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Individual, Independent, and Joint Associations of Toxic Metals and Manganese on Hypertensive Disorders of Pregnancy: Results from the MIREC Canadian Pregnancy Cohort

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Background: Toxic metals, such as lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg), may be associated with a higher risk of gestational hypertension and preeclampsia, whereas manganese (Mn) is an essential metal that may be protective.

Objectives: We estimated the individual, independent, and joint associations of Pb, Cd, As, Hg, and Mn on the risk of developing gestational hypertension and preeclampsia in a cohort of Canadian women.

Methods: Metal concentrations were analyzed in first and third trimester maternal blood ($n = 1{,}560$). We measured blood pressure after 20 wk gestation to diagnose gestational hypertension, whereas proteinuria and other complications defined preeclampsia. We estimated individual and independent (adjusted for coexposure) relative risks (RRs) for each doubling of metal concentrations and examined interactions between toxic metals and Mn. We used quantile g-computation to estimate the joint effect of trimester-specific exposures.

Results: Each doubling of third trimester Pb (RR = 1.54; 95% CI: 1.06, 2.22) and first trimester blood As (RR = 1.25; 95% CI: 1.01, 1.58) was independently associated with a higher risk of developing preeclampsia. First trimester blood As (RR = 3.40; 95% CI: 1.40, 8.28) and Mn (RR = 0.63; 95% CI: 0.42, 0.94) concentrations were associated with a higher and lower risk, respectively, of developing gestational hypertension. Mn modified the association with As such that the deleterious association with As was stronger at lower concentrations of Mn. First trimester urinary dimethylarsinic acid concentrations were not associated with gestational hypertension (RR = 1.31; 95% CI: 0.60, 2.85) or preeclampsia (RR = 0.92; 95% CI: 0.68, 1.24). We did not observe overall joint effects for blood metals.

Discussion: Our results confirm that even low blood Pb concentrations are a risk factor for preeclampsia. Women with higher blood As concentrations combined with lower Mn in early pregnancy were more likely to develop gestational hypertension. These pregnancy complications impact maternal and neonatal health. Understanding the contribution of toxic metals and Mn is of public health importance. https://doi.org/10.1289/EHP10825

Introduction

Gestational hypertension and preeclampsia are major contributors to maternal[1,2] and newborn[3,4] morbidity and mortality. Women with these conditions have an increased risk of developing hypertension and other cardiovascular diseases later in life.[5–7] Based on recent reviews, toxic metals such as lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) are associated with a higher risk of developing these conditions.[8–10] However, these reviews have also noted that this evidence is derived from small samples using nonlongitudinal study designs with a single exposure biomarker and at higher levels of exposure than most pregnant women in developed countries experience today.[8,10–12] Further studies are needed to address these limitations.

Oxidative stress is one of several mechanisms through which Pb, Cd, As, and Hg may operate,[13,14] either by generating reactive oxygen species[15,16] or by interfering with antioxidant enzymes, such as superoxide dismutase (SOD).[17–21] In contrast, manganese (Mn), an essential metal necessary for optimal fetal and placental development,[22] may reduce the risk of preeclampsia[23,24] through its antioxidant capacity as a constituent of the enzyme SOD (i.e., MnSOD).[25,26] Reduced concentrations of SOD have been observed in both the plasma[27] and placentae[27,28] of women with preeclampsia vs. women with otherwise healthy pregnancies. Epidemiological[29,30] and experimental[31,32] evidence supports the potential for interaction between toxic metals and Mn. However, evidence for interactions with perinatal outcomes is mixed.[33–36] Further knowledge of potential metal interactions may be beneficial for elucidating mechanisms of action and developing public health interventions.[37]

In addition, studies are needed to estimate the health effects of chemical mixtures,[38] especially for chemicals with suspected common or opposing mechanisms of action. As noted in a 2022 review,[39] two studies have examined associations between mixtures of toxic metals and preeclampsia. In a case–control study of pregnant women in China using weighted-quantile sum regression, Wang et al. reported that each tertile sum increase in blood metal concentrations was associated with a higher risk of preeclampsia[40]; the largest weights were for chromium, Hg, Pb, and As. Using data from a nested case–control study, Bommarito et al. used principal components analysis to examine associations between late-pregnancy urinary concentrations of metals and preeclampsia.[41] None of the metals were associated with preeclampsia individually, but the authors found that the mixture of Cd, Mn, and Pb was positively associated with preeclampsia among individuals with low levels of essential metals (copper, selenium,

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and zinc). Although not specific to gestational hypertension, Yim et al.[39] noted in their review that several papers have identified associations between mixtures of toxic metals and hypertension in nonpregnant adults.

Our primary objective was to estimate individual, independent, and joint associations for blood Pb, Cd, As, Hg, and Mn in both early and late pregnancy with the risk of developing gestational hypertension or preeclampsia in a large prospective pregnancy cohort. Examining and comparing the individual, independent, and joint associations will improve our ability to disentangle the potential health effects of these metals and reduce the potential for coexposure confounding.[42] To expand on our primary analysis of blood As, we also examined associations with methylated As metabolites and organic As species measured in urine. Fetal sex differences have been identified in studies examining prenatal exposure to toxic metals and pregnancy/birth outcomes.[33,43–51] Placental antioxidant defense mechanisms, implicated in the development of hypertensive disorders of pregnancy,[27,28] may be reduced for male fetuses.[52] Therefore, we explored the potential for effect modification by fetal sex. Finally, in a sensitivity analysis, we estimated associations between changes in these metals and measured blood pressure from the first to third trimesters.

Methods

Study Design and Participants

We analyzed data from the Maternal–Infant Research on Environmental Chemicals (MIREC) study, a prospective Canadian pregnancy cohort. Detailed information on study design, recruitment, and inclusion/exclusion criteria have been published.[53] We recruited 2001 pregnant women from 10 sites across Canada during the first trimester between 2008 and 2011. Women were excluded if they were <18 years of age, at >14 wk gestation, reported illicit drug use, or could not communicate in either English or French. Women were also excluded if their fetus had known fetal abnormalities or fetal chromosomal malformations or if the woman currently had any of the following conditions: molar pregnancy, threatened spontaneous abortion (women with previous bleeding in the first trimester were included if the site documented a viable fetus at the time of recruitment), renal disease with altered renal function, epilepsy, any collagen disease (e.g., lupus erythematosus, scleroderma), active and chronic liver disease (hepatitis), heart disease, serious pulmonary disease, cancer, or hematological disorder (women with anemia or thombophilias were included).

Women who withdrew from the study ($n=18$), experienced a miscarriage or stillbirth ($n=74$), were carrying multiple fetuses ($n=48$), or with chronic hypertension ($n=57$) were excluded from this analysis, leaving an eligible sample size of 1,804 participants. In addition, participants who did not provide both first and third trimester blood samples ($n=244$, including 14 participants with gestational hypertension and 8 participants with preeclampsia) were excluded to facilitate comparisons across trimesters, resulting in a final analytical sample of 1,560 participants with complete outcome data. This study was approved by the research ethics boards of Health Canada/Public Health Agency of Canada and Sainte-Justine University Hospital, as well as those of all MIREC-affiliated study sites. Informed consent was obtained from all participants.

Measurement of Toxic Metals and Mn

We collected maternal whole blood samples during first (6–13 wk gestation) and third (27–40 wk gestation) trimester clinic visits, as well as a urine sample during the first trimester clinic visit. The Laboratoire de Toxicologie, Institut National de Santé Publique du Québec (Quebec City, Quebec, Canada), which has ISO/CEI 17025 accreditation of the Standards Council of Canada for human toxicology analysis, performed the metal analyses. The accuracy and precision of the analyses are evaluated on a regular basis through the laboratory's participation in external quality assessment programs. Internal quality assurance was ensured by running validated reference materials after calibration after every 10th sample and at the end of each analytical sequence. Whole blood was analyzed using inductively coupled plasma mass spectrometry (ICP-MS; PerkinElmer ELAN ICP-MS DRC II, Norwalk CT, USA), as previously described.[53–55] Limits of detection (LODs) and the percentages >LOD are provided in Table S1.

