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Maternal and fetal exposure to cadmium, lead, manganese and mercury: The MIREC study

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HIGHLIGHTS

- Distribution of metals between maternal and cord blood differed by metal.
- Cadmium was rarely detected in cord blood (19%) or meconium (3%).
- Median cord Pb and Hg significantly higher than in maternal blood.
- Manganese increased over the course of the pregnancy and highest in cord blood.
- Higher Ca and vitamin D intake during pregnancy may lower maternal and cord Pb.

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ABSTRACT

Given the susceptibility of the fetus to toxicants, it is important to estimate their exposure. Approximately 2000 pregnant women were recruited in 2008–2011 from 10 cities across Canada. Cd, Pb, Mn and total Hg were measured in maternal blood from the 1st and 3rd trimesters, umbilical cord blood, and infant meconium. Nutrient intakes of vitamin D, iron, and calcium (Ca) were assessed using a food frequency questionnaire and a dietary supplement questionnaire.

Median concentrations in 1st trimester maternal blood (n = 1938) were 0.20, 8.79 and 0.70 μg/L for Cd, Mn and Hg, respectively, and 0.60 μg/dL for Pb. While the median difference between the paired 1st and 3rd trimester concentrations of Cd was 0, there was a significant decrease in Pb (0.04 μg/dL) and Hg (0.12 μg/L) and an increase in Mn (3.30 μg/L) concentrations over the course of the pregnancy. While Cd was rarely detected in cord blood (19%) or meconium (3%), median Pb (0.77 μg/dL), Mn (31.87 μg/L) and Hg (0.80 μg/L) concentrations in cord blood were significantly higher than in maternal blood. Significant negative associations were observed between estimated Ca intake and maternal Cd, Pb, Mn and Hg, as well as cord blood Pb. Vitamin D intake was associated with lower maternal Cd, Pb, and Mn as well as Pb in cord blood.

Even at current metal exposure levels, increasing dietary Ca and vitamin D intake during pregnancy may be associated with lower maternal blood Pb and Cd concentrations and lower Pb in cord blood.
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1. Introduction

Various metals, including cadmium (Cd), lead (Pb), manganese (Mn), and mercury (Hg) are found in our environment and food,

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and may be ingested or inhaled. Metal concentrations in the Canadian general population have shown some temporal variability. Comparing cycles of the Canadian Health Measures Survey between 2007 and 2013, there was no significant difference in blood Cd or Hg, but Pb concentrations declined (geometric means 1.3 to 1.1 μg/dL) (Health Canada, 2015). Geometric means of blood Mn concentrations increased between 2007 and 2011 (9.2–9.8 μg/L) (Health Canada, 2013b). While levels of metals such as Pb in most environmental media and products have declined over the past few decades, Canadians can still be exposed to Pb in food, drinking water, air, dust, soil and manufactured products (Health Canada, 2013a), to Cd from cigarette smoke and diet (Järup and Åkesson, 2009), to Hg from certain fish species and dental amalgam fillings (Health Canada, 2009), and to Mn from a variety of foods and from nutritional supplements, but also in air, water and soil (ATSDR, 2012). Manganese is also a required trace element and an important antioxidant nutrient in pregnancy (Mistry and Williams, 2011).

A recent review of the epidemiologic literature has reported associations between maternal exposures to: Pb and low birth weight, preterm birth, stillbirths, spontaneous abortions and hypertension; Cd and low birth weight, neurological dysfunction and low Apgar score; and Hg and spontaneous abortions and neurotoxic effects (Rahman et al., 2016). Furthermore, the toxicity of Pb appears to increase in the presence of higher levels of Cd, Mn or Hg (reviewed in Claus Henn et al., 2014). Elevated concentrations of blood Mn during pregnancy have been associated with gestational hypertension (Vigeh et al., 2013) and may also affect birth weight (Zota et al., 2009).

The placenta, which acts as an interface between maternal circulation and the fetus, also protects the embryo by filtering the passage of pollutants. However, while a number of metals such as Pb and Hg can cross the placental barrier, it is less clear for Cd (Caserta et al., 2013).

Nutrient intake may impact the toxicokinetics of metals by reducing the absorption or toxicity of toxic metals such as Pb and Cd (Hong et al., 2014; Liu et al., 2013; Nishijo et al., 2004; Gulson et al., 2016; Ettinger et al., 2009; Åkesson et al., 2002). Analysis of maternal and infant biospecimens for chemical biomarkers provides an opportunity to estimate the extent of exposure for both the pregnant woman and her fetus to environmental chemicals during critical periods of development. There are limited data available which prospectively track exposure to current levels of metals across pregnancy and at birth in a large population. To address this dearth, the Maternal-Infant Research on Environmental Chemicals (MIREC) Study was designed to generate national-level data on maternal and newborn exposure to priority environmental contaminants, including metals (Arbuckle et al., 2013).

The goals of this analysis were to describe exposure of pregnant women and their infants to these metals and to examine the possible effects of nutrient intake on metal levels. The MIREC Study offers a unique opportunity to do this as metals were measured in 1st and 3rd trimester blood samples, umbilical cord blood and meconium in a large national-level Canadian pregnancy cohort. In addition, given the health concerns about excess exposure to these metals, determining whether current levels are moderated by maternal nutrient intakes will make an important contribution to possible public health interventions.

2. Methods

2.1. Study population

A cohort of pregnant women (n = 2001) was recruited over 4 years (2008–2011) from obstetric and prenatal clinics in ten cities across Canada, as part of the Maternal-Infant Research on Environmental Chemicals (MIREC) Study. Eligibility criteria included: less than 14 weeks gestation; ability to consent and to communicate in English or French; age 18 years or older; planning on delivering locally; and agreeing to participate in the cord blood collection component of the MIREC Study. Women with a medical history of any of the following were excluded from the study: major chronic disease; threatened abortion; and illicit drug use. Details on the cohort have been previously described (Arbuckle et al., 2013).

The study was reviewed and approved by the Health Canada Research Ethics Board and the ethics committees at the participating hospitals and research centers across Canada. Potential participants were provided with information on the objectives and design of the study and asked to sign informed consent prior to participation.

2.2. Data collection and determination of nutrient intake

Questionnaires were administered during the 1st and 3rd trimester study visits to collect information on the characteristics of the participants (e.g., maternal age and education, household income, pre-pregnancy body mass index (BMI), parity, country of birth, and smoking status). The season in which the blood was collected and whether it was a fasting sample were also noted.

At the second trimester visit, between 16 and 21 weeks of pregnancy, a semi-quantitative food frequency questionnaire (FFQ) with a one-month recall period was administered to obtain a ranking of iron (Fe), calcium (Ca) and vitamin D intakes for each individual. The methodology used to obtain the nutrient intake has been previously described (Morisset et al., 2016). Briefly, for each food item, information on the frequency and serving size consumed were collected and each food was matched to corresponding foods in the 2010 Canadian Nutrient File (Health Canada, 2010a). The serving size, gram weight and the amount of nutrient were calculated for each serving of the food item reported.

Participants also completed a nutrient supplement questionnaire around 16 weeks gestation that asked for a detailed list of dietary supplements taken in the last 30 days (brand name and description of product, amount taken each time and frequency). Supplement Fe, Ca and vitamin D content were derived using Health Canada's Drug Product database (http://www.hc-sc.gc.ca/dhp-mps/prodpharma/databasdon/index-eng.php) and/or the detailed ingredients list from the product monograph. Intakes from food and supplements were summed to derive quartile estimates of total intake.

2.3. Biospecimen collection and metal analysis

Maternal 1st (6–13 wks) and 3rd (32–34 wks) trimester whole blood was collected in 6 mL K2EDTA tubes and aliquotted into 5 mL Sarstedt® tubes (Sarstedt AG & Company, Nümbrecht, Germany) and frozen at −20 °C. Venous umbilical cord blood was collected using a Sarstedt® S- Monovette, inverted 8–10 times, transferred to 5 mL Sarstedt® tubes and frozen at −20 °C. Mère Hélène® bioliners (Mère Hélène, Quebec Canada) were inserted in the diapers and meconium was transferred to 50 mL Sarstedt® tubes and frozen at −20 °C. Biospecimens were shipped frozen to the Centre de Toxicologie du Québec, Institut National de Santé Publique du Québec (INSPQ), Quebec QC, Canada for analysis.

