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Maternal blood metal levels and fetal markers of metabolic function ☆

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ABSTRACT

Exposure to metals commonly found in the environment has been hypothesized to be associated with measures of fetal growth but the epidemiological literature is limited. The Maternal–Infant Research on Environmental Chemicals (MIREC) study recruited 2001 women during the first trimester of pregnancy from 10 Canadian sites. Our objective was to assess the association between prenatal exposure to metals (lead, arsenic, cadmium, and mercury) and fetal metabolic function. Average maternal metal concentrations in 1st and 3rd trimester blood samples were used to represent prenatal metals exposure. Leptin and adiponectin were measured in 1363 cord blood samples and served as markers of fetal metabolic function. Polytomous logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (CI) for the association between metals and both high ($\geq$90%) and low ($\leq$10%) fetal adiponectin and leptin levels. Leptin levels were significantly higher in female infants compared to males. A significant relationship between maternal blood cadmium and odds of high leptin was observed among males but not females in adjusted models. When adjusting for birth weight *z*-score, lead was associated with an increased odd of high leptin. No other significant associations were found at the top or bottom 10th percentile in either leptin or adiponectin models. This study supports the proposition that maternal levels of cadmium influence cord blood adipokine levels in a sex-dependent manner. Further investigation is required to confirm these findings and to determine how such findings at birth will translate into childhood anthropometric measures.

1. Introduction

Early pregnancy is a critical window of fetal development and exposure to environmental contaminants during this time period may adversely impact the pregnancy as well as neonatal, early childhood, and later life outcomes (Gluckman et al., 2008; Selevan et al., 2000). Previous studies have suggested that prenatal exposure to metals, such as lead, mercury, cadmium, and arsenic, may adversely affect fetal growth (Drouillet-Pinard et al., 2010; Gundacker et al., 2010; Kippler et al., 2012; Lin et al., 2011; Menai et al., 2012; Schell et al., 2009; Xie et al., 2013; Zhu et al., 2010). Maternal exposure to lead, mercury, and arsenic creates direct exposure to the developing fetus as these chemicals can pass through the placenta into fetal circulation (Barr et al., 2007; Klaasen, 2010; Needham et al., 2011). Cadmium, which does not directly enter fetal circulation, can promote potentially adverse effects on the fetus by accumulating in the placenta and altering normal placental processes and function (Barr et al., 2007; Roels et al., 1978). Studies in North American populations have demonstrated that exposure is ubiquitous with the majority of women having detectable concentrations of metals in their blood or urine (Health Canada, 2010; NHANES, 2013). Epidemiologic literature is suggestive of an inverse association between maternal exposure to certain metals, particularly lead, and infant growth (Gonzalez-

☆This study received ethical approval from Health Canada, St. Justine's Hospital (Montreal, QC), and the IWK Health Centre (Halifax, NS). All study participants provided informed consent.

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Cossio et al., 1997; Schell et al., 2009; Xie et al., 2013; Zhu et al., 2010), but longitudinal analyses in North American populations without relatively high levels of exposure is limited.

Metabolic function can be examined by measuring leptin and adiponectin levels in blood, two hormones produced by adipocytes that play critical roles in metabolic function (Karakosta and Chatzi, 2011; Trujillo and Scherer, 2005; Walsh et al., 2014). Elevated levels of both leptin and adiponectin in umbilical cord blood are correlated with high birth weight and may provide insight on future risk of childhood obesity (Karakosta and Chatzi, 2011; Mantzoros et al., 2009). In adults, elevated leptin levels are associated with increased adipose tissue mass, insulin resistance (Mantzoros et al., 2009), and, in pregnant women having large for gestational age infants (Retnakaran et al., 2012). In contrast, low adiponectin levels in adulthood have been implicated in insulin resistance, type 2 diabetes, and metabolic syndrome (Mazaki-Tovi et al., 2005; Trujillo and Scherer, 2005). Examining the relationship between maternal metal concentrations and biomarkers of fetal metabolic function may provide insight into the susceptibility of fetal metabolic development to exogenous *in utero* exposures. The objectives of the present study were to assess the relationship between maternal blood levels of lead, arsenic, cadmium, and mercury and umbilical cord blood levels of leptin and adiponectin among the cohort of mother–infants pairs enrolled in the Maternal–Infant Research on Environmental Chemicals (MIREC) study.