Urine samples were analyzed for the As species arsenate, arsenite, dimethylarsinic acid (DMA), monomethylarsonic acid (MMA), and arsenobetaine by high-performance liquid chromatography coupled with ICP-MS (Varian Inc., Palo Alto CA, USA), as previously described.[54,56] The LOD for all urinary As species was 0.75 μg As/L. DMA and arsenobetaine were detectable in 1,337 (86%) and 733 (50%) samples (Table S1), respectively, and were included in the present analysis. Arsenobetaine was dichotomized as above or below the LOD because 787 (50.4%) samples had undetectable concentrations. Arsenate, arsenite, and MMA were detected in few samples (26–249 samples, or 1.7%–16.0%; Table S1), so these data were not considered further. In descriptive analyses, arsenobetaine and DMA were standardized by specific gravity (SG) and measured in thawed urine samples by refractometry (Atago U.S.A. Inc., Bellevue WA) using the following formula[57]: $P_c = P_i[(SG_m - 1)/(SG_i - 1)]$, where P_c is the SG-adjusted metabolite concentration, P_i is the observed metabolite concentration, SG_i is the SG of the ith urine sample, and SG_m is the median SG for the MIREC cohort. Arsenobetaine and DMA were not standardized for SG in regression models; instead SG included as a covariate in these models.

Definitions of Gestational Hypertension and Preeclampsia

Systolic and diastolic blood pressure (SBP and DBP, respectively) were assessed by clinical staff using a sphygmomanometer at three prenatal clinic visits (at 6–13, 14–26, and 27–40 wk gestation). SBP and DBP were measured twice, about 1 min apart, and were averaged. Using the Society of Obstetricians and Gynaecologists of Canada guidelines,[58] women were considered as having gestational hypertension if SBP was ≥140 mmHg or DBP was ≥90 mmHg at a gestational age of 20 wk or later. Women with preexisting (i.e., chronic) hypertension, diagnosed if hypertension was present at a gestational age of <20 wk, were excluded from this analysis even if they developed superimposed preeclampsia. Preeclampsia was defined as the presence of gestational hypertension with the addition of either *a*) proteinuria (defined as protein dipstick test ≥1+ or proteinuria in urine ≥300 mg/24 h or ≥0.3 g/L) or *b*) related maternal "adverse conditions," including abdominal right upper quadrant pain, headaches, visual disturbances, shortness of breath with SpO2 <97%, elevated creatinine, or "adverse complications," such as abruptio placenta, disseminated intravascular coagulation, pulmonary edema, convulsions/eclampsia, stroke or coma, blood transfusion, elevated liver enzyme levels, and/or platelet count $<50 \times 10^9$/L. This information was collected by clinical staff at the study visits or was abstracted from medical charts following delivery. Participants who did not meet any of these conditions were considered normotensive. Gestational age in weeks was based on last menstrual period and ultrasound dating. Last menstrual period was the preferred method. If these two methods differed by >7 d, gestational age was determined using ultrasound owing to concerns over recall and reliability of the last menstrual period estimate. Participants were categorized into three outcome groups for

the present analysis: normotensive, gestational hypertension (without preeclampsia), and preeclampsia.

Measurement of Fine Particulate Matter

Fine particulate matter [PM $\leq$2.5 μm in aerodynamic diameter ($PM_{2.5}$)] was considered in our analyses as a confounder of the associations between metals and hypertensive disorders of pregnancy. $PM_{2.5}$ is a complex mixture of airborne particles, including toxic metals,[59] and has been associated with the development of gestational hypertension and preeclampsia.[60,61] Coexposure to components of $PM_{2.5}$ other than metals may confound associations between toxic metals and gestational hypertension or preeclampsia. Air pollution exposures were assigned to each participant based on the first three digits of their postal code (Forward Sortation Area) at delivery. As previously described,[62] these estimates were produced by combining satellite-based retrievals of aerosol optical depth with chemical transport model output to develop estimates of near-surface $PM_{2.5}$ concentrations. These estimates were further refined using geographically weighted regression to produce 1-km annual concentrations of $PM_{2.5}$. Temporal resolution was added to the surface data by using daily values from National Air Pollution Surveillance monitoring stations located within a 30-km centroid of the participants' Forward Sortation Area.[63]

Covariates

We identified covariates *a priori* based on previous assessments of risk factors for gestational hypertension and preeclampsia among Canadian women[64]; previously identified predictors of blood and urine concentrations of Pb, Cd, As, Hg, and Mn among pregnant women[54,55]; as well as previous reports of associations between these metals and gestational hypertension/preeclampsia.[8,9,24] We used a directed acyclic graph to identify the minimally sufficient set of confounders[65] (Figure S1). Participants completed in-person questionnaires during each trimester to gather sociodemographic and lifestyle information. We abstracted clinical data from medical charts.

Covariates included maternal age at delivery (in years, continuous), parity (nulliparous vs. multiparous), level of education [university (i.e., bachelor's degree), college (i.e., 2-y diploma, less than college)], maternal smoking status at the first and third trimester (never, quit before pregnancy, quit during pregnancy, currently smoking), country of birth (Canada vs. elsewhere), prepregnancy body mass index [BMI; determined as weight in kilograms divided by height in meters (squared), continuous], self-reported race and ethnicity (White vs. other), and trimester-specific estimates of exposure to $PM_{2.5}$. Participants self-reported their race and ethnicity from the following options: White, Chinese, South Asian, Black, Filipino, Southeast Asian, Latin American, Arab, West Asian, Japanese, Korean, and Aboriginal. For the purpose of this analysis, participants who exclusively identified their race or ethnicity as White were coded as being White, whereas participants selecting any other category or combination of categories were coded as other. Disaggregated data on race and ethnicity are presented descriptively in Table S2 by collapsing the categories above into those participants who exclusively reported their race or ethnicity as White, Black, Indigenous, Chinese, or Latin American, as well as other, including those response options with <20 respondents as well as anyone selecting multiple response options.

In analyses for blood Hg, we also adjusted for the potential confounding effect of fish consumption. Fish is a common source of Hg exposure as well as a primary source of omega-3 fatty acids, protein, vitamin D, and other essential minerals important for optimal fetal development and maternal health.[66] During the first and third trimester, women completed a food frequency questionnaire indicating daily, weekly, or monthly consumption of 25 species of fish. We summarized these data according to the frequency (i.e., weekly vs. monthly consumption) of consuming all fish species vs. only those species with higher levels of Hg (i.e., tuna, marlin, orange roughy, shark, swordfish, mackerel, and escolar[67,68]). We adjusted for the reported consumption of fish species high in Hg on an ordinal scale ranging from none to $\geq$6 times per month.

Statistical Analysis

Descriptive statistics. We performed statistical analyses using SAS Enterprise Guide (version 7.1; SAS institute, Cary, NC) and R (version 3.6.2; R Development Core Team). Participant characteristics and metal concentrations are presented using frequencies or median and interquartile range (IQR), as appropriate, by study outcome group. Relationships among metals within and between trimesters were assessed using Spearman and intra-class correlations (ICCs), respectively.

Individual associations. We derived relative risks (RRs) and 95% confidence intervals (95% CIs) using Poisson regression with robust variance estimation[69] for the association between metals and the development of gestational hypertension or preeclampsia (in separate models). Analyses were adjusted for the aforementioned covariates as well as for reported consumption of fish high in Hg in the analyses of Hg and As. For models of urinary As species, we adjusted for the aforementioned covariates (including fish consumption) as well as for urinary SG. Given the lognormal distribution of the observed data and the high frequency of detection, for blood metals and urinary DMA concentrations below the LOD, we assigned a value of the LOD divided by the square root of 2.[70,71] Metal concentrations were $\log_2$ transformed to normalize the distribution and to allow for interpretation of parameter estimates as per a doubling (i.e., 2-fold increase) of concentration. Missing covariate data were imputed using multiple imputation with the fully conditional specification method using a logistic model for categorical variables and a linear model for continuous variables (5 imputations),[72,73] and iteration-specific parameter estimates were combined to provide appropriate variance estimation.

We examined the potential for interactions between toxic metals and Mn on the multiplicative scale by using product terms. We visualized statistically significant continuous interactions using predicted probabilities from a multivariable binomial model. We also calculated the relative excess risk due to interaction (RERI)[74,75] to assess additive interactions for each 2-fold increase in toxic metal concentrations and each 2-fold decrease in Mn concentration.[76] A RERI >0 indicates the presence of an additive interaction between a toxic metal and Mn.

