A metabolome-wide association study of in utero metal and trace element exposures with cord blood metabolome profile: Findings from the Boston Birth Cohort

Mingyu Zhang\textsuperscript{a,b}, Jessie P Buckley\textsuperscript{a,c}, Liming Liang\textsuperscript{d,e}, Xiumei Hong\textsuperscript{f}, Guoying Wang\textsuperscript{f}, Mei-Cheng Wang\textsuperscript{g}, Marsha Wills-Karp\textsuperscript{c}, Xiaobin Wang\textsuperscript{h}, Noel T Mueller\textsuperscript{a,b,*}

\textsuperscript{a}Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
\textsuperscript{b}Welch Center for Prevention, Epidemiology, and Clinical Research, Johns Hopkins University, Baltimore, MD, USA
\textsuperscript{c}Department of Environmental Health and Engineering, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
\textsuperscript{d}Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA
\textsuperscript{e}Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA, USA
\textsuperscript{f}Center on the Early Life Origins of Disease, Department of Population, Family and Reproductive Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
\textsuperscript{g}Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
\textsuperscript{h}Department of Pediatrics, Johns Hopkins School of Medicine, Baltimore, MD, USA

Abstract

**Background:** Exposure to metals lead (Pb), mercury (Hg), and cadmium (Cd) and trace elements selenium (Se) and manganese (Mn) has been linked to the developmental origins of cardiometabolic diseases, but the mechanisms are not well-understood.

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\*Corresponding author at: Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, 2024 E. Monument St, Room 2-636, Baltimore, MD 21205, USA. noeltmueller@jhu.edu (N.T. Mueller).

CRediT authorship contribution statement

Mingyu Zhang: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing. Jessie P Buckley: Methodology, Writing – review & editing. Liming Liang: Data curation, Funding acquisition, Methodology, Writing – review & editing. Xiumei Hong: Data curation, Funding acquisition, Writing – review & editing. Guoying Wang: Data curation, Funding acquisition, Writing – review & editing. Mei-Cheng Wang: Funding acquisition, Writing – review & editing. Marsha Wills-Karp: Funding acquisition, Writing – review & editing. Xiaobin Wang: Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing. Noel T Mueller: Conceptualization, Methodology, Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.106976.
Objective: Conduct a metabolome-wide association study to understand how in utero exposure to Pb, Hg, Cd, Se, and Mn affects the metabolic programming of fetuses.

Methods: We used data from the Boston Birth Cohort, which enrolled mother-child pairs from Boston, MA. We measured metals and trace elements in maternal red blood cells (RBCs) collected 24–72 h after delivery, and metabolites in cord blood collected at birth. We used multivariable linear regression to examine associations of metals and trace elements with metabolites and Bonferroni correction to account for multiple comparisons. We assessed non-linear associations of metals and trace elements with metabolites using restricted cubic spline plots.

Results: This analysis included 670 mother-child pairs (57% non-Hispanic Black and 24% Hispanic). After Bonferroni correction, there were 25 cord metabolites associated with at least one of the metals or trace elements. Pb was negatively associated with the xenobiotic piperine, Cd was positively associated with xenobiotics cotinine and hydroxycotinine, and Hg was associated with 8 lipid metabolites (in both directions). Se and Mn shared associations with 6 metabolites (in both directions), which mostly included nucleotides and amino acids; Se was additionally associated with 7 metabolites (mostly amino acids, nucleotides, and carnitines) and Mn was additionally associated with C36:4 hydroxy phosphatidylcholine. Restricted cubic spline plots showed that most associations were linear.

Discussion: Maternal RBC metal and trace element concentrations were associated in a dose-dependent fashion with cord blood metabolites. What remains to be determined is whether these metals- and trace elements-associated changes in cord metabolites can influence a child’s risk of cardiometabolic diseases.

Keywords
Heavy metals; Lead; Mercury; Cadmium; Trace elements; Selenium; Manganese; Metabolome; Metabolomics; Metabolome-wide association study; Child health; Environmental health

1. Introduction

In utero exposure to metals and trace elements may be involved in the developmental origins of cardiometabolic diseases. Lead (Pb), mercury (Hg), and cadmium (Cd) are toxic heavy metals that have no known biological role in the human body, but can cause adverse health effects even at trace levels (Buckley et al., 2020). Exposure to these metals has been linked with higher risk of cardiometabolic diseases in human adults (Chowdhury et al., 2018), and they have been identified as chemicals of major public health concern by the World Health Organization (WHO) (WHO, 2010) and the United States (U.S.) Centers for Disease Control and Prevention (CDC)’s National Biomonitoring Program (Centers for Disease Control and Prevention, 2021). Accumulating evidence also suggests that in utero exposure to these heavy metals may affect offspring cardiometabolic health, including being associated with higher risk of elevated blood pressure and overweight or obesity (Wang et al., 2019a; Wang et al., 2019b; Farzan et al., 2018; Gump et al., 2005; Skröder et al., 2016; Zhang et al., 2012; Gregory et al., 2016; Kalish et al., 2014; Sørensen et al., 1999; Thurston et al., 2007; Chatzi et al., 2019; Hawkesworth et al., 2013; Kupsco et al., 2019; Howe et al., 2021). Selenium (Se) and manganese (Mn), in contrast, are two essential trace elements for human health. Although evidence is inconclusive on the associations of Se...
and Mn with adult cardiometabolic health, our previous study found that in utero exposure to these two elements may protect against child high blood pressure (Zhang et al., 2021). However, mechanisms by which these metals and trace elements may affect cardiometabolic programming in utero are not well-understood.