2. Material and methods

2.1. Study design

Details of the MIREC study have been previously reported (Arbuckle, et al., 2013). Briefly, 2001 women were recruited from 10 Canadian sites during their first trimester and consented to provide urine and blood samples. Women were eligible for inclusion if they were <14 weeks gestation at the time of recruitment, ≥ 18 years of age, able to communicate in French or English, and planning on delivering at a local hospital. Women with known fetal or chromosomal anomalies in the current pregnancy and women with serious medical complications were excluded from the study (Arbuckle et al., 2013). Of the 2001 women recruited into the MIREC study, 18 withdrew and asked that all their data and biospecimens be destroyed. Of the remaining 1983 subjects, 1363 had infants with a cord blood sample. For this analysis, 103 were excluded for: multiple birth, pre-term birth, cord blood samples unsuitable for analysis, missing metal data or unknown infant sex, resulting in an analytical sample size of 1260.

2.2. Metal exposure

Chemical analysis of blood samples were carried out at the Laboratoire de Toxicologie, Institut National de Santé Publique du Québec (INSPQ) (Québec, QC, Canada), accredited by the Standards Council of Canada. Lead, arsenic, mercury, and cadmium were measured in maternal whole blood collected during the 1st and 3rd trimesters using inductively coupled plasma mass spectrometry (PerkinElmer ELAN ICP-MS DRC II). Metal concentrations from the two time points were averaged to create an estimate of gestational exposure. In the case where the value for one time point was missing, the other value was used. All samples below the level of detection (LOD) were imputed as one half the level of detection. We also conducted an analysis to determine whether samples collected in the third trimester would have a different influence on results compared to the first trimester measurements.

2.3. Fetal markers of metabolic function

Leptin and adiponectin were measured in plasma from 1363 stored umbilical cord blood samples by ELISA using kits from Meso Scale Discovery (MSD) (Rockville, MD, USA) at Mt. Sinai Laboratory (Toronto, ON, Canada). Repeated analysis was performed on all samples with a coefficient of variation (CV) greater than 15%. The inter- and intra-assay CVs, respectively, were 11.8% and 9.3% for leptin and 8% and 9% for adiponectin. All samples were above the limit of detection.

2.4. Covariates

Data on covariates were extracted from questionnaires and hospital charts by trained research staff. We examined the following variables as potential confounders: maternal age at delivery (≤ 24, 25–29, 30–34, ≥ 35 years), pre-pregnancy body mass index (BMI) according to WHO guidelines (World Health Organization, 2006), parity (nulliparous, parous), maternal education (high school diploma or less, some college or trade school, undergraduate university degree, graduate university degree), household income ($\leq 30,000, 30,001–50,000, 50,001–100,000, $\geq 100,000$), ethnicity (Caucasian/non-Caucasian), and maternal smoking (never or quit before pregnancy, quit when pregnancy confirmed, current smoker).

2.5. Statistical analysis

Umbilical cord blood levels of leptin and adiponectin were categorized into the 10th and 90th percentiles, as the previous literature has shown that both low and high levels of both of these biomarkers are associated with potentially adverse outcomes (Mantzoros et al., 2009; Trujillo and Scherer, 2005; Walsh et al., 2014). Due to differing leptin levels between male and female infants, the binary leptin variables were derived using sex specific cut-off points: 10th percentile (males 1.8, females 3.5 ng/mL) and 90th percentile (males 31.2, females 54.6 ng/mL). Adiponectin levels did not vary by sex, thus, sex-specific cut-offs were not necessary.

Descriptive statistics for maternal demographics, weight-related characteristics, and pregnancy characteristics were calculated according to levels of leptin and adiponectin using frequency distributions and chi-square tests of significance for the difference between the low ($\leq$ 10%ile), moderate ($>10–<90%$) and elevated ($\geq$ 90%ile) leptin and adiponectin groups.

