birth length (β = −1.13, 95% CI = −2.03, −0.24)

Table 1.

Weighted[a] demographic characteristics of the study population (n = 390).

Demographic characteristic	N	Weighted %
Maternal age (years)		
≤24	41	11.2
25–29	76	19.9
30–34	159	40.3
35+	114	28.6
Race/ethnicity		
White	234	59.7
African American	58	15.0
Other	98	25.3
Education		
High school degree or less	53	13.3
Technical college	59	15.2
Junior college or some college	116	30.9
≥College graduate	152	40.6
Missing	10	
Health insurance		
Private/Health Maintenance Organization/self-pay	317	82.7
Public	64	17.3
Missing	9	
Prepregnancy body mass index (kg/m^2)		
<25	216	57.2
25–30	96	25.9
>30	68	16.9
Missing	10	
Tobacco use		
None in pregnancy	360	94.0
Some in pregnancy	25	6.0
Missing	5	
Alcohol use		
None in pregnancy	366	95.3
Some in pregnancy	15	4.7
Missing	9	
Parity		
Nulliparous	171	44.1
Parous	219	55.9
Use of assisted reproductive technology		
No	355	90.7
Yes	35	9.3
Sex of neonate		
Male	220	55.0
Female	170	45.0

[a]Weights were used to account for original case–control study design.

Table 2.

Weighted[a] distribution of specific gravity–corrected urinary trace metals from ~26 weeks of gestation in ppb (n = 390).

Metals	LOD	N (%) <LOD	25th	50th	75th
As	0.30	0 (0)	9.66	18.0	32.5
Ba	0.10	4 (1.00)	0.96	1.95	3.36
Be	0.04	356 (91.3)	0.01	0.02	0.04
Cd	0.06	218 (55.9)	0.04	0.08	0.13
Cr	0.40	330 (84.6)	0.10	0.21	0.39
Cu	2.50	32 (8.20)	6.88	9.08	12.1
Hg	0.05	32 (8.20)	0.27	0.51	0.97
Mn	0.08	6 (1.50)	0.51	0.73	1.14
Mo	0.30	0 (0)	37.1	51.3	69.7
Ni	0.80	54 (13.9)	1.88	2.84	3.97
Pb	0.10	92 (23.6)	0.15	0.35	0.62
Se	5.00	3 (0.77)	29.7	37.2	45.8
Sn	0.10	24 (6.15)	0.35	0.62	1.22
Tl	0.02	61 (15.6)	0.08	0.13	0.18
U	0.01	342 (87.7)	0.004	0.007	0.01
W	0.20	309 (79.2)	0.09	0.14	0.23
Zn	2.00	0 (0)	146.8	245.9	367.5

[a]Weights were used to account for original case–control study design.
ppb indicates parts per billion.