For As and Hg models, we explored adjusting these models for reported consumption of 25 species of fish rather than only species of fish high in Hg. In analyses for blood Cd, we explored adjusting for trimester-specific concentrations of plasma cotinine,[77] the predominant metabolite of nicotine, instead of, and along with, self-reported smoking status. Higher levels of plasma cotinine reflect exposure from not only primary tobacco smoke but also from secondhand exposure. Finally, we explored restricting the model with first trimester blood As to those with arsenobetaine concentrations <1 μg/L ($n = 812$ normotensive, $n = 64$ with gestational hypertension, and $n = 23$ with preeclampsia), as well as separately adjusting for first trimester urinary concentrations of arsenobetaine to control for organic As species and metabolites that originate from fish rather than from inorganic As.[78–80]

To evaluate the dose–response relationship, these exposures were also analyzed according to tertiles. The adverse effects of Mn tend to be observed at extreme high and low levels of exposure.[25] Therefore, in a separate sensitivity analysis, we categorized

participants into three groups and estimated the effect of having low (<10th percentile) or high (>90th percentile) Mn concentrations relative to the middle group (10th–90th percentiles). A previous analysis of participants in the MIREC study using this approach reported that Mn concentrations <10th percentile were associated with low birth weight.[81]

Infant sex, determined by chart review, was explored as an effect modifier. Although parity is another important effect modifier, it was not considered in this paper because only 10 multiparous women developed preeclampsia.

Independent and joint associations. To account for potential confounding by coexposure to multiple toxic metals, we examined the independent associations with each blood metal while adjusting for other trimester-specific blood metals. To estimate the joint associations for exposure to these metals, we used quantile g-computation, a generalized linear model based implementation of g-computation.[82] This method estimates the parameters of a marginal structural model that characterizes the change in the expected potential outcome for a simultaneous, 1-quartile increase in all of the exposures in the specified mixture. This approach provides an estimate of the overall joint effect along with weights that can be interpreted as adjusted, independent effect sizes for quantized exposures. When the weights are in the same direction, they will sum to one and can be directly compared relatively to one another. If the weights are in different directions, they represent partial negative and positive effects, which cannot be directly compared and will not sum to one. Log_2-transformed metal concentrations were rescaled with a mean of zero and standard deviation (SD) of one prior to being included in the model. We ran the quantile g-computation model on each individual imputed data set ($m = 5$) that was generated for the regression analyses and averaged the results to obtain our final estimates. We also ran the quantile g-computation models for the toxic metals only.

Analyses of measured blood pressure. We examined the associations between changes in individual log_2-transformed blood metals and changes in continuous measures of SBP and DBP between first and third trimester using adjusted linear mixed models with a first-order autoregressive [AR(1)] covariance matrix. We explored the potential for nonlinearity using 3-knot restricted cubic splines. Given that the use of antihypertensive medications would directly impact participants' blood pressure measures and could mask potential associations, participants who reported using these medications at a gestational age of 20 wk or later ($n = 32$) were excluded from the blood pressure analyses only. These participants were not excluded from the categorical analyses for gestational hypertension and preeclampsia.

Results

Descriptive Results

Of the 1,560 participants, 1,403 (89.9%) women were normotensive, 114 (7.3%) developed gestational hypertension (without preeclampsia), and 43 (2.8%) developed preeclampsia (Table 1). There were no cases of eclampsia in the cohort. Gestational age was determined using last menstrual period for 1,390 women and using ultrasound dating for 170 women. Thirty-three of the 43 women who developed preeclampsia had proteinuria and, of the remaining 10 women, 8 had elevated liver enzymes and 2 received a blood transfusion. Participants included in this analysis were similar to those excluded from the analysis (Table S2), with the exception of a higher proportion of missing values for characteristics assessed in the third trimester because participation in both first and third trimester visits was a criterion for inclusion in this analysis. Overall, the percentage missing was <1% for most covariates, except for prepregnancy BMI, for which 114 (7%) participants had missing data. The relative efficiency for imputing prepregnancy BMI with five imputations was 0.995, suggesting that there was little to be gained from additional imputations.

Median (IQR) values for blood metal concentrations and urinary As species are presented in Table 2. Relative to the other groups, women with preeclampsia had slightly higher first trimester concentrations of blood As and urinary arsenobetaine and lower blood concentrations of Mn. Trimester-specific Spearman correlations among blood metals were generally weak; the strongest correlation was observed between Hg and As ($r = 0.38$ in both trimesters; Table S3). ICCs between trimesters were high for most blood metals (ICC = 0.61–0.76), except for blood As (ICC = 0.35). First trimester blood As was moderately correlated with first trimester urinary DMA (Spearman $r = 0.22$) and arsenobetaine (Spearman $r = 0.48$); urinary DMA and arsenobetaine were also moderately correlated with each other (Spearman $r = 0.45$).

Individual Models

In single-metal multivariable models (Table 3), women were at a higher risk of developing preeclampsia with each doubling of third trimester Pb concentrations (RR = 1.44; 95% CI: 0.99, 1.98) or first trimester blood As (RR = 1.24; 95% CI: 1.01, 1.51). Women were at a higher risk of developing gestational hypertension with each doubling of first trimester As concentrations (RR = 3.26; 95% CI: 1.11, 9.58) and at a lower risk with each doubling of Mn concentrations (RR = 0.67; 95% CI: 0.44, 0.99). For these two metals, we observed an interaction on the multiplicative scale (product term RR = 0.68; 95% CI: 0.48, 0.96), as well as an excess RR due to interaction on the additive scale for developing gestational hypertension (RERI = 0.46; 95% CI: 0.03, 0.89). Figure 1 displays the predicted probabilities of developing gestational hypertension according to As concentrations. At the mean concentration of Mn, the probability of developing gestational hypertension aligned with the prevalence of diagnoses in this sample (7.3%) and was stable across values of As. The deleterious association with As was observed at lower concentrations of Mn (−1 SD). There were no other interactions between blood metals and Mn (Table S4).

The associations for As and Hg were similar when we adjusted for reported consumption of 25 species of fish (Table S5) rather than only fish high in Hg. Associations for Cd were similar when we adjusted for trimester-specific plasma cotinine concentrations with, or without, smoking status (Table S6).

Additionally adjusting for urinary arsenobetaine attenuated the association between first trimester blood As and gestational hypertension (RR = 2.58; 95% CI: 1.10, 6.05) and had little impact on the association for blood Mn (RR = 0.68; 95% CI: 0.47, 0.98) or their interaction (RR = 0.68; 95% CI: 0.48, 0.97). Similarly, this had little effect on the association between first trimester blood As and preeclampsia (RR = 1.27; 95% CI: 1.02, 1.58). When we restricted the analysis to those women with <1 μg/L arsenobetaine concentrations, the associations with gestational hypertension for first trimester blood As (RR = 15.1; 95% CI: 3.69, 61.9), first trimester blood Mn (RR = 0.43; 95% CI: 0.23, 0.77), and their interaction (product term RR = 0.43; 95% CI: 0.28, 0.66) were considerably stronger, but more imprecise. We observed a similar association between first trimester As and preeclampsia in this restricted analysis (RR = 1.41; 95% CI: 0.99, 2.01). Finally, neither urinary DMA concentrations nor arsenobetaine detection were associated with the risk of developing the outcomes (Table 3). We also observed null associations for urinary DMA concentrations with gestational hypertension (RR = 1.34; 95% CI: 0.40, 4.50) and preeclampsia (RR = 0.85; 95% CI: 0.48, 1.50) when restricting the analysis to those women with arsenobetaine concentrations <1 μg/L. We also did not observe interactions between urinary As species and Mn (Table S4).

Table 1. Participant descriptive characteristics [*n* (%) or median (IQR)] by study outcome group among 1,560 Canadian women in the MIREC study (2008–2011).