The geometric means (GM) and standard deviations (SD) of the metals according to the outcome categories of leptin and adiponectin were determined. Separate models were developed for leptin and adiponectin using polytomous regression to examine the odds of both high ($\geq$ 90%) and low ($\leq$ 10%) levels of the markers of metabolic function. Polytomous logistic regression is an extension of simple logistic regression that facilitates analysis of multinomial outcomes (Ananth and Kleinbaum, 1997). The cut-off points in the present study were selected to capture infants with elevated and suppressed adipokine values. But, in recognition of the fact that the choice of these cut-off points was somewhat arbitrary, we conducted a sensitivity analysis to examine the outcome categories at the 25th and 75th percentiles.

Next, metals were categorized in quartiles and, since no metal had more than 25% below the LOD, all levels below LOD were included in the lowest quartile for each metal. Due to the lack of linearity in quartile association estimates, we did not examine the chemical exposures as continuous variables. In the multivariate models, we included variables that were selected *a priori* (maternal age) or significantly associated with the adipokines at a p-value <0.1 in order to facilitate identification of a common set

of potential confounders across metals. Effect modification between infant sex and metal exposure was assessed in each model using the likelihood ratio test to determine if model fit improved with the inclusion of the product term. Results were stratified by sex if the likelihood ratio test was significant at $p < 0.1$. In addition, in order to determine whether any association we observed between metals and leptin or adioponectin were independent of fetal fat mass, we conducted an analysis adjusting for birth *z*-score (as a surrogate of fat mass).

For metals with significant associations in either the main or sensitivity analysis, we produced locally weighted scatterplot smoothing (LOESS) plots to facilitate visualization of associations observed in the categorical analyses. The smoothing criterion for the displayed plots was selected as the value that would minimize the corrected Akaike's information criterion (AIC).

All analyses were done completed using SAS v.9.2 (SAS Institute, Inc., Cary, NC, USA). This study received ethical approval from the IWK Health Centre (Halifax, NS), Health Canada, and Ste. Justine's Hospital (Montreal, QC).

3. Results

Median (IQR) leptin concentrations were significantly higher among female infants (16.0 (26.3) ng/mL) than in males (8.7 (13.6) ng/mL) and ranged from 0.086 to 243 ng/mL. Median adiponectin concentrations did not differ by sex (males 16.7 (12.7), females 16.7 (12.6) μg/mL) and ranged from 0.19 to 239 μg/mL. The Pearson correlation coefficient between leptin and adiponectin levels was 0.45 among males and 0.53 among females.

The majority of study participants were over 30 years of age at the time of pregnancy, were of normal BMI, were university educated, had a household income greater than $50,000, never smoked and were Caucasian (Table 1).

The geometric mean (SD) levels of the average of first and third trimester metals are as follows: lead = 0.88 (1.61) μg/dL, arsenic = 1.14 (1.98) μg/L, mercury = 0.86 (2.84) μg/L, and cadmium = 0.32 (2.13) μg/L. The Pearson correlation coefficient for the first and third trimester blood metal levels as follows: lead = 0.70, arsenic = 0.36, cadmium = 0.60, mercury = 0.75 (Pearson correlation coefficient). Geometric means according to high and low levels of leptin and adiponectin are presented in Table 2.

Multivariate models assessing the association between metals and leptin were adjusted for maternal age, pre-pregnancy BMI, and parity. The adiponectin models were adjusted for age and education. No statistically significant associations were observed between lead, arsenic or mercury and either low or high leptin levels (Table 3). Adjustment for birth weight *z*-score resulted in an elevated, borderline significant odds ratio of elevated leptin in the fourth quartile of maternal lead levels (OR = 1.7 95% CI: 1.0–2.9) (Table 3). Effect modification by sex was observed in the relationship between cadmium and leptin levels (p-value < 0.1). The highest quartile of maternal blood cadmium was associated with a significantly increased risk of high ($\geq 90\%$) leptin (OR = 4.3, 95% CI: 1.8–10.0) among males in the adjusted model (Table 4). There were no significant associations between any of the metals and adiponectin in crude or adjusted analyses (Table 5).

In sensitivity analyses, the highest quartile of maternal cadmium was associated with increased odds of high ($\geq 75\%$) leptin (aOR = 1.7, 95% CI: 1.0–2.9) in males. No significant associations were observed between cadmium and leptin in females or between leptin and lead, mercury or arsenic. No significant associations were observed between any of the metals and adiponectin in the sensitivity analysis using the upper and lower 25%ile of adiponectin. All results using third trimester measurements compared to first trimester measurements were similar in magnitude and statistical significance.

