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Maternal and cord blood manganese (Mn) levels and birth weight: The MIREC birth cohort study

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ABSTRACT

Epidemiological studies have hypothesized that both insufficient and excess blood manganese (Mn) levels during pregnancy are associated with reduced fetal growth. This literature is characterized by inconsistent results and a limited focus on women with exposures representative of the general North American population. We examined the relationship between maternal and cord blood Mn levels and fetal growth among women enrolled in the Maternal-Infant Research on Environmental Chemicals Study (MIREC). Mothers with singleton, term infants and complete maternal first and third trimester blood Mn data were eligible for inclusion in the present study (n = 1519). Mean birth weight and odds ratios of small for gestational age (SGA) births according to maternal and cord blood Mn levels (low (< 10), referent (10– < 90), high (≥90) percentiles) were estimated. We also evaluated the association between the ratio of cord and maternal blood Mn and birth weight. Women with low (< 0.82 μg/dL) maternal blood third trimester Mn levels had infants that weighed an average of 64.7 g (95% CI: −142.3,12.8) less than infants born to women in the referent exposure group. This association was strengthened and became statistically significant when adjusted for toxic metals (lead, mercury, arsenic, and cadmium) [−83.3 g (95% CI: −162.4, −4.1)]. No statistically significant associations were observed in models of maternal first trimester or cord blood Mn. A one unit increase in the cord/maternal blood Mn ratio was associated with a 29.4 g (95% CI: −50.2, −8.7), when adjusted for maternal and neonatal characteristics. Our findings motivate additional research regarding the relation between Mn exposure and fetal growth. Further inquiry is necessary to determine whether an exposure threshold exists, how growth related effects of maternal and fetal Mn may differ, and how concurrent exposure to other toxic metals may impact the association between Mn and growth.

1. Introduction

Manganese (Mn), a naturally occurring metal and an essential micronutrient, is necessary for optimal fetal development (Mistry and Williams, 2011). Experimental studies demonstrate that insufficient developmental Mn exposure may impair growth, bone formation, immune function, metabolism (Aschner and Aschner, 2005; US ATSDR, 2012; Yoon et al., 2011), and neurodevelopment (Erikson et al., 2008). Similarly, epidemiologic research has reported that prenatal Mn deficiency is associated with adverse effects on neurodevelopment (Chung et al., 2015) and growth (Wood, 2009). Excess Mn has been associated with adverse childhood neurological outcomes as demonstrated in reviews of both experimental (Erikson et al., 2008) and human studies (Bjørklund et al., 2017). In addition, excess Mn has been associated with both reduced (Molina et al., 2012; Sanchez et al., 1993; Torrente et al., 2002) and excess birth weight (Betharia and Maher, 2012) in

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experimental studies.

In humans, it is well known that maternal blood Mn concentrations rise throughout pregnancy (Arbuckle et al., 2016), most likely to meet the increased demand for micronutrients necessary for fetal growth (Tholin et al., 1995; Zota et al., 2009); higher levels in umbilical cord blood have also been reported (Arbuckle et al., 2016; Zota et al., 2009). Mn is rapidly metabolized and cleared (US ATSDR, 2012); trimester specific measurements, therefore, are likely representative of recent exposure. The observed increases in Mn throughout pregnancy are primarily driven by pregnancy-related physiological changes (ie increased absorption) rather than increased exposure (Tholin et al., 1995). The main Mn sources in the general population typically come from diet and naturally occurring Mn in air, soil, and water. Anthropogenic sources of Mn, such as from power plants and other industrial operations, also contribute to environmental Mn exposure (US ATSDR, 2012; Health Canada, 2013).

Considering putative Mn adverse effects at low and high exposure, epidemiologists have hypothesized that the relation between Mn and birth weight is characterized by an inverse U-shaped curve. Cohort studies from the US (Zota et al., 2009), China (Chen et al., 2014; Guan et al., 2013), and Korea (Eum et al., 2014) and one case-control study from China (Xia et al., 2016) have reported curvilinear associations between either maternal or cord blood Mn levels and birth weight. Further, in a retrospective cohort study conducted in California, USA, elevated outdoor airborne Mn concentrations were associated with low birth weight (Basu et al., 2014). Similar associations between Mn and birth weight were not found in cohort studies from China (Yu et al., 2013) and Costa Rica (Mora et al., 2015) or a Spanish cross-sectional study (Bermúdez et al., 2015). One of the challenges of this limited body of literature is the inconsistency in timing (3rd trimester versus delivery) and the matrix of Mn measurement (urinary, whole blood, and ambient air). Also, studies with exposure levels representative of the general North American population are limited (Basu et al., 2014; Takser et al., 2004).

