ORIGINAL RESEARCH

Trace Minerals, Heavy Metals, and Preeclampsia: Findings from the Boston Birth Cohort

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***Background*—**Preeclampsia is a leading contributor to maternal and perinatal morbidity and mortality. In mice experiments, manganese (Mn) and selenium (Se) are protective whereas cadmium (Cd) is promotive for preeclampsia. Epidemiologic findings on these chemical elements have been inconsistent. To confirm experimental findings in mice, we examined associations of trace minerals (Mn and Se) and heavy metals (Cd, lead [Pb], and mercury [Hg]) with preeclampsia in a birth cohort.

***Methods and Results*—**A total of 1274 women from the Boston Birth Cohort (enrolled since 1998) had complete data on the exposures and outcome. We measured Mn, Se, Cd, Pb, and Hg from red blood cells collected within 24 to 72 hours after delivery. We ascertained preeclampsia diagnosis from medical records. We used Poisson regression with robust variance models to estimate prevalence ratios (PRs) and 95% CIs. A total of 115 (9.0%) women developed preeclampsia. We observed evidence of a dose–response trend for Mn (*P* for trend<0.001) and to some extent for Cd (*P* for trend=0.009) quintiles. After multivariable adjustment, a 1 SD increment in Mn was associated with 32% lower risk of developing preeclampsia (PR=0.68; 95% CI, 0.54–0.86), whereas a 1 SD increment in Cd was associated with 15% higher risk of preeclampsia (PR=1.15; 95% CI, 0.98–1.36). Null associations were observed for Se, Pb, and Hg.

***Conclusions*—**Findings from our cohort, consistent with evidence from mice experiments and human studies, indicate that women with lower blood concentration of Mn or higher Cd are more likely to develop preeclampsia. **(*J Am Heart Assoc*. 2019;8: e012436. DOI: 10.1161/JAHA.119.012436.)**

Key Words: cadmium • epidemiology • manganese • metal • preeclampsia/pregnancy • trace mineral

Preeclampsia is a pregnancy-specific complication chiefly characterized by new-onset hypertension and proteinuria after 20 weeks of gestation, and accompanied by signs of damage to other organ systems, such as hemolysis, elevated liver enzymes, and low platelet count (HELLP syndrome).[1] It is a leading contributor to maternal and perinatal morbidity and mortality, accounting for an estimated 50 000 to 60 000 deaths of mothers and their offspring per year worldwide.[2,3] In addition, the American Heart Association recently added preeclampsia as a risk factor for future cardiovascular disease in women,[4] increasing risk at a similar magnitude as diabetes mellitus.[5] In the United States, the incidence of preeclampsia has increased by 25% during the past 2 decades.[6] Though strategies to prevent preeclampsia have been studied extensively, pharmacologic prevention is at best minimally effective among general pregnant women,[7] with the only exception that low-dose aspirin may be effective in preventing preeclampsia in women at high risk.[8]

Several lines of evidence suggest that essential trace minerals (eg, manganese [Mn] and selenium [Se]) and toxic heavy metals (cadmium [Cd], lead [Pb], and mercury [Hg]) may have opposing roles in the development of preeclampsia. Mn has antioxidant effects and has been shown in experimental mouse models to reduce risk of preeclampsia.[9–11] Yet epidemiologic studies on Mn and preeclampsia have been limited to small case–control studies with inconsistent results.[12–17] It has also been shown that increasing the antioxidant Se in pregnancy reduced oxidative stress and

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Accompanying Tables S1 through S9, Figure S1 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.119.012436

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Clinical Perspective

What Is New?

- Higher manganese concentration, measured in maternal red blood cells obtained shortly after delivery, was associated with lower risk of preeclampsia.
- Higher cadmium concentration in maternal red blood cells was associated with higher risk of preeclampsia.
- No significant associations of selenium, lead, and mercury with preeclampsia were identified.

What Are the Clinical Implications?

- The inverse association between manganese and preeclampsia provides new insight into a potentially modifiable way to prevent preeclampsia.
- The hazardous effect of cadmium highlights the need to limit exposure to toxic metals for pregnant women in eliminating risk of preeclampsia.

improved preeclampsia features in rats[18] and preeclampsia biomarkers in clinical trials[19]; however, larger human studies are needed.

In contrast, the heavy metal Cd has been shown to induce oxidative stress,[20] endothelial dysfunction,[21] and immune abnormality[22] in mice. Moreover, a recent literature review of epidemiologic studies considered Cd and Pb as possible risk factors for preeclampsia,[23] but the sample sizes of the observational studies reviewed were small. Furthermore, a hazardous association of Hg with preeclampsia was also reported by 1 recent study of 124 adult dental staff[24]; however, this study was small and needs to be replicated.

Overall, the majority of the epidemiologic studies on the association of trace minerals and heavy metals with preeclampsia have been small and they have not controlled for potentially confounding factors (ie, factors that affect both chemical elements and preeclampsia[25]). They have also been conducted in settings (eg, places outside the United States[12–15,17] or among dental staff[24]) that may not be generalizable to exposure levels for typical pregnancies in the United States. Furthermore, no studies, to our knowledge, have measured concentrations of aforementioned trace minerals and metals in red blood cells (RBCs), a biomarker believed to be more precise and stable in reflecting the concentration of these chemical elements compared with serum, plasma, and whole blood in pregnant women.[26–29]

In light of these literature gaps, we examined the associations of trace minerals (Mn and Se) and heavy metals (Cd, Pb, and Hg) with preeclampsia in women from the Boston Birth Cohort, using concentrations measured in RBCs obtained shortly after delivery. We hypothesized that higher Mn and Se concentration in RBCs are associated with lower risk of preeclampsia, whereas higher Cd, Pb, and Hg concentration are associated with higher risk of preeclampsia, after appropriate adjustment.

Methods

The data, analytic methods, and study materials that support the findings of this study will be available from Dr Xiaobin Wang (xwang82@jhu.edu) on reasonable request.

Study Population

The Boston Birth Cohort was initially designed as a molecular epidemiology study on environmental and genetic determinants on low birth weight and preterm birth.[30] Pregnant women were recruited at delivery since 1998 at Boston Medical Center on a rolling basis. Multiple-gestation pregnancies, pregnancies resulting from in vitro fertilization, deliveries induced by maternal trauma, or newborns with major birth defects were not included.

After informed consent was obtained, a face-to-face interview using a standardized questionnaire was conducted within 24 to 72 hours after delivery to obtain information including sociodemographic characteristics, smoking status, and alcohol consumption during pregnancy, as well as medical and reproductive history. Electronic medical records were reviewed to obtain clinical information including prenatal care and pregnancy complications. Blood samples were collected from all participants during enrollment.

Of the 1448 participants who had complete information on chemical elements, 174 were excluded from this analysis for the following reasons: 98 had chronic hypertension before pregnancy, 76 had missing covariates included in the statistical model (62 on prepregnancy height and/or weight, 10 on smoking status during pregnancy, 3 on education achievement, and 1 on parity) (Figure S1). After these exclusions, 1274 participants were left for the current analysis. The Institutional Review Board of Boston Medical Center and Johns Hopkins Bloomberg School of Public Health approved this study.