2.3.1. Instrumentation

All biological samples (blood, cord blood and meconium) were analyzed using a single-quadrupole PerkinElmer (Waltham, MA USA) Elan DRCII ICP-MS (inductively coupled plasma-mass spectrometry) system operated in standard mode. The system was equipped with a cyclonic spray chamber, an Elemental Scientific

MicroFlow PFA-ST nebulizer and a quartz torch. Lens and gas flow parameters of the ICP-MS were daily optimized to maximize sensitivity while maintaining oxides and double charges low (CeO/Ce $\leq$ 3.2%; Ba++/Ba $\leq$ 3%). Sample preparation and analysis were carried out in the INSPQ laboratory that was specifically designed and dedicated to trace element analysis.

The analytical methods were fully developed and validated for monitoring purposes following 17025 ISO guidelines. The method limits of detection (LOD) for all elements (shown in Table 1) were determined by using a signal to noise ratio of 3 from 10 consecutive measurements of a representative sample. When several detection limits were present, the highest LOD is presented in the tables.

2.3.2. Field blanks

Prior to the biospecimen collection, all collection materials were screened for the elements of interest using a validated method for trace metals. During the field work, field blanks were utilized to assess potential risks of contamination by mimicking the process of collection and handling of biospecimens at recruitment sites, transportation, storage and laboratory analysis, using steril.O reagent grade deionized distilled water as the matrix. Results showed that the field blanks were free of contamination, with the exception of Mn; a value of 14 nmol/L was subtracted from each Mn value prior to recording the result.

2.3.3. Blood and cord blood analysis

Maternal blood samples (500 μL) were diluted 20-fold and cord blood (250 μL) 40-fold in a diluent containing 0.5% (v/v) NH_4OH and 0.1% (v/v) octylphenol ethoxylate. External calibration curves were prepared by diluting 20-fold (for blood) and 40-fold (for cord blood) respectively, the corresponding volume of human blood (from healthy volunteers) with diluent and then spiking with different volumes of 1 mg/L multi-elements standard solution (SCP Science, PlasmaCal ICP-MS Verification Standard 1; 5% HNO_3, #141-110-011). The internal standards for the calibration curve and the blood samples analysis were ^{89}Y (for ^{55}Mn), ^{103}Rh (for ^{114}Cd), ^{159}Tb (for ^{208}Pb) and ^{195}Pt (for ^{202}Hg). Internal quality assurance was ensured by running validated reference materials (QMEQAS08B05, QMEQAS08B08, QM-B-Q1108 and QM-B-Q1201 - human blood from the QMEQAS inter-laboratory comparison scheme) after calibration, after every 10th sample and at the end of each analytical sequence.

2.3.4. Meconium analysis

Meconium was homogenized and then samples (500 mg) were predigested at room temperature in a Teflon bomb for 3 h under acidic conditions (2 mL HNO_3). The bombs were then sealed and placed at 110 °C for 18 h to complete the digestion. The digest was diluted 50-fold with an aqueous solution (diluent) containing 0.002% (m/v) L-(+)-cysteine and 100 μgL-1 Au. External calibration curves were prepared in 2% (v/v) HNO_3 containing 0.002% (m/v) L-(+)-cysteine and 100 μgL-1 Au by spiking with different volumes of 1 mg/L multi-elements standard solution (SCP Science, PlasmaCal ICP-MS Verification Standard 1; 5% HNO_3, #141-110-011). The internal standards for calibration standards and meconium samples were ^{89}Y (for Mn), ^{103}Rh (for Cd), ^{159}Tb (for Pb) and ^{195}Pt (for Hg). Internal quality assurance was ensured by running standard reference materials (SRM 1577 b - bovine liver from NIST) and validated reference materials (QM-H-Q1101 - human hair from the QMEQAS external quality assessment scheme) after calibration, after every 10th sample and at the end of each analytical sequence.

2.4. Statistical analysis

Substitution methods for dealing with laboratory results below the method's LOD, such as LOD/2 or LOD/$\sqrt{2}$ may lead to increased bias and an underestimation of the error variance, resulting in low power for statistical hypothesis testing (Cole et al., 2009; Leith et al., 2010; May et al., 2011; Helsel, 2012; Nysen et al., 2012). To mitigate these issues, we applied survival analysis techniques for right censored data to the left-censored scenario to better account for the values below the LOD using either parametric maximum likelihood estimation (MLE) or nonparametric Kaplan-Meier (KM) and generalized Wilcoxon tests (Helsel, 2012). Maximum likelihood depends on distributional assumptions, such as log-normality and may be inefficient with small sample sizes; in the latter cases, nonparametric methods were used for analysis. As most of the metals had some values below the LOD, both censoring methods were implemented for descriptive statistics and hypothesis testing. The geometric mean from a lognormal random variable with censoring was calculated using the MLE method, while the median was calculated using the Kaplan-Meier approach. The Greenwood estimate of variance was used for determination of Kaplan-Meier confidence intervals. The parametric and nonparametric censoring methods were used only for metals with at least 50% of

Table 1
Descriptive statistics for metals in maternal, cord blood and meconium using censoring methods.

Metal	Sample	N	LOD	%<LOD	Min	Median	95th percentile	Max	KM median	95% CI		GM MLE	95% CI	
Cadmium (Cd) (μg/L)	1st trimester	1938	0.0450	2.63	ND	0.2023	1.124	5.6199	0.2023	0.1953	0.2093	0.2197	0.2118	0.2278
	3rd trimester	1673	0.0450	3.89	ND	0.2023	0.7418	4.2711	0.2023	0.1957	0.2089	0.2005	0.1934	0.2078
	Cord blood	1420	0.1124	80.77	ND	ND	0.2248	4.0463	NA	NA	NA	NA	NA	NA
	Meconium [a]	1591	0.004	97.36	ND	ND	ND	0.1300	NA	NA	NA	NA	NA	NA
Lead (Pb) (μg/dL)	1st trimester	1938	0.1036	0	0.1554	0.6009	1.409	5.1803	0.6009	0.5851	0.6167	0.6205	0.6075	0.6338
	3rd trimester	1673	0.1036	0.18	ND	0.5595	1.3759	4.1442	0.5595	0.5447	0.5742	0.5728	0.5589	0.5870
	Cord blood	1419	0.2072	2.61	ND	0.7667 [b]	1.6991	5.1803	0.7667	0.7443	0.7890	0.7483	0.7284	0.7688
	Meconium [a]	1591	0.004	79.38	ND	ND	0.0085	0.48	NA	NA	NA	NA	NA	NA
Manganese (Mn) (μg/L)	1st trimester	1938	0.5495	0	2.0330	8.7912	14.8352	29.1209	8.7912	8.6462	8.9362	8.7974	8.6733	8.9232
	3rd trimester	1673	0.5495	0	2.4725	12.6374	20.3297	33.5165	12.6374	12.4138	12.8609	12.2608	12.0679	12.4568
	Cord blood	1419	0.5495	0	5.4945	31.8681 [b]	54.9451	98.9011	31.8681	31.1883	32.5479	31.4586	30.8846	32.0434
	Meconium [a]	1591	0.01	0	0.24	4.9	15	40	4.9	4.6816	5.1184	4.4996	4.3279	4.6782
Total mercury (Hg) (μg/L)	1st trimester	1938	0.1204	9.80	ND	0.7021	2.8084	10.0301	0.7021	0.6624	0.7418	0.6133	0.5842	0.6438
	3rd trimester	1673	0.1204	11.24	ND	0.5617	2.2869	12.4373	0.5617	0.5287	0.5947	0.4965	0.4726	0.5216
	Cord blood	1419	0.4012	28.19	ND	0.8024 [b]	3.6108	14.2427	0.8024	0.7428	0.8620	0.7659	0.7264	0.8075
	Meconium [a]	1591	0.01	75.11	ND	ND	0.025	0.14	NA	NA	NA	NA	NA	NA

LOD: limit of detection.
ND: not detected (<LOD).
NA: not calculated as more than 70% of the observations were < LOD.
[a] Unit for levels of heavy metals in meconium is μg/g.
[b] Significantly higher than maternal median concentrations ($p < 0.0001$).

the data above the LOD as recommended by Helsel (2012). Due to the non-normality of the data, Wilcoxon signed rank test or sign test were used to test whether the medians of the two measurements (1st and 3rd trimesters) were equal.