As Mn is an essential nutrient and is widely occurring in food, all women have detectable concentrations of Mn. Biomonitoring studies in North America report geometric mean blood Mn concentrations in women of 9.5 μg/L (Health Canada, 2013) and 10.6 μg/L (Oulhote et al., 2014). In 2013, 6.7% of births in Canada were low birth weight (< 2500 g), a predictor of multiple adverse child health outcomes (Statistics Canada, 2016). Considering the potential public health burden of reduced fetal growth and in light of the inconsistent findings regarding Mn-related effects, we examined the association between *in utero* Mn exposure and fetal growth in a Canadian birth cohort. Mn exposure was assessed via maternal and cord blood Mn concentrations. We evaluated fetal growth using measures of birth weight and small for gestational age (SGA).

2. Methods

2.1. Study population

A total of 1983 women were recruited to the Maternal-Infant Research on Environmental Chemicals (MIREC) study from 10 Canadian sites between 2008–2011 during their first trimester of pregnancy, as previously described (Arbuckle et al., 2013, 2016). Whole blood samples were collected during the first and third trimesters as well as venous umbilical cord blood at delivery for environmental chemical analysis. Women were eligible for inclusion if they were < 14 weeks gestation at time of recruitment, ≥18 years of age, able to communicate in French or English, and planning to deliver in a participating hospital. Women with known fetal or chromosomal anomalies in the current pregnancy or with serious medical complications were excluded from the study (Arbuckle et al., 2013, 2016). Of the 1983 women with available questionnaire and chemical data, 1648 mothers had complete maternal first and third trimester blood Mn data. Among these women, 1519 participants had a singleton, live, term birth and were included in the present study. Of these, 1214 women had complete cord blood Mn data.

Data on maternal and neonatal variables were extracted from questionnaires administered by research personnel, and from hospital charts by trained research nurses and staff. Ethics approval was obtained from the Health Canada ethics review board as well as ethics review boards at participating hospitals at all study sites. Eligible women signed informed consent forms prior to participating in the study.

2.2. Measurement of maternal and cord blood Mn concentrations

Maternal and cord blood samples were analyzed for Mn using a single-quadrupole Perkin Elmer inductively coupled mass spectrometry at the Centre de toxicologie du Quebec, Institut national de santé publique du Québec (INSPQ), Quebec, QC, Canada following 17,025 ISO guidelines as previously described (Arbuckle et al., 2016). Internal quality control (IQS) was ensured by analyzing non-certified reference materials from the Quebec Multielement External Quality Assessment Scheme (QMEQAS) (QM-B-Q1108, QM-B-Q1201) after calibration, every 10th sample, as well as at the end of each analytical sequence. The QMEQAS program is operated by the Centre de toxicologie du Québec (Institut national de santé publique du Québec, Quebec, Canada). IQC recoveries ranged from 89 to 120%. Other metals such as cadmium (Cd), arsenic (As), lead (Pb) and mercury (Hg) were analyzed using the same analytical method and instrumentation as was used for Mn.

2.3. Outcome assessment: birth weight and small for gestational age (SGA)

Infant weight (g) at birth was abstracted from study participants' medical charts. SGA births were defined as below the 10th percentile of birth weight distribution for each gestational week and sex, based on Canadian standards (Kramer et al., 2001).

Potential confounders were identified as variables previously reported to be associated with birth weight or Mn concentrations (Zota et al., 2009). These included maternal age at delivery (≤24, 25–29, 30–34, ≥35 y), pre-pregnancy body mass index (BMI) according to WHO guidelines (World Health Organization, 2018), parity (nulliparous, parous), maternal education (high school diploma or less, some college or trade school, undergraduate university degree, graduate university degree), household income ($ ≤30,000, 30,001–50,000, 50,001–100,000, ≥100,000), race (Caucasian/non-Caucasian), maternal smoking (never or quit before pregnancy, quit when knew pregnant, current smoking), gestational age at time of blood sampling, and hemoglobin level (Wood, 2009). Pb, Cd, As, and Hg were also considered potential confounders (Arbuckle et al., 2016; Govarts et al., 2016)