Exposures

The primary exposures for this study were concentrations of Mn, Se, Cd, Pb, and Hg in RBCs. Detailed laboratory methods for measurements had been described previously.[29] Briefly, plasma and RBCs were separated by centrifugation and kept frozen at −80°C in vials that were certified to be free of those elements after collection. They were transported on dry ice to the Public Health and Environmental Laboratories in the Department of Health of the State of New Jersey, USA and

were measured using inductively coupled plasma mass spectrometry. The number and percentage of samples with Mn, Se, Cd, Pb, and Hg concentrations below the limits of detection were 2 (0.2%), 0 (0%), 115 (9.0%), 0 (0%), and 143 (11.2%), respectively. For samples below limits of detection, the concentrations were re-assigned as the limits of detection divided by the square root of 2.[31]

Outcome

In assessing outcome, we extracted physician diagnoses directly from medical records. Preeclampsia was defined based on the criteria at the time of diagnosis as newly diagnosed hypertension (a systolic blood pressure of 140 mm Hg or higher or diastolic blood pressure of 90 mm Hg or higher) and proteinuria (excretion of 300 mg or more in a 24-hour collection, or 0.3 g/g by urine protein: creatinine ratio, or +1 by dipstick if quantitative methods are unavailable) occurring after 20 weeks of gestation.[32] HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count), a subtype of preeclampsia, was also included in the outcome definition. One participant developed eclampsia and was assigned as having the outcome. Those who did not have a medical diagnosis of preeclampsia or HELLP syndrome in their medical records were considered as not having the condition.

Covariates

We extracted age at delivery and parity from electronic medical records. Information on prepregnancy weight and height, self-reported race, education achievement, smoking status, and alcohol consumption during pregnancy were obtained from a questionnaire administered postpartum. Prepregnancy body mass index was calculated as weight in kilograms divided by height in meters squared. Postpartum plasma folate concentrations were measured using chemiluminescent immunoassay with diagnostic kits (Shenzhen New Industries Biomedical Engineering Co., Ltd, Shenzhen, China) and the interassay coefficient of variation was reported to be <4% by previous studies.[33,34]

Statistical Analysis

We described participants' characteristics using percentages for categorical variables, means and standard deviations (SD) for normally distributed variables, and median and interquartile range (IQR) for nonnormally distributed variables. Pearson χ^2, ANOVA, and permutation test were used for comparison of categorical, normally, and nonnormally distributed continuous variables, respectively. We used box and whisker plots to display the median, IQR, and range of each trace mineral and heavy metal, and permutation test to determine differences in their distributions by preeclampsia status. We used Spearman rank-order correlation coefficients to quantify the interelement correlation for each pairwise combination of chemical elements.

We used Poisson regression with robust variance models to examine the prevalence ratio of preeclampsia in relation to concentration of each chemical element. In all models, chemical element concentrations were treated both as categorical variables by quintiles to explore potential nonlinear effects on preeclampsia and continuous variables scaled to 1 SD increment. *P* values for overall trend were obtained by coding concentration categories as ordinal variables in the regression models.[35,36]

To assess confounding, we began with an unadjusted model (model 1) and then added confounders (model 2) defined as covariates known to be associated with both chemical elements and preeclampsia but not in the causal pathway.[25] Confounders included age at delivery (continuous), self-reported race (black, nonblack), education achievement (below high school, high school, college or above), parity (nulliparous, multiparous), prepregnancy body mass index (continuous), and smoking status during pregnancy (never, former, current smoker). In supplemental analyses, we additionally adjusted for postpartum plasma folate concentration, as a surrogate of maternal nutrition, and also adjusted for other trace minerals and metals to further reduce potential bias caused by confounding.

We considered self-reported race, smoking status during pregnancy, and parity as potential effect measure modifiers. We tested effect measure modification on the multiplicative scale by including an interaction term between the exposure and each effect measure modifier in our final multivariable adjusted model. *P* values for interaction were obtained from these models. We also conducted subgroup analyses stratified by each effect measure modifier.

All tests were based on a 2-sided $P<0.05$ as evidence of statistical significance. Data management and analyses were performed using Stata 14.2 (Stata Statistical Software: Release 14, 2015. College Station, TX: StataCorp LP.).

Results

Participant Characteristics

Of the 1274 women included in the analysis, 115 (9.0%) developed preeclampsia. Women with preeclampsia were more likely to have higher prepregnancy body mass index and higher educational achievement (high school or above; Table 1). Babies born to preeclamptic mothers were more likely to have shorter gestation and lower birth weight. The distributions of trace minerals and metals according to

Table 1. Characteristics of Mother–Child Pairs in the Boston Birth Cohort By Preeclampsia Status (n=1274)

Variable, n (%)*	Normal	Preeclampsia	*P* Value
N	1159	115	
Maternal characteristics			
Age at delivery, years, mean (SD)	27.99 (6.31)	29.12 (6.17)	0.07
Black race	671 (57.9%)	68 (59.1%)	0.80
Education level			0.02
Middle school or below	318 (27.4%)	21 (18.3%)	
High school	417 (36.0%)	56 (48.7%)	
College or above	424 (36.6%)	38 (33.0%)	
Nulliparous	526 (45.4%)	60 (52.2%)	0.16
Married	362 (31.2%)	40 (34.8%)	0.58
Smoking status during pregnancy			0.09
Never smoker	929 (80.2%)	99 (86.1%)	
Former smoker	105 (9.1%)	11 (9.6%)	
Current smoker	125 (10.8%)	5 (4.3%)	
Alcohol consumption during pregnancy	108 (9.3%)	7 (6.1%)	0.18
Prepregnancy BMI (kg/m^2), median (IQR)	24.88 (21.64, 29.18)	26.58 (23.46, 31.62)	0.003
Pregestational/gestational diabetes mellitus	123 (10.6%)	18 (15.7%)	0.25
Offspring characteristics			
Boys	599 (51.7%)	49 (42.6%)	0.06
Gestational age (wks), median (IQR)	39.00 (37.29, 40.14)	36.29 (32.57, 38.57)	<0.001
Birth weight (kg), median (IQR)	3.12 (2.67, 3.51)	2.40 (1.50, 3.17)	<0.001

BMI indicates body mass index; IQR, interquartile range; SD, standard deviation.
*Unless otherwise indicated.

preeclampsia status, shown in Figure 1, indicated that preeclamptic participants had statistically significantly lower Mn concentration than normal counterparts. The interelement correlation coefficients were small to modest, ranging from −0.03 to 0.34 (Table S1). Participant characteristics by Mn, Se, Cd, Pb, and Hg quintiles are provided in Tables S2 through S6, respectively.

Mn and Preeclampsia

In Table 2, we show unadjusted and multivariable-adjusted associations of trace minerals and metals with preeclampsia risk. We observed evidence of a dose–response trend across Mn quintiles (*P* for trend<0.001; Figure 2). After multivariable adjustment, the fourth and fifth quintiles of Mn were associated with 0.46 (95% CI, 0.26–0.81) and 0.33 (95% CI, 0.17–0.65) times lower risk of preeclampsia compared with the first quintile, respectively. In linear modeling, each 1 SD (15.52 μg/L) increment of Mn was associated with 0.68 (95% CI, 0.54–0.86) times lower risk of preeclampsia (Table 2).

Cd and Preeclampsia

Higher Cd levels were associated with greater preeclampsia risk, in somewhat of a dose-dependent manner (*P* for trend=0.009) (Figure 2). Compared with the first quintile, the fourth and fifth quintiles were associated with 1.97 (95% CI, 1.08–3.59) and 1.86 (95% CI, 0.98–3.50) times greater risk after adjustment, respectively. In linear modeling, each 1 SD (0.69 μg/L) increment of Cd was associated with 1.15 (95% CI, 0.98–1.36) times higher risk of preeclampsia (Table 2). Estimates for both Mn and Cd were not materially changed after additional adjustment for maternal plasma folate concentration (Table S7) or upon additional adjustment for other trace minerals or metals measured in RBCs (Tables S8 and S9).