Spearman correlations for each metal were calculated between each sampling period and matrix and the intraclass correlation coefficients (ICCs) were calculated to examine within-woman variability between the 1st and 3rd trimester metal concentrations.

Statistical hypothesis testing was used to test for significant differences among demographic groups. The maximum likelihood estimation (MLE) was employed to account for left–censored repeated measures, analogous to Jin et al. (2011) and Thiebaut and Jacqmin-Gadda (2004) using the NLMIXED procedure in SAS. Hypothesis testing was performed using both the 1st and 3rd trimester data for metals having at least 50% of the observations above the LOD using likelihood ratio tests which follow a chi-square distribution. The Bonferroni multiple comparison technique was used when overall tests were significant. In addition, geometric mean maternal and cord blood metal concentrations were calculated by quartile categories of selected nutrients (Ca, Fe and vitamin D intake and maternal blood Mn). To test significant differences among nutrient groups, analysis of variance was performed. When the overall F-test for variables which have more than two groups was significant, Scheffé multiple pair-wise comparisons were used to determine which groups were significantly different from one another. In addition, simple linear regression analyses were performed to examine the relationships between metal concentrations and each nutrient. A residual analysis was implemented to verify the statistical assumptions of normality and constant variance. When these assumptions were not satisfied for the log-transformed data, nonparametric regression was used. Statistical analysis was performed using SAS (Statistical Analysis System) Enterprise Guide 4.2 and R (R Core Development Team). For the censoring methods, functions from the R packages NADA and SURVIVAL and SAS procedure NLMIXED were used for analysis. Unless otherwise indicated, a 5% significance level ($\alpha = 0.05$) was implemented throughout.

3. Results

3.1. Descriptive statistics on metals in maternal-fetal unit

Among the 2001 women recruited, 18 withdrew, and 1st trimester maternal blood metal data were available for 1938 women. A description of the study population is shown in Supplemental Material Table S1. The majority of the women were 30 years or older, had never smoked, were primigravida or secundigravida, were born in Canada, had a normal or underweight BMI and were well educated. The male:female birth ratio was 1.096.

Most of the women had detectable concentrations of Pb, Cd, Mn and total Hg in their blood (Table 1). Maternal blood Cd concentrations ranged from below the LOD to 5.62, with a median of 0.20 μg/L. Cadmium was not detected in 80% of the cord blood and 97% of the meconium samples. GM concentrations of Pb in maternal blood were 0.62 and 0.57 μg/dL for 1st and 3rd trimesters, respectively, and 0.75 μg/dL in cord blood. Manganese concentrations increased over the course of the pregnancy and were highest in cord blood. Total mercury was detected in approximately 90% of maternal blood samples but only 25% of meconium samples (even with a more sensitive LOD in meconium than in blood). Median cord blood Pb, total Hg and Mn concentrations were statistically higher than maternal concentrations at 1st and 3rd trimesters.

The relative comparison of metal concentrations in the maternal and cord blood provides an indication of the exposure of the fetus, which may experience more or less exposure based both on the mother's exposure and the extent of placental transfer. Moderate to high correlations were observed between 1st and 3rd trimester maternal blood metal concentrations, as well as between maternal and cord blood for all metals except Cd (Supplemental Material Table S2). Linear regression analysis also showed positive associations between log maternal and cord blood metal levels, with somewhat stronger associations between cord and 3rd trimester than between cord and 1st trimester blood metals (Supplemental Figs. S1 and S2). Supplemental Fig. S3 shows the cumulative distribution for the difference (A) and ratio (B) between maternal 3rd trimester and cord blood concentrations (ug/L) for the four metals. Observing the differences (A), it appears that Cd and Hg concentrations tend to equilibrate in maternal and cord blood while Pb and Mn concentrations show greater variability between mother and fetus/infant; however, the greater variability of differences between mother and fetus/infant for Pb and Mn (compared with Cd and Hg) is probably because concentrations of Pb and Mn are much larger than those of Cd and Hg. The ratio (B) of maternal-to-cord blood concentrations indicates that the extent of transfer varies considerably both among and between the metals, with the median ratios varying from about 3.2 for Cd to 0.4 for Mn.

The intraclass correlation coefficients and 95% confidence intervals between 1st and 3rd trimesters were 0.81 (0.79, 0.82), 0.73 (0.71, 0.76), 0.29 (0.24, 0.33) and 0.60 (0.57, 0.63) for Cd, Pb, Mn and Hg, respectively. While the median difference between the paired 1st and 3rd trimester concentrations of Cd was 0.0 μg/L, there was a significant decrease in Pb (0.04 μg/dL) and Hg (0.12 μg/L) and an increase in Mn (3.30 μg/L) concentrations over the course of the pregnancy (Supplemental Material Table S3).

For this study, we considered maternal blood Pb, Cd, and Hg concentrations at or exceeding 4 μg/dL, 5.1 μg/L and 8 μg/L, respectively, reportable and a copy of the individual's results were shared with the participant's health care provider for follow-up. Less than 1% of the women had blood Pb, Cd or Hg concentrations at or exceeding the MIREC reporting levels.

3.2. Factors associated with maternal blood metal levels

Women less than 25 years of age had significantly higher blood Cd concentrations, while older women had higher blood Pb; GM concentrations for Hg increased with increasing age (Table 2). Women who had never smoked had significantly lower blood Cd and Pb, whereas Mn levels were higher in never smokers and Hg was lowest in current smokers or who had recently quit. Higher blood Cd concentrations were observed in women who were less educated and had lower household incomes. Obese women had higher blood Mn and lower Hg. Mercury geometric mean blood concentrations were also positively associated with household income. Maternal Cd and Pb blood concentrations were significantly lower when collected in the spring compared to summer months.

Maternal country of birth outside Canada was significantly associated with elevated levels of all metals (Table 2). A further examination showed that women born in Canada had significantly lower GM Cd than women born in Asian countries, lower Pb than women born in Africa, Asia, the Caribbean, Eastern Europe and Europe, lower Mn than women born in Asia, and lower Hg than women born in Asia, the Caribbean, Central or South America and Europe (Supplemental Material, Table S4).

3.3. Factors associated with fetal exposure

Among the three metals with sufficient detection in cord blood, Mn and Hg were significantly higher in male infants than in females (Table 3). Similar to maternal blood concentrations, cord blood Hg was significantly lower in current smokers. Of the metals, only Mn

Table 2
Univariate predictors of maternal blood metal concentrations (μg/L) based on data from 1st and 3rd trimesters (n = 3611).