Fig. 1 and 2 depict LOESS curves for the associations between leptin and cadmium (males only) and lead. The smoothing parameter was set to 0.471 and 0.998 in the lead and cadmium plots respectively as these values were associated with a minimal AIC value. Fig. 1 depicts the association between cadmium and leptin, demonstrating a trend towards increasing leptin levels at high maternal cadmium concentrations for male infants. Fig. 2 depicts the association between lead and leptin, showing a non-linear relationship between lead and leptin that plateaus at the highest concentrations.

4. Discussion

In this analysis of the association between maternal blood levels of metals and cord blood levels of leptin and adiponectin, we observed that elevated maternal blood concentrations of cadmium were significantly associated with high cord blood leptin levels among their male infants. An analysis based on continuous measures of leptin showed a similar association with cadmium levels, and suggests that leptin increases only at the higher cadmium levels within this population. On average, the maternal level of cadmium exposure (median level = 0.28 μg/L) in MIREC participants was well below the intervention level of 5 μg/L defined by OSHA (2004). Only a limited number of participants had cadmium exposure levels that approached this cut-off value. Our finding suggests that maternal cadmium concentrations substantially lower than the cut-off value (the 4th quartile cut-off was 0.44 μg/L) are associated with elevated cord blood leptin levels in males. No significant associations were observed between leptin and cadmium among females, nor did we observe any statistically significant associations between maternal metal levels and either low or high adiponectin.

While there have been no epidemiological studies examining the relationship between cadmium and cord blood leptin concentrations, there is evidence from the toxicology literature that placental cadmium concentrations are associated with decreased placental leptin synthesis (Stasenko et al., 2010) and that leptin and adiponectin expression is reduced in response to cadmium exposure (Kawakami et al., 2013). A Bangladeshi cohort study reported a significant inverse association between maternal blood cadmium levels (median = 0.63 μg/L) and birth weight among girls but not boys (Kippler et al., 2012), inverse association between maternal cadmium blood levels (median = 0.8 μg/L) and birth weight was observed among smokers, but not non-smokers, in a French birth cohort. Results were not stratified by sex in this study (Menai et al., 2012). A Taiwanese birth cohort study reported a significant inverse association between cord blood, but not maternal (median = 1.05 μg/L), cadmium and head circumference at birth. Associations between both cord blood and maternal cadmium levels were inversely yet not significantly associated with other growth parameters at birth (Lin et al., 2011). The inverse association between cadmium and fetal growth may be explained by the fact that cadmium has been shown to interfere with the placental transfer of micronutrients such as zinc to the fetus (Kippler et al., 2010). This explanation is consistent with evidence that cadmium accumulates in the placenta and that cord blood levels have been shown to be lower than maternal blood levels (Barr et al., 2007; Kuhnert et al., 1982).

In light of this previous research and evidence that fetal cadmium exposure is mitigated by the placenta, the observed finding of a significant association between relatively low maternal cadmium levels and elevated cord blood leptin concentrations among males is novel and warrants further investigation.

Table 1
Maternal demographic characteristics according to cord blood levels of leptin (ng/mL) and adiponectin (μg/mL). (MIREC study, 2008-2011).