Effect Measure Modification

In subgroup analyses, associations of (1-SD increment in) Mn and Cd with preeclampsia were consistent across strata of self-reported race, smoking status during pregnancy, and

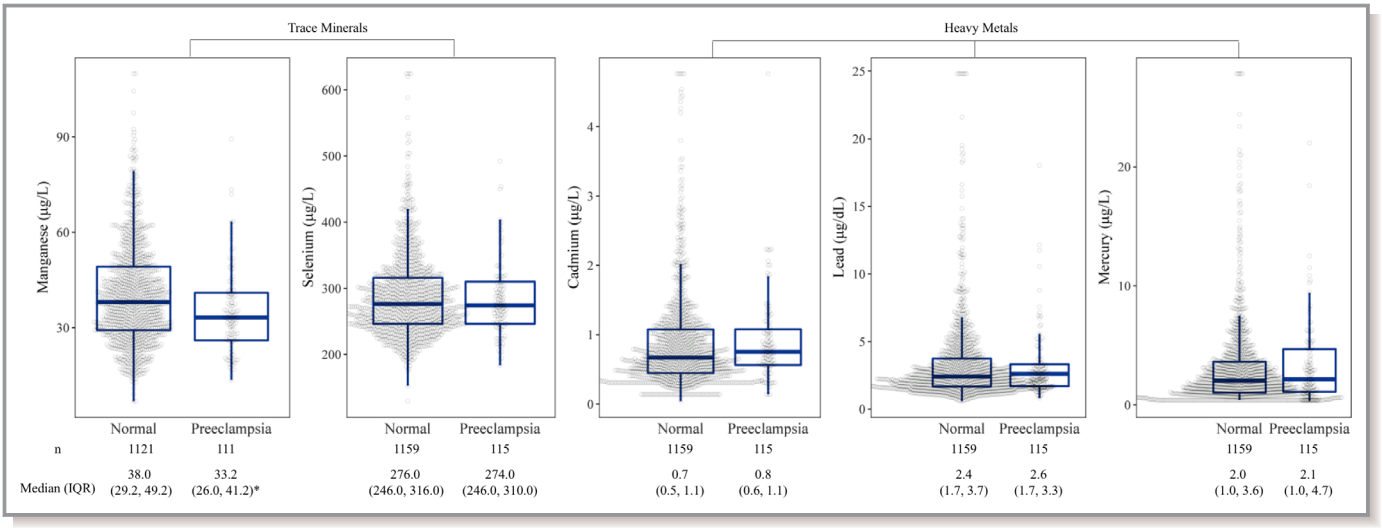

Figure 1. The distributions of trace minerals and heavy metals measured in red blood cells among pregnant women from the Boston Birth Cohort by preeclampsia status. *Statistically significant difference between women diagnosed with preeclampsia versus not ($P<0.001$) based on permutation test.

parity (Figure 3). Furthermore, all *P* values for interaction on the multiplicative scale were not statistically significant, suggesting no evidence of effect measure modification.

Other Trace Minerals, Metals, and Preeclampsia

We did not observe significant associations (linear or nonlinear) of Se, Pb, and Hg with preeclampsia (Table 2). The fifth quintile of Se, Pb, and Hg was associated with a prevalence ratio of 0.93 (95% CI: 0.52–1.66), 0.90 (95% CI: 0.48–1.68), and 1.12 (95% CI: 0.72–2.05) as compared with the first quintile, respectively. There was also no evidence that race, smoking status, or parity modified these associations.

Discussion

In this multiethnic, predominantly urban and low-income cohort of pregnant women with high burden of preeclampsia, higher Mn levels (measured in RBCs) were associated, in a dose–response fashion, with lower preeclampsia risk. Independently, Cd had a positive association with preeclampsia. These associations persisted after controlling for potential confounders and were consistent across levels of race, smoking status during pregnancy, and parity. The other chemical elements measured in our study (Se, Pb, and Hg) were not associated with preeclampsia.

Our findings contribute to an emerging literature base on the association of the essential trace mineral Mn with preeclampsia. Consistent with our findings on Mn, 3 small case–control studies conducted in Saudi Arabia (n=120, 40 cases),[14] Bangladesh (n=108, 50 cases),[15] and South Africa (n=66, 43 cases)[12] all reported significantly lower Mn concentration in serum among women with preeclampsia compared with healthy controls. Furthermore, in a prospective cohort study of 620 pregnant women in Iran, Goodarzi Khoigani et al[16] reported that Mn intake in the third trimester, estimated using 48-hour dietary recalls, was significantly lower among women who developed preeclampsia compared with those who did not. In contrast, in a case–control study of 396 participants (31 preeclampsia cases) in Iran, Vigeh et al[17] reported that Mn concentration in umbilical cord blood, but not maternal blood, was associated with higher odds of preeclampsia after adjustment. However, their study was limited by having few cases of preeclampsia and Mn concentration measured in whole blood, which, unlike in RBCs, is subject to the extent of plasma volume expansion in pregnant women with and without preeclampsia.[26,27]

Our findings on Cd are also largely consistent with previous studies from around the world. A nested case–control study of 172 participants (86 cases) in the United States reported that each 1 ng/g increment in placenta Cd was associated with an odds ratio of 1.50 (95% CI: 1.10–2.20) after adjustment.[37] Similarly, another case–control study of 102 participants (51 cases) in China reported that the odds ratio comparing the third tertile with the first tertile of blood Cd in the third trimester was 7.83 (95% CI: 1.64–37.26) after adjustment.[38] In DR Congo, Elongi Moyene et al[13] also found significantly higher urinary Cd concentration among 88 women with preeclampsia compared with 88 controls that were matched on age, parity, and duration of pregnancy. However, Maduray et al[12] did not detect such association using either serum or hair Cd in a case–control study conducted in 43 preeclamptic and 23 normotensive

Table 2. Prevalence Ratios and 95% CIs for Preeclampsia in Relationship to Concentrations of Trace Minerals and Heavy Metals in RBCs, Before and After Adjustment for Potential Confounders (n=1274)

	n	Cases (%)	Model 1*		Model 2†	
			PR (95% CI)	*P* Value	PR (95% CI)	*P* Value
RBC manganese (μg/L)‡						
Quintile 1 (6.86–27.00)	248	33 (13.3%)	Ref.		Ref.	
Quintile 2 (27.20–33.80)	250	26 (10.4%)	0.78 (0.48–1.27)	0.32	0.75 (0.46–1.23)	0.25
Quintile 3 (34.00–41.20)	246	25 (10.2%)	0.76 (0.47–1.24)	0.28	0.68 (0.41–1.11)	0.12
Quintile 4 (41.40–51.80)	244	16 (6.6%)	0.49 (0.28–0.87)	0.01	0.46 (0.26–0.81)	0.008
Quintile 5 (52.00–109.80)	244	11 (4.5%)	0.34 (0.17–0.65)	0.003	0.33 (0.17–0.65)	0.001
P trend			<0.001		<0.001	
Per 1 SD (15.52) increment	N.A.		0.70 (0.57–0.86)	0.001	0.68 (0.54–0.86)	0.001
RBC selenium (μg/L)						
Quintile 1 (129.22–241.12)	255	22 (8.6%)	Ref.		Ref.	
Quintile 2 (242.00–264.00)	269	23 (8.5%)	0.99 (0.57–1.73)	0.97	0.94 (0.54–1.63)	0.82
Quintile 3 (266.00–290.00)	243	28 (11.5%)	1.33 (0.79–2.27)	0.28	1.31 (0.77–2.22)	0.32
Quintile 4 (291.51–326.00)	257	21 (8.2%)	0.95 (0.53–1.68)	0.85	0.93 (0.51–1.68)	0.80
Quintile 5 (328.00–624.00)	250	21 (8.4%)	0.97 (0.55–1.72)	0.93	0.93 (0.52–1.66)	0.80
P trend			0.89		0.81	
Per 1 SD (61.55) increment	N.A.		0.95 (0.79–1.13)	0.54	0.93 (0.78–1.12)	0.46
RBC cadmium (μg/L)						
Quintile 1 (0.04–0.39)	256	17 (6.6%)	Ref.		Ref.	
Quintile 2 (0.40–0.59)	256	18 (7.0%)	1.06 (0.56–2.01)	0.86	1.09 (0.58–2.06)	0.77
Quintile 3 (0.59–0.80)	253	24 (9.5%)	1.43 (0.79–2.59)	0.24	1.48 (0.81–2.71)	0.21
Quintile 4 (0.80–1.18)	255	31 (12.2%)	1.83 (1.04–3.22)	0.04	1.97 (1.08–3.59)	0.03
Quintile 5 (1.19–4.76)	254	25 (9.8%)	1.48 (0.82–2.68)	0.19	1.86 (0.98–3.50)	0.06
P trend			0.04		0.009	
Per 1 SD (0.69) increment	N.A.		1.03 (0.90–1.19)	0.65	1.15 (0.98–1.36)	0.09
RBC lead (μg/dL)						
Quintile 1 (0.58–1.56)	255	22 (8.6%)	Ref.		Ref.	
Quintile 2 (1.57–2.10)	258	22 (8.5%)	0.99 (0.56–1.74)	0.97	1.05 (0.59–1.85)	0.87
Quintile 3 (2.12–2.80)	254	26 (10.2%)	1.19 (0.69–2.04)	0.53	1.19 (0.68–2.10)	0.54
Quintile 4 (2.81–4.28)	253	23 (9.1%)	1.05 (0.60–1.84)	0.85	1.03 (0.57–1.88)	0.91
Quintile 5 (4.30–24.80)	254	22 (8.7%)	1.00 (0.57–1.77)	0.99	0.90 (0.48–1.68)	0.74
P trend			0.99		0.75	
Per 1 SD (3.07) increment	N.A.		0.95 (0.80–1.13)	0.57	0.91 (0.74–1.11)	0.36
RBC mercury (μg/L)						
Quintile 1 (0.30–0.89)	255	23 (9.0%)	Ref.		Ref.	
Quintile 2 (0.90–1.60)	256	23 (9.0%)	1.00 (0.57–1.73)	0.99	0.94 (0.54–1.61)	0.82
Quintile 3 (1.59–2.58)	254	17 (6.7%)	0.74 (0.41–1.35)	0.33	0.69 (0.38–1.26)	0.23
Quintile 4 (2.60–4.28)	257	21 (8.2%)	0.91 (0.51–1.59)	0.73	0.84 (0.47–1.49)	0.55
Quintile 5 (4.30–27.80)	252	31 (12.3%)	1.36 (0.82–2.27)	0.23	1.21 (0.72–2.05)	0.47
P trend			0.34		0.58	
Per 1 SD (3.60) increment	N.A.		1.06 (0.92–1.22)	0.45	1.03 (0.88–1.20)	0.71