Variable	Normotensive ($n = 1{,}403$)	Gestational hypertension ($n = 114$)[a]	Preeclampsia ($n = 43$)
Maternal age (y)	32 (29, 36)	32 (28, 55)	31 (27, 36)
Prepregnancy BMI[b]			
Under/normal weight	879 (62.7)	46 (40.4)	12 (27.9)
Overweight	270 (19.2)	26 (22.8)	8 (18.6)
Obese	157 (11.2)	29 (25.4)	19 (44.2)
Missing	97 (6.9)	13 (11.4)	4 (9.3)
Place of birth			
Canada	1,126 (80.3)	101 (88.6)	38 (88.4)
Other	277 (19.7)	13 (11.4)	5 (11.6)
Race/ethnicity			
White	1,171 (83.5)	103 (90.3)	38 (88.4)
Black	38 (2.7)	6 (5.3)	1 (2.3)
Indigenous	29 (2.1)	1 (0.9)	2 (4.6)
Chinese	32 (2.3)	0	0
Latin American	42 (3.0)	0	1 (2.3)
Other	91 (6.5)	4 (3.5)	1 (2.3)
Education			
High school diploma or less	186 (13.3)	11 (9.7)	15 (34.9)
Some college/trade school	309 (22.0)	37 (32.5)	11 (25.6)
University degree	906 (64.6)	66 (57.9)	17 (39.5)
Missing	2 (0.1)	0	0
Smoking status—first trimester			
Never	868 (61.9)	74 (64.9)	22 (51.2)
Quit before pregnancy	378 (26.9)	28 (24.6)	12 (27.9)
Quit during pregnancy	82 (5.8)	7 (6.1)	7 (16.3)
Current smoker	75 (5.4)	5 (4.4)	2 (4.7)
Smoking status—third trimester			
Never	866 (61.7)	74 (64.9)	22 (51.2)
Quit before pregnancy	372 (26.5)	28 (24.6)	12 (27.9)
Quit during pregnancy	94 (6.7)	8 (7.0)	7 (16.3)
Current smoker	68 (4.8)	4 (3.5)	2 (4.7)
Missing	3 (0.2)	0	0
Parity			
Nulliparous	607 (43.3)	52 (45.6)	33 (76.7)
Multiparous	796 (56.7)	62 (54.4)	10 (23.4)
Consumption of fish high in Hg—first trimester[c]			
None	574 (40.9)	38 (33.3)	13 (30.2)
At least once/month	385 (27.4)	36 (31.6)	17 (39.5)
Once/month	166 (11.8)	13 (11.4)	3 (7.0)
2–3 times/month	73 (5.2)	6 (5.3)	2 (4.7)
4–5 times/month	126 (9.0)	13 (11.4)	4 (9.3)
≥6 times/month	74 (5.3)	7 (6.1)	4 (9.3)
Missing	5 (0.4)	1 (0.9)	0 (0)
Consumption of fish high in Hg—third trimester[c]			
None	625 (44.5)	47 (41.2)	22 (51.2)
At least once/month	344 (24.5)	32 (28.1)	9 (20.9)
Once/month	163 (11.6)	11 (9.3)	4 (9.3)
2–3 times/month	74 (5.3)	4 (3.5)	3 (7.0)
4–5 times/month	136 (9.7)	13 (11.4)	5 (11.6)
≥6 times/month	58 (4.1)	6 (5.3)	0 (0)
Missing	3 (0.2)	1 (0.9)	0 (0)
SBP (mmHg)	125 (118, 135)	146 (139, 151)	152 (144, 169)
DBP (mmHg)	82 (77, 88)	93 (88, 100)	100 (93, 108)

Note: Categorical values are presented using frequency (column percentage) and continuous values as median (IQR). %, percentage; BMI, body mass index; DBP, highest measured diastolic blood pressure after 20 wk gestation; first trimester, 6–13 wk gestation; Hg, mercury; IQR, interquartile range; MIREC, Maternal–Infant Research on Environmental Chemicals; SBP, highest measured systolic blood pressure after 20 wk gestation; third trimester, 27–40 wk gestation.

[a]Includes participants with gestational hypertension (not preexisting hypertension) but without preeclampsia.

[b]Under/normal weight, overweight, and obese, with cutoffs at 25 and 30 kg/m^2, respectively.[83]

[c]Includes reported consumption of tuna, marlin, orange roughy, shark, swordfish, mackerel, and escolar.

We observed a monotonic dose–response pattern for the aforementioned associations when exposures were analyzed according to tertiles (Table S7). In addition, participants in the second tertile of third trimester blood Pb concentrations had a higher risk of developing gestational hypertension, but this association did not follow a monotonic dose–response pattern. Mn exposure categorized according to the 10th and 90th percentiles was not associated with either condition (Table S8). We observed effect modification by fetal sex for first trimester Mn only (Table S9), whereby each doubling of concentration was associated with a lower risk of developing gestational hypertension among women carrying male (RR = 0.47; 95% CI: 0.29, 0.75), but not female, fetuses [RR = 1:45 (95% CI: 0.58, 3.62)]; [interaction RR = 2:42 (95% CI: 1.07, 5.46)]. No other sex-specific differences were observed.

Independent Models

In multivariable models estimating independent associations (i.e., adjusting for other metals), the associations for third trimester Pb and first trimester As remained relatively unchanged (Table 4). Similarly, the associations for first trimester As and Mn with

Table 2. Median (IQR) maternal concentrations of blood metals [first trimester (6–13 wk gestation) and third trimester (27–40 wk gestation)] and urinary As species (first trimester) among 1,560 Canadian women in the MIREC study (2008–2011).

	Normotensive ($n = 1{,}403$)	Gestational hypertension ($n = 114$)[a]	Preeclampsia ($n = 43$)
First trimester urine (μg As/L)[b]			
DMA	2.43 (1.60, 3.97)	2.27 (1.51, 3.33)	2.32 (1.62, 3.38)
Arsenobetaine	<LOD (<LOD, 3.90)	0.51 (<LOD, 2.78)	1.56 (<LOD, 6.96)
First trimester blood (μg/dL)			
Pb	0.62 (0.46, 0.85)	0.57 (0.41, 0.75)	0.54 (0.41, 0.81)
Cd	0.20 (0.13, 0.30)	0.19 (0.12, 0.27)	0.19 (0.15, 0.31)
As	0.82 (0.52, 1.20)	0.75 (0.51, 1.05)	0.90 (0.58, 1.42)
Hg	0.72 (0.34, 1.36)	0.64 (0.34, 1.20)	0.66 (0.18, 1.04)
Mn	8.79 (7.14, 10.99)	8.24 (7.14, 10.44)	8.24 (7.14, 9.34)
Third trimester blood (μg/dL)			
Pb	0.56 (0.41, 0.79)	0.53 (0.44, 0.77)	0.58 (0.44, 0.91)
Cd	0.20 (0.13, 0.29)	0.18 (0.12, 0.27)	0.19 (0.13, 0.26)
As	0.70 (0.41, 1.12)	0.57 (0.39, 0.97)	0.67 (0.49, 1.05)
Hg	0.56 (0.28, 1.02)	0.43 (0.20, 0.82)	0.42 (0.12, 0.78)
Mn	12.64 (9.89, 15.38)	12.36 (9.89, 15.38)	11.54 (9.34, 15.38)

Note: As, arsenic; Cd, cadmium; DMA, dimethylarsinic acid; Hg, mercury; IQR, interquartile range (25th and 75th percentiles); LOD, limit of detection; MIREC, Maternal–Infant Research on Environmental Chemicals; Mn, manganese; Pb, lead.
[a]Includes participants with gestational hypertension but without preeclampsia.
[b]Specific-gravity standardized values.

gestational hypertension were retained, as were the interactions on both the multiplicative (product term RR = 0.68; 95% CI: 0.48, 0.96) and additive (RERI = 0.43; 95% CI: 0.03, 0.85) scales. None of the other metals in either trimester were independently associated with the risk of developing the outcomes.

Table 3. Adjusted RR (95% CI) for individual associations between first trimester (6–13 wk gestation) blood metals and urinary As species concentrations, as well as third trimester (27–40 wk gestation) blood metal concentrations, and gestational hypertension without preeclampsia ($n = 114$) or with preeclampsia ($n = 43$) vs. having normal blood pressure among 1,560 Canadian women in the MIREC study (2008–2011).