Characteristic	N	Leptin (row %)				Adiponectin (row %)			
		≤ 10%	10–90%	≥ 90%	p-Value	≤ 10%	10-90%	≥ 90%	p-Value
Maternal age									
≤ 24	60	13.3	80.0	6.7	0.73	10.0	80.0	10.0	0.90
25–29	270	10.0	77.8	12.2		10.7	77.8	11.5	
30–34	451	8.0	83.4	8.7		10.0	80.7	9.3	
≥ 35	479	11.7	78.1	10.2		10.0	80.0	10.0	
Pre-pregnancy BMI									
Underweight (< 18.5)	27	22.2	70.4	7.4	< 0.01	22.2	70.4	7.4	0.28
Normal (18.5–24.9)	720	13.0	79.6	7.5		11.1	78.6	10.3	
Overweight (25–29.9)	272	4.0	83.8	12.1		8.8	80.5	10.7	
Obese (≥ 30)	171	6.4	76.0	17.5		8.2	83.0	8.8	
Missing	70								
Parity									
Nulliparous	529	9.3	76.9	13.8	< 0.01	9.3	79.6	11.2	0.19
Parous	729	10.7	82.2	7.1		10.8	79.8	9.3	
Missing	2								
Education									
High school diploma or less	105	10.5	79.1	10.5	0.71	10.5	81.0	8.6	0.10
Some college, or trade school	362	10.8	77.1	12.2		11.9	79.0	9.1	
Undergraduate university degree	480	10.0	81.5	8.5		9.6	80.6	9.8	
Graduate university degree	311	9.3	81.4	9.3		9.0	78.8	12.2	
Missing	2								
Household Income ($CAD)									
≤ 30,000	91	14.3	74.7	11.0	0.80	13.2	78.0	8.8	0.36
30,001–50,000	116	5.2	84.5	10.3		7.8	84.5	7.8	
50,001–100,000	516	10.9	77.9	11.2		11.1	77.5	11.4	
≥ 100,000	488	9.2	82.2	8.6		8.6	81.8	9.6	
Missing	49								
Ethnicity									
Caucasian	1087	9.8	80.4	9.8	0.89	10.6	78.7	10.8	0.60
Not Caucasian	173	11.6	77.5	11.0		7.5	86.7	5.8	
Maternal smoking									
Never or quit before pregnancy	1109	10.1	80.6	9.3	0.23	10.1	79.8	10.1	0.78
Quit when pregnancy confirmed	90	8.9	76.7	14.4		12.2	80.0	7.8	
Current smoker	61	11.5	73.8	14.8		8.2	78.7	13.1	
Infant sex									
Male	675	10.1	80.0	9.9	0.99	9.6	81.6	8.8	0.50
Female	585	10.1	80.0	9.9		10.8	77.7	11.6	
Cord blood adiponectin (μg/mL)									
≤ 10%	123	43.8	50.0	6.3	< 0.01	–	–	–	–
10–90%	1005	6.8	84.4	8.9					
≥ 90%	127	2.4	75.6	22.1					
Cord blood leptin (ng/mL)									
≤ 10%	127	–	–	–	–	44.1	53.5	2.4	< 0.01
< 90%	1008					6.6	84.1	9.5	
≥ 90%	125					6.4	71.2	22.4	

Table 2
Geometric mean blood concentration of metals according to categories of cord leptin (ng/mL) and adiponectin (μg/mL) (MIREC study, 2008–2011) (n=1260).

Metal	% > LOD[a]	Leptin			Adiponectin		
		≤ 10%	10–90%	≥ 90%	≤ 10%	10–90%	≥ 90%
Lead (μg/dL)	100	0.89 (1.61)	0.88 (1.61)	0.93 (1.65)	0.88 (1.61)	0.89 (1.61)	0.86(1.63)
Mercury (μg/L)	90.6	0.93 (2.93)	0.87 (2.85)	0.74 (2.69)	0.86 (2.88)	0.86 (2.82)	0.83 (3.00)
Arsenic (μg/L)	93.6	1.11 (2.13)	1.15 (1.95)	1.10 (2.04)	1.14 (2.11)	1.14 (1.94)	1.17 (2.14)
Cadmium (μg/L)	97.4	0.32 (2.22)	0.31 (2.07)	0.36 (2.50)	0.30 (2.04)	0.32 (2.15)	0.31 (2.10)

[a] % > LOD based on first trimester measurements (lead=0.1 μg/dL, mercury=0.12 μg/L, arsenic=0.22 μg/L, cadmium=0.04 μg/L)

Table 3
Odds ratio of high (≥ 90%) and low (≤ 10%) leptin (ng/mL) and prenatal exposure to quartiles of maternal blood metals (MIREC study, 2008–2011) ($n=1188$).