N.A. indicates not applicable; PR, prevalence ratio; RBC, red blood cell; Ref, reference group.

*Model 1 was an unadjusted model.

†Model 2 was adjusted for age at delivery (continuous), self-reported race (black, nonblack), education (below high school, high school, college or above), parity (nulliparous, multiparous), prepregnancy body mass index (continuous), and smoking status during pregnancy (never, former, current).

‡There are 42 missing values for manganese (n=1232).

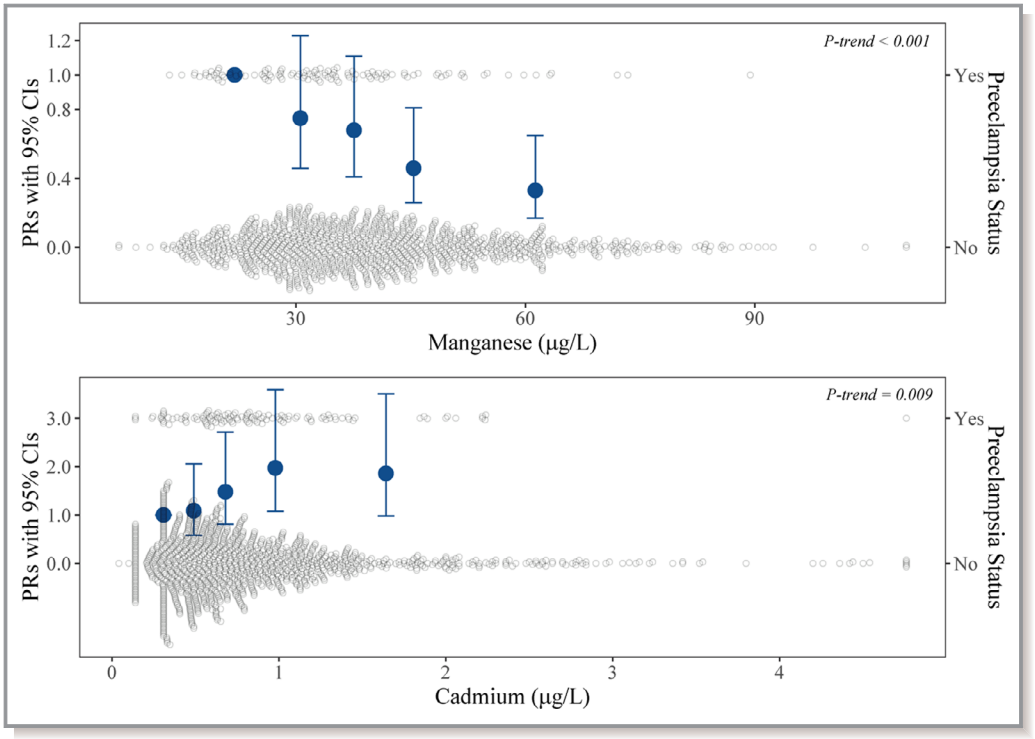

Figure 2. Prevalence ratios (PRs) and 95% CIs of preeclampsia in relationship to quintiles of manganese and cadmium measured in red blood cells. Quintile points were set at the median value of each quintile. Models adjusted for age at delivery, self-reported race, education, parity, prepregnancy body mass index, and smoking status during pregnancy.

pregnant women in South Africa. The null finding may have been because of the small sample size, lack of adjustment for potential confounders, and/or the aforementioned effect of plasma volume expansion on metal concentrations measured in serum.

In our study, we did not find evidence that Pb was associated with preeclampsia. However, Pb has been linked to increased risk of preeclampsia for more than a century, and a recently published systematic review and meta-analysis reported that an increment of 1 μg/dL blood Pb was associated with a 1.6% higher likelihood of preeclampsia.[39] Thus, considering the preponderance of evidence linking Pb and preeclampsia, null findings from our single study should be interpreted cautiously. One reason for the lack of association in our study could be that Pb concentration in our sample was lower than the majority of the studies reviewed. This could be partially because of the substantial decline in blood Pb concentration of the entire US population over the past 40 years,[40,41] whereas that half of the reviewed studies were conducted before year 2000 in low- and middle-income countries.

Our findings also do not support associations of Hg, measured in RBCs, with risk of preeclampsia. A single prospective cohort study of 64 pregnant dental staff and 60 pregnant administrators conducted by El-Badry et al in Egypt reported that the dental staff had significantly higher urinary Hg concentration and higher odds of preeclampsia compared with the administrators.[24] However, this study was small (n=124) and concentrations of Hg in the dental staff may be higher than what pregnant women are typically exposed to.

As for Se, 1 randomized clinical trial conducted in 166 pregnant Iranian women suggested that Se supplementation may be associated with a lower incidence of preeclampsia; however, they only had 3 preeclampsia events in the control arm and 0 in the active treatment arm.[42] In another randomized trial of 230 pregnant women in the United Kingdom, Se supplementation improved a preeclampsia biomarker (serum soluble vascular endothelial growth factor receptor 1), implying that Se may prevent preeclampsia.[19] However, these trials have not been definitive, and thus the null association observed in our study cannot be ruled out. Other explanations for the incongruent findings from ours and prior studies include the different time points studied, differences in the population studied, and differences in the covariate adjustment.