Characteristic	N[a]	Cadmium			Lead		
		p-value	Pairwise[b]	GM (95% CI)	p-value	Pairwise[b]	GM (95% CI)
Maternal age							
<25	244	0.0001	A	0.2655 (0.2254, 0.3128)	<0.0001	A	0.5383 (0.4858, 0.5965)
25–29	848		B	0.2021 (0.1848, 0.2209)		A	0.5433 (0.5137, 0.5747)
30–34	1290		B	0.2026 (0.1885, 0.2177)		A	0.5807 (0.5550, 0.6076)
≥35	1229		B	0.2218 (0.2060, 0.2388)		B	0.6729 (0.6423, 0.7048)
Parity							
0	1604	0.0222	A	0.2209 (0.2082, 0.2343)	0.0070	A	0.6198 (0.5970, 0.6435)
1	1447		B	0.2015 (0.1894, 0.2143)		B	0.5773 (0.5551, 0.6005)
2+	557		AB	0.2212 (0.2002, 0.2444)		AB	0.5921 (0.5557, 0.6309)
Maternal smoking status							
Current/quit during pregnancy	427	<0.0001	A	0.5933 (0.5398, 0.6520)	0.0007	A	0.6281 (0.5854, 0.6739)
Former	974		B	0.2081 (0.1953, 0.2217)		A	0.6284 (0.5992, 0.6590)
Never	2205		C	0.1752 (0.1680, 0.1828)		B	0.5790 (0.5608, 0.5978)
Maternal education							
High school or less	309	<0.0001	A	0.3586 (0.3152, 0.4081)	<0.0001	A	0.6076 (0.5588, 0.6608)
College courses or diploma	1031		B	0.2200 (0.2049, 0.2362)		B	0.5489 (0.5241, 0.5749)
University degree	2267		C	0.1948 (0.1856, 0.2044)		A	0.6210 (0.6018, 0.6409)
Place of birth							
Elsewhere	677	0.0281	A	0.2294 (0.2129, 0.2471)	<0.0001	A	0.7489 (0.7152, 0.7843)
Canada	2934		B	0.2091 (0.2018, 0.2167)		B	0.5679 (0.5555, 0.5806)
Pre-pregnancy BMI (kg/m^2)							
<25 (underweight-normal)	2141	0.2540		0.2155 (0.2049, 0.2266)	0.0001	A	0.6171 (0.5974, 0.6374)
25–29 (overweight)	720			0.2073 (0.1902, 0.2259)		B	0.5641 (0.5337, 0.5962)
≥30 (obese)	487			0.1999 (0.1800, 0.2221)		B	0.5526 (0.5164, 0.5913)
Household income							
≤ $50,000	618	<0.0001	A	0.2634 (0.2404, 0.2887)	0.0022	A	0.6389 (0.6021, 0.6781)
$50,001–100,000	1433		B	0.2056 (0.1934, 0.2184)		B	0.5764 (0.5541, 0.5996)
> $100,000	1398		B	0.1941 (0.1825, 0.2064)		AB	0.6023 (0.5786, 0.6269)
Season blood collected							
Fall	995	0.0007	AB	0.2105 (0.1984, 0.2234)	<0.0001	A	0.6184 (0.5965, 0.6411)
Winter	826		B	0.2180 (0.2046, 0.2323)		B	0.5885 (0.5663, 0.6115)
Spring	947		A	0.2002 (0.1885, 0.2127)		B	0.5731 (0.5525, 0.5945)
Summer	843		B	0.2247 (0.2110, 0.2394)		A	0.6117 (0.5888, 0.6355)
Fasting status							
No	3532	0.0706		0.2122 (0.2054, 0.2192)	0.0952		0.5971 (0.5849, 0.6095)
Yes	69			0.2427 (0.2097, 0.2811)			0.6418 (0.5891, 0.6992)

Characteristic	N[a]	Manganese			Total mercury		
		p-value	Pairwise[b]	GM (95% CI)	p-value	Pairwise[b]	GM (95% CI)
Maternal age							
<25	244	0.8624		10.3125 (9.6259, 11.0481)	<0.0001	A	0.2650 (0.2123, 0.3309)
25–29	848			10.1628 (9.7915, 10.5481)		B	0.4064 (0.3606, 0.4580)
30–34	1290			10.1808 (9.8784, 10.4923)		C	0.5505 (0.5000, 0.6061)
≥35	1229			10.2915 (9.9785, 10.6143)		D	0.7873 (0.7136, 0.8686)
Parity							
0	1604	0.0277	A	10.0181 (9.7758, 10.2664)	0.3286		0.5687 (0.5236, 0.6178)
1	1447		B	10.3829 (10.1190, 10.6537)			0.5451 (0.4998, 0.5945)
2+	557		AB	10.4172 (9.9938, 10.8585)			0.5156 (0.4484, 0.5929)
Maternal smoking status							
Current/quit during pregnancy	427	0.0002	A	9.5655 (9.1269, 10.0251)	<0.0001	A	0.4112 (0.3523, 0.4798)
Former	974		AB	10.0944 (9.7837, 10.4150)		B	0.6084 (0.5483, 0.6751)
Never	2205		B	10.4176 (10.2029, 10.6369)		B	0.5579 (0.5201, 0.5984)
Maternal education							
High school or less	309	0.8995		10.2576 (9.7031, 10.8438)	<0.0001	A	0.3147 (0.2623, 0.3775)
College courses or diploma	1031			10.1732 (9.8673, 10.4886)		B	0.4506 (0.4078, 0.4979)
University degree	2267			10.2415 (10.0321, 10.4553)		C	0.6529 (0.6102, 0.6986)
Place of birth							
Elsewhere	677	<0.0001	A	10.9977 (10.6650, 11.3408)	<0.0001	A	0.9061 (0.8191, 1.0022)
Canada	2934		B	10.0534 (9.9063, 10.2027)		B	0.4913 (0.4680, 0.5158)
Pre-pregnancy BMI (kg/m^2)							
<25 (underweight-normal)	2141	0.0256	A	10.1639 (9.9496, 10.3829)	<0.0001	A	0.6155 (0.5737, 0.6605)
25–29 (overweight)	720		A	10.0994 (9.7365, 10.4759)		B	0.5253 (0.4658, 0.5924)
≥30 (obese)	487		B	10.7119 (10.2445, 11.2007)		C	0.3998 (0.3449, 0.4635)
Household income							
≤ $50,000	618	0.3504		10.2688 (9.8727, 10.6809)	<0.0001	A	0.4246 (0.3728, 0.4836)
$50,001–100,000	1433			10.2995 (10.0357, 10.5701)		B	0.5071 (0.4653, 0.5528)
> $100,000	1398			10.0816 (9.8200, 10.3501)		C	0.6795 (0.6227, 0.7414)
Season blood collected							
Fall	995	0.0004	A	10.1519 (9.8500, 10.4630)	<0.0001	A	0.5580 (0.5165, 0.6029)
Winter	826		AC	9.8634 (9.5429, 10.1947)		AB	0.5142 (0.4736, 0.5583)
Spring	947		AB	10.5369 (10.2163, 10.8676)		AB	0.5295 (0.4895, 0.5728)
Summer	843		AB	10.3193 (9.9872, 10.6623)		AC	0.6026 (0.5554, 0.6538)

Table 2 (*continued*)

Characteristic	N[a]	Manganese			Total mercury		
		p-value	Pairwise[b]	GM (95% CI)	p-value	Pairwise[b]	GM (95% CI)
Fasting status							
No	3532	0.4685		10.2256 (10.0886, 10.3645)	0.5120		0.5492 (0.5249, 0.5747)
Yes	69			9.9111 (9.1132, 10.7789)			0.5824 (0.4875, 0.6958)

[a] Number of blood samples.
[b] Results of statistical hypothesis testing of differences by group characteristics: groups with same letter indicate levels are statistically similar, whereas groups with different letters represent significant differences.

was detected in sufficient amounts in meconium to test for associations and neither infant sex, maternal smoking or season of collection were significantly associated with Mn concentrations in meconium (data not shown).

3.4. Nutrient-metal associations

Although most of the r-squares of the simple regressions were ≤1%, a number of significant trends were observed between nutrients and 3rd trimester metal concentrations in blood (Supplemental Tables). Maternal blood Cd, Pb and Hg concentrations increased significantly with quartiles of blood Mn concentrations (Table 4) and in the regression analysis (Supplemental Table S5). No significant association was observed between maternal Mn concentration in the 3rd trimester and Pb and Hg concentrations in cord blood; however, a significant positive association was observed between maternal blood Mn and cord blood Mn.

Ca intake was negatively associated with 3rd trimester maternal blood Cd, Pb, Mn and Hg, as well as cord blood Pb, according to the regression analysis (Supplemental Table S6). The GMs of maternal metal concentrations of Pb were significantly different by quartiles of Ca intake; however, we could not state which group GM was significantly lower than the other group means based on the Scheffé test (Table 5).