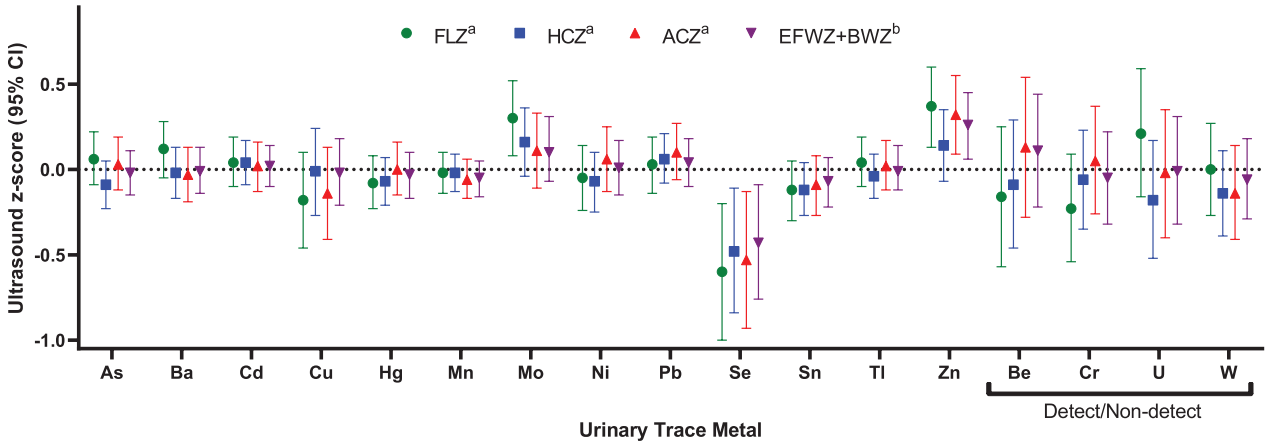

Figure 1. Adjusted (adjusted for coexposures, specific gravity, maternal age, prepregnancy BMI, race/ethnicity, education, infant sex, gestational age at time of ultrasound; adjusted for coexposures, specific gravity, maternal age, prepregnancy BMI, race/ethnicity, education, infant sex, gestational age at time of ultrasound, gestational age at delivery) differences in *z*-score for repeated measures of femur length, head circumference, abdominal circumference, and combination of estimated fetal weight and birth weight per interquartile range increase of each urinary trace metal. All models included a random intercept for participant and a random slope for gestational age at sample collection and were weighted to account for original case–control study design. ACZ indicates abdominal circumference *z* score; BWZ, birth weight *z* score; EFWZ, estimated fetal weight *z* score; FLZ, femur length *z* score; HCZ, head circumference *z* score.

Table 3.

Adjusted,[a,b] differences in *z* scores for repeated measures of femur length, head circumference, abdominal circumference, and combination of estimated fetal weight and birth weight by principal components.

	FLZ[a]	HCZ[a]	ACZ[a]	EFWZ + BWZ[b]
Metal	**Observations = 396; n = 264**	**Observations = 394; n = 264**	**Observations = 396; n = 264**	**Observations = 770; n = 374**
PC1[c]: "Toxic"	0.03 (–0.07, 0.14)	0.01 (–0.10, 0.09)	0.08 (–0.02, 0.19)	0.02 (–0.08, 0.11)
PC2[d]: "Essential"	0.03 (–0.08, 0.15)	–0.06 (–0.16, 0.04)	–0.02 (–0.13, 0.10)	0.00 (–0.10, 0.10)
PC3[e]: "Seafood-related"	–0.09 (–0.19, 0.02)	**–0.14 (–0.23, –0.05)**	–0.09 (–0.19, 0.01)	**–0.10 (–0.19, –0.01)**

Bold values indicate $P < 0.05$. All models included a random intercept for participant and a random slope for gestational age at sample collection and were weighted to account for original case–control study design.

[a]Adjusted for maternal age, prepregnancy BMI, race/ethnicity, education, infant sex, gestational age at time of ultrasound.

[b]Adjusted for maternal age, prepregnancy BMI, race/ethnicity, education, infant sex, gestational age at time of ultrasound or gestational age at delivery.

[c]PC 1: Pb, Cd, Mn.

[d]PC 2: Cu, Se, Zn.

[e]PC 3: As, Hg, Sn.

ACZ indicates abdominal circumference *z* score; BWZ, birth weight *z* score; EFWZ, estimated fetal weight *z* score; FLZ, femur length *z* score; HCZ, head circumference *z* score; PC, principal component.

but did not detect associations between other trace metals and these outcomes.

PCA identified three meaningful PCs from the urinary trace metals data. PC 1 had high loadings from Cd, Mn, and Pb; PC 2 had higher loadings from Cu, Se, and Zn; and PC 3 had high loadings from As, Hg, and Sn.[30] We identified PC 1 as the "toxic" group because Cd and Pb are toxic metals and high exposure to Mn is considered toxic.[14,18,39] PC 2 was identified as the "essential" group because Cu, Se, and Zn are all essential trace metals.[15,17] PC 3 was identified as the "seafood-related" group because these metals are associated with coastal seafood consumption.[16,40,41] We calculated unadjusted (eTable 5; http://links.lww.com/EE/A74) and adjusted (Table 3) differences in *z* scores for each set of longitudinal outcomes with all PCs in the model. We observed null or weak associations across all measures for PC 1 and 2. PC 3 was inversely associated with head circumference *z* score (β = –0.14, 95% CI = –0.23, –0.05) and the combined EFW and BW *z* score (β = –0.10, 95% CI = –0.19, –0.01).