There are several possible biologic mechanisms underlying the opposing associations of Mn and Cd with preeclampsia, some of which may operate through a shared pathway. According to in vivo and animal models,

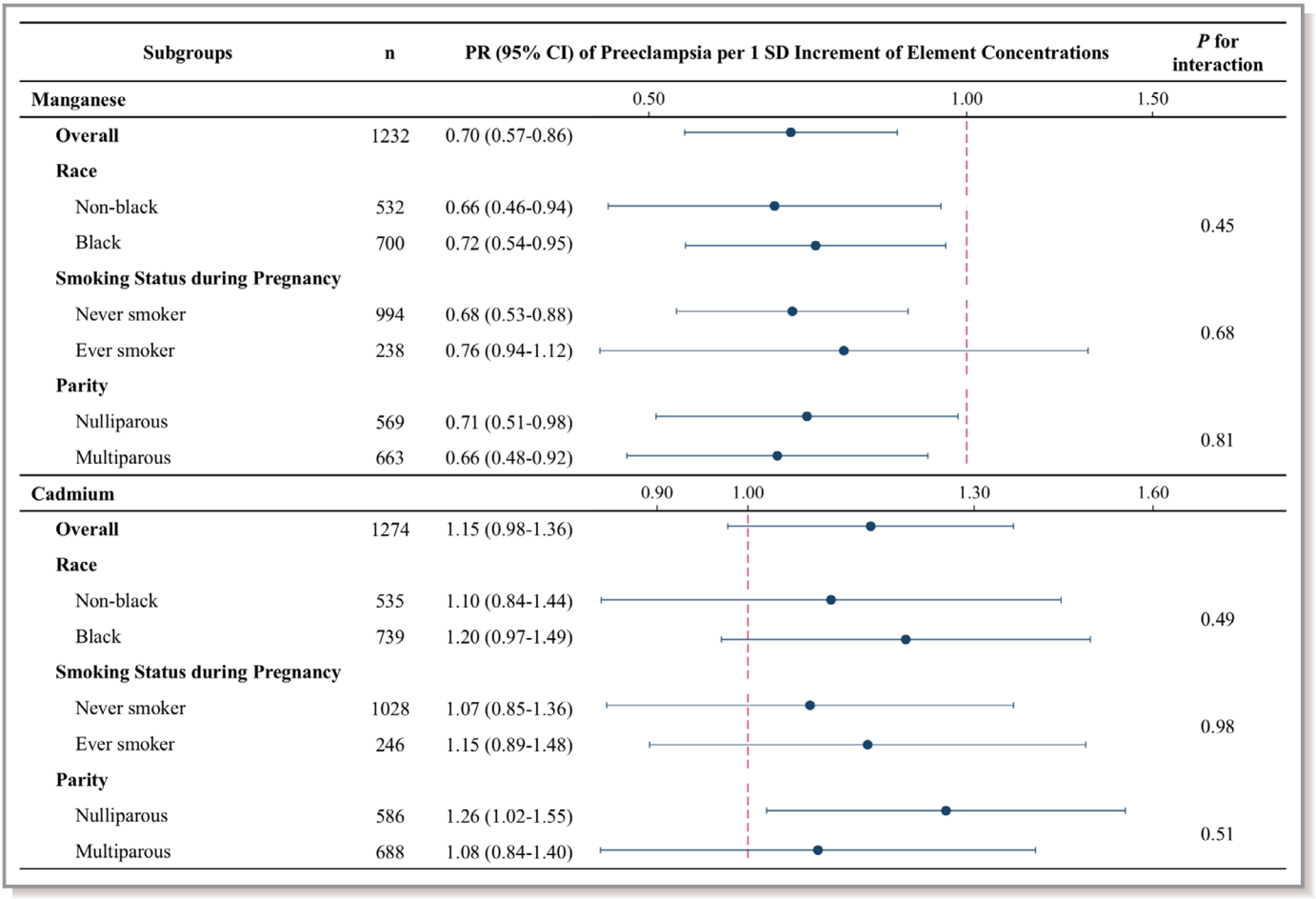

Figure 3. Subgroup analysis of the association of manganese and cadmium in red blood cells with preeclampsia, by potential effect measure modifiers. Model adjusted for age at delivery, self-reported race (when not testing race), education, parity (when not testing parity), prepregnancy body mass index, and smoking status during pregnancy (when not testing smoking status). PR indicates prevalence ratio; SD, standard deviation.

Mn and Cd could modulate reactive oxygen species and oxidative stress, factors on the pathway to preeclampsia.[43,44] Mn is an essential component of manganese superoxide dismutase, which is located within mitochondria and serves as the first line of defense against superoxide and free radical.[9,10] In contrast, Cd may interfere with enzymes of the cellular antioxidant system and induce oxidative stress.[20] Therefore, low concentration of Mn, as well as high concentration of Cd, could cause accumulation of reactive oxygen species and oxidative stress, which may eventually lead to preeclampsia. Other potential mechanisms include endothelial dysfunction[10,21] and immune abnormality.[22] Additionally, Cd concentrates in the kidney and has been shown to induce proteinuria and renal dysfunction from studies both in animal and general population.[45,46]

A major strength of our study is the measurement of trace minerals and heavy metals in RBCs, which has been purported to be a more precise and stable biomarker than other hematological indices, including whole blood, serum, and plasma, in pregnant women.[26–29] Therefore, using RBCs may reduce measurement error. Furthermore, because of the long biological half-lives of those chemical elements in the human body, RBCs are believed to reflect their long-term body burden. Second, the extensive covariate information afforded by the Boston Birth Cohort enabled us to control for a variety of confounders and stratify by potential modifiers in the analysis. Finally, the participant diversity of our cohort improves the external validity of the conclusion.

There are also limitations of the current study that merit mention. First, we only had blood available at delivery, which precluded us from firmly establishing a temporal association between concentrations of trace minerals and metals with preeclampsia. No study has been done to establish the exact exposure period reflected by RBCs by comparing with other widely accepted biomarkers for those chemical elements. Therefore, while we assume that chemical elements measured in our study reflect pregnancy levels before development of preeclampsia, we cannot rule out the possibility that the

concentrations were influenced by preeclampsia. Second, misclassification of preeclampsia could exist, since the diagnosis was extracted directly from electronic medical records. It is reasonable to consider the misclassification as independent and nondifferential, which would bias the observed association towards the null. Third, because of the lack of information on the onset time of preeclampsia in our cohort, we were unable to distinguish between early- and late-onset preeclampsia, which may differ with respect to pathogenesis and clinical managements.[47] Therefore, we cannot specifically discern whether trace minerals and metals examined in our analysis have different associations with early- versus late-onset preeclampsia. Finally, because our study is observational, we cannot rule out the possibility of residual or unmeasured confounding of the reported associations.

Conclusions

Our finding on Mn, an essential trace mineral, provides new insight into a potentially modifiable way to prevent preeclampsia, while the observation of a potentially hazardous effect of Cd on preeclampsia reinforces the recommendations by the American College of Obstetricians and Gynecologists that healthcare professionals provide useful information and necessary interventions for pregnant women to limit exposure to toxic metals.[48] Nevertheless, the associations observed in our study need to be replicated by longitudinal studies with measurements of trace minerals and metals in both RBCs and other biomarkers, as well as their original sources (eg, diet). If replicated, randomized clinical trials are needed to determine whether the associations are causal. Moreover, further research with information on characteristic features of preeclampsia (such as severity of blood pressure and proteinuria) and clinical subtypes of preeclampsia (eg, early-onset versus late-onset) is needed to elucidate the potential heterogeneity of mechanisms that are responsible for the effect of trace minerals and metals, which is in line with recent research recommendations by the National Institutes of Health.[7]

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Disclosures

None.

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SUPPLEMENTAL MATERIAL

Table S1. Spearman rank-order correlation coefficients among trace minerals and heavy metals (n=1274).

Elements	Manganese	Cadmium	Lead	Mercury	Selenium
Manganese*	—				
Cadmium	-0.03	—			
Lead	0.08†	0.34†	—		
Mercury	0.05	0.10†	0.32†	—	
Selenium	0.20†	0.11†	0.25†	0.28†	—

* There are 42 missing values for manganese (n=1232).

