Urinary trace metals in association with fetal ultrasound measures during pregnancy

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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.7–11 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, and 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.19,24 Examining the effect of chemicals on fetal growth

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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.

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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 <13 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 2012.12

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) ICAPPRO inductively coupled plasma mass spectrometry and a Tokai-Accuspray 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.31,34 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, pregravid 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 0.4112 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.\textsuperscript{47} 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.\textsuperscript{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).\textsuperscript{30} Urinary concentrations of Cu, Mn, Ni, and Zn were slightly higher than those from NHANES or the Canadian Health Measures Survey, and levels of other trace metals were comparable.\textsuperscript{30} We also compared metal concentrations between individuals who had an ultrasound at visit 3 and at visit 4 and those who did not (Table 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 (Table 2; http://links.lww.com/EE/A74) and adjusted (Figure 1, Table 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) (Table 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 ($\beta = −1.13$, 95% CI = −2.03, −0.24)

### Table 1.

| 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     |

\*Weights were used to account for original case–control study design.

### Table 2.

| 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     | 0.25| 32 (8.20)  | 6.88 | 9.08 | 12.1 |
| Hg     | 0.05| 32 (8.20)  | 0.27 | 0.51 | 0.97 |
| Mo     | 0.08| 6 (1.50)   | 0.51 | 0.73 | 1.14 |
| Mn     | 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 |
| Ti     | 0.02| 61 (15.6)  | 0.08 | 0.13 | 0.18 |
| U      | 0.01| 242 (67.7) | 0.004| 0.007| 0.01 |
| W      | 0.20| 399 (79.2) | 0.09 | 0.14 | 0.23 |
| Zn     | 2.00| 0 (0)      | 146.8| 245.9| 367.5 |

\*Weights were used to account for original case–control study design. ppb indicates parts per billion.
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.10 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 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 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 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); 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

![Image of ultrasound z-score distribution]

**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.

| Metal | FLZa | HCZa | ACZa | EFWZ + BWZa |
|-------|------|------|------|-------------|
| PC1c: “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) |
| PC2d: “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) |
| PC3e: “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.

aAdjusted for maternal age, prepregnancy BMI, race/ethnicity, education, infant sex, gestational age at time of ultrasound.

bAdjusted for maternal age, prepregnancy BMI, race/ethnicity, education, infant sex, gestational age at time of ultrasound or gestational age at delivery.
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.7,24

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.21,24,64,65 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.31

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.2,55

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 supports DNA, RNA, cellular replication, and regulating hormones.31

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-estimate 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. Second, there can be error in ultrasound measurements due to intra- and interobserver variability. 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. 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.

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