The study of metabolomics affords a unique opportunity to understand such mechanisms. Metals and trace elements have been linked with changes in metabolome profiles in human adults (Li et al., 2020; Eguchi et al., 2018; Suvagandha et al., 2014; Dudka et al., 2014; Kelly et al., 2020; Ellis et al., 2012). For example, Kelly et al. (2020) examined the plasma metabolomic signatures of Pb exposure (measured in blood and toenails) and found that Pb may affect oxidative stress and immune dysfunction. Ellis et al. (2012) found that urinary Cd was positively associated with levels of urinary citrate and the oxidative stress biomarker 8-oxo-deoxyguanosine. Other studies have found changes in lipids, amino acids, nucleic acids, and other metabolites in response to metal exposures, which may reflect energy and hormone metabolism, inflammation, oxidative stress, among other pathways involved in the pathogenesis of cardiometabolic diseases (Li et al., 2020; Eguchi et al., 2018; Suvagandha et al., 2014; Dudka et al., 2014).

As summarized in a recent review article (Dai et al., 2020), few studies examined the effects of pregnancy metal exposures on maternal and fetal metabolome. In cross-sectional analyses of pregnant women in China, Li et al. (2019) (n = 246) and Wang et al. (2018) (n = 232) examined associations of urinary Cd, Se, and Mn with urinary metabolites in pregnancy and identified pathways related to oxidative stress, amino acid and purine metabolism, and tricarboxylic acid cycle. Maternal urinary metabolites, however, may not reflect fetal exposures, and whether these pathway changes relate to offspring health is not clear. Contrastingly, cord blood more directly represents fetal exposures, and its metabolites may reflect metabolic responses in the developing fetus (Herrera et al., 2006; Cleal et al., 2018). Small cohort studies in Bangladesh (n = 35) (Wei et al., 2017) and in Mexico (n = 50) (Laine et al., 2017) found that maternal urinary and cord blood arsenic levels were associated with differences in cord metabolomic profiles that include fatty acids, amino acids, and lipids. To our knowledge, no study has examined whether in utero Pb, Hg, Cd, Se, or Mn exposure is associated with cord blood metabolites. Such associations may elucidate the molecular pathways through which these metals and trace elements may affect cardiometabolic programming and later health outcomes and related modifiable intervention targets for children exposed to metals in utero.

Considering this literature gap, we conducted a metabolome-wide association study to delineate the associations of in utero exposure to Pb, Hg, Cd, Se and Mn with cord blood metabolomic profiles in an ongoing prospective birth cohort that comprises a predominantly urban, low-income, minority population in the U.S. We hypothesized that Pb, Hg, and Cd are associated with higher levels of oxidative stress and inflammation related metabolites, while Se and Mn are associated with lower levels of such metabolites.
2. Material and methods

2.1. Study population

We used data from the Boston Birth Cohort, an ongoing prospective cohort that started enrolling mother-child pairs in 1998 at the Boston Medical Center. Mothers who had multiple gestation pregnancies or who gave birth to neonates with major birth defects were excluded from participation. Research staff approached and recruited eligible mothers 24–72 h after delivery and collected their socioeconomic and demographic information with a standardized postpartum questionnaire. Samples to measure maternal metals and trace elements and cord blood metabolites were selected at random from those under follow up in the Boston Birth Cohort. We measured metals and trace elements in 1,501 maternal blood samples and metabolites in 1,000 cord blood samples. This analysis included 670 mother-child pairs with data on both maternal metals and trace elements and cord blood metabolites. Participants were enrolled between December 2002 and October 2013.

The Boston Birth Cohort study was approved by the Institutional Review Boards of the Boston Medical Center and the Johns Hopkins Bloomberg School of Public Health. All mothers provided written informed consent for their participation into the study.