2.4. Statistical analysis

Descriptive statistics were calculated for all variables. All Mn samples were above the limit of detection. Mn was not log-transformed as data were not characterized by skewed distributions. We calculated the mean difference in birth weight according to categories of maternal and neonatal characteristics. For continuous variables, we calculated the change in birth weight per unit increase in the continuously measured characteristic (e.g. gestational age, hemoglobin). To graphically depict the relationship between blood Mn and birth weight as continuous variables, we used unadjusted generalized additive models (GAM). Based on previous literature showing inverse U-shaped relationships between birth weight and Mn (Chen et al., 2014; Ettinger et al., 2009; Eum et al., 2014; Xia et al., 2016), we categorized Mn concentrations in maternal and cord blood into three groups: < 10th (low), 10– < 90th (middle), and ≥90th percentiles (high). We calculated the mean

difference in birth weight between infants whose mothers had low or high concentrations and those in the referent (middle) group. Separate multiple regression models were performed using Mn measured in the first trimester, third trimester, or cord blood as the independent variable. The models were adjusted for the following variables, selected based on the univariate analysis and those previously recommended (Zota et al., 2009): maternal age, parity, pre-pregnancy BMI, household income, infant sex, maternal hemoglobin, ethnicity, gestational age at delivery, and maternal smoking. In addition to adjustment for maternal and neonatal characteristics, we analyzed a second model that also adjusted for Pb, Cd, As, and Hg. Prior to modeling the confounding effects, spearman correlation coefficients between Mn and the other metals were calculated. Furthermore, we included gestational age at the time of blood sampling and interactions terms between Mn and both hemoglobin and infant sex in the models. To further assess potential differences according to infant sex, we also conducted a stratified analysis.

Logistic regression was used to evaluate the association between Mn categories and odds of SGA. We calculated descriptive statistics of maternal and cord blood Mn according to SGA status and determined whether differences in concentrations between appropriate for gestational age (AGA) and SGA infants were statistically significant. These models were adjusted for the same set of confounders, including toxic metals, used in the analysis of birth weight as a continuous variable.

In addition, we conducted an exploratory analysis using the ratio of cord blood to third trimester maternal blood Mn levels as the exposure of interest (Kopp and Kumbartski, 2012). This approach has been applied in literature related to Mn neurotoxicity (Henn et al., 2017) as an indicator of placental transfer. The relationship between the ratio and birth weight was examined using locally weighted scatterplot smoothing (LOESS) and linear regression.

Analyses were performed using SAS v. 9.3. All results with a p-value < 0.05 were considered statistically significant.

3. Results

Mean (SD) birth weight was 3509 g (452.6 g) and 5.7% of births were classified as SGA. Median (range) maternal first and third trimester levels were 0.9 (0.2–2.9) and 1.3 (0.2–3.4) μg/dL respectively. Median (range) cord blood Mn levels were 3.2 (0.6–8.8) μg/dL. The mean (range) gestational week at the time of blood sampling in the first and third trimester was 12 (6–19) and 33 (27–41) weeks respectively.

Maternal and infant characteristics are shown in Table 1. The majority of mothers were older than thirty years at the time of pregnancy with normal BMI, Caucasian, did not smoke during pregnancy, and had a moderate or high household income. Non-Caucasian race, female infant sex, and primiparity were associated with reduced birth weight. Pre-pregnancy BMI, maternal age, and gestational age were associated with infant birth weight (p-value < 0.1).

3.1. Relation between maternal and cord blood Mn and birth weight

The relation between maternal third trimester blood Mn concentration and birth weight is depicted in Fig. 1. Increased Mn levels corresponded to higher birth weight, but only when Mn levels were below 0.8 μg/dL; the association plateaus when Mn levels are between 0.8 and 2.0. At the highest Mn concentrations (> 2.5 μg/dL), representing only 1% of the study population, increasing Mn concentrations were associated with increased birth weight. The GAM models for the first trimester and cord blood Mn were mostly linear and did not demonstrate any evident inflection points (data not shown).