Contaminant	Low leptin			High leptin		
	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)	Adjusted OR[b] (95% CI)	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)	Adjusted OR[b] (95% CI)
Lead (μg/dL)						
≤ 0.63	1.0	1.0	1.0	1.0	1.0	1.0
0.64 to ≤ 0.87	1.1 (0.6–1.8)	1.1 (0.6–1.8)	0.9 (0.5–1.6)	1.1 (0.6–1.8)	1.1 (0.6–1.9)	1.2 (0.7–2.1)
0.88 to ≤ 1.20	0.8 (0.4–1.3)	0.7 (0.4–1.3)	0.6 (0.3–1.1)	0.8 (0.4–1.3)	0.9 (0.5–1.6)	1.0 (0.6–1.8)
> 1.20	1.3 (0.8–2.2)	1.2 (0.7–2.0)	0.9 (0.5–1.5)	1.3 (0.8–2.1)	1.4 (0.8–2.3)	1.7 (1.0–2.9)
Arsenic (μg/L)						
≤ 0.77	1.0	1.0	1.0	1.0	1.0	1.0
0.78 to ≤ 1.13	0.9 (0.6–1.6)	1.0 (0.6–1.6)	1.0 (0.6–1.8)	1.0 (0.6–1.7)	1.0 (0.6–1.6)	1.0 (0.6–1.8)
1.14 to ≤ 1.72	0.6 (0.3–1.0)	0.6 (0.3–1.0)	0.6 (0.3–1.1)	0.8 (0.5–1.4)	0.8 (0.5–1.3)	0.8 (0.5–1.5)
> 1.72	1.0 (0.6–1.6)	0.9 (0.5–1.5)	0.9 (0.5–1.5)	0.8 (0.5–1.4)	0.8 (0.5–1.4)	0.9 (0.5–1.6)
Mercury (μg/L)						
≤ 0.46	1.0	1.0	1.0	1.0	1.0	1.0
0.47 to ≤ 0.99	1.0 (0.6–1.7)	0.9 (0.5–1.5)	1.0 (0.5–1.7)	1.3 (0.8–2.1)	1.4 (0.8–2.3)	1.4 (0.8–2.5)
1.0 to ≤ 1.86	1.1 (0.6–1.8)	0.9 (0.5–1.5)	0.8 (0.5–1.5)	0.9 (0.5–1.5)	0.8 (0.5–1.5)	0.9 (0.5–1.6)
> 1.86	0.9 (0.5–1.5)	0.7 (0.4–1.2)	0.6 (0.3–1.1)	0.7 (0.4–1.3)	0.7 (0.4–1.3)	0.8 (0.4–1.4)

[a] Adjusted for maternal age, parity, pre-pregnancy BMI.
[b] Adjusted for maternal age, parity, pre-pregnancy BMI, birth weight *z*-score.

Our finding of a sex-dependent association between maternal cadmium and cord blood leptin levels, however, is consistent with evidence that cadmium exposure has been shown to have a sex-specific effect on DNA methylation. Methylated sites in girls were associated with organ development changes where those changes observed in boys were found in cell death (Kippler et al., 2013). The sex-dependent nature of cadmium related effects is hypothesized to be due to its estrogenic properties (Garcia-Morales et al., 1994; Henson and Chedrese, 2004; Johnson et al., 2003).

Moreover, it is worth noting that though smoking is a known source of exposure to cadmium, this variable was not identified as a confounder in the present analysis and, therefore, not included in our statistical models. The lack of influence of smoking on the associations between cadmium and cord blood cytokine levels may be due to the low smoking rates among MIREC study participants.

Though no identified literature has examined the association between prenatal lead exposure and leptin levels, epidemiological studies have reported an inverse relationship between lead and newborn growth (Gonzalez-Cossio et al., 1997; Schell et al., 2009; Xie et al., 2013; Zhu et al., 2010). Inverse associations between maternal lead levels and birth weight have been observed in birth cohort studies from NY state (Schell et al., 2009; Zhu et al., 2010), Mexico (Gonzalez-Cossio et al., 1997), and China (Xie et al., 2013). The median level of maternal blood lead in these studies was 3 μg/dL in the New York state birth cohort (selected based on risk factors for lead exposure, namely poverty and residence in older, poorly maintained housing) (Schell et al., 2009), 3.2 μg/dL in the Chinese birth cohort (Xie et al., 2013), and 2 μg/dL in a birth cohort from NY study using data from metals registry (Zhu et al., 2010). The Mexican cohort examined tibial bone lead levels (Gonzalez-Cossio et al., 1997). The observed inverse associations between maternal lead levels and birth weight may be explained by lead interference with calcium transport and function (Bellinger, 2005; Pounds, 1984). We observed that the highest quartile of lead exposure was associated with increased odds of elevated leptin after adjustment for birth weight *z*-score. This finding, which warrants further investigation, raises interesting questions regarding the role of lead in fetal growth. On the one hand, previous research has demonstrated that lead is inversely related to growth, particularly