Sensitivity analyses

In sensitivity analyses, we first examined associations using datasets that were imputed for missing ultrasound measures of estimated fetal weight. We observed a similar pattern across all measurements with similar precision, although effect estimates were attenuated slightly toward the null, with several associations from our primary models losing statistical significance (eTable 6; http://links.lww.com/EE/A74). For example, the association between Se and estimated fetal weight and birth weight described above changed from –0.43 (95% CI = –0.76, –0.09) to –0.34 (95% CI = –0.67, 0.00). Second, we created single pollutant models where we did not adjust for coexposures and observed similar patterns of associations (eTable 7; http://links.lww.com/EE/A74). As in the multipollutant model, we observed a pattern of decreased *z* scores across all measures associated with urinary Se, while urinary Zn remained positively associated with femur length *z* score, abdominal circumference *z* score, and the combined EFW and BW *z* scores. We also observed inverse associations for all metals with head circumference *z* score, although effect estimates tended to be more precise. Third, we examined associations between tertiles of urinary trace metals generally considered essential and estimated fetal weight combined with birth weight. We did not observe the U-shaped curve in the tertile analysis (eFigure 1 and eTable 8; http://links.lww.com/EE/A74); rather, all associations appeared to be linear. For example, individuals in tertile 1 of urinary Se had higher estimated fetal weight and birth weight *z* scores compared with those in tertile 2, and individuals in tertile 3 had lower estimated fetal weight and birth weight *z* scores compared with those in tertile 2 (P for trend = 0.04). Finally, we limited our analysis to only term births, birth ≥37 weeks gestation, and

did not include inverse probability weightings (eTable 9; http://links.lww.com/EE/A74). The associations between Se, Zn, Mo, and femur length *z* score were consistent with our main findings. The other associations remained in the same direction but were less precise.

Discussion

Overall, we observed the strongest associations between fetal growth and urinary Se, Zn, and Mo, with increases in *z* scores across all measures for Zn and Mo but decreases in fetal growth measures in association with Se. Additionally, in our mixture analysis using PCA, we observed that a principal component based on metals with seafood consumption as a common exposure route was inversely associated with fetal growth. Finally, urinary concentrations of toxic metals were not associated with fetal growth in our study population.

Although Se, Zn, and Mo are essential metals, they can be toxic at higher doses.[42–44] Our findings for an adverse effect of Se are difficult to interpret, as Se is traditionally seen as beneficial, with toxic effects observed at high doses,[45,46] and the urinary concentrations in our population are similar to those observed in other pregnant and nonpregnant populations in the United States.[12,47] Previous epidemiologic evidence has consistently shown that lower levels of Se are associated with adverse birth outcomes in studies measuring exposure biomarkers in maternal serum, plasma, toenails, or cord blood.[23,24,48,49] The only other studies of maternal urinary Se concentrations in pregnancy found no association with birth weight, although urinary concentrations were similar to what we observed in our study population.[12,49] We also did not observe U-shaped associations, i.e., worse outcomes at lower as well as higher exposure levels, between Se and fetal growth as we might have expected based on this prior literature. One possible explanation for this is that, because our study population is well educated, we do not observe insufficiency in this study population and thus are only able to detect adverse effects with levels at the higher end of the spectrum.

Our findings for Zn are more in the expected direction because it is an essential metal. While there is some inconsistency across studies, low (i.e., insufficient) serum or blood Zn concentrations during pregnancy have generally been associated with decreased birth weight and other adverse birth outcomes (i.e., higher levels are better).[7,21,22,50] Consistent with these findings, we observed higher fetal growth *z* scores across all measures in association with urinary Zn. Zn is actively transported across the placenta during pregnancy and it is necessary for synthesizing DNA and RNA, cellular replication, and regulating hormones.[51]