Infants born to women with maternal first or third trimester Mn concentrations in the lowest decile weighed less than infants of mothers in the referent group (those in the 10^{th} to $< 90^{th}$ percentile of Mn concentrations) (Table 2). The magnitude of this association was strongest in the third trimester Mn model; women with low Mn concentrations had, on average, infants that weighed 64.7 g less than infants born to women in the referent group (95% CI: −142.3, 12.8). Women with high third trimester Mn levels had infants that weighed 17.4 g (-54.8, 89.8) more than infants born to women in the referent group. Infants with high cord blood levels (≥4.84 μg/dL), on average, weighed 65.7 g less than those in the referent group (95% CI: −148.5,17.2); while infants with low cord blood levels weighed 31.2 g (−52.3,114.8) more than those in the referent group (Table 2). We did not observe any interaction between either hemoglobin or infant sex and blood Mn concentrations based on the significance of product terms and stratified analyses. Adjusting for gestational age at the time of

Table 1
Maternal and neonatal characteristics and their univariate associations with birth weight (g), n = 1519.

Covariate	N (%)	Birth weight difference (g) (95% CI)
Maternal age (yrs)		
≤24[a]	75 (4.9)	0
25–29	316 (20.8)	150.0 (36.1–263.8)
30–34	531 (35.0)	133.2 (23.8–242.5)
≥35	597 (39.3)	151.5 (42.9–260.1)
Household income ($ CAD)		
≤30,000[a]	110 (7.6)	0
30,001–50,000	137 (9.4)	81.0 (−31.6 to 193.6)
50,001–100,000	609 (41.9)	79.1 (−12.1 to 170.2)
> 100,000	598 (41.1)	95.5 (4.3–186.8)
Ethnicity		
Caucasian[a]	1303 (85.8)	0
Non-Caucasian	216 (14.2)	−144.3 (−209.2 to −79.5)
Parity		
0[a]	665 (43.8)	0
1	606 (39.9)	74.3 (24.5–124.0)
≥2	247 (16.3)	34.6 (−31.4 to 100.7)
Pre-Pregnancy BMI[a]		
Underweight (< 18.5)	38 (2.7)	0
Normal (18.5–24.9)	883 (62.4)	154.3 (8.8–299.7)
Overweight (25–29.9)	298 (21.1)	243.9 (92.7–395.1)
Obese (≥30)	197 (13.9)	310.0 (154.5–465.6)
Maternal Smoking		
Never + quit before pregnancy[a]	1340 (88.2)	0
Quit when knew pregnancy	107 (7.0)	2.8 (−86.5 to 91.9)
Current	72 (4.7)	−70.8 (−178.2 to 36.6)
Infant sex		
Male[a]	800 (52.7)	0
Female	719 (47.3)	−115.1 (−160.4 to −69.8)
	Mean (SD)	
Gestational Age (wks)	39.2 (1.2)	140.2 (121.8–158.6)
Hemoglobin (g/L)	125.0 (9.9)	1.6 (−0.8 to 4.0)

[a] = referent category; Data missing for income (n = 65), parity (n = 1), BMI (n = 103), hemoglobin (n = 89); P-values < 0.1 for age, ethnicity, parity, sex, BMI, gestational age.

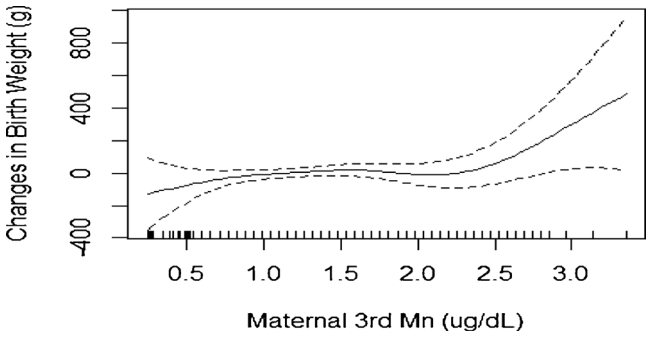

Fig. 1. GAM plot of the association between continuous maternal third trimester Mn and differences in birth weight.

Table 2
Associations between maternal and cord blood Mn and birth weight (g).