2.2. Maternal heavy metals and trace elements

We measured heavy metal and trace element concentrations in maternal red blood cells (RBCs) collected 24–72 h after delivery. Tubes used for blood collection were standard clinical blood collection purple top tubes with EDTA as an anticoagulant; there is no known contaminations by metals. As detailed in a previous publication (Chen et al., 2014), we demonstrated a high degree of transplacental passage of these metals and trace elements from maternal to fetal circulation. Given that RBC’s lifespan is about 120 days and that the half-lives of the metals included in this study range from 2 to 4 months, maternal RBC metals and trace elements are a reliable proxy of fetal exposure in second to third trimester (Zhang et al., 2021). Plasma and RBCs were separated by centrifugation and kept frozen at −80°C. Aliquots (0.5 ml) were transported on dry ice to the Public Health and Environmental Laboratories in Trenton, NJ. For RBC sample aliquot, storage, and shipping, we used lab 2.0 ml freestanding micro tube with cap and graduation (Thermo Fisher Scientific, Inc., Waltham, MA); these tubes were certified by the lab to have been manufactured in a Class 100,000 cleanroom environment and to be metal-free. Samples were measured using inductively coupled plasma mass spectrometry (ICP-MS) on an Agilent 8900 QQQ (Agilent Technologies Inc., Santa Clara, CA). Metals and trace elements were measured in the same run, and the intra-assay coefficients of variation (CV) were < 5.0%. Table S1 shows the summary data on the metal and trace element concentrations and the number (%) of samples below the limits of detection (LOD). We assigned samples below the LOD a value of LOD divided by square root of 2.

2.3. Cord blood metabolites

Cord blood was collected at birth, and the metabolites were analyzed at the Broad Institute of MIT and Harvard, MA. We used two liquid chromatography-tandem mass spectrometry (LC-MS) techniques: 1) hydrophilic interaction liquid chromatography in the
positive ionization mode (HILIC-pos) analyses of water-soluble metabolites, and 2) C8 chromatography with positive ion mode (C8-pos) analyses of polar and non-polar plasma lipids. The experimental designs, including the LC-MS parameters, methods for sample analysis and normalization, selection and inclusion of the blind duplicates, and metabolite extraction processes, have been described in detail in a previous publication by the Broad Institute lab (Roberts et al., 2012); we strictly followed these protocols. We included multiple quality control and quality assurance steps. First, specimens were randomly located and were analyzed in a blinded fashion. Second, a pooled study reference sample composed of all study samples was randomly inserted across samples (per 20–30 samples); the CV for each metabolite was calculated using the reference samples, and metabolites with CV > 20% were excluded from the downstream analyses. Third, 51 pairs of blind duplicate pooled samples were included to ensure data accuracy and reliability; these samples demonstrated extremely high levels of pairwise correlation (median: 0.9995, interquartile range: 0.9990 to 0.9998; correlation coefficients for each pair are provided in Table S2). Fourth, as internal standard metabolites, the same concentrations of 2 metabolites (phenylalanine-d8 and valine-d8) and 1 metabolite (C24:0 PC) were respectively included in all study samples for HILIC-pos and C8-pos analyses; the CVs of these internal standard metabolites were < 5%. Fifth, internal standard peak was monitored to ensure system performance throughout the analyses.

Of the 397 known metabolites quantified, we removed 16 metabolites with CV > 20% and 3 metabolites that were internal controls, leaving 378 metabolites in the final analysis. We imputed non-detectable values as one half of the minimal value and used inverse normal transformation to render an approximately normal distribution of the metabolites and control for the effects of potential outliers. Table S3 provides a list of all metabolites included in this analysis with their methods of quantification and their CVs.

2.4. Covariates

Data on maternal age at delivery, preeclampsia, chronic diabetes mellitus, gestational diabetes mellitus, child sex, birth weight, and gestational age at birth were extracted from the electronic medical records. Data on maternal pre-pregnancy weight, height, race/ethnicity, educational level, cigarette smoking history, and second-hand smoking exposure during pregnancy were determined from the standardized Maternal Postpartum Questionnaire administered at enrollment (i.e., 24–72 h after delivery). In the questionnaire, mothers were asked their intake of various foods (including fish) during pregnancy, and we used these data to calculate a Mediterranean diet score as published previously (Rhee et al., 2021). We calculated maternal pre-pregnancy body mass index (BMI) as weight (kg) divided by height (m) squared and classified mothers as underweight (BMI < 18.5 kg/m²), normal weight (18.5 to 24.9 kg/m²), overweight (25 to 29.9 kg/m²), and obese (≥30 kg/m²). We defined low birth weight as birth weight < 2,500 g and preterm birth as gestational age at birth < 37 weeks.

2.5. Statistical analysis

We used multivariable linear regression models to examine the associations of metals and trace elements with cord metabolites. We modelled metal and trace element concentrations
as continuous variables scaled to per standard deviation (SD) increment. We used Bonferroni correction to account for multiple comparisons (two-sided p-value < 1.3 × 10^{-4} as statistically significant, calculated as 0.05 divided by 378 metabolites). For metabolites that were associated with metals or trace elements after Bonferroni correction, we used restricted cubic spline plots (with 3 knots) to examine the dose–response and/or non-linear associations. As Bonferroni correction is conservative due to correlation between metabolites and may result in false negative findings, we additionally used the false discovery rate (FDR) approach to control for a low proportion of false positive results (Benjamini and Hochberg, 1995; White et al., 2019) and presented these results in the Supplementary Materials.