There are several studies on Mo and associations with male reproductive health outcomes,[52–54] but there is limited epidemiologic evidence on effects in pregnancy. Two previous studies observed no associations between maternal urinary Mo and infant birth weight or fetal anthropometry, and we observed increased fetal growth across all of our measurements.[12,55] Animal studies, on the other hand, have been mostly consistent in finding no changes in birth outcomes associated with maternal exposure to Mo.[56] Mo is an essential micronutrient that serves as an enzyme cofactor and has a low degree of deficiency and toxicity in humans.[56]

In contrast to previous studies, we observed no association between known toxic metals in our study, such as Cd, Hg, and Pb, and fetal growth, though Hg had patterns of negative associations across most measures. Detection of Cr was slightly associated with femur length *z* score but was not associated with the other fetal growth measures. Several studies have found associations between prenatal exposure to toxic metals, such as Pb and Cd, and decreased birth weight or increased odds of being born small for gestational age.[10,19,49,57–59] Additionally, Goodrich et al.[12] observed inverse associations between urinary As, Pb, and Ba measured in first trimester urine and decreased femur length but not other fetal growth measures. Results for the relationship between prenatal Hg exposure and low birth weight have been less consistent.[27,28,60,61] Most of the studies that observed adverse effects on fetal growth were conducted in populations with high exposure of heavy metals in mothers during pregnancy, whereas our study population had lower exposures of these toxic metals.[30,47,62] This could explain the absence of any associations with toxic metals here.

For the most part, combinations of "toxic," "essential," and "seafood-related" metals were not associated with fetal growth in our analysis applying PCA, which was consistent with results from our multipollutant model. We observed no associations between "toxic" metals, the component with higher loading from Pb, Cd, and Mn, and fetal growth, which was consistent with the null or weak associations observed for these metals in the multipollutant model. The "essential" PC association (loaded by Cu, Se, and Zn) with fetal growth was also null, which we expected since we observed Se and Cu acting in opposite directions of Zn in the multipollutant model. On the other hand, As, Hg, and Sn (comprising the "seafood-related" PC) showed modest inverse associations with fetal growth in the multipollutant model and were associated with lower head circumference and weight *z* scores in the PCA model. Previous studies have found inconsistent associations for Hg and As and birth outcomes when analyzed individually.[27,60,63–65] Thus, by looking at these metals as a mixture, we may better understand the total effect that cannot be observed with models of individual metals.

The use of urinary biomarkers to estimate exposure to metals in the environment should be considered carefully in the interpretation of our results. Urinary Se is an indicator of short-term exposure and studies have shown correlation between dietary Se intake, the primary route of human exposure, and daily urinary excretion.[66–69] Plasma and serum are used more commonly and also reflect recent intake of Se, whereas whole blood represents intermediate to long-term exposure.[67] For Zn, urinary concentrations have also been associated with dietary intake and are also considered reliable indicators of zinc status.[70,71] Finally, for Mo, Urinary Mo is mostly an indicator of long-term exposure and is the primary route of excretion, and is thus a good biomarker for epidemiologic studies.[56,72,73] Null associations in our study could be attributable to the use of urine when it is not ideal for assessing exposure. Among the toxic metals that we would have expected to be associated with decreased fetal growth, urine is an appropriate marker for chronic exposure to Cd, but blood is preferable for Pb.[17,18,74] Urinary Pb may be reflective of long-term exposure but is generally preferred for occupational studies.[75] Finally, urine is the preferred matrix for assessing chronic exposure to As but we did not have information on speciation which informs exposure source and toxicity.[16]

It should be noted that ultrasounds from visits 3 and 4 were not part of the research study design. Instead, they were collected clinically, and therefore we do not have ultrasound measures for all participants and we may over-represent pregnancies that were suspected to have fetal growth restriction or other health issues, such as gestational diabetes, that necessitated multiple ultrasounds.[9] This may limit the generalizability of our primary findings to higher-risk pregnancies, although it would not create bias. When we created models with imputed data for the missing ultrasound data from visits 3 and 4, we observed that effect estimates were attenuated slightly toward the null, with some associations losing statistical significance. Because missing data were more likely to exist for healthier cases, we can interpret this difference to indicate that these associations, e.g., between Se and decreased fetal growth, may be stronger in higher-risk pregnancies. It may be important to consider the vulnerability of the individual in future work examining associations between exposure to metals in pregnancy and adverse birth outcomes.