Mn percentile (μg/dL)	N (%)	Unadjusted		Model 1[a]	Model 2[b]
		Mean birth weight (g)	Birth weight difference (g) (95% CI)	Birth weight difference (g) (95% CI)	Birth weight difference (g) (95% CI)
Maternal 1st trimester					
< 10 (< 0.60)	127 (10.0)	3489.8	−32.5 (−115.0 to 50.0)	−22.2 (−97.8 to 53.3)	−37.3 (−113.8 to 39.3)
10– < 90 (0.60– < 1.32)	1010 (79.2)	3522.3	0	0	0
≥90 (≥1.32)	138 (10.8)	3471.0	−51.3 (−130.8 to 28.2)	−36.5 (−110.6 to 37.6)	−29.8 (−104.3 to 44.7)
Maternal 3rd trimester					
< 10 (< 0.82)	119 (9.3)	3451.5	−69.1 (−154.0 to 15.8)	−4.7 (−142.3 to 12.8)	−83.3 (−162.4 to −4.1)
10– < 90 (0.82– < 1.81)	1010 (79.2)	3520.6	0	0	0
≥90 (≥1.81)	146 (11.5)	3515.3	−5.2 (−82.8 to 72.3)	17.4 (−54.8 to 89.8)	25.4 (−47.6 to 98.4)
Cord blood					
< 10 (< 2.03)	101 (9.8)	3565.7	38.0 (−53.7 to 129.6)	31.2 (−52.3 to 114.8)	24.2 (− to 108.2)
10– < 90 (2.03– < 4.84)	830 (80.3)	3527.8	0	0	0
≥90 (≥4.84)	103 (10.0)	3475.8	−51.9 (−142.8 to 38.9)	−65.7 (−148.5 to 17.2)	−64.55 (−147.7 to 18.7)

Abbreviations: *Mn* manganese, *g* grams, *95% CI* 95th percentile confidence interval.
[a] Model 1: adjusted for maternal age, parity, BMI, income, infant sex, hemoglobin, smoking, ethnicity, gestational age.
[b] Model 2: adjusted for Model 1 covariates + lead, cadmium, mercury, arsenic (measured at the same time as Mn (i.e. 1st 1st trimester Mn models were adjusted for 1st trimester Pb, Cd, Hg, and As).

blood sampling did not result in any notable changes in parameter estimates in either the first or third trimester maternal blood Mn birth weight models.

3.2. Mn-birth weight models adjusted for other metals

Correlation coefficients between Mn, measured during the first trimester, third trimester, and in cord blood, and other metals, measured at the same time point as Mn, were relatively weak, with the strongest correlation observed between third trimester Mn and cadmium ($r = 0.23$, $p < 0.05$) (Table S1).

In the first trimester Mn analysis adjusted for other metals, women in the lowest decile of Mn had infants that weighed 37.3 g (95% CI: −113.8–39.3 g) less than infants born to women in the referent exposure group (Table 2). In the third trimester models, adjustment for toxic metals resulted in a statistically significant difference in birth weight; women with low Mn (< 10th percentile) had infants that weighed an average of 83.3 g less than the referent group (95% CI: −162.4 to −4.1), representing an 18.6 g greater difference than the model not adjusted for toxic metals. The reduction in birth weight, based on the toxic metal adjusted model, represents less than 20% of the standard deviation of mean birth weight in the total population. The only notable change in cord blood results in the models adjusted for additional metals was a marginal reduction in the birth weight difference between low and referent cord blood Mn levels (Table 2). Adjusting logistic regression models for lead, arsenic, mercury, or cadmium had no effect on SGA results for either maternal or cord blood data (data not shown).

3.3. Relation between maternal and cord blood Mn and odds of SGA

We did not observe any notable differences in maternal or cord blood Mn concentrations according to SGA status. Median (IQR) maternal first and third trimester Mn concentrations among SGA infants were 0.9 (0.7–1.2) and 1.2 (1.0–1.5) μg/dL respectively. Among appropriate for gestational age (AGA) infants, median (IQR) maternal first and third trimester Mn concentrations were 0.9 (0.7–1.0) and 1.3 (1.0–1.5) μg/dL respectively. Median cord blood Mn concentrations were 3.2 (2.5–4.2) and 3.1 (2.5–4.0) μg/dL among SGA and AGA infants respectively. All odds ratios of association between first and third trimester maternal Mn concentrations and SGA were close to the null value (Table 3). Compared to the referent group, mothers with high cord blood Mn levels were 1.8 times more likely to have SGA infants (95% CI: 0.8, 3.9).