	Gestational hypertension	Preeclampsia
First trimester urine (μg As/L)		
DMA	1.31 (0.60, 2.85)	0.92 (0.68, 1.24)
Arsenobetaine		
<LOD	1.00	1.00
≥LOD	1.12 (0.77, 1.63)	1.20 (0.66, 2.18)
First trimester blood (μg/dL)		
Pb	0.91 (0.68, 1.20)	1.05 (0.69, 1.59)
Cd	0.90 (0.75, 1.09)	0.91 (0.65, 1.28)
As	3.26 (1.11, 9.58)	1.24 (1.01, 1.51)
Hg	0.97 (0.85, 1.12)	0.93 (0.74, 1.18)
Mn	0.67 (0.44, 0.99)	0.87 (0.46, 1.66)
Third trimester blood (μg/dL)		
Pb	1.05 (0.81, 1.37)	1.44 (0.99, 2.08)
Cd	0.96 (0.81, 1.13)	1.02 (0.80, 1.29)
As	0.91 (0.79, 1.04)	0.97 (0.78, 1.21)
Hg	0.88 (0.77, 1.01)	0.85 (0.67, 1.08)
Mn	0.99 (0.65, 1.51)	0.94 (0.49, 1.81)

Note: RRs represent a doubling (per $\log_2$ increase) in whole blood or urinary concentration derived from Poisson regression models with robust variance estimation with multiple imputation ($m = 5$) for missing covariate information. For arsenobetaine, RRs are modeled as ≥LOD (0.75 μg As/L) vs. <LOD (reference). All models are adjusted for maternal age (continuous), education (university, college, less than college), first or third trimester-specific smoking status (never, quit before pregnancy, quit during pregnancy, currently smoking), prepregnancy BMI (continuous), parity (nulliparous vs. multiparous), race/ethnicity (White vs. other), $PM_{2.5}$ (continuous), and country of birth (Canada vs. elsewhere). Models for As and Hg are additionally adjusted for reported consumption of fish high in Hg during the 30 d prior to the first or third trimester visit (none, at least once/month, once/month, 2–3 times/month, 4–5 times/month, ≥6 times/month). Models for urinary As species are additionally adjusted for specific gravity. For gestational hypertension, models for first trimester blood As and Mn are additionally adjusted for their multiplicative (product) interaction term (product term RR = 0.68; 95% CI: 0.48, 0.96). For gestational hypertension, models for first trimester urinary DMA are additionally adjusted for first trimester Mn [main effect RR = 0.91; 95% CI: (0.54, 1.54)], as well as the multiplicative (product) interaction term between urinary DMA and Mn [RR = 0.91; 95% CI: (0.71, 1.17)]. As, arsenic; BMI, body mass index; Cd, cadmium; CI, confidence interval; DMA, dimethylarsinic acid; Hg, mercury; LOD, limit of detection; MIREC, Maternal–Infant Research on Environmental Chemicals; Mn, manganese; Pb, lead; $PM_{2.5}$, particulate matter ≤2.5 μm in aerodynamic diameter; RR, relative risk.

Mixture Models

Using quantile g-computation, we did not observe an association for a simultaneous 1-quartile increase in metal concentrations within each trimester on the risk of developing either condition (Table 5); the overall joint relative risks were all close to the null. Blood As was the only chemical with a positive weight (i.e., contributing to a higher RR) in the first trimester model for preeclampsia, whereas Pb had the highest positive weight in the third trimester models; however, interpreting these weights in the context of null overall effects is difficult and should be done with caution. We reran the quantile g-computation models for the toxic metals only (excluding Mn) and observed similar null overall effect estimates (Table S10).

Models for Measured Blood Pressure

Changes in either blood Pb or Hg concentrations were not prospectively associated with changes in measured blood pressure between the first and third trimester (Table S11). Changes in blood Cd concentrations were positively associated with DBP, but not SBP. Changes in As concentrations were positively associated with SBP, whereas changes in Mn concentration were negatively associated with SBP; neither were associated with DBP. The magnitude of all of these associations were weak (i.e., <1 mmHg change from first to third trimester per doubling of exposure from first to third trimester). Using 3-knot restricted cubic splines, we did not observe any nonlinear associations with measured blood pressure (Figure S2). There were no interactions between toxic metals and Mn for either SBP or DBP (Table S12), and we did not observe effect modification by fetal sex for any of the blood metals (Table S13).

Discussion

We examined the individual, independent, and joint associations between exposure to four toxic metals and Mn and the risk of developing gestational hypertension or preeclampsia in a pan-Canadian sample of pregnant women. We showed that both third trimester Pb and first trimester As blood concentrations were independently associated with a higher risk of developing preeclampsia. We also showed that higher first trimester As and Mn concentrations were associated with higher and lower risks, respectively, of developing gestational hypertension. Similar directions of association were observed in the prospective analysis of changes in metals on changes in SBP (but not DBP), although the magnitude of these associations was small. In addition, we observed an interaction

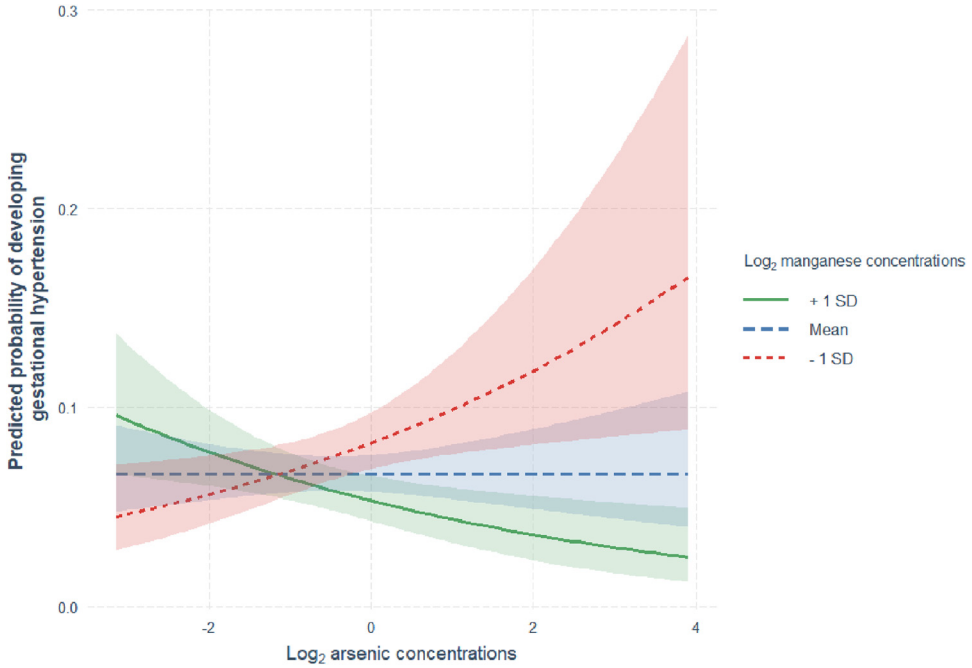

Figure 1. Interaction effect and 95% confidence intervals of first trimester (6–13 wk gestation) blood As concentrations on the predicted probability of developing gestational hypertension by levels of first trimester blood Mn while holding all covariates centered at their mean among 1,560 Canadian women in the MIREC study (2008–2011). Predicted probabilities are derived from a multivariable binomial model. Lines intersect at the mean $\log_2$ concentration of As. All models are adjusted for maternal age (continuous), education (university, college, less than college), first trimester-specific smoking status (never, quit before pregnancy, quit during pregnancy, currently smoking), prepregnancy BMI (continuous), parity (nulliparous vs. multiparous), race/ethnicity (White vs. other), $PM_{2.5}$ (continuous), country of birth (Canada vs. elsewhere), and reported consumption of fish high in Hg during the 30 d prior to the first trimester visit (none, at least once/month, once/month, 2–3 times/month, 4–5 times/month, ≥6 times/month). For gestational hypertension, models for first trimester As and Mn are additionally adjusted for their multiplicative (product) interaction term. Note: As, arsenic; BMI, body mass index; Hg, mercury; MIREC, Maternal–Infant Research on Environmental Chemicals; Mn, manganese; $PM_{2.5}$, particulate matter ≤2.5 μm in aerodynamic diameter; SD, standard deviation.

between first trimester concentrations of As and Mn on both the multiplicative and additive scales such that the deleterious association for a 2-fold change in As concentration on the risk of gestational hypertension was strongest among women with lower Mn concentrations. The tertile-specific associations support a monotonic, or linear, dose–response relationship for these associations. Neither first nor third trimester Cd and Hg concentrations were associated with either condition.