Although not consistent across quartiles (Table 6), higher estimates of Fe intake were significantly associated with higher blood Hg concentrations in the regression analysis (Supplemental Table S7). The 3rd quartile of Fe intake was associated with significantly lower blood Mn than the 1st quartile of intake.

Vitamin D intake was significantly associated with lower maternal blood Cd, Pb, and Mn, and lower Pb in cord blood in the regression analysis (Supplemental Table S8). Compared to the 4th quartile of vitamin D intake, the 1st quartile was associated with significantly higher 3rd trimester blood Cd and Mn concentrations (Table 7).

4. Discussion

4.1. Metals in the maternal-fetal unit

Only limited mother-child paired data have been published on the partitioning of Pb, Cd, Hg and Mn in maternal and cord blood and the sample sizes in many of these studies have been relatively small (n < 115) (Hu et al., 2015; Chen et al., 2014; García-Esquinas et al., 2013; Kopp et al., 2012; Kim et al., 2015). There are even less data available for meconium.

Our results demonstrate that the distribution of these four metals between maternal and cord blood differed by metal. Cadmium was detected in over 96% of maternal bloods, but rarely detected in cord blood samples. In contrast, Pb and Mn were detected in almost all maternal and cord blood samples, while Hg was detected in about 90% of maternal and 72% of cord blood samples. It is difficult, however, to make a direct comparison of percentages detected in various matrices given the different LODs of each method. Median cord blood Pb, Hg and Mn concentrations were all statistically higher than maternal concentrations at 1st and 3rd trimesters, especially for Mn.

The concept of placental transfer of mobilised maternal skeletal stores of Pb is supported by the similarity of isotopic ratios in maternal and cord blood reported in a recent study (Gulson et al., 2016). A German study of mother-infant pairs reported similar median Pb concentrations in maternal and cord blood, and median cord Cd below the limit of quantification; however, median cord Hg was about threefold higher than maternal Hg (1.48 vs. 0.44 μg/L) (Kopp et al., 2012). A Korean study of 104 mother-child pairs also reported higher Hg (and methylmercury) in cord than in maternal blood, but not for Pb or Cd; median ratios of cord to 2nd trimester

Table 3
Predictors of cord blood metal concentrations (μg/L) (n = 1415).

Characteristic	Manganese			Lead			Total mercury		
	p-value	Pairwise[a]	GM (95% CI)	p-value	Pairwise[a]	GM (95% CI)	p-value	Pairwise[a]	GM (95% CI)
Infant gender									
Male (n = 751)	0.0002	A	32.4737 (31.6664, 33.3016)	0.5037		0.7549 (0.7273, 0.7836)	0.0065	A	0.8217 (0.7649, 0.8827)
Female (n = 664)		B	30.2640 (29.4658, 31.0838)			0.7411 (0.7124, 0.7710)		B	0.7118 (0.6595, 0.7683)
Maternal smoking									
Current (n = 152)	0.1745		30.6826 (28.6623, 32.8453)	0.7235		0.7716 (0.6973, 0.8538)	0.0067	A	0.6245 (0.5147, 0.7579)
Former (n = 371)			30.6651 (29.3493, 32.0400)			0.7601 (0.7121, 0.8113)		B	0.8434 (0.7450, 0.9548)
Never (n = 833)			31.8170 (30.9004, 32.7609)			0.7469 (0.7152, 0.7801)		B	0.7922 (0.7290, 0.8608)
Season of collection									
Fall (n = 339)	0.8491		31.8722 (30.2916, 33.5353)	<0.0001	AC	0.8488 (0.7884, 0.9138)	0.2943		0.8262 (0.7153, 0.9544)
Winter (n = 321)			31.2758 (29.6850, 32.9519)		A	0.7879 (0.7304, 0.8499)			0.7331 (0.6316, 0.8510)
Spring (n = 392)			31.2917 (29.8426, 32.8111)		AB	0.7129 (0.6655, 0.7637)			0.7300 (0.6372, 0.8364)
Summer (n = 364)			31.1967 (29.7033, 32.7651)		B	0.6706 (0.6245, 0.7202)			0.7890 (0.6862, 0.9071)

[a] Results of statistical hypothesis testing of differences by group characteristics: groups with same letter indicate levels are statistically similar, whereas groups with different letters represent significant differences.

Table 4
Maternal blood manganese quartiles and geometric means of other metals from 3rd trimester.

Metal concentration in maternal blood 3rd trimester	Potential predictor		N	p-value	Pairwise[a]	GM	95% CI of GM	
	Manganese concentration in maternal blood 3rd trimester							
	Category	Range (μg/L)						
Cadmium (μg/L)	Q1	[<LOD, 9.889]	357	<0.0001	A	0.1651	0.1523	0.1789
	Q2	[9.890, 12.6373]	564		AB	0.1847	0.1733	0.1969
	Q3	[12.6374, 15.3845]	322		B	0.2036	0.1871	0.2215
	Q4	[15.3846, 32.9588]	430		C	0.2435	0.2263	0.2620
Lead (μg/dL)	Q1	[<LOD, 9.889]	357	<0.0001	A	0.4946	0.4692	0.5214
	Q2	[9.890, 12.6373]	564		B	0.5571	0.5342	0.5810
	Q3	[12.6374, 15.3845]	322		BC	0.5944	0.5623	0.6284
	Q4	[15.3846, 32.9588]	430		C	0.6499	0.6194	0.6820
Mercury (μg/L)	Q1	[<LOD, 9.889]	357	0.0024	A	0.4307	0.3859	0.4806
	Q2	[9.890, 12.6373]	564		A	0.4559	0.4178	0.4975
	Q3	[12.6374, 15.3845]	322		AB	0.5057	0.4506	0.5676
	Q4	[15.3846, 32.9588]	430		B	0.5575	0.5045	0.6160

[a] Results of statistical hypothesis testing of differences by group characteristics: groups with same letter indicate levels are statistically similar, whereas groups with different letters represent significant differences.

Table 5
Estimated quartiles of maternal calcium intake during 2nd trimester (n = 527 missing) and geometric means of maternal blood metals in the 3rd trimester.

Metal concentration in maternal blood 3rd trimester	Potential predictor		N	p-value	Pairwise[a]	GM	95% CI of GM	
	Total calcium intake (mg/day)							
Cadmium (μg/L)	Q1	[147.48, 878.22]	283	0.6050		0.2072	0.1893	0.2269
	Q2	[878.23, 1154.69]	285			0.1974	0.1803	0.2160
	Q3	[1154.70, 1507.47]	291			0.1898	0.1735	0.2075
	Q4	[1507.48, 7318.97]	287			0.1976	0.1806	0.2163
Lead (μg/dL)	Q1	[147.48, 878.22]	283	0.0274	A	0.6182	0.5812	0.6575
	Q2	[878.23, 1154.69]	285		A	0.6060	0.5699	0.6445
	Q3	[1154.70, 1507.47]	291		A	0.5613	0.5281	0.5965
	Q4	[1507.48, 7318.97]	287		A	0.5538	0.5209	0.5888
Manganese (μg/L)	Q1	[147.48, 878.22]	283	0.4215		12.4830	12.0074	12.9774
	Q2	[878.23, 1154.69]	285			12.5615	12.0846	13.0572
	Q3	[1154.70, 1507.47]	291			12.2597	11.7989	12.7384
	Q4	[1507.48, 7318.97]	287			12.0413	11.5857	12.5148
Mercury (μg/L)	Q1	[147.48, 878.22]	283	0.1061		0.5596	0.4947	0.6330
	Q2	[878.23, 1154.69]	285			0.4743	0.4195	0.5363
	Q3	[1154.70, 1507.47]	291			0.4578	0.4054	0.5169
	Q4	[1507.48, 7318.97]	287			0.4772	0.4222	0.5393

[a] Results of statistical hypothesis testing of differences by group characteristics: groups with same letter indicate levels are statistically similar, whereas groups with different letters represent significant differences.