Table 3
Associations between maternal and cord blood Mn and odds of small for gestational age.

Mn percentile (μg/dL)	N (%)	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)
Maternal 1st trimester			
< 10 (< 0.60)	127 (10.0)	0.8 (0.4,2.0)	0.7 (0.3,1.7)
10– < 90 (0.60– < 1.32)	1010 (79.2)	1.0	1.0
≥90 (≥1.32)	138 (10.8)	1.0 (0.5,2.2)	1.0 (0.5,2.3)
Maternal 3rd trimester			
< 10 (< 0.82)	119 (9.3)	1.1 (0.5,2.4)	1.0 (0.4,2.3)
10– < 90 (0.82– < 1.81)	1010 (79.2)	1.0	1.0
≥90 (≥1.81)	146 (11.5)	1.1 (0.6,2.4)	1.2 (0.5,2.5)
Cord blood			
< 10 (< 2.03)	101 (9.8)	1.0 (0.4,2.5)	1.0 (0.4,2.7)
10– < 90 (2.03– < 4.84)	830 (80.3)	1.0	1.0
≥90 (≥4.84)	103 (10.0)	1.8 (0.8,3.7)	1.8 (0.8,3.9)

Abbreviations: *Mn* manganese, *OR* odds ratio, *95% CI* 95th percentile confidence interval.
[a] Adjusted for maternal age, parity, pre-pregnancy BMI, income, hemoglobin, smoking, ethnicity.

3.4. Cord-maternal ratio and birth weight

The ratio of cord to maternal third trimester blood Mn levels ranged from 0.5 to 12.3. We observed an inverse linear relationship between this ratio and birth weight (Figure S1), suggesting that as the cord to maternal blood ratio increases, birth weight decreases. In a multiple linear regression model, adjusted for the same maternal and neonatal covariates as in the previous analysis, a one unit increase in the cord/maternal ratio was associated with a 29.4 g (95% CI: −50.2, −8.7) decrease in birth weight.

4. Discussion

In this population of Canadian pregnant women, we examined the relation between maternal and cord blood Mn and birth weight. We observed an association between low third trimester Mn and reduced birth weight that became statistically significant when adjusted for

toxic metals. No statistically significant associations were observed in models of maternal first trimester or cord blood Mn. Our exploratory analysis of the relation between the cord to maternal third trimester Mn ratio and birth weight suggested that a higher ratio was associated with lower birth weight.

Both insufficient and excess Mn concentrations have been shown to affect mechanisms underlying fetal growth. Mn is necessary for bone formation and is a cofactor for enzymes in bone tissue (Palacios, 2006). In animal studies, Mn deficiency has been associated with reduced bone concentration (Bolze et al., 1985), body weight (Clegg et al., 1998) and with disruptions in insulin homeostasis (Clegg et al., 1998). Compared to controls, chicks with Mn deficient diets were observed to have significantly lower bone concentrations (Bolze et al., 1985). In addition, rats fed a Mn deficient diet were observed to have 20% lower body weight and 33% lower levels of insulin growth factor. Authors of this study hypothesized that Mn-induced alteration in insulin growth factor hormone may underlie the observed reductions in weight (Clegg et al., 1998). Excess Mn adversely affected iron metabolism in a study of pregnant rats (Molina et al., 2012), providing a potential mechanism for the effect of high Mn concentrations on fetal growth. Further, recent mechanistic research has explored the potential pathways underlying the effects of metals, including Mn, on birth outcomes. Certain matrix metalloproteinases (MMPs), which are markers for inflammatory and oxidative stress, have been shown to be associated with both Mn (Au et al., 2016) and birth weight (Kumarathasan et al., 2016). This literature suggests that Mn may affect birth weight through a MMP mediated pathway. Additionally, a recent study of nutrient levels in preterm infants reported that Mn may minimize free radical formation and protect against oxidative damage, both of which may represent additional mechanisms underlying fetal growth (Bocca et al., 2017). Given that Mn is integral to numerous physiological functions, perturbations in Mn may affect fetal growth through multiple pathways. While it is not possible to extrapolate concentrations used in experimental literature to humans, this body of literature does suggest that a Mn-related effect on fetal growth is biologically plausible.