† $P<0.05$.

Table S2. Characteristics of participants in the Boston Birth Cohort by RBC manganese level (n=1232).

Variable, n (%)*	RBC Manganese Level (μg/L)					*P* value
	Quintile 1 (6.86-27.00)	Quintile 2 (27.20-33.80)	Quintile 3 (34.00-41.20)	Quintile 4 (41.40-51.80)	Quintile 5 (52.00-109.80)	
n	248	250	246	244	244	
Age at Delivery (years), mean (SD)	29.23 (6.49)	28.13 (6.47)	28.28 (6.35)	27.76 (6.17)	27.21 (5.94)	0.008
Black Race	161 (64.9%)	158 (63.2%)	137 (55.7%)	130 (53.3%)	114 (46.7%)	<0.001
Education Level						
Middle school or below	63 (25.4%)	69 (27.6%)	52 (21.1%)	66 (27.0%)	83 (34.0%)	
High school	95 (38.3%)	87 (34.8%)	94 (38.2%)	93 (38.1%)	82 (33.6%)	0.16
College or above	90 (36.3%)	94 (37.6%)	100 (40.7%)	85 (34.8%)	79 (32.4%)	
Nulliparous	118 (47.6%)	112 (44.8%)	120 (48.8%)	111 (45.5%)	108 (44.3%)	0.83
Married Participants	66 (26.6%)	77 (30.8%)	85 (34.6%)	81 (33.2%)	77 (31.6%)	0.58
Smoking Status During Pregnancy						
Never smoker	169 (68.1%)	199 (79.6%)	206 (83.7%)	213 (87.3%)	207 (84.8%)	<0.001
Former smoker	169 (68.1%)	199 (79.6%)	206 (83.7%)	213 (87.3%)	207 (84.8%)	

Current smoker	53 (21.4%)	25 (10.0%)	18 (7.3%)	13 (5.3%)	17 (7.0%)	
Alcohol Consumption During Pregnancy	31 (12.5%)	28 (11.2%)	16 (6.5%)	17 (7.0%)	19 (7.8%)	0.23
Pre-pregnancy BMI, median (IQR)	25.28 (7.06)	25.03 (8.16)	25.34 (8.39)	24.67 (7.77)	24.73 (6.72)	0.92
Pre-gestational/Gestational Diabetes	35 (14.1%)	27 (10.8%)	29 (11.8%)	24 (9.8%)	25 (10.2%)	0.56
RBC Cadmium (μg/L), median (IQR)	0.68 (0.80)	0.65 (0.53)	0.63 (0.58)	0.68 (0.69)	0.69 (0.54)	0.54
RBC Lead (μg/dL), median (IQR)	2.21 (1.53)	2.29 (1.75)	2.38 (1.87)	2.45 (2.16)	2.48 (2.57)	0.07
RBC Mercury (μg/L), median (IQR)	1.78 (2.66)	2.03 (2.84)	2.07 (2.62)	2.11 (2.46)	2.18 (2.44)	0.44
RBC Selenium (μg/L), median (IQR)	264.00 (52.00)	270.00 (62.00)	276.00 (52.00)	286.00 (74.00)	299.00 (82.00)	<0.001
Preeclampsia	33 (13.3%)	26 (10.4%)	25 (10.2%)	16 (6.6%)	11 (4.5%)	0.006

SD, standard deviation; IQR, interquartile range; BMI, body mass index; RBC, red blood cell.

*Unless otherwise indicated.

Table S3. Characteristics of participants in the Boston Birth Cohort by RBC selenium level (n=1274).

Variable, n (%)*	RBC Selenium Level (μg/L)					*P* value
	Quintile 1 (129.22-241.12)	Quintile 2 (242.00-264.00)	Quintile 3 (266.00-290.00)	Quintile 4 (291.51-326.00)	Quintile 5 (328.00-624.00)	
n	255	269	243	257	250	
Age at Delivery (years), mean (SD)	28.04 (6.07)	28.23 (6.48)	27.80 (6.23)	27.90 (6.33)	28.48 (6.41)	0.77
Black Race	110 (43.1%)	129 (48.0%)	150 (61.7%)	165 (64.2%)	185 (74.0%)	<0.001
Education Level						
Middle school or below	75 (29.4%)	71 (26.4%)	48 (19.8%)	73 (28.4%)	72 (28.8%)	0.33
High school	88 (34.5%)	105 (39.0%)	95 (39.1%)	96 (37.4%)	89 (35.6%)	
College or above	92 (36.1%)	93 (34.6%)	100 (41.2%)	88 (34.2%)	89 (35.6%)	
Nulliparous	113 (44.3%)	129 (48.0%)	119 (49.0%)	110 (42.8%)	115 (46.0%)	0.62
Married Participants	74 (29.0%)	85 (31.6%)	70 (28.8%)	82 (31.9%)	91 (36.4%)	0.27
Smoking Status During Pregnancy						
Never smoker	201 (78.8%)	204 (75.8%)	197 (81.1%)	218 (84.8%)	208 (83.2%)	0.02

Former smoker	22 (8.6%)	27 (10.0%)	18 (7.4%)	20 (7.8%)	29 (11.6%)	
Current smoker	32 (12.5%)	38 (14.1%)	28 (11.5%)	19 (7.4%)	13 (5.2%)	
Alcohol Consumption During Pregnancy	28 (11.0%)	35 (13.0%)	24 (9.9%)	16 (6.2%)	12 (4.8%)	0.04
Pre-pregnancy BMI, median (IQR)	24.46 (8.24)	24.94 (7.55)	24.69 (8.23)	25.00 (6.96)	25.69 (7.28)	0.67
Pre-gestational/Gestational Diabetes	31 (12.2%)	27 (10.0%)	33 (13.6%)	22 (8.6%)	28 (11.2%)	0.45
RBC Manganese† (μg/L), median (IQR)	34.40 (18.10)	34.20 (16.80)	35.40 (16.40)	40.80 (20.20)	42.80 (21.40)	<0.001
RBC Cadmium (μg/L), median (IQR)	0.59 (0.50)	0.67 (0.60)	0.70 (0.61)	0.71 (0.56)	0.81 (0.68)	<0.001
RBC Lead (μg/dL), median (IQR)	2.06 (1.53)	2.12 (1.32)	2.44 (2.07)	2.62 (2.21)	3.00 (2.82)	<0.001
RBC Mercury (μg/L), median (IQR)	1.36 (2.60)	1.61 (2.20)	2.26 (2.65)	2.18 (2.64)	3.10 (3.27)	<0.001
Preeclampsia	22 (8.6%)	23 (8.6%)	28 (11.5%)	21 (8.2%)	21 (8.4%)	0.68

SD, standard deviation; IQR, interquartile range; BMI, body mass index; RBC, red blood cell.

* Unless otherwise indicated.

† There are 42 missing values for manganese (n=1232).

Table S4. Characteristics of participants in the Boston Birth Cohort by RBC cadmium level (n=1274).