Table 6
Estimated quartiles of maternal iron intake during 2nd trimester (n = 527 missing) and geometric means of maternal blood metals in the 3rd trimester.

Metal concentration in maternal blood 3rd trimester	Potential predictor		N	p-value	Pairwise[a]	GM	95% CI of GM	
	Total iron intake (mg/day)							
Cadmium (μg/L)	Q1	[2.33, 11.85]	276	0.6929		0.2036	0.1857	0.2232
	Q2	[11.86, 33.72]	289			0.2018	0.1845	0.2208
	Q3	[33.73, 37.15]	288			0.1895	0.1732	0.2074
	Q4	[37.16, 337.52]	293			0.1970	0.1802	0.2153
Lead (μg/dL)	Q1	[2.33, 11.85]	276	0.8415		0.5848	0.5493	0.6227
	Q2	[11.86, 33.72]	289			0.5878	0.5528	0.6250
	Q3	[33.73, 37.15]	288			0.5705	0.5365	0.6066
	Q4	[37.16, 337.52]	293			0.5927	0.5577	0.6299
Manganese (μg/L)	Q1	[2.33, 11.85]	276	0.0177	AB	12.9825	12.4833	13.5016
	Q2	[11.86, 33.72]	289		A	12.2184	11.7591	12.6956
	Q3	[33.73, 37.15]	288		AC	11.9039	11.4556	12.3696
	Q4	[37.16, 337.52]	293		A	12.2818	11.8232	12.7582
Mercury (μg/L)	Q1	[2.33, 11.85]	276	0.0539		0.4741	0.4186	0.5371
	Q2	[11.86, 33.72]	289			0.4793	0.4243	0.5414
	Q3	[33.73, 37.15]	288			0.4490	0.3974	0.5072
	Q4	[37.16, 337.52]	293			0.5648	0.5004	0.6374

[a] Results of statistical hypothesis testing of differences by group characteristics: groups with same letter indicate levels are statistically similar, whereas groups with different letters represent significant differences.

Table 7
Estimated quartiles of maternal vitamin D intake during 2nd trimester (n = 527 missing) and geometric means of maternal blood metals in the 3rd trimester.

Metal concentration in maternal blood 3rd trimester	Potential predictor		N	p-value	Pairwise[a]	GM	95% CI of GM	
	Total vitamin D intake (IU/day)							
Cadmium (μg/L)	Q1	[9.13, 388.88]	280	0.0187[b]	A	0.2133	0.1947	0.2336
	Q2	[388.89, 582.92]	286		AB	0.1935	0.1768	0.2117
	Q3	[582.93,723.55]	286		AB	0.2001	0.1829	0.2190
	Q4	[723.56, 7966.11]	294		B	0.1861	0.1703	0.2035
Lead (μg/dL)	Q1	[9.13, 388.88]	280	0.0975		0.6101	0.5733	0.6492
	Q2	[388.89, 582.92]	286			0.5883	0.5532	0.6256
	Q3	[582.93,723.55]	286			0.5924	0.5570	0.6299
	Q4	[723.56, 7966.11]	294			0.5483	0.5161	0.5826
Manganese (μg/L)	Q1	[9.13, 388.88]	280	0.0059	AB	12.9505	12.4566	13.4640
	Q2	[388.89, 582.92]	286		A	12.2218	11.7605	12.7012
	Q3	[582.93,723.55]	286		A	12.4581	11.9878	12.9467
	Q4	[723.56, 7966.11]	294		AC	11.7624	11.3244	12.2174
Mercury (μg/L)	Q1	[9.13, 388.88]	280	0.4147		0.4684	0.4137	0.5302
	Q2	[388.89, 582.92]	286			0.4660	0.4122	0.5269
	Q3	[582.93,723.55]	286			0.4982	0.4406	0.5633
	Q4	[723.56, 7966.11]	294			0.5302	0.4698	0.5985

[a] Results of statistical hypothesis testing of differences by group characteristics: groups with same letter indicate levels are statistically similar, whereas groups with different letters represent significant differences.
[b] The analysis is based on the non-parametric Kruskal Wallis test; the confidence intervals were calculated on the parametric analysis.

maternal blood were 0.72, 0.03 and 1.49 for Pb, Cd, and total Hg, respectively (Kim et al., 2015). Similarly, a South African study also reported higher Hg in cord blood than in maternal blood (Rudge et al., 2009). An estimate of the mean cord to maternal blood ratio for methylmercury and inorganic mercury based on a meta-analysis was 1.89 and 1.01, respectively (Ou et al., 2014). Mercury in cord blood is primarily methylmercury (Hackett and Kelman, 1983; Kim et al., 2015), and cord blood has about the same level of inorganic Hg as in maternal blood (Ou et al., 2014). The methylmercury fraction (usually >98%) of total Hg binds to hemoglobin (Hb) in erythrocytes (Rudge et al., 2009) and can pass easily through the placenta to reach the fetus. As Hb levels decrease over the course of pregnancy (Abbassi-Ghanavati et al., 2009) and are higher in cord than maternal blood (Lao et al., 1991), this may explain the disparity between maternal and cord Hg levels, provided fish consumption remains unchanged and plasma volume expansion is considered. The difference in Hg levels could simply represent a passive equilibrium that is due to binding of methylmercury in Hb rather than an active transfer of metals.

In our study, cord blood median concentrations of Mn (31.87 μg/L) were over threefold higher than 1st trimester (8.79 μg/L) and 2.5 times higher than 3rd trimester maternal concentrations (12.64 μg/L). The German study also reported that Mn was about 70% higher in cord (28.8 μg/L) than maternal blood samples collected at delivery (17.0 μg/L), although our 3rd trimester median Mn concentrations were considerably higher than the German concentrations (Kopp et al., 2012). Similar results were also reported in a US mother-infant pair study (Zota et al., 2009) which reported a 45% increase between maternal Mn concentrations at delivery and those in cord blood with a correlation of 0.38. It has been hypothesized that Mn crosses the placenta via active transport; however, other explanations for the higher Mn concentrations in cord blood, such as lower or restricted elimination of the element by the fetus or inability of the fetus to utilize Mn, may account for this observation (Nandakumaran et al., 2015). Mn was the only metal that was detected in 100% of the meconium samples in the MIREC Study which would suggest that the fetus is able to eliminate Mn. While the correlations between maternal or cord blood Mn with meconium Mn were statistically significant, they were low (ranging from 0.13 for 1st trimester to 0.25 for 3rd trimester blood vs. meconium).

4.2. Changes in metal levels throughout pregnancy

In populations with higher mean blood Pb concentrations (≥2 μg/dL), Pb concentrations in the same subjects tend to display a U-shaped curve over three trimesters due to mobilisation of Pb from the bone to meet increasing demands for Ca (Gulson et al., 2016; Hertz-Picciotto et al., 2000; Schell et al., 2000; Rothenberg et al., 1994). However, as seen in our study, populations with a mean blood Pb concentration <1.2 μg/dL do not display this pattern (Sowers et al., 2002; Rabito et al., 2014). In addition to variations in exogenous exposure, blood Pb concentrations can vary due to changes in hematocrit and Ca levels, plasma volume and to mobilisation of Pb from bones during pregnancy (Gulson et al., 2016). However, several studies have reported that correcting blood Pb concentrations for hematocrit did not change their findings (Gulson et al., 2016; Hertz-Picciotto et al., 2000; Schell et al., 2000). The extent of exposure may also determine the relationship between hematocrit and blood Pb concentrations in pregnancy. La-Llave-León et al. (2015) reported that Hb level, hematocrit, and red blood cell count were significantly higher in pregnant women with a blood Pb concentration ≥ 5 μg/dL than in the group with lower blood Pb concentrations. The extent of plasma volume expansion during pregnancy may also impact the concentration of Pb measured in blood later in pregnancy. Maternal blood volume increases (approximately 45%) starting around 6–8 weeks of gestation and peaks at 32 weeks (Hytten and Paintin, 1963); however, health conditions such as fetal growth restriction or preeclampsia may be associated with lower plasma volume expansion (Salas et al., 2006) and thus affect the interpretation of the results.