Authors of studies from the US, China, and Korea have reported inverted U-shaped relationships between blood Mn levels and birth weight (Chen et al., 2014; Eum et al., 2014; Zota et al., 2009). In these studies, inflection points were observed at levels ranging from 3 to 4.2 μg/dL (Chen et al., 2014; Eum et al., 2014; Zota et al., 2009). In the MIREC cohort, the maternal 3rd trimester blood Mn concentration at the 95th percentile was 2.03 μg/dL, and only three observations exceeded 3 μg/dL. Median blood concentrations (1.3 μg/dL maternal) were also lower in our study population compared to populations from the US and China, where medians ranged from 2.2 (Eum et al., 2014; Zota et al., 2009) to 5 μg/dL (Chen et al., 2014). Maternal 3rd trimester concentrations at the 5th percentile in the MIREC cohort were also lower than the 5th percentile in a US cohort study (0.71 vs 1.3 μg/dL); higher concentrations among participants in this US study may be explained, in part, by their residential proximity to a Superfund hazardous waste site (Chen et al., 2014). Hence, we would have expected to see an association between the low blood Mn levels observed in the present study and lower birth weight.

In contrast, studies from Costa Rica (Mora et al., 2015) and China (Guan et al., 2013) reported no association between maternal Mn and birth weight. Median maternal blood concentrations in these studies ranged from 2.6 (Mora et al., 2015) to 5.1 μg/dL (Guan et al., 2013). In light of overlapping exposure distributions among populations with and without significant associations, including the present study, the association between Mn and birth weight may be influenced by unmeasured individual factors such as co-occurrence of other micronutrients, deficiencies in nutrients such as iron, stress (Vorhees et al., 2014), genetics, ethnicity, and geographic region (O'Neal and Zheng, 2015).

Similar to previous work (Guan et al., 2013; Takser et al., 2004; Zota et al., 2009), we observed that cord blood Mn concentrations were higher than maternal blood concentrations. The growth related effects of Mn may differ between maternal and cord blood measurements of Mn. Both cohort (Chen et al., 2014; Zota et al., 2009) and case-control (Guan et al., 2013) studies have reported different directions and magnitudes of associations depending on maternal versus cord blood Mn measurement. Authors of an Iranian study reported that maternal Mn concentrations were significantly lower among intrauterine growth restricted (IUGR) cases than AGA infants, whereas cord blood Mn concentrations were significantly higher among IUGR cases (Vigeh et al., 2008). One of the largest cross-sectional studies reported that high cord serum Mn (> 5.0 μg/L) was associated with an elevated threefold odds of the high ponderal index (birth weight g/birth length cm^3) (Yu et al., 2013). Though confidence intervals were included the null value in most models, our findings suggest that low (< 10th percentile) maternal and high (≥ 90th percentile) cord blood levels are associated with lower birth weight. These observed effects are consistent with the inverse relationship between the cord to maternal Mn ratio and birth weight since the combination of low maternal Mn blood levels and high cord blood Mn levels will result in an increased ratio of the two measures. Considering the limited research attention devoted to the association between this ratio and birth weight as well as uncertainty regarding the interpretation of this measure, we present the findings of this analysis as exploratory. The ratio was originally described in an analysis of metal partitioning between the maternal and fetal unit (Kopp and Kumbartski, 2012). These authors raise the possibility that infants with discordant maternal and cord blood levels may be a distinct subpopulation (Kopp and Kumbartski, 2012). Simultaneous examination of both measures of in utero Mn levels (e.g., maternal and cord blood) may allow identification of potential exposure related adverse health effects unique to this subpopulation. We identified one case-control study that explored the association between this ratio and birth weight (Vigeh et al., 2008). In line with our findings, these authors reported that the cord to maternal third trimester Mn ratio was inversely related to birth weight. Further research is needed to understand if the relative concentrations of cord to maternal blood Mn have a more profound impact on fetal growth than cord blood concentrations alone. Variation in the degree of placental transfer and resulting cord blood concentrations may be influenced by factors not measured in our study such as transport gene polymorphisms (Ng et al., 2015). It is also possible that the ratio was influenced by the time of maternal third trimester blood sampling (ranging from 27 to 41 weeks). Given that Mn continues to rise throughout pregnancy, women sampled earlier in the third trimester may have had lower maternal Mn concentrations and cord to maternal blood ratio.