Interpretation of Mixture Results

We did not observe an overall joint effect for these metals in the quantile g-computation models, despite observing individual effects. This finding could be explained by the modest correlation among blood metal concentrations and limited likelihood of observing simultaneous, or joint, changes in these exposures. Although these toxic metals and Mn have multiple shared sources of exposure (e.g., drinking water and air), they also have independent, or at least more prominent, sources of exposure. These include Pb-based paints,[84] as well as resorption from bone stores during pregnancy[85] for Pb, cigarette smoke for Cd,[86] shellfish[87] and rice[88] consumption for As, and predatory fish consumption[66] and dental amalgams[89] for Hg. Our results are similar to two recent cross-sectional analyses using quantile g-computation. Xu et al. did not find an association between a mixture of toxic metals and Mn on the prevalence of hypertension (prevalence ratio = 0.96; 95% CI: 0.73, 1.27) among adults involved in cleanup activities for the *Deepwater Horizon* oil spill.[90] Similarly, in another study, Xu et al. did not find an association between a mixture of toxic metals and Mn measured in ambient air and the prevalence of hypertension (prevalence ratio = 1.02; 95% CI: 0.99, 1.04) among 47,595 women enrolled in the Sister Study cohort across the United States.[91]

Pb and Preeclampsia

After adjusting for coexposure to other metals, each doubling of third trimester blood Pb concentration was associated with a 58% higher risk of developing preeclampsia. Pb is an established risk factor for preeclampsia,[9] and our results extend this literature by providing evidence for the deleterious effects of Pb at concentrations comparable to, or even lower than, those typically experienced in the general population. For example, the median Pb concentration among MIREC participants was slightly lower than that observed among similarly aged women sampled around the same time in the Canadian Health Measures Survey (median blood Pb concentration = 0.86 μg/dL).[92]

In contrast, we did not observe associations with first trimester blood Pb concentrations with either condition. In this sample of women, average blood Pb concentrations declined by 10% between the first and third trimesters among women with normal blood pressure, which could be the result of plasma volume expansion,[93] but concentrations increased by 6% among women with preeclampsia. This temporal pattern could be influenced by the lower plasma volume expansion that women with preeclampsia experience in pregnancy,[94] which may be due to a loss of intravascular water volume into interstitial areas.[95] This temporal pattern is also similar to findings from an analysis of repeated blood Pb samples during pregnancy by Sowers et al.[96] These authors showed that, relative to women without preeclampsia, early pregnancy blood Pb levels were similar among women who would eventually present with preeclampsia but that blood Pb levels were consistently higher among these women at every time point afterward, even after adjusting for calcium intake.[96] Another possible explanation is the resorption of Pb from bone during pregnancy. Ninety percent of Pb is stored in bone[97] and

Table 4. Adjusted RR (95% CI) for the association between first trimester (6–13 wk gestation) and third trimester (27–40 wk gestation) blood metal concentrations and gestational hypertension without preeclampsia ($n = 114$) or with preeclampsia ($n = 43$) vs. having normal blood pressure while controlling for other trimester-specific blood metals in the model among 1,560 Canadian women in the MIREC study (2008–2011).

	Gestational hypertension	Preeclampsia
First trimester (μg/dL)		
Pb	0.95 (0.72, 1.24)	1.07 (0.70, 1.64)
Cd	0.92 (0.76, 1.12)	0.93 (0.66, 1.30)
As	3.20 (1.09, 9.37)	1.26 (1.01, 1.60)
Hg	1.00 (0.87, 1.15)	0.89 (0.69, 1.14)
Mn	0.72 (0.46, 1.12)	0.84 (0.43, 1.65)
Third trimester (μg/dL)		
Pb	1.10 (0.84, 1.45)	1.58 (1.08, 2.3)
Cd	0.96 (0.81, 1.13)	0.98 (0.77, 1.25)
As	0.93 (0.80, 1.08)	1.03 (0.81, 1.31)
Hg	0.89 (0.77, 1.03)	0.80 (0.62, 1.04)
Mn	1.00 (0.64, 1.55)	0.84 (0.43, 1.66)

Note: RRs represent a doubling (per $\log_2$ increase) in whole blood concentration derived from Poisson regression models with robust variance estimation with multiple imputation ($m = 5$) for missing covariate information. All models are adjusted for maternal age (continuous), education (university, college, less than college), first or third trimester-specific smoking status (never, quit before pregnancy, quit during pregnancy, currently smoking), prepregnancy BMI (continuous), parity (nulliparous vs. multiparous), race/ethnicity (White vs. other), $PM_{2.5}$ (continuous), country of birth (Canada vs. elsewhere), and reported consumption of fish high in Hg during the 30 d prior to the first or third trimester visit (none, at least once/month, once/month, 2–3 times/month, 4–5 times/month, ≥6 times/month) and other trimester-specific blood metal concentrations. For gestational hypertension, models for first trimester As and Mn are additionally adjusted for the multiplicative (product) interaction term between As and Mn (product term RR = 0.68; 95% CI: 0.48, 0.96). As, arsenic; BMI, body mass index; Cd, cadmium; CI, confidence interval; Hg, mercury; MIREC, Maternal–Infant Research on Environmental Chemicals; Mn, manganese; Pb, lead; $PM_{2.5}$, particulate matter ≤2.5 μm in aerodynamic diameter; RR, relative risk.

bone serves as a novel source of maternal Pb exposure during pregnancy,[85] especially during the third trimester.[98] It is possible that women who developed preeclampsia had higher levels of bone but not blood Pb in early pregnancy. Bone Pb reflects decades of prior exposure,[99] whereas blood Pb reflects only exposure within the past month.[84] This hypothesized toxicokinetic pattern of Pb in women with preeclampsia may help explain why we did not observe an association between first trimester Pb and preeclampsia.

As and Hypertensive Disorders of Pregnancy

Most of the previous evidence of the association between As and gestational hypertension or preeclampsia is limited to small nonlongitudinal studies with exposure assessed late in pregnancy or at delivery. Evidence from these studies is equivocal. One case–control study ($n = 88$ cases, 88 matched controls) in the Democratic Republic of the Congo found that women with preeclampsia had higher concentrations of urinary As,[100] whereas a Mexican case–control study ($n = 104$ cases, 202 unmatched controls) observed no such difference.[101] A South African case–control study ($n = 23$ cases, 43 unmatched controls) had mixed findings with slightly higher hair As concentrations in women with preeclampsia but much higher serum concentrations of As in controls.[102] In a larger case–control study of pregnant women in China ($n = 427$ cases, 427 matched controls), Wang et al. showed that As concentrations were associated with a higher risk of preeclampsia both individually and as a mixture.[40] Using prospective data from a large sample of pregnant women, we showed that each doubling of As concentrations in early pregnancy was associated with >3 times the risk of developing gestational hypertension, as well as a 23% higher risk of developing preeclampsia. These results are in line with evidence from nonpregnant populations highlighting As as a risk factor for developing hypertension[103] and other cardiovascular diseases.[104] Moreover, our finding is consistent with evidence previously generated from this same cohort demonstrating associations between As, in multiple matrices and forms, and gestational diabetes[51,105] or small-for-gestational age birth.[106]

Previous studies analyzing repeated measures of As exposure during pregnancy have provided equivocal evidence for a relevant window of susceptibility, with studies reporting that both earlier[107] or later[46,108] measures may be more relevant for pregnancy complications. It is plausible that early pregnancy may be the more relevant critical window of exposure for total blood As given that As methylation efficiency increases throughout pregnancy, which may reduce the toxicity of total blood As in later pregnancy.[109] However, at least some of the toxic effect of total As is likely derived from methylated urinary metabolites.[110,111] In our analysis, adjusting for urinary arsenobetaine attenuated, but did not nullify, the association between first trimester blood As and gestational hypertension, and we observed considerably stronger, although more imprecise, associations when restricting this analysis to women with <1 μg/L urinary arsenobetaine concentrations. This suggests that the associations with blood As were not driven by fish consumption. We did not observe an association with either condition for DMA, which is consistent with a recent analysis of first trimester urinary As species and hypertensive disorders of pregnancy from a Chinese birth cohort.[112] In both this study and that by Wang et al.,[112] estimates of exposure could be imprecise given that As species were analyzed in a single spot urine sample for which the within-person variance has been shown to be larger than the between-person variance[46] and reflects exposure to As over only a short interval.[113] MMA, arsenate, and arsenite had low rates of detection in our study. The LODs in our study are similar to or lower than national-level surveys conducted around the same time as our study in Canada[92] and the United States.[114,115] Studies

Table 5. Associations between a simultaneous 1-quartile increase in the trimester-specific blood metal mixture and adjusted RR (95% CI) for gestational hypertension (without preeclampsia) or preeclampsia among 1,560 Canadian women in the MIREC study (2008–2011).