Our study faced several additional limitations as well. Although we were able to measure a large panel of metals, we only analyzed metal concentrations from one time point during

pregnancy, which does not give us the whole picture of exposure because these metal concentrations can change throughout gestation because of metabolic changes caused by the pregnancy.[76] Second, there can be error in ultrasound measurements due to intra- and interobserver variability.[77] Finally, we were also unable to capture additional information on dietary intake which could influence urinary concentrations of trace metals as well as fetal growth.

Our study had several strengths that make our findings an important contribution to the literature on trace metals exposure in pregnancy and fetal growth. We were able to analyze a large panel of 17 trace metals from pregnant mothers and utilized repeated measures of fetal growth during pregnancy, rather than only measures at birth, in a relatively large study population. Though multiple measures of metal concentrations throughout pregnancy would have given us a picture of exposure during pregnancy, a previous study of pregnant women in Australia found high correlation between metal concentrations in the first and third trimester which suggests that the metal input does not change as much throughout pregnancy and the differences in concentration may be from metabolic changes.[76] And though urine may not be the ideal biomarker for all of the metals, it is a valid biomarker and valuable for several of them. In addition, we utilized a mixtures approach to assess predictor groups of exposure in relation to fetal growth measures, whereas previous studies have only analyzed metals in single-pollutant models.

Conclusions

In conclusion, we observed associations between reduced fetal growth and urinary concentrations of Se, as well as increased growth with urinary Zn and Mo. PCA identified three meaningful groups of metals, and we observed associations between the "seafood-related" component and reduced fetal growth as well. Further research is necessary to understand the surprising inverse association between Se and fetal growth, with particular attention to which matrix (i.e., whole blood, serum, plasma, or urine) is most relevant for estimating maternal exposure in pregnancy.