Variable, n (%)*	RBC Cadmium Level (μg/L)					*P* value
	Quintile 1 (0.04-0.39)	Quintile 2 (0.40-0.59)	Quintile 3 (0.59-0.80)	Quintile 4 (0.80-1.18)	Quintile 5 (1.19-4.76)	
n	256	256	253	255	254	
Age at Delivery (years), mean (SD)	25.92 (6.16)	26.92 (6.52)	28.45 (5.75)	28.70 (6.20)	30.48 (5.91)	<0.001
Black Race	107 (41.8%)	141 (55.1%)	144 (56.9%)	176 (69.0%)	171 (67.3%)	<0.001
Education Level						
Middle school or below	91 (35.5%)	71 (27.7%)	53 (20.9%)	58 (22.7%)	66 (26.0%)	0.01
High school	83 (32.4%)	86 (33.6%)	101 (39.9%)	99 (38.8%)	104 (40.9%)	
College or above	82 (32.0%)	99 (38.7%)	99 (39.1%)	98 (38.4%)	84 (33.1%)	
Nulliparous	135 (52.7%)	135 (52.7%)	102 (40.3%)	117 (45.9%)	97 (38.2%)	<0.001
Married Participants	58 (22.7%)	76 (29.7%)	97 (38.3%)	97 (38.0%)	74 (29.1%)	0.003
Smoking Status During Pregnancy						
Never smoker	230 (89.8%)	226 (88.3%)	221 (87.4%)	209 (82.0%)	142 (55.9%)	<0.001
Former smoker	25 (9.8%)	21 (8.2%)	20 (7.9%)	22 (8.6%)	28 (11.0%)	

Current smoker	1 (0.4%)	9 (3.5%)	12 (4.7%)	24 (9.4%)	84 (33.1%)	
Alcohol Consumption During Pregnancy	23 (9.0%)	20 (7.8%)	25 (9.9%)	22 (8.6%)	25 (9.8%)	0.82
Pre-pregnancy BMI, median (IQR)	25.01 (8.94)	24.70 (7.52)	25.53 (7.69)	24.88 (6.56)	24.84 (6.90)	0.61
Pre-gestational/Gestational Diabetes	37 (14.5%)	21 (8.2%)	26 (10.3%)	27 (10.6%)	30 (11.8%)	0.30
RBC Manganese† (µg/L), median (IQR)	38.60 (20.40)	37.10 (16.30)	37.80 (21.20)	37.40 (19.60)	36.40 (21.20)	0.43
RBC Lead (µg/dL), median (IQR)	1.79 (1.11)	2.20 (1.54)	2.42 (2.01)	2.92 (2.22)	3.03 (2.78)	<0.001
RBC Mercury (µg/L), median (IQR)	1.60 (2.06)	2.01 (2.55)	2.22 (2.44)	2.38 (3.09)	2.06 (2.69)	<0.001
RBC Selenium (µg/L), median (IQR)	275.00 (68.00)	272.00 (62.00)	270.00 (68.00)	286.00 (70.00)	280.00 (74.00)	<0.001
Preeclampsia	17 (6.6%)	18 (7.0%)	24 (9.5%)	31 (12.2%)	25 (9.8%)	0.18

SD, standard deviation; IQR, interquartile range; BMI, body mass index; RBC, red blood cell.

* Unless otherwise indicated.

† There are 42 missing values for manganese (n=1232).

Table S5. Characteristics of participants in the Boston Birth Cohort by RBC lead level (n=1274).

Variable, n (%)*	RBC Lead Level (μg/dL)					*P* value
	Quintile 1 (0.58-1.56)	Quintile 2 (1.56-2.10)	Quintile 3 (2.12-2.80)	Quintile 4 (2.81-4.28)	Quintile 5 (4.30-24.80)	
n	255	258	254	253	254	
Age at Delivery (years), mean (SD)	25.82 (5.77)	26.18 (5.93)	28.42 (6.25)	29.63 (6.29)	30.44 (5.95)	<0.001
Black Race	101 (39.6%)	133 (51.6%)	141 (55.5%)	171 (67.6%)	193 (76.0%)	<0.001
Education Level						
Middle school or below	101 (39.6%)	133 (51.6%)	141 (55.5%)	171 (67.6%)	193 (76.0%)	
High school	86 (33.7%)	101 (39.1%)	90 (35.4%)	99 (39.1%)	97 (38.2%)	0.79
College or above	96 (37.6%)	86 (33.3%)	93 (36.6%)	89 (35.2%)	98 (38.6%)	
Nulliparous	140 (54.9%)	136 (52.7%)	100 (39.4%)	102 (40.3%)	108 (42.5%)	<0.001
Married Participants	64 (25.1%)	65 (25.2%)	69 (27.2%)	91 (36.0%)	113 (44.5%)	<0.001
Smoking Status During Pregnancy						
Never smoker	204 (80.0%)	195 (75.6%)	190 (74.8%)	200 (79.1%)	239 (94.1%)	<0.001
Former smoker	33 (12.9%)	34 (13.2%)	29 (11.4%)	16 (6.3%)	4 (1.6%)	

Current smoker	18 (7.1%)	29 (11.2%)	35 (13.8%)	37 (14.6%)	11 (4.3%)	
Alcohol Consumption During Pregnancy	29 (11.4%)	25 (9.7%)	24 (9.4%)	25 (9.9%)	12 (4.7%)	0.28
Pre-pregnancy BMI, median (IQR)	24.81 (9.03)	24.34 (6.71)	25.20 (7.14)	25.00 (7.06)	25.65 (6.56)	0.36
Pre-gestational/Gestational Diabetes	30 (11.8%)	15 (5.8%)	31 (12.2%)	33 (13.0%)	32 (12.6%)	0.10
RBC Manganese† (µg/L), median (IQR)	36.00 (19.00)	37.60 (19.80)	34.30 (15.60)	39.10 (20.10)	40.40 (21.00)	0.002
RBC Cadmium (µg/L), median (IQR)	0.49 (0.41)	0.61 (0.42)	0.73 (0.66)	0.82 (0.57)	0.95 (0.74)	<0.001
RBC Mercury (µg/L), median (IQR)	1.22 (1.78)	1.74 (1.91)	2.19 (3.05)	2.44 (2.93)	3.07 (3.55)	<0.001
RBC Selenium (µg/L), median (IQR)	260.00 (60.00)	266.00 (54.00)	278.00 (60.00)	284.00 (68.00)	300.00 (88.00)	<0.001
Preeclampsia	22 (8.6%)	22 (8.5%)	26 (10.2%)	23 (9.1%)	22 (8.7%)	0.96

SD, standard deviation; IQR, interquartile range; BMI, body mass index; RBC, red blood cell.

* Unless otherwise indicated.

† There are 42 missing values for manganese (n=1232).

Table S6. Characteristics of participants in the Boston Birth Cohort by RBC mercury level (n=1274).

Variable, n (%)*	RBC Mercury Level (μg/L)					*P* value
	Quintile 1 (0.30-0.89)	Quintile 2 (0.90-1.60)	Quintile 3 (1.59-2.58)	Quintile 4 (2.60-4.28)	Quintile 5 (4.30-27.80)	
n	255	256	254	257	252	
Age at Delivery (years), mean (SD)	26.40 (6.39)	27.45 (6.00)	27.42 (6.03)	29.29 (6.35)	29.91 (6.11)	<0.001
Black Race	122 (47.8%)	146 (57.0%)	154 (60.6%)	175 (68.1%)	142 (56.3%)	<0.001
Education Level						
Middle school or below	73 (28.6%)	64 (25.0%)	69 (27.2%)	65 (25.3%)	68 (27.0%)	0.67
High school	90 (35.3%)	103 (40.2%)	101 (39.8%)	86 (33.5%)	93 (36.9%)	
College or above	92 (36.1%)	89 (34.8%)	84 (33.1%)	106 (41.2%)	91 (36.1%)	
Nulliparous	135 (52.9%)	120 (46.9%)	116 (45.7%)	112 (43.6%)	103 (40.9%)	0.08
Married Participants	67 (26.3%)	69 (27.0%)	81 (31.9%)	86 (33.5%)	99 (39.3%)	0.02
Smoking Status During Pregnancy						
Never smoker	193 (75.7%)	191 (74.6%)	196 (77.2%)	222 (86.4%)	226 (89.7%)	<0.001
Former smoker	28 (11.0%)	28 (10.9%)	30 (11.8%)	17 (6.6%)	13 (5.2%)	