Studies that have conducted prospective sampling of Mn levels during pregnancy are scarce. An early Australian study reported a significant increase in mean whole blood Mn at 10–20 weeks (8.24 μg/L), 25 weeks (9.43 μg/L), and 34 weeks (12.64 μg/L) gestation (Spencer, 1999). A study of Costa Rican women reported that blood Mn concentrations increased significantly between the 2nd and 3rd trimesters of pregnancy and calculated an ICC of 0.44 for all three trimesters (Mora et al., 2014). Our study also reported a significant increase in blood Mn between the 1st and 3rd trimesters, with an ICC of 0.29. Approximately 66% of Mn in blood is bound to the Hb protein in erythrocytes and Mn levels in maternal erythrocytes have been shown to increase significantly over the

three trimesters (Tsai et al., 2015). Elevated blood Mn concentrations later in pregnancy may be attributable to increased intestinal absorption, which has been reported in later stages of pregnancy in a rat model (Kirchgessner et al., 1982). While Hb levels decrease from 1st to 3rd trimester (Abbassi-Ghanavati et al., 2009), Mn levels may increase because of increased absorption and possibly increased binding capacity towards Hb to ensure sufficiency.

An analysis of maternal blood Hg in the ELEMENT Study reported no significant difference across the three trimesters and an ICC of 0.37 (Basu et al., 2014), compared to a significant decrease in maternal Hg in the 3rd trimester and an ICC of 0.60 in MIREC. Blood volume expansion later in pregnancy may be responsible for lower concentrations of both Hg and Hb (along with other constituents), although a reduction in fish consumption in the 3rd trimester could also explain the downward trend.

We found no significant difference in maternal Cd concentrations over the pregnancy. A Chinese study also reported consistent blood Cd concentrations across all three trimesters (Liu et al., 2013).

4.3. Comparison of levels of various metals in MIREC and other studies

A comparison of blood metal concentrations in pregnant women from various countries, as well as women of reproductive age in Canada, showed that MIREC women tended to have among the lowest geometric means for Pb, Cd and Hg (Fig. S4). With the exception of a study in Costa Rica (Mora et al., 2015), maternal Mn concentrations in MIREC and other international studies of pregnant women were similar and comparable to a Canadian population-based national survey of women aged 20–39 years sampled over the same time period (9.8–11 μg/L) (Health Canada, 2010b, 2013b) (Fig. S5). The normal range of blood Mn concentrations in the general population is 4–15 μg/L (ATSDR, 2012). Concentrations in MIREC pregnant women ranged from 2 to 33.5 μg/L and up to 98.9 μg/L in corresponding cord blood samples.

Studies which have measured metals in meconium are rare. A Japanese study reported trace levels of Pb and to a lesser extent Cd in meconium (Yang et al., 2013), similar to the results reported in MIREC. In contrast, studies in high pollution areas of Turkey (Turker et al., 2006), and the Philippines (Ostrea et al., 2002), as well as China (Peng et al., 2015) and Taiwan (Jiang et al., 2014) reported higher metal levels than our study. Concentrations of Mn in meconium from earlier studies also appear to be substantially higher (35.8 μg/g at 38–42 weeks gestation) (Haram-Mourabet et al., 1998) than the concentrations measured in MIREC (median 4.9 μg/g).

4.4. Maternal smoking and blood Mn

Consistent inverse associations have been observed between maternal smoking and blood Mn concentrations. In MIREC and in a study of pregnant women from Costa Rica (Mora et al., 2014), smoking was associated with significantly lower maternal blood Mn concentrations. Similar associations were observed in cord blood, where maternal smoking was associated with a non-significant but slightly lower GM Mn concentration in our study and in other smaller studies (Mistry and Williams, 2011; Jones et al., 2010). These results are surprising as, in addition to other metals such as Hg, Cd, Pb, and As (Afridi et al., 2015), cigarette smoke also contains Mn (Pappas et al., 2014), which might be expected to result in higher maternal and cord blood Mn concentrations. Several mechanisms may explain why blood Mn concentrations are lower in smokers. Oxidative stress is a major pathophysiological factor in the multiple adverse effects of smoking during pregnancy (Stone et al., 2014). As Mn is an essential nutrient that plays a key role in cellular adaptation to oxidative stress (Aguirre and Culotta, 2012), Mn cellular uptake may increase in smokers, resulting in lower Mn in blood and reduced availability for fetal transfer in pregnant smokers. In addition, the other metals in smoke such as Cd can compete with Mn for blood transporters which would lead to lower blood concentrations. The divalent metal transporter 1 (DMT1) has an affinity not only for Mn but also for other divalent cations including Fe, cobalt, nickel, Cd, Pb, copper, and zinc (Kayaalti et al., 2015).

4.5. Association between relative nutrient intake and maternal and cord blood metal levels

In our study, the GM maternal blood Pb concentration in the 3rd trimester was about 11% lower in the highest compared to the lowest quartile of relative Ca intake and regression analysis showed negative correlations between maternal Ca intake and both maternal and cord blood Pb. In addition to differences in sources and degree of exposure, nutrient intake may explain some of the variability in metal concentrations observed in various studies of pregnant women. Ca intake has been negatively associated with maternal Pb concentration, although the effect size has been small (Gulson et al., 2016; Hong et al., 2014; Taylor et al., 2013; Suarez-Ortegón et al., 2013; Jiang et al., 2011; Zentner et al., 2008). When blood Ca concentrations are low during pregnancy, more mobilisation from bone is required to meet fetal demands leading to more Pb also being mobilised from bone (Gulson et al., 2016). In a randomized placebo-controlled trial in Mexico City where GM blood Pb was about 4.0 μg/dL, a 1200 mg daily calcium carbonate supplement was associated with modest reductions in blood Pb when administered during pregnancy (Ettinger et al., 2009). While Ca was negatively associated with maternal Cd concentrations in the regression analysis indicating a small negative linear relationship, there was no significant difference in the GMs of Cd when quartiles of Ca intake were examined. One other study reported no association between Ca intake and 3rd trimester blood Cd (Suarez-Ortegón et al., 2013).

Vitamin D intake was negatively associated with maternal blood Cd, Pb and Mn in the regression analysis but only for Cd did the GMs for the categories vary significantly with the highest quartile of intake resulting in about 13% reduction in the GM for Cd compared to the lowest quartile of intake. Regression analysis also suggested that vitamin D intake was negatively associated with cord blood Pb. There is limited evidence in the literature of associations between vitamin D and blood metals. Negative associations have been observed between maternal dietary intakes of vitamin D and cord blood Pb (Schell et al., 2003) and 25(OH)D concentration in Chinese children (Chang et al., 2014). However, an analysis of premenopausal women in the U.S. NHANES found no association between vitamin D intake and blood Pb (Jackson et al., 2010). Studies examining vitamin intake and blood Cd are rare with a path analysis in a cross-sectional study of Korean women showing that depressed vitamin D levels increased the risk of iron deficiency anemia, and that this anemia increased Cd concentrations in blood (Suh et al., 2016).

Given the coincidence of sources in maternal diets and the strong interdependence of both Ca and vitamin D, it is not surprising to observe similar effects on blood metal concentrations.

Higher maternal Fe intake has been associated with lower cord blood Pb (Schell et al., 2003) and small reductions in maternal blood Pb (Taylor et al., 2013; Jiang et al., 2011). Maternal blood Cd was negatively associated with Fe intake in one study (Nishijo et al., 2004) but showed no association in another (Suarez-Ortegon et al., 2013). However, no such relationships between Fe intake and blood Pb or Cd were observed in our study. Women tend to have higher

blood Cd concentrations than men (Health Canada, 2013b) which may be explained by increased intestinal absorption of dietary Cd when Fe stores are low (Berglund et al., 1994) and especially during pregnancy (Akesson et al., 2002). In our regression analysis, Fe intake was positively associated with maternal blood Hg, which agrees with a cross-sectional study of Inuit women from northern Canada that observed that ferritin was positively correlated with blood Hg (Plante et al., 2011). This positive association may be explained by shared major sources of Fe and Hg in the diet of northern populations. The effect of Fe on Hg levels could also potentially be impacted by maternal Hb levels. We did not have reliable complete blood counts to test this hypothesis.