Conflicts of interest statement

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

REFERENCES

1. Barker DJ, Eriksson JG, Forsén T, Osmond C. Fetal origins of adult disease: strength of effects and biological basis. Int J Epidemiol. 2002;31:1235–1239.
2. McCormick MC. The contribution of low birth weight to infant mortality and childhood morbidity. N Engl J Med. 1985;312:82–90.
3. McIntire DD, Bloom SL, Casey BM, Leveno KJ. Birth weight in relation to morbidity and mortality among newborn infants. N Engl J Med. 1999;340:1234–1238.
4. Albu AR, Anca AF, Horhoianu VV, Horhoianu IA. Predictive factors for intrauterine growth restriction. J Med Life. 2014;7:165–171.
5. Haram K, Søfteland E, Bukowski R. Intrauterine growth restriction: effects of physiological fetal growth determinants on diagnosis. Obstet Gynecol Int. 2013;2013:708126.
6. Mayer C, Joseph KS. Fetal growth: a review of terms, concepts and issues relevant to obstetrics. Ultrasound Obstet Gynecol. 2013;41:136–145.
7. Zheng T, Zhang J, Sommer K, et al. Effects of environmental exposures on fetal and childhood growth trajectories. Ann Glob Health. 2016;82:41–99.
8. Ferguson KK, Meeker JD, Cantonwine DE, et al. Environmental phenol associations with ultrasound and delivery measures of fetal growth. Environ Int. 2018;112:243–250.
9. Ferguson KK, Meeker JD, Cantonwine DE, Chen YH, Mukherjee B, McElrath TF. Urinary phthalate metabolite and bisphenol A associations with ultrasound and delivery indices of fetal growth. Environ Int. 2016;94:531–537.
10. Rodosthenous RS, Burris HH, Svensson K, et al. Prenatal lead exposure and fetal growth: smaller infants have heightened susceptibility. Environ Int. 2017;99:228–233.
11. Mikelson CK, Troisi J, LaLonde A, et al. Placental concentrations of essential, toxic, and understudied metals and relationships with birth outcomes in Chattanooga, TN. Environ Res. 2019;168:118–129.
12. Goodrich JM, Ingle ME, Domino SE, et al. First trimester maternal exposures to endocrine disrupting chemicals and metals and fetal size in the Michigan Mother-Infant Pairs study. J Dev Orig Health Dis. 2019;10:447–458.
13. Wigle DT, Arbuckle TE, Turner MC, et al. Epidemiologic evidence of relationships between reproductive and child health outcomes and environmental chemical contaminants. J Toxicol Environ Health B Crit Rev. 2008;11:373–517.
14. Agency for Toxic Substances and Disease Registry (ATSDR). Public Health Statement: Cadmium; 2015.
15. ATSDR. Toxicological Profile for Copper. In: Services USDoHaH. Atlanta, GA: Public health Service; 2004.
16. ATSDR. Toxicological Profile for Arsenic. In: Services USDoHaH. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 2007.
17. ATSDR. Toxicological Profile for Zinc. In: Services USDoHaH. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 2005.
18. ATSDR. Toxicological Profile for Lead. In: Services USDoHaH. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. 2007.
19. Johnston JE, Valentiner E, Maxson P, Miranda ML, Fry RC. Maternal cadmium levels during pregnancy associated with lower birth weight in infants in a North Carolina cohort. PLoS One. 2014;9:e109661.
20. Al-Saleh I, Shinwari N, Mashhour A, Rabah A. Birth outcome measures and maternal exposure to heavy metals (lead, cadmium and mercury) in Saudi Arabian population. Int J Hyg Environ Health. 2014;217:205–218.
21. Neggers YH, Cutter GR, Acton RT, et al. A positive association between maternal serum zinc concentration and birth weight. Am J Clin Nutr. 1990;51:678–684.
22. Goldenberg RL, Tamura T, Neggers Y, et al. The effect of zinc supplementation on pregnancy outcome. JAMA. 1995;274:463–468.
23. Bogden JD, Kemp FW, Chen XH, Stagnaro-Green A, Stein TP, Scholl TO. Low-normal serum selenium early in human pregnancy predicts lower birth weight. Nutrition Research. 2006;26:497–502.
24. Mistry HD, Kurlak LO, Young SD, et al. Maternal selenium, copper and zinc concentrations in pregnancy associated with small-for-gestational-age infants. Matern Child Nutr. 2014;10:327–334.
25. Claus Henn B, Ettinger AS, Schwartz J, et al. Early postnatal blood manganese levels and children's neurodevelopment. Epidemiology. 2010;21:433–439.
26. Vollet K, Haynes EN, Dietrich KN. Manganese exposure and cognition across the lifespan: contemporary review and argument for biphasic dose-response health effects. Curr Environ Health Rep. 2016;3:392–404.
27. Ballester F, Iñiguez C, Murcia M, et al. Prenatal exposure to mercury and longitudinally assessed fetal growth: Relation and effect modifiers. Environ Res. 2018;160:97–106.
28. Drouillet-Pinard P, Huel G, Slama R, et al. Prenatal mercury contamination: relationship with maternal seafood consumption during pregnancy and fetal growth in the 'EDEN mother-child' cohort. Br J Nutr. 2010;104:1096–1100.
29. Ferguson KK, McElrath TF, Meeker JD. Environmental phthalate exposure and preterm birth. JAMA Pediatr. 2014;168:61–67.
30. Kim SS, Meeker JD, Carroll R, et al. Urinary trace metals individually and in mixtures in association with preterm birth. Environ Int. 2018;121(pt 1):582–590.
31. Hadlock FP, Harrist RB, Sharman RS, Deter RL, Park SK. Estimation of fetal weight with the use of head, body, and femur measurements–a prospective study. Am J Obstet Gynecol. 1985;151:333–337.
32. Cantonwine DE, Ferguson KK, Mukherjee B, et al. Utilizing longitudinal measures of fetal growth to create a standard method to assess the impacts of maternal disease and environmental exposure. PLoS One. 2016;11:e0146532.
33. Hornung RW, Reed LD. Estimation of Average Concentration in the Presence of Nondetectable Values. Applied Occupational and Environmental Hygiene. 1990;5:46–51.
34. Hewett P, Ganser GH. A comparison of several methods for analyzing censored data. Ann Occup Hyg. 2007;51:611–632.