We defined confounders as covariates expected to be associated with the exposure (metal/trace element) and cord metabolites but not on the underlying pathway based on a priori information from the literature. We adjusted for confounders that included maternal age at delivery (continuous), self-reported race/ethnicity (non-Hispanic White; non-Hispanic Black; Hispanic; others [including Asian, Pacific Islander, and mixed-race]), educational level (middle school or below; high school graduate or some college; college graduate and above), pre-pregnancy BMI (underweight; normal weight; overweight; obese), preeclampsia (no; mild; severe), diabetes mellitus (no; chronic; gestational), cigarette smoking history (never smoked; quit smoking before pregnancy; smoked during pregnancy), and second-hand smoking exposure during pregnancy (yes; no). In the sensitivity analyses, we additionally adjusted for the following covariates: 1) preterm birth (yes; no) and low birth weight (yes; no), 2) Mediterranean diet score (continuous), and 3) fish intake during pregnancy (none; ≤1 d/wk; 1–2 d/wk; 3–5 d/wk; 6–7 d/wk; do not know). These variables were not adjusted in the primary models because preterm birth and low birth weight were more likely to be causal intermediates than confounders, and maternal diet data was only collected once postpartum and may be subject to recall bias. We adjusted for maternal preeclampsia and diabetes mellitus in the primary models because they could have affected maternal metal and trace element levels; however, since the other way around could also be true, we excluded these two covariates from the multivariable-adjusted models in a sensitivity analysis.

To consider child sex as a biological variable (Arnegard et al., 2020), we examined whether the associations of metals and trace elements with metabolites differed by child sex (male vs. female). We also examined if the associations differed by maternal race/ethnicity (Hispanic vs. non-Hispanic Black), because we observed previously that the associations of in utero exposure to Mn and Hg with child blood pressure differed between children born to non-Hispanic Black vs. Hispanic mothers (Zhang et al., 2021). We did not conduct subgroup analyses in the non-Hispanic White (n = 30) or Others (n = 91) groups due to the small sample size. We included a product term of the metal/trace element (SD-scaled) with child sex or maternal race/ethnicity and used the p-values for the product terms as the interaction p-values. We considered a two-sided p-value < 2.6 × 10^{-4} (calculated as 0.10 divided by 378 metabolites) as statistically significant.

We used the multiple imputation by chained equations method (10 imputations, each with 10 iterations) to impute a small proportion of missing covariate data that were included in
the main linear regression analyses (0.4% for maternal educational level, 1.3% for maternal smoking status, 5.1% for maternal pre-pregnancy BMI, 4.5% for second-hand smoking exposure during pregnancy, 0.1% for Mediterranean diet score; Table 1). Covariates used to impute these data included all covariates in the main analyses and the sensitivity analyses (except metals/trace elements or metabolites). Each reported regression parameter estimate was calculated as the average of the 10 estimates derived from imputed datasets. Standard errors of the regression parameter estimates and the p-values were computed using Rubin’s rules (Rubin, 2004).

We conducted analyses using Stata 15.1 (Stata Corp) and R 4.0.3 (R Foundation for Statistical Computing).

3. Results

Table 1 shows the characteristics of the 670 mother-child pairs included in this analysis. Of the mothers, 385 (57%) were non-Hispanic Black, 164 (24%) were Hispanic, 179 (27%) had education level as middle school or below, 338 (50%) were overweight or obese, and 62 (9%) smoked during pregnancy. Of the children, 322 (48%) were female, 115 (17%) were born preterm, and 108 (16%) were born low birth weight. Spearman correlations of each pair of metals ranged from −0.02 to 0.35 (Fig. S1). Table S4 shows the comparison of the characteristics of pairs included in (n = 670) vs. excluded from (n = 1,161) this analysis due to missing maternal metal/trace element or cord metabolite data. Compared to children included, those excluded were slightly more likely to have been born preterm or low birth weight; all other maternal and child characteristics were comparable between the two groups. Table S5 and Table S6 provide the measured concentrations (i.e., peak areas from the LC-MS) and the Pearson correlation matrix for all 378 cord metabolites included in the analysis.

Table 2 and Fig. 1 show the 25 cord metabolites that were significantly associated with at least one of the metals or trace elements after Bonferroni correction (i.e., p-value < 1.3 × 10⁻⁴). Pb was negatively associated with the xenobiotic piperine, and Cd was positively associated with xenobiotics cotinine and hydroxycotinine, two nicotine metabolites. Hg was associated with 8 lipid metabolites, i.e., negatively associated with C22:5 CE, C22:4 LPC, C22:5 LPC, and C20:4 LPE and positively associated with C56:7 TAG, C56:8 TAG, C58:11 TAG, and C60:12 TAG. Se and Mn had overlapping associations with 6 metabolites: negative associations with guanidinoacetic acid (GAA) and imidazole propionate (ImP) and positive associations with hypoxanthine, inosine, cadaverine, and 8-hydroxy-deoxyguanosine (8-OHdG). Additionally, Se was associated with 7 metabolites: negatively with asparagine, hydroxyproline, C6 carnitine, C7 carnitine, C18:0 LPE B, and adenosine, and positively with xanthosine, and Mn was positively associated with C36:4 hydroxy-PC. Results did not markedly change when we additionally adjusted for preterm birth, low birth weight, Mediterranean diet score, or fish intake during pregnancy, or when we excluded covariates preeclampsia or diabetes mellitus from the regression models (Table S7). Most associations of metals and trace elements with metabolites were approximately linear, as shown in the restricted cubic spline plots in Fig. S2 to Fig. S6 (respectively for Pb, Hg, Cd, Se, and Mn). Multivariable adjusted estimates, nominal p-values, and FDR for
the associations of metals and elements with cord metabolites included in this analysis are provided in Table S8 (for Pb, Hg, Cd) and Table S9 (for Se and Mn).