Current smoker	28 (11.0%)	28 (10.9%)	30 (11.8%)	17 (6.6%)	13 (5.2%)	
Alcohol Consumption During Pregnancy	25 (9.8%)	26 (10.2%)	22 (8.7%)	26 (10.1%)	16 (6.3%)	0.58
Pre-pregnancy BMI, median (IQR)	24.37 (7.65)	24.87 (7.98)	24.91 (7.87)	25.34 (7.63)	25.41 (6.65)	0.30
Pre-gestational/Gestational Diabetes	28 (11.0%)	25 (9.8%)	30 (11.8%)	21 (8.2%)	37 (14.7%)	0.25
RBC Manganese† (μg/L), median (IQR)	36.80 (20.00)	35.40 (17.80)	40.80 (20.40)	38.80 (19.40)	36.20 (19.40)	0.01
RBC Cadmium (μg/L), median (IQR)	0.58 (0.68)	0.64 (0.71)	0.68 (0.55)	0.72 (0.62)	0.77 (0.65)	0.02
RBC Lead (μg/dL), median (IQR)	1.90 (1.25)	2.14 (1.57)	2.24 (1.75)	2.82 (2.67)	2.98 (2.75)	<0.001
RBC Selenium (μg/L), median (IQR)	258.00 (56.00)	266.00 (56.00)	280.00 (74.00)	290.00 (74.00)	294.00 (78.00)	<0.001
Preeclampsia	23 (9.0%)	23 (9.0%)	17 (6.7%)	21 (8.2%)	31 (12.3%)	0.27

SD, standard deviation; IQR, interquartile range; BMI, body mass index; RBC, red blood cell.

* Unless otherwise indicated.

† There are 42 missing values for manganese (n=1232).

Table S7. Prevalence ratios and 95% confidence intervals for preeclampsia in relationship to manganese and cadmium in RBCs, before and after adjustment for folate.

	Model 2*		Model 3†	
	PR (95% CI)	*P* value	PR (95% CI)	*P* value
RBC manganese (μg/L)				
Per 1 SD (15.52) increment	0.68 (0.54-0.86)	0.001	0.61 (0.48-0.77)	<0.001
Quintile 1 (6.86-27.00)	Ref.		Ref.	
Quintile 2 (27.20-33.80)	0.75 (0.46-1.23)	0.25	0.87 (0.51-1.49)	0.62
Quintile 3 (34.00-41.20)	0.68 (0.41-1.11)	0.12	0.65 (0.38-1.11)	0.11
Quintile 4 (41.40-51.80)	0.46 (0.26-0.81)	0.008	0.44 (0.24-0.83)	0.01
Quintile 5 (52.00-109.80)	0.33 (0.17-0.65)	0.001	0.22 (0.09-0.54)	0.001
P-trend	<0.001		<0.001	
RBC cadmium (μg/L)				
Per 1 SD (0.69) increment	1.15 (0.98-1.36)	0.09	1.13 (0.93-1.37)	0.20
Quintile 1 (0.04-0.39)	Ref.		Ref.	
Quintile 2 (0.40-0.59)	1.09 (0.58-2.06)	0.77	1.49 (0.69-3.24)	0.31
Quintile 3 (0.59-0.80)	1.48 (0.81-2.71)	0.21	1.79 (0.84-3.81)	0.13
Quintile 4 (0.80-1.18)	1.97 (1.08-3.59)	0.03	2.14 (0.99-4.63)	0.05
Quintile 5 (1.19-4.76)	1.86 (0.98-3.50)	0.06	2.13 (0.98-4.61)	0.06
P-trend	0.009		0.03	

PR, prevalence ratio; CI, confidence interval; SD, standard deviation; RBC, red blood cell; Ref, reference group.

* Model 2 was adjusted for age at delivery (continuous), self-reported race (black, non-black), education (below high school, high school, college or above), parity (nulliparous, multiparous), pre-pregnancy BMI (continuous), and smoking status during pregnancy (never, former, current). (n=1232 for manganese, n=1274 for cadmium)

† Model 3 was further adjusted for folate (continuous), in addition to model 2. (n=1042 for manganese, n=1082 for cadmium)

Table S8. Prevalence ratios and 95% confidence intervals for preeclampsia in relationship to manganese in RBCs, before and after adjustment for other trace minerals and heavy metals (n=1232).

	Model 2*		Model 4†		Model 5‡	
	PR (95% CI)	*P* value	PR (95% CI)	*P* value	PR (95% CI)	*P* value
RBC manganese (μg/L)						
Per 1 SD (15.52) increment	0.68 (0.54-0.86)	0.001	0.67 (0.53-0.85)	0.001	0.68 (0.54-0.87)	0.002
Quintile 1 (6.86-27.00)	Ref.		Ref.		Ref.	
Quintile 2 (27.20-33.80)	0.75 (0.46-1.23)	0.25	0.74 (0.46-1.23)	0.25	0.76 (0.46-1.24)	0.27
Quintile 3 (34.00-41.20)	0.68 (0.41-1.11)	0.12	0.66 (0.40-1.09)	0.10	0.68 (0.41-1.11)	0.13
Quintile 4 (41.40-51.80)	0.46 (0.26-0.81)	0.008	0.45 (0.25-0.79)	0.006	0.46 (0.26-0.82)	0.008
Quintile 5 (52.00-109.80)	0.33 (0.17-0.65)	0.001	0.32 (0.16-0.64)	0.001	0.34 (0.17-0.68)	0.002
P-trend	<0.001		<0.001		<0.001	

PR, prevalence ratio; CI, confidence interval; SD, standard deviation; RBC, red blood cell; Ref, reference group.

* Model 2 was adjusted for age at delivery (continuous), self-reported race (black, non-black), education (below high school, high school, college or above), parity (nulliparous, multiparous), pre-pregnancy BMI (continuous), and smoking status during pregnancy (never, former, current). † Model 4 was further adjusted for cadmium (continuous), in addition to model 2. ‡ Model 5 was further adjusted for lead (continuous) and selenium (continuous), in addition to model 2, because they were correlated with manganese according to Spearman rank-order correlation coefficients.

Table S9. Prevalence ratios and 95% confidence intervals for preeclampsia in relationship to cadmium in RBCs, before and after adjustment for other trace minerals and heavy metals (n=1274).

	Model 2*		Model 4†		Model 5‡	
	PR (95% CI)	*P* value	PR (95% CI)	*P* value	PR (95% CI)	*P* value
RBC cadmium (μg/L)						
Per 1 SD (0.69) increment	1.15 (0.98-1.36)	0.09	1.15 (0.97-1.37)	0.10	1.19 (1.01-1.39)	0.04
Quintile 1 (0.04-0.39)	Ref.		Ref.		Ref.	
Quintile 2 (0.40-0.59)	1.09 (0.58-2.06)	0.77	1.19 (0.62-2.27)	0.59	1.11 (0.59-2.08)	0.76
Quintile 3 (0.59-0.80)	1.48 (0.81-2.71)	0.21	1.65 (0.89-3.07)	0.11	1.53 (0.83-2.82)	0.17
Quintile 4 (0.80-1.18)	1.98 (1.08-3.59)	0.03	2.24 (1.20-4.19)	0.01	2.10 (1.14-3.84)	0.02
Quintile 5 (1.19-4.76)	1.86 (0.98-3.50)	0.06	2.06 (1.07-3.99)	0.03	2.06 (1.08-3.90)	0.03
P-trend	0.009		0.003		0.003	

PR, prevalence ratio; CI, confidence interval; SD, standard deviation; RBC, red blood cell; Ref, reference group.

* Model 2 was adjusted for age at delivery (continuous), self-reported race (black, non-black), education (below high school, high school, college or above), parity (nulliparous, multiparous), pre-pregnancy BMI (continuous), and smoking status during pregnancy (never, former, current). † Model 4 was further adjusted for manganese (continuous), in addition to model 2. (n=1232). ‡ Model 5 was further adjusted for lead (continuous), mercury (continuous), and selenium (continuous), in addition to model 2, because they were correlated with cadmium according to Spearman rank-order correlation coefficients.

Figure S1. Flow chart of sample selection for the current analysis.