35. Jiang Y, Scott AJ, Wild CJ. Secondary analysis of case-control data. Stat Med. 2006;25:1323–1339.
36. O'Rourke N, Hatcher L. A Step-by-Step Approach for Using SAS for Factor Analysis and Structural Equation Modeling. 2nd ed. Cary, NC: SAS Institute Inc.; 2013.
37. Ferguson KK, Yu Y, Cantonwine DE, McElrath TF, Meeker JD, Mukherjee B. Foetal ultrasound measurement imputations based on growth curves versus multiple imputation chained equation (MICE). Paediatr Perinat Epidemiol. 2018;32:469–473.
38. van Buuren S, Groothuis-Oudshoorn K. mice: multivariate imputation by chained equations in R. J Stat Softw. 2011;45:1–67.
39. Williams M, Todd GD, Roney N, et al. Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profiles. In: Toxicological Profile for Manganese. Atlanta (GA): Agency for Toxic Substances and Disease Registry (US); 2012.
40. ATSDR. Toxicological Profile for Mercury. In: Services USDoHaH. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 1999.
41. ATSDR. Toxicological profile for Tin. In: Services USDoHaH. Atlanta, GA: Public Health Service; 2005.
42. Jablonska E, Vinceti M. Selenium and human health: witnessing a copernican revolution? J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 2015;33:328–368.
43. Maret W, Sandstead HH. Zinc requirements and the risks and benefits of zinc supplementation. J Trace Elem Med Biol. 2006;20:3–18.
44. Vinceti M, Filippini T, Wise LA. Environmental selenium and human health: an update. Curr Environ Health Rep. 2018;5:464–485.
45. Rayman MP. Selenium and human health. Lancet. 2012;379:1256–1268.
46. Institute of Medicine (US) Panel on Micronutrients. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington, DC: National Academies Press; 2001.
47. CDC. Fourth Report on Human Exposure to Environmental Chemicals, Updated Tables. 2018. Available at: https://www.cdc.gov/exposurereport/. Accessed 4 January 2018.
48. Everson TM, Kappil M, Hao K, et al. Maternal exposure to selenium and cadmium, fetal growth, and placental expression of steroidogenic and apoptotic genes. Environ Res. 2017;158:233–244.
49. Sun H, Chen W, Wang D, Jin Y, Chen X, Xu Y. The effects of prenatal exposure to low-level cadmium, lead and selenium on birth outcomes. Chemosphere. 2014;108:33–39.
50. Caulfield LE, Zavaleta N, Shankar AH, Merialdi M. Potential contribution of maternal zinc supplementation during pregnancy to maternal and child survival. Am J Clin Nutr. 1998;68(2 suppl):499S–508S.
51. Wilson RL, Grieger JA, Bianco-Miotto T, Roberts CT. Association between maternal zinc status, dietary zinc intake and pregnancy complications: a systematic review. Nutrients. 2016;8:641.
52. Meeker JD, Rossano MG, Protas B, et al. Cadmium, lead, and other metals in relation to semen quality: human evidence for molybdenum as a male reproductive toxicant. Environ Health Perspect. 2008;116:1473–1479.
53. Meeker JD, Rossano MG, Protas B, et al. Environmental exposure to metals and male reproductive hormones: circulating testosterone is inversely associated with blood molybdenum. Fertil Steril. 2010;93:130–140.
54. Lewis RC, Johns LE, Meeker JD. Exploratory analysis of the potential relationship between urinary molybdenum and bone mineral density among adult men and women from NHANES 2007-2010. Chemosphere. 2016;164:677–682.
55. Shirai S, Suzuki Y, Yoshinaga J, Mizumoto Y. Maternal exposure to low-level heavy metals during pregnancy and birth size. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2010;45:1468–1474.
56. ATSDR. Toxicological Profile for Molybdenum: Draft for Public Comment. Atlanta, Georgia: ATSDR; 2017.