When conducting subgroup analyses for all metal/trace element–cord metabolite pairs, we observed that most associations were consistent between male vs. female children and between children born to Hispanic vs. non-Hispanic Black mothers (Table S10 to Table S14). After Bonferroni correction (i.e., p-value < 2.6 × 10⁻⁴), no association differed by child sex and only the association between Pb and piperine differed by maternal race/ethnicity; the inverse association was stronger among children born to non-Hispanic Black mothers, and the association was positive among those born to Hispanic mothers (Table S10).

4. Discussion

In this U.S. urban, racially diverse, low-income population, we used a metabolome-wide association approach to identify 25 unique cord metabolites, including xenobiotics, lipids, amino acids, and nucleotides, that were associated with in utero exposure to metals Pb, Cd, and Hg and trace elements Se and Mn after adjustment for potential confounders and correction for multiple comparisons. Most associations were linear and did not differ by child sex or maternal race/ethnicity. To our knowledge, ours is the first study to examine the association of in utero exposure to Pb, Cd, Hg, Se, and Mn with cord blood metabolome.

We found that Se and Mn were both associated with lower levels of cord GAA and ImP, which have both been linked with adverse cardiometabolic health in adults. GAA is a natural biosynthesis precursor of creatine and both GAA and creatinine are involved in cellular energy metabolism (Brosnan and Brosnan, 2007; Ostojic, 2015). Higher GAA levels are also associated with cardiometabolic risk factors, including higher total homocysteine levels and insulin hypersecretion (Ostojic et al., 2013; Alsever et al., 1970). A study by Ostojic et al. (2018) in 151 healthy adults found higher serum GAA levels associated with higher insulin, total homocysteine, and percent body fat. ImP is a microbiologically-produced metabolite from histidine that may contribute to type 2 diabetes (T2D) pathogenesis. Koh et al. (2018) found that ImP levels were higher in mice with T2D, and that ImP impairs glucose tolerance and insulin signaling levels. ImP has also been shown to attenuate the glucose-lowering effect of metformin (Koh et al., 2020). In a European cohort, Molinaro et al. (2020) also found that patients with prediabetes and diabetes had higher serum ImP levels than non-diabetic controls. ImP level has also been found to be higher in adults with low bacterial gene richness (diversity) and lower relative abundance of the Bacteroides 2 enterotype, and each of these microbiome signatures have been linked with development of obesity and T2D (Molinaro et al., 2020; Lim, 2020; Wang et al., 2020). To our knowledge, no animal or human studies have examined the associations of Mn or Se with GAA or ImP.

We also found that Se and Mn were both associated with higher hypoxanthine, inosine, cadaverine, and 8-OHdG levels. Inosine and hypoxanthine are in the purine catabolism pathway and lead to the production of uric acid (Kutzing and Firestein, 2008), which is associated with risk of cardiovascular diseases in adults and children (Feig et al., 2008; Sautin and Johnson, 2008; Scheepers et al., 2017; Moulin-Mares et al., 2021). Prior studies
reported an inverse association of serum Se (Martí del Moral et al., 2011; Ruggiero et al., 2007) (mean level of ~ 70 μg/L in both studies) and urinary Mn (Sun et al., 2011) (low-level occupational exposure; exposure level was unclear) with uric acid in human adults, yet we did not find such associations in our study. As for cadaverine, a metabolite formed by bacterial decarboxylation of the amino acid lysine, it is unclear why it was inversely associated with both Se and Mn; we also did not find any literature on the association of cadaverine with metal exposure or with cardiometabolic health. Metabolite 8-OHdG is an established marker of oxidative DNA damage caused by excess reactive oxygen species, and its higher blood and urine levels are associated with human cardiovascular diseases and T2D (Kroese and Scheffer, 2014; Di Minno et al., 2016; Dong et al., 2008). This finding is opposite to our hypotheses on Mn and Se, which were formed on the basis that Mn and Se typically have antioxidant roles in human physiology (Tinggi, 2008; Chen et al., 2018). However, it is consistent with prior studies, specifically a study of 20 boilermakers occupationally exposed to particulate matters (positive association of Mn with urinary 8-OHdG) (Kim et al., 2004) and a study of 53 Chinese college students (positive associations of urinary Mn and Se with 8-OHdG; mean level was 2.113 μg/L for Mn and 22.00 μg/L for Se) (Lu et al., 2016).