Although we observed a significant association between quartiles of Fe intake and maternal blood Mn, no consistent trend was observed. During conditions of low Fe, abnormal absorption (Finley, 1999) and accumulation of Mn can occur because both share and compete for many protein transporters responsible for absorption (Fitsanakis et al., 2010). On the other hand, when Mn concentrations change, the homeostasis and deposition of Fe and other transition metals are affected (Fitsanakis et al., 2010). Iron/folic acid supplement use has been associated with decreased concentrations of blood Mn in late pregnancy in a small Australian study (Callan et al., 2013). However a small clinical trial of Fe supplementation in pregnancy reported no significant difference in blood Mn, although Fe supplemented women had significantly higher concentrations of blood Hb and serum ferritin compared to the placebo group (Tholin et al., 1995). Another study reported that taking Fe supplements throughout pregnancy increased Mn levels (Spencer, 1999). Increased Mn absorption during pregnancy or change in how the body distributes or metabolizes Mn during pregnancy may explain the increased blood Mn concentrations in pregnancy compared to the non-pregnant state (Tholin et al., 1995). The wide range of maternal Mn concentrations in whole blood (2–33.5 μg/L) observed in MIREC may reflect different diets, use of supplements and varying degrees of Fe deficiency status among the population, although the latter could not be measured.

In our study, increasing maternal blood Mn concentrations were associated with higher blood Cd, Hg and Pb concentrations in the 3rd trimester. Several studies have reported a relationship between blood Mn and levels of other metals. In an analysis of NHANES data, a non-linear relationship was observed between blood Mn with Cd, Pb and Hg concentrations (Oulhote et al., 2014). A Taiwanese study has reported that an increase in maternal blood Mn at birth from the 25th to 75th percentile (15.8–28.4 μg/L) appeared to decrease the placental transfer of Pb in cord blood by 0.12 μg/dL (Lin et al., 2010). However, although our quartiles of Mn were lower, we found no significant difference between quartiles of maternal Mn in the 3rd trimester and cord blood Pb. Maternal blood Pb concentrations have also been associated with dose-dependent decreases in cord Mn (Kopp et al., 2012). The mechanism by which Pb reduces Mn (and Fe) in the fetal unit remains to be ascertained.

4.6. Strengths and limitations

A major strength of this study is the serial collection of biospecimens over the pregnancy in several matrices as well as key information on important covariates that might affect exposure levels in this population. Although the study population was relatively large and geographically diverse, similar to many observational studies, participants were on average from a higher socioeconomic stratum (Arbuckle et al., 2013). This limits our ability to generalize the findings to other population groups. Our analysis did not examine genetic variation within the population which could play a significant role in determining metal absorption and distribution. There is also uncertainty associated with the estimation of dietary nutrient intake. The FFQ may not have captured all potential sources of these nutrients, therefore may have underestimated some nutrient intakes. Also, although the FFQ was designed to obtain relative total intakes of Fe, Ca and vitamin D for Canadian pregnant women, the estimated intakes from diet and supplements are not sufficient to determine whether individuals met the daily recommended intake for these nutrients in pregnancy. Furthermore, there may be other important vitamins (e.g., vitamin C), minerals (e.g., selenium, phosphorus, magnesium, zinc) and dietary factors (e.g., fat) that are important for metal toxicokinetics. However, using these categorized estimates for a few key nutrients, we demonstrated in a large sample of pregnant women from across Canada that estimated intakes from diet and supplements were associated with some maternal blood metal concentrations.

4.7. Summary, recommendations and conclusions

The MIREC Study offers a unique opportunity to estimate fetal exposure to environmental chemicals of interest *in utero*: 1) indirectly, by assessing the nature and degree of maternal exposure through measurement of metals in blood samples and 2) directly, by measuring metal concentrations in cord blood and meconium. In general, with the exception of 3rd trimester Mn, blood metal concentrations were lower in MIREC than in the general population of women aged 20–39 in Canada. As our study and others (Spencer, 1999; Tsai et al., 2015; Mora et al., 2015) have shown, blood Mn concentrations increase over the course of the pregnancy, although guidelines on appropriate levels in pregnancy are not available.

Our results suggest placental transfer of Cd is limited, Pb can cross easily and Hg and Mn may accumulate in cord blood. This latter conclusion is further strengthened by examining metal concentrations in meconium, where Cd was detected in only 3%, Pb in 20%, Hg in 25% and Mn in 100% of the samples. There are likely different modes of action for placental transfer of Pb and Hg, with one group suggesting more active transport of Hg to the fetus, while Pb transfer may be more passive (Gundacker et al., 2010). However, given the binding of methylmercury to Hb, active transport of Hg may be less plausible. Maternal-fetal transfer of metals is a major source of early life exposure for Cd, Hg and Pb (Chen et al., 2014). Large variability in maternal and fetal exposure levels were observed in our study, suggesting both individual differences in exposure and heterogeneity in placental transfer. Nonetheless, minimizing maternal exposure will reduce fetal exposure.

Given the probability of maternal-fetal metal transfer and the heightened health concerns due to the sensitivity of a developing fetus to the presence of such metals, more effort needs to be expended to both establish national/international guidelines and to identify vulnerable populations and individuals at risk. A recent paper has reiterated the need for international consensus on levels of concern for Pb, Cd and Hg exposure during pregnancy, which requires ongoing monitoring of levels in pregnant women (Taylor et al., 2014) and prospective studies of potential health effects in the pregnant woman and her child. In addition, normative concentrations for blood Mn during pregnancy need to be established (Kopp et al., 2012).

To date, no agency has proposed guidelines for blood Hg concentrations in pregnant women. In Canada, a provisional blood guidance value of 8 μg/L for methylmercury has been proposed for children, pregnant women and women of childbearing age (Legrand et al., 2010). The U.S. EPA reference dose for methylmercury is 0.1 μg/kg/day (https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=20873&CFID=55941601&CFTOKEN=41278506, accessed April 6, 2016) associated with a cord blood methylmercury value of 5.8 μg/L (National Research Council, 2000). Canadian academic researchers have concluded that a proposed

periconceptional blood Hg screening program for women planning a pregnancy would be highly cost-effective from a societal perspective (Gaskin et al., 2015). These researchers used an intervention level equal to the threshold for adverse neurodevelopmental effects of 3.4 μg/L in maternal blood and recommended that women replace high Hg fish in their diet with low Hg/high polyunsaturated fatty acid fish (Gaskin et al., 2015). Fewer than 5% of the MIREC women had a blood Hg concentration exceeding 3.4 μg/L. American (U.S. FDA, 2014) and Canadian (Health Canada, 2011) agencies have recommended that pregnant women and women of childbearing age limit consumption of certain types of fish that may have higher Hg concentrations.

The U.S. Occupational Safety and Health Administration blood Cd threshold for intervention for employees is 5 μg/L (https://www.osha.gov/Publications/osha3136.pdf, accessed August 11, 2015) and the threshold for Pb intervention in pregnant women promoted by the U.S. CDC is 5 μg/dL (http://www.cdc.gov/nceh/lead/publications/leadandpregnancy2010.pdf, accessed August 11, 2015). No threshold for intervention for blood Mn could be found.

Our results, consistent with some of the literature, provide some support that at even at current levels, higher Ca and vitamin D intake can be associated with lower maternal blood Pb and Cd concentrations. However, the potential benefits of increasing intake of vitamin D (or Ca) on maternal metal concentrations needs to be further studied, given that others have suggested that excessive vitamin D intake may enhance absorption of toxic metals (Schwalfenberg and Genuis, 2015; Moon, 1994).

Future research on this prospective cohort and other similar cohorts will generate the potential health effects data needed to develop policy on blood metal concentrations in pregnancy and to establish normative concentrations of blood Mn during pregnancy.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2016.08.023.

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