57. Sabra S, Malmqvist E, Saborit A, Gratacós E, Gomez Roig MD. Heavy metals exposure levels and their correlation with different clinical forms of fetal growth restriction. PLoS One. 2017;12:e0185645.
58. Jelliffe-Pawlowski LL, Miles SQ, Courtney JG, Materna B, Charlton V. Effect of magnitude and timing of maternal pregnancy blood lead (Pb) levels on birth outcomes. J Perinatol. 2006;26:154–162.
59. Taylor CM, Golding J, Emond AM. Adverse effects of maternal lead levels on birth outcomes in the ALSPAC study: a prospective birth cohort study. BJOG. 2015;122:322–328.
60. Gundacker C, Fröhlich S, Graf-Rohrmeister K, et al. Perinatal lead and mercury exposure in Austria. Sci Total Environ. 2010;408:5744–5749.
61. Guo BQ, Cai SZ, Guo JL, et al. Levels of prenatal mercury exposure and their relationships to neonatal anthropometry in Wujiang City, China. Environ Pollut. 2013;182:184–189.
62. Health Canada. Report on Human Biomonitoring of Environmental Chemicals in Canada: Results of the Canadian Health Measures Survey Cycle 1 (2007-2009). Ottawa, Canada: 2010.
63. Rahman A, Vahter M, Smith AH, et al. Arsenic exposure during pregnancy and size at birth: a prospective cohort study in Bangladesh. Am J Epidemiol. 2009;169:304–312.
64. Kwok RK, Kaufmann RB, Jakariya M. Arsenic in drinking-water and reproductive health outcomes: a study of participants in the Bangladesh Integrated Nutrition Programme. J Health Popul Nutr. 2006;24:190–205.
65. Davis HT, Aelion CM, Lawson AB, Cai B, McDermott S. Associations between land cover categories, soil concentrations of arsenic, lead and barium, and population race/ethnicity and socioeconomic status. Sci Total Environ. 2014;490:1051–1056.
66. Sanz Alaejos M, Díaz Romero C. Urinary selenium concentrations. Clin Chem. 1993;39:2040–2052.
67. Hays SM, Macey K, Nong A, Aylward LL. Biomonitoring equivalents for selenium. Regul Toxicol Pharmacol. 2014;70:333–339.
68. Longnecker MP, Stram DO, Taylor PR, et al. Use of selenium concentration in whole blood, serum, toenails, or urine as a surrogate measure of selenium intake. Epidemiology. 1996;7:384–390.
69. ATSDR. Toxicological profile for Selenium. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 2003.
70. Lowe NM, Fekete K, Decsi T. Methods of assessment of zinc status in humans: a systematic review. Am J Clin Nutr. 2009;89:2040S–2051S.
71. Wieringa FT, Dijkhuizen MA, Fiorentino M, Laillou A, Berger J. Determination of zinc status in humans: which indicator should we use? Nutrients. 2015;7:3252–3263.
72. Turnlund JR, Keyes WR, Peiffer GL. Molybdenum absorption, excretion, and retention studied with stable isotopes in young men at five intakes of dietary molybdenum. Am J Clin Nutr. 1995;62:790–796.
73. Turnlund JR, Keyes WR, Peiffer GL, Chiang G. Molybdenum absorption, excretion, and retention studied with stable isotopes in young men during depletion and repletion. Am J Clin Nutr. 1995;61:1102–1109.
74. ATSDR. Toxicological Profile for Tungsten. In: Services USDoHaH. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 2005.
75. Barbosa F Jr, Tanus-Santos JE, Gerlach RF, Parsons PJ. A critical review of biomarkers used for monitoring human exposure to lead: advantages, limitations, and future needs. Environ Health Perspect. 2005;113:1669–1674.
76. Fort M, Cosín-Tomás M, Grimalt JO, Querol X, Casas M, Sunyer J. Assessment of exposure to trace metals in a cohort of pregnant women from an urban center by urine analysis in the first and third trimesters of pregnancy. Environ Sci Pollut Res Int. 2014;21:9234–9241.
77. Dudley N. A systematic review of the ultrasound estimation of fetal weight. Ultrasound Obstet Gynecol .2005;25:80–89.