		Mixture weights[a]				
	RR (95% CI)	Pb	Cd	As	Hg	Mn
First trimester (6–13 wk gestation)						
Gestational hypertension	0.79 (0.56, 1.11)	−0.45	−0.16	−0.21	1	−0.17
Preeclampsia	1.07 (0.61, 1.85)	−0.40	0.02	0.98	−0.34	−0.25
Third trimester (27–40 wk gestation)						
Gestational hypertension	0.85 (0.62, 1.16)	0.61	−0.20	−0.23	−0.57	0.39
Preeclampsia	0.99 (0.60, 1.66)	0.93	−0.44	0.07	−0.55	−0.01

Note: RRs represent a simultaneous 1-quartile change in all exposures derived from quantile g-computation models with multiple imputation ($m = 5$) for missing covariate information. All models are adjusted for maternal age (continuous), education (university, college, less than college), first or third trimester-specific smoking status (never, quit before pregnancy, quit during pregnancy, currently smoking), prepregnancy BMI (continuous), parity (nulliparous vs. multiparous), race/ethnicity (White vs. other), $PM_{2.5}$ (continuous), country of birth (Canada vs. elsewhere), and reported consumption of fish high in Hg during the 30 d prior to the first or third trimester visit (none, at least once/month, once/month, 2–3 times/month, 4–5 times/month, ≥6 times/month). As, arsenic; BMI, body mass index; Cd, cadmium; CI, confidence interval; Hg, mercury; MIREC, Maternal–Infant Research on Environmental Chemicals; Mn, manganese; Pb, lead; $PM_{2.5}$, particulate matter ≤2.5 μm in aerodynamic diameter; RR, relative risk.
[a]Positive mixture weights indicate contributions to higher risk of conditions and sum to positive one; negative weights contribute to lower risk of conditions and sum to negative one.

with more sensitive LODs[116] (i.e., 0.1 vs. 0.75 μg As/L, as in our study) have reported higher detection rates. The use of more sensitive laboratory methods would be beneficial in future studies to better characterize exposure to these As species.

Mn and Gestational Hypertension

Mn is an essential metal but can be toxic at high levels of exposure[25]; associations with health are thought to be characterized by an inverted U-shaped dose–response pattern. Several prospective studies have shown that infant birth weight is higher with increasing Mn blood levels up to inflection points ranging from 21 to 42 μg/L,[117–119] after which birth weight is then lower with increasing levels. The levels of Mn in the present study's sample were within the range where beneficial effects on birth weight have been observed,[81] but nearly all participants in the present study had levels below these inflection points.

Within the context of this exposure range, our results suggest that higher concentrations of Mn were associated with a lower risk of developing gestational hypertension. This is in line with evidence in nonpregnant adults, which shows that Mn (measured in urine,[120] blood,[121] toenails,[122] estimated via diet recall,[123] or through occupational exposure[124]) is associated with lower blood pressure. The likely mechanism for the association is through the antioxidant enzyme MnSOD, which scavenges reactive oxygen species, such as superoxide anions, that are associated with hypertension.[125] However, we did not observe associations with preeclampsia, which is contrary to the existing literature. Using data from two birth cohorts, Liu et al. showed that higher levels of Mn in red blood cells (RBCs) in the first trimester and at delivery were protective against developing preeclampsia.[23,24] Mn measured in RBCs is somewhat comparable to whole blood (about 66% of Mn in whole blood is bound to RBCs[126]), and because of the expected life span of RBCs ($\sim$120 d[127]), these measures likely correspond to exposures around the first and third trimesters. These authors examined preeclampsia only, rather than separating preeclampsia from gestational hypertension, limiting a direct comparison with this work.

The low number of cases of preeclampsia in our study ($n = 43$) may have contributed to null association between Mn and preeclampsia observed in our study. The number of cases of preeclampsia was higher in the analysis from the Boston Birth Cohort study ($n = 115$),[24] which likely provided more statistical power for the observed negative association (per SD change in Mn, PR = 0.68; 95% CI: 0.54, 0.86). However, the number of cases in the analysis from Project Viva was similar to our study ($n = 48$),[23] and the authors observed a negative association (third vs. first tertile RR = 0.50; 95% CI: 0.25, 0.99).

We found that the association between Mn and gestational hypertension was present among women carrying male fetuses only. Evidence for fetal sex-specific effects of maternal levels of Mn is limited and contradictory. Studies examining higher levels of Mn exposure have identified inverted U-shaped associations with risk of small-for-gestational age among male infants,[119] as well as head circumference[33] and low birth weight[128] among female infants. These sex-specific differences could be explained by sexually dimorphic placental antioxidant defense mechanisms. One study of human placentas showed that MnSOD concentrations were markedly reduced in the placentas for male vs. female fetuses.[52] Similar to much of this literature, our sex-specific findings are based on a modest number of cases and will require replication in future work.

Interaction between As and Mn

To our knowledge, this is the first time that an interaction between As and Mn has been observed for hypertensive disorders of pregnancy. Ultimately, this finding will need to be replicated in separate prospective pregnancy cohorts, but it is plausible based on epidemiological and experimental literature. Authors of a Bangladeshi birth cohort have observed interactions between prenatal exposure to As and Mn in regard to child neurodevelopment.[30] However, the evidence for interaction in studies examining birth outcomes is equivocal, with some studies observing interactions[33,34] and others not observing interactions.[35,36] One possible explanation for this interaction is the potential effect of As on MnSOD. In a study of nonpregnant Taiwanese adults,[29] higher urinary As concentrations were associated with higher odds of developing hypertension, and this association was strongest among individuals with a single nucleotide polymorphism that reduces MnSOD activity. Our finding is supported by experimental work in rodents demonstrating that exposure to As can reduce MnSOD activity[31,32] and that this effect can be attenuated with coexposure to Mn.[32] In addition, Biswas et al. also found that serum concentrations of the liver enzymes aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase were elevated among mice exposed to As but not elevated among mice that were coexposed to both As and Mn.[32] Elevations in these liver enzymes are one component of the definitions of both preeclampsia and Hemolysis, Elevated Liver enzymes and Low Platelets (HELLP) syndrome.

Cd and Hypertensive Disorders of Pregnancy

We did not observe associations between Cd and gestational hypertension or preeclampsia in our study. The epidemiological evidence for Cd is limited to a few nonlongitudinal studies that have demonstrated mixed results. Liu et al. identified an association between Cd measured in RBCs and preeclampsia, although the association was small and crossed the null (prevalence ratio = 1.15; 95% CI: 0.98, 1.36).[24] In a case–control study among South African women, those with or without preeclampsia had similar Cd concentrations measured in both serum (0.10 vs. 0.05 mg/L, respectively) and hair (3.75 vs. 3.96 μg/g, respectively).[102] Two case–control studies of women with higher exposure to Cd found that those with preeclampsia had higher concentrations of Cd in urine (geometric mean = 1.78 vs. 0.53 μg/L),[100] as well as in maternal blood (median = 1.21 vs. 1.09 μg/L)[129] or placental tissue (median = 4.28 vs. 3.61 μg/kg).[129] Neither of these studies adjusted for relevant confounders in their analysis, although one study matched participants for maternal age, gestational age, and parity.[100] A larger case–control study observed a protective association between blood Cd and preeclampsia [high vs. low tertiles odds ratio (OR) = 0.64; 95% CI: 0.45, 0.91] with a monotonic dose–response pattern across tertiles that persisted after adjusting for several other toxic metals.[40] Finally, a nested case–control study observed a deleterious association between placental Cd and preeclampsia (OR = 1.50; 95% CI: 1.10, 2.20).[130] These authors found that the association for Cd was stronger in women with lower placental selenium (OR = 2.0; 95% CI: 1.1, 3.5) and null in women with higher placental selenium (OR = 0.98; 95% CI: 0.5, 1.9).