We also observed associations of several metabolites with the heavy metals Pb and Cd. Cotinine and hydroxycotinine, two nicotine metabolites, were strongly positively associated with Cd levels, which is consistent with the literature on cigarette smoke as a major source of Cd exposure (Ashraf, 2012). Interestingly, these associations persisted after adjustment for self-reported maternal smoking and second-hand smoking status, but it is likely that the persistence of this association reflects residual confounding by exposure to cigarette smoke, as self-report of cigarette smoking is subject to recall bias and underreporting (Liber and Warner, 2018). The inverse Pb – piperine association in our study is quite intriguing, as it is consistent with a previous study that found piperine protected against lead acetate-induced nephrotoxicity and renal damage in rats (Sudjarwo et al., 2017). As a major component of black pepper that contributes to the pungent flavor, piperine has been found in in vitro studies to reduce insulin resistance, inflammation, and oxidative stress and to possess anti-mutagenic and anti-tumor effects (Derosa et al., 2016; Srinivasan, 2007). Studies have also found that piperine reduced Cd-induced oxidative stress in cultured human peripheral blood lymphocytes (Verma et al., 2020) and that the hepatic renal Cd levels were decreased in mice when treated with piperine (Khandelwal et al., 2008). Consistent with these studies, we also observed an inverse association of Cd and piperine, although it did not reach statistical significance after Bonferroni correction. Experimental studies are warranted to test the hypothesis that piperine blocks the negative health effects of Pb and Cd in humans.

Many of the other metabolites associated with metals in our study have also been found to be linked with cardiometabolic health in adults. To highlight a few, lipids accounted for all metabolites associated with Hg exposure, and many of the lipid metabolites have been linked with cardiometabolic health outcomes (Jiang et al., 2018; Balasubramanian et al., 2020; Chen et al., 2019; Dickerman et al., 2020). Of the metabolites associated with Se, asparagine (inversely associated with Se) is a precursor of aspartate, a main constituent of soy protein that has been linked with a lower risk of ischemic heart disease and diastolic blood pressure in a Mendelian randomization study of 0.34 million participants (Zhao et
In a study among patients with chronic kidney diseases, higher levels of C7 carnitine (inversely associated with Se) and xanthosine (positively associated with Se) were both associated with a higher risk of the occurrence of cardiovascular events (Ganda et al., 2017). C7 carnitine has also been associated with left ventricular diastolic dysfunction in the Women’s Interagency HIV study (Bravo et al., 2020) but not with T2D or cardiovascular mortality in other studies (Guasch-Ferre et al., 2019; Yeri et al., 2019). For C36:4 hydroxy-PC (positively associated with Mn), it is an oxidized lipid that has been linked with risk of cardiovascular diseases and coronary heart disease in the PREDIMED (Prevención con Dieta Mediterránea) trial (Paynter et al., 2018).

While a discussion of all the metabolites associated with metals and trace elements in our study is beyond the scope of this manuscript, we have instead focused our attention on metabolites that showed the strongest associations with metals and trace elements in our analyses. Nonetheless, we have also provided estimates for all the associations of each metal or trace element with each metabolite in Table S8 and Table S9 in hope that future studies could replicate and/or compare their findings to ours. When comparing findings, one should note: the possible differences in the biomarkers used (in this analysis, metals and trace elements were measured in maternal RBCs, and metabolites were measured in cord blood); the time points at biomarker collection (in this analysis, maternal blood were collected 24–72 h after delivery, and cord blood were collected at birth); and the exposure or internal dose levels of metals/trace elements in the study population (concentrations of metals and trace elements for this analysis are provided in Table S1).

Our study has limitations. First, although we adjusted for a comprehensive set of confounders in the main analyses (including maternal smoking and second-hand smoking exposure), and we additionally adjusted for maternal Mediterranean diet scores in the sensitivity analyses, there is possibility of residual (e.g., inaccurate report of cigarette smoke exposure or dietary intake and lack of time-varying information on these variables during pregnancy) and unmeasured (e.g., other lifestyle factors that could have affected metals/trace elements and metabolites) confounding. Second, we did not have repeated measures or trimester-specific metal and trace element data, so we were not able to identify the critical window of susceptibility or to assess the impact of cumulative exposure in utero. Third, we used Bonferroni correction to adjust for multiple comparisons, so there are likely false negative findings in our analysis. However, even with the stringent threshold, there could still be false positive findings as well. Finally, our analyses only included 378 known metabolites, which precluded us from evaluating the whole pathways that may have been affected by metals and trace elements; there could also be yet-to-be confirmed and/or unknown metabolites that we were not able to include in this analysis.