An additional challenge to interpreting Mn exposure levels is the lack of health-based guidelines. Reference ranges for blood concentrations have been reported for healthy adults (0.4–1.5 μg/dL) (US ATSDR, 2012; National Services Scotland, 2018.) and pregnant women (3^{rd} trimester blood, 0.49–2.03 μg/dL) (National Services Scotland, 2018.). There were very few MIREC study participants with levels outside the reference range for pregnant women (0.5% < 0.49 μg/dL; 4.6% > 2.03 μg/dL). Considering that birth weight-related effects have been observed at concentrations within the reference range (Chen et al., 2014; Zota et al., 2009), further efforts to identify a threshold for intervention may be warranted if an etiologic effect is established.

In our study, we were able to examine the impact of maternal blood Cd, As, Hg, and Pb, on the association between Mn and birth weight. We observed that adjusting for these metals strengthened the association between low Mn and birth weight, particularly in the model of third trimester maternal Mn levels. In analyses examining the individual effects of each metal, we observed that adjustment for Pb resulted in the largest change in Mn-birth weight model estimates. Understanding the underlying mechanisms and implications of these findings is a challenge. It is well established that Pb, even at levels < 10 μg/dL, is associated with reduced birth weight (Gonzalez-Cossio et al., 1997; Zhu et al., 2010). A previous analysis of MIREC data reported that maternal Pb concentrations increased with quartiles of

maternal Mn concentrations (Arbuckle et al., 2016). Similarly, the National Health and Nutrition Examination Survey reported that blood Mn increased with blood Pb levels, and the association was strongest at low Pb levels (Oulhote et al., 2014). Pb and Mn are positively correlated, but the strength of this correlation is relatively weak (0.11–0.19) indicating that some women with low Mn may have high Pb values. As these two metals may have different birth weight-related effects at low concentrations, the models not adjusted for toxic metals may bias the Mn-birth weight estimates towards the null. We speculate that adjustment for toxic metals minimized this bias as observed by a stronger magnitude of effect when comparing birth weight between low and referent maternal third trimester Mn concentrations. Though we did not explore the interaction between Mn and metals, authors of experimental studies have reported that there may be a pharmacological interaction between Mn and Pb (Betharia and Maher, 2012; Stackelberg et al., 2015). Also, Mn, particularly in combination with other nutrients such as zinc, copper, and magnesium, may exacerbate the birth weight-related effects of Pb and Cd (Luo et al., 2017). Though the complexities of the relations among Mn, other metals and birth weight preclude complete explanation of our findings, it appears that not controlling for concurrent toxic metal exposure masks the potential effect of low Mn on birth weight.

4.1. Strengths and limitations

Our study is one of the few North American investigations of the relation between Mn and birth weight with exposure levels representative of the general population and relatively large sample size. Our analyses benefited from the availability of data on Mn levels in two blood compartments representing maternal and fetal blood, as well as biomarkers of exposure to other metals and extensive information on maternal and infant characteristics. Despite these strengths, it is possible that the results are biased due to unmeasured confounders. For example, though we adjusted for several toxic metals, the mixture of commonly occurring pollutants may have a more profound effect on birth weight than individually modeled chemicals (Govarts et al., 2016). Due to the lack of Mn health-based guidelines for pregnant women, we used arbitrary cut-offs at the 10th and 90th percentiles to represent extremes of the exposure distribution that may be associated with adverse birth outcomes. Considering that the inflection points in the GAM curve for third trimester Mn and birth weight were similar to our cutoff points, categorizing Mn values represented a reasonable analytical approach. Had we used inflection points from the GAM model, our exposed groups would have been smaller and, therefore, had lower power to detect statistical associations. Considering that the MIREC cohort is comprised of relatively healthy women of moderate to high socioeconomic status, our ability to generalize our findings to populations with a different demographic composition is limited.

5. Conclusions

In this cohort of Canadian women, we observed an association between low third trimester Mn and reduced birth weight, particularly in models adjusted for toxic metals. No statistically significant associations were observed in models using measures of Mn in maternal first trimester or cord blood. Our findings motivate additional research regarding the relation between Mn exposure and fetal growth. Further inquiry is necessary to determine whether an exposure threshold exists, how growth related effects of maternal versus fetal Mn differs, and how concurrent exposure to other toxic metals may impact Mn related effects.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijheh.2018.05.015.

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