Our results are generally consistent with the studies with similarly low blood concentrations of Cd.[24,102] Further research is needed to elucidate associations between Cd and hypertensive disorders of pregnancy. It may be helpful for this research to consider potential interactions with trace metals other than Mn, including selenium and zinc, which may be implicated in the development of hypertensive disorders of pregnancy.[131]

Hg and Hypertensive Disorders of Pregnancy

We also did not observe associations between total blood Hg and gestational hypertension or preeclampsia in our study. A previous analysis from this cohort found no association between the presence or replacement of dental amalgams, which is a source of

inorganic Hg exposure, and gestational hypertension.[132] In the Boston Birth Cohort, Liu et al. found no association between preeclampsia and Hg measured in RBCs,[24] which is more reflective of exposure to methylmercury.[133] Moreover, our findings are generally consistent with meta-analyses of the published literature demonstrating null associations between Hg and cardiovascular diseases, including hypertension, at the lower levels typically observed in the general population.[134,135] However, a separate meta-analysis by Hu et al.[135] supports an association between Hg and hypertension at higher levels of exposure (blood Hg >4.88 μg/L). In their case–control study, Wang et al. found that women in the highest tertile of blood Hg concentrations (≥1.89 μg/L, or 2–3 times higher than in our study) had higher odds of having preeclampsia.[40] Similarly, a prospective occupational study of pregnant dental staff with high exposure to Hg through dental amalgams and dental administrators without such exposure found that the dental staff had higher urinary concentrations of Hg and higher odds of developing preeclampsia (RR = 3.67; 95% CI: 1.25, 10.78).[18] The dental staff also had lower blood concentrations of two antioxidant enzymes, including SOD. The null associations for Hg observed in our study are consistent with previous analyses of similarly low levels of exposure.

Interpretations of Time Windows of Exposure When Using Whole Blood as a Matrix

Whole blood is a widely used matrix for biomonitoring of metals,[136] but this likely reflects different time windows of exposure for different metals. For Pb, Cd, Hg, and Mn, the concentrations reported in our paper provide reasonable estimates of trimester-specific exposures. For Pb, whole blood is the most commonly used matrix for biomonitoring[84] and represents exposure in the previous 30 d.[99] For Cd, whole blood is considered the most valid marker of recent exposure,[86] with a half-life of 3–4 months.[137] Following long-term, low-dose exposure, blood Cd can serve as a good reflection of Cd body burden.[138,139] Whole blood is commonly used to measure total Hg, with a half-life ranging from 44[140] to 80 d.[141,142] Although there is no accepted matrix for measuring Mn, whole blood Mn is thought to be a good indicator of environmental exposure,[143] with a half-life ranging from 2 to 5 wk.[144–146] However, urine is the preferred matrix for measuring total As, although with a half-life of 4 d.[147] Whole blood provides a measure of As exposure from several hours prior to collection,[148] which may not reflect longer term exposure.[113] With continuous exposure, such as through drinking water, blood As concentrations may reach a steady state.[147,149] Use of total blood As is a limitation of our study and likely contributes to nondifferential exposure misclassification, reducing the precision of the observed associations. Analyses from several other pregnancy cohorts have identified deleterious associations for perinatal health or birth outcomes with total As in whole blood,[34,35,40,150] although others have not.[36] Given that As has a relatively low rate of bioaccumulation, even in urine[151] when compared with Pb, Cd, or Hg, future studies should employ repeated measures to better characterize chronic exposures. Future studies should ideally include high-quality speciation with sensitive methods to disentangle inorganic and organic As to elucidate sources of exposure that will facilitate translating findings into public health action.

Studies of chemical mixtures, including metals, have typically used a single matrix to evaluate exposure,[39] which can be imperfect if the chemicals have different pharmacokinetic properties. Using repeated, paired blood and urine samples from Project Viva, Ashrap et al. recently showed that measuring metals in either blood or urine are equally good approaches for assessing metals as a mixture in relation to the risk of preterm birth.[152]

Strengths and Limitations

This study has several strengths. We examined exposure to toxic metals and Mn at two time points during pregnancy using data from a large prospective pregnancy cohort. This allowed us to establish temporality, to compare time windows of exposure, and to control our analyses for several potential static and time-varying confounders. As part of this investigation, we used a novel statistical approach to estimate the joint effect of a mixture of metals. In contrast to a similar method, weighted-quantile sum regression, quantile g-computation does not require that the exposures have the same direction of association (directional homogeneity), does not require sample splitting into training/validation sets, and is computationally more efficient. In addition, we examined associations with urinary As species alongside total blood As concentrations. Finally, the richness of the exposure information in the MIREC cohort allowed us to leverage trimester-specific air pollution exposure data to account for potential confounding of $PM_{2.5}$, which has not typically been done in studies examining blood metals and perinatal health.

This study has some limitations. First, concentrations of toxic metals were similar, or slightly lower, in this sample of women compared with nationally representative Canadian data from similarly aged women collected around the same time as this cohort (e.g., median Pb = 0.86 μg/dL, Cd = 0.28 μg/L, Hg = 0.69 μg/L; median urinary DMA = 6.7 μg/L).[92] These results should be interpreted within the context of these low exposure levels. For instance, the health effects of As and Mn may be more apparent in populations with lower exposure to Pb,[153] as is the case with our study. There is limited evidence examining associations in contemporary populations with low levels of exposure, and our work contributes to filling this gap in the literature. Second, in this study, we examined total blood Hg rather than the potentially unique contribution of inorganic and methylmercury; however, because most of the Hg in humans is in the methyl form,[154] total blood Hg should reflect mostly methyl Hg exposure. Wells et al. showed that total blood Hg at delivery was not associated with maternal blood pressure at delivery[155] but, rather, that methyl and inorganic Hg were associated with higher, and lower, blood pressures, respectively. Our results for total blood Hg are in line with those from Wells et al.,[155] but a direct comparison with our results is difficult because it is not uncommon for blood pressure to increase transiently during early labor.[156,157] Third, there is growing evidence to suggest that essential trace metals, such as calcium,[158] selenium,[131] copper,[131,159] and zinc,[159] may be implicated in blood pressure changes or development of hypertension/preeclampsia in pregnancy. We did not measure these essential trace metals in this study. Fourth, there may have been random error in the measurement of blood pressure, but this would be expected to be nondifferential because participants and study staff were not aware of the participants' blood metal concentrations. Fifth, change in paternal partner is another potential risk factor for preeclampsia[160,161] that could confound associations. We did not collect information on paternal partners from prior pregnancies. Sixth, although women with chronic kidney disease were excluded from enrollment, it is possible that alteration in kidney function could confound the association between first trimester blood As concentrations and gestational hypertension, if this were differential. Finally, we likely had greater statistical power to detect associations with gestational hypertension than we did for preeclampsia because of the larger numbers of cases, which is expected based on the prevalence of these conditions. Owing to the relatively small number of women with preeclampsia in our cohort ($n = 43$), we were unable to estimate associations separately for women with early vs. late onset preeclampsia, which should be prioritized in future work.

Conclusion

Our results confirm that even low blood Pb concentrations are a risk factor for preeclampsia. This work contributes to a growing body of evidence supporting the deleterious role of As on the risk of developing gestational hypertension and preeclampsia, as well as the protective role of Mn for gestational hypertension. We observed an interaction between As and Mn in early pregnancy such that the deleterious association with higher As concentrations was stronger at lower concentrations of Mn. This paper highlights the importance of considering individual, independent, and mixture analyses to examine multiple chemical associations and reinforces the value of incorporating multiple chemicals and multiple measures of exposure throughout pregnancy.

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