Our study also has several novelties and strengths. First, we used data from a prospective birth cohort of a diverse group of participants (57% non-Hispanic Black, 24% Hispanic) who, despite bearing a disproportionately high burden of heavy metal exposure and cardiometabolic diseases, have been underrepresented in prior research on this topic. Second, we measured metals and trace elements in maternal RBCs, which, compared to plasma, better reflect maternal-fetal transfer of metals, represent in utero exposure, and are less influenced by hemodilution (Chen et al., 2014). Third, we examined cord blood
metabolites, which directly represents fetal metabolic responses to heavy metals Pb, Cd, Hg, and trace elements Mn and Se, and we also used a metabolome-wide association study approach to identify multiple novel metabolites associated with these exposures. What remains to be determined is whether these associations of metals and trace elements with cord metabolites have measurable consequences for a child’s risk of cardiometabolic diseases; this is especially important given the small effect sizes of the associations observed in this analysis.

5. Perspectives

In this study, we found associations of in utero exposure to metals Pb, Hg, Cd and trace elements Se and Mn with cord metabolite levels. Many of these metabolites have been previously found to be associated with cardiometabolic disease outcomes in human populations. Given the mounting evidence that in utero metal/trace element exposures may be involved in the developmental origins of cardiometabolic disease outcomes, our findings provide important insight on the molecular pathways by which these metals and trace elements may alter metabolic programming and affect offspring health outcomes. If confirmed by future studies, our findings may be used to inform potential therapeutic targets for children exposed to heavy metals (e.g., if the xenobiotic piperine may reduce blood Pb levels). Future studies should confirm our findings and examine how cord blood metabolites may be associated with childhood cardiometabolic health outcomes (e.g., high blood pressure and overweight or obesity) and whether they mediate or modify the intergenerational associations of in utero metal/trace element exposures with these outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

| Abbreviation | Definition          |
|--------------|---------------------|
| Pb           | lead                |
| Hg           | mercury             |
| Cd           | cadmium             |
| Se           | selenium            |
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Fig. 1.
Volcano plots of associations of lead (Panel A), mercury (Panel B), cadmium (Panel C), selenium (Panel D), and manganese (Panel E) with cord metabolites after Bonferroni correction for multiple comparisons among mother-child pairs in the Boston Birth Cohort (n = 670). Models adjusted for maternal age at delivery, race/ethnicity, educational level, pre-pregnancy body mass index, preeclampsia, diabetes mellitus, cigarette smoking history, and second-hand smoking exposure during pregnancy.
Table 1

Characteristics of the mothers and children included in this analysis (n = 670).

| Maternal characteristics                                      |          |
|---------------------------------------------------------------|----------|
| Age at delivery (years), mean (SD)                            | 28.7 (6.7) |
| Race/ethnicity †, n (%)                                       |          |
| Non-Hispanic Black                                            | 385 (57) |
| Non-Hispanic White                                            | 30 (4)   |
| Hispanic                                                      | 164 (24) |
| Others                                                        | 91 (14)  |
| Educational level †, n (%)                                    |          |
| Middle school or below                                        | 179 (27) |
| High school graduate or some college                          | 405 (61) |
| College graduate and above                                    | 83 (12)  |
| Missing                                                       | 3        |
| Pre-pregnancy BMI categories †, n (%)                         |          |
| Underweight                                                   | 19 (3)   |
| Normal weight                                                 | 279 (44) |
| Overweight                                                    | 186 (29) |
| Obese                                                         | 152 (24) |
| Missing                                                       | 34       |
| Cigarette smoking history †, n (%)                            |          |
| Never smoked                                                  | 547 (83) |
| Quit smoking before pregnancy                                 | 52 (8)   |
| Smoked during pregnancy                                       | 62 (9)   |
| Missing                                                       | 9        |
| Second-hand smoking exposure during pregnancy †, n (%)        |          |
| No                                                            | 503 (79) |
| Yes                                                           | 136 (21) |
| Missing                                                       | 31       |
| Preeclampsia, n (%)                                           |          |
| No                                                            | 616 (92) |
| Mild                                                          | 22 (3)   |
| Severe                                                        | 32 (5)   |
| Diabetes mellitus, n (%)                                      |          |
| No                                                            | 576 (86) |
| Gestational diabetes mellitus                                 | 54 (8)   |
| Chronic diabetes mellitus                                     | 40 (6)   |
| Mediterranean diet score †, median (IQR)                      | 25.0 (22.0, 27.0) |

| Child characteristics                                          |          |
|----------------------------------------------------------------|----------|
| Child sex, n (%)                                               |          |
|                      |   |   |
|----------------------|---|---|
| Female               | 322 (48) |   |
| Male                 | 348 (52) |   |
| Preterm birth, n (%) |   |   |
| No                   | 555 (83) |   |
| Yes                  | 115 (17) |   |
| Low birth weight, n (%) |   |   |
| No                   | 562 (84) |   |
| Yes                  | 108 (16) |   |

Abbreviations: SD, standard deviation; IQR, interquartile range; BMI, body mass index.

* Percentages do not add up to 100% due to rounding.

† For categorical covariates with missing observations, missing observations were not included in the denominator when deriving percentages for the categories with known values. For the continuous variable Mediterranean diet score, there was 1 missing. Missing data were imputed using the multiple imputation by chained equations method in subsequent analyses.
Table 2

Cord metabolites that were significantly associated with metals or trace elements after Bonferroni correction among mother-child pairs in the Boston Birth Cohort (n = 670). Estimates show the differences in inverse normal transformed metabolite concentrations per standard deviation (SD) increment in metal/trace element concentrations.

| Cord metabolites | Metabolite class | Adjusted-β | Nominal P-value | FDR  |
|------------------|------------------|-----------|----------------|------|
| **Lead**         |                  |           |                |      |
| Piperine         | Xenobiotic       | −0.18     | 3.73 × 10⁻⁶    | 0.001|
| **Mercury**      |                  |           |                |      |
| C22:5 CE        | CE               | −0.21     | 8.69 × 10⁻⁸    | <0.001|
| C22:4 LPC       | LPC              | −0.17     | 1.43 × 10⁻⁵    | 0.001|
| C22:5 LPC       | LPC              | −0.15     | 9.09 × 10⁻⁵    | 0.004|
| C20:4 LPE       | LPE              | −0.17     | 2.98 × 10⁻⁵    | 0.002|
| C56:7 TAG       | TAG              | 0.16      | 4.35 × 10⁻⁵    | 0.002|
| C56:8 TAG       | TAG              | 0.17      | 9.95 × 10⁻⁶    | 0.001|
| C58:11 TAG      | TAG              | 0.17      | 2.67 × 10⁻⁵    | 0.002|
| C60:12 TAG      | TAG              | 0.19      | 8.04 × 10⁻⁷    | <0.001|
| **Cadmium**     |                  |           |                |      |
| Cotinine        | Xenobiotic       | 0.13      | 7.77 × 10⁻⁵    | 0.015|
| Hydroxycotinine | Xenobiotic       | 0.15      | 2.76 × 10⁻⁶    | 0.001|
| **Selenium**    |                  |           |                |      |
| Asparagine      | AA               | −0.19     | 3.95 × 10⁻⁷    | <0.001|
| Guanidinoacetic acid | AA | −0.27 | 7.47 × 10⁻¹² | <0.001|
| Hydroxyproline  | AA               | −0.16     | 3.05 × 10⁻⁵    | 0.001|
| C6 carnitine    | AC               | −0.15     | 5.69 × 10⁻⁵    | 0.002|
| C7 carnitine    | AC               | −0.18     | 4.00 × 10⁻⁶    | <0.001|
| Imidazole propionate | Imidazole | −0.18 | 7.34 × 10⁻⁷ | <0.001|
| C18:0 LPE B     | LPE              | −0.20     | 1.38 × 10⁻⁷    | <0.001|
| Adenosine       | Nucleotide       | −0.17     | 6.10 × 10⁻⁶    | <0.001|
| Hypoxanthine    | Nucleotide       | 0.17      | 8.31 × 10⁻⁶    | <0.001|
| Inosine         | Nucleotide       | 0.21      | 2.83 × 10⁻⁸    | <0.001|
| Xanthosine      | Nucleotide       | 0.14      | 4.38 × 10⁻⁵    | 0.001|
| Cadaverine      | Polyamine        | 0.22      | 5.10 × 10⁻⁹    | <0.001|
| 8-hydroxy-deoxyguanosine | Purine nucleotide | 0.18 | 7.50 × 10⁻⁷ | <0.001|
| **Manganese**   |                  |           |                |      |
| Guanidinoacetic acid | AA | −0.23 | 2.43 × 10⁻⁹ | <0.001|
| Imidazole propionate | Imidazole | −0.17 | 2.93 × 10⁻⁶ | <0.001|
| Hypoxanthine    | Nucleotide       | 0.15      | 9.50 × 10⁻⁵    | 0.005|
| Inosine         | Nucleotide       | 0.18      | 1.20 × 10⁻⁶    | <0.001|
| C36:4 hydroxy-PC | PC              | 0.15      | 3.49 × 10⁻⁵    | 0.002|
| Cord metabolites           | Metabolite class | Adjusted-$\beta^*$ | Nominal P-value | FDR       |
|----------------------------|------------------|--------------------|-----------------|-----------|
| Cadaverine                 | Polyamine        | 0.21               | $8.44 \times 10^{-9}$ | <0.001    |
| 8-hydroxy-deoxyguanosine   | Purine nucleotide| 0.16               | $1.59 \times 10^{-5}$ | 0.001     |

Abbreviations: SD, standard deviation; FDR, false discovery rate; CE, cholesterol ester; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; TAG, triacylglycerol; AA, amino acid; PC, phosphatidylcholine.

*Models adjusted for maternal age at delivery, race/ethnicity, educational level, pre-pregnancy body mass index, preeclampsia, diabetes mellitus, cigarette smoking history, and second-hand smoking exposure during pregnancy.