Table 4
Odds ratio of high (≥ 90%) and low (≤ 10%) leptin (ng/mL) and prenatal exposure to quartiles of maternal blood cadmium, stratified by infant sex (MIREC study, 2008–2011) ($n=1188$).

Contaminant	Low leptin			High leptin		
	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)	Adjusted OR[b] (95% CI)	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)	Adjusted OR[b] (95% CI)
Cadmium (μg/L)						
Males (n=639)						
≤ 0.20	1.0	1.0	1.0	1.0	1.0	1.0
0.21 to ≤ 0.29	1.0 (0.5–2.0)	1.0 (0.5–2.0)	1.0 (0.5–2.1)	2.1 (0.9–5.1)	2.3 (0.9–5.7)	2.4 (0.9–6.0)
0.30 to ≤ 0.44	0.7 (0.3–1.4)	0.7 (0.3–1.4)	0.7 (0.3–1.5)	1.9 (0.8–4.6)	2.2 (0.9–5.4)	2.4 (0.9–6.0)
> 0.44	1.0 (0.5–2.1)	1.0 (0.5–2.0)	0.9 (0.4–2.0)	3.9 (1.7–8.8)	4.3 (1.8–10.0)	4.5 (1.9–10.8)
Females (n=549)						
≤ 0.20	1.0	1.0	1.0	1.0	1.0	1.0
0.21 to ≤ 0.29	1.8 (0.8–3.9)	1.7 (0.8–3.9)	1.7 (0.7–3.8)	1.2 (0.6–2.4)	1.2 (0.6–2.6)	1.2 (0.6–2.6)
0.30 to ≤ 0.43	1.0 (0.4–2.4)	0.9 (0.4–2.1)	0.8 (0.3–2.1)	0.7 (0.3–1.6)	0.8 (0.3–1.7)	0.8 (0.3–1.7)
> 0.43	1.6 (0.7–3.6)	1.5 (0.7–3.5)	1.2 (0.5–2.9)	0.8 (0.4–1.7)	0.8 (0.3–1.7)	0.8 (0.3–1.9)

[a] Adjusted for maternal age, parity, pre-pregnancy BMI.
[b] adjusted for maternal age, parity, pre-pregnancy BMI, birth weight *z*-score.

Table 5
Odds ratio of high (≥ 90%) and low (≤ 10%) adiponectin (μg/mL) and prenatal exposure to quartiles of maternal blood metals (MIREC study, 2008–2011) (n = 1258).

Contaminant	Low adiponectin			High adiponectin		
	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)	Adjusted OR[b] (95% CI)	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)	Adjusted OR[b] (95% CI)
Lead (μg/dL)						
≤ 0.63	1.0	1.0	1.0	1.0	1.0	1.0
0.64 to ≤ 0.87	1.3 (0.8–2.2)	1.3 (0.8–2.2)	1.3 (0.8–2.2)	0.9 (0.5–1.5)	0.8 (0.5–1.4)	0.9 (0.5–1.5)
0.88 to ≤ 1.20	0.9 (0.5–1.6)	0.9 (0.5–1.5)	0.8 (0.5–1.4)	1.1 (0.7–0.9)	1.0 (0.6–1.7)	1.1 (0.7–1.9)
> 1.20	1.1 (0.6–1.9)	1.1 (0.7–1.9)	1.1 (0.6–1.9)	0.8 (0.5–1.4)	0.8 (0.5–1.3)	0.9 (0.5–1.5)
Arsenic (μg/L)						
≤ 0.77	1.0	1.0	1.0	1.0	1.0	1.0
0.78 to ≤ 1.13	0.8 (0.5–1.4)	0.8 (0.5–1.3)	0.8 (0.5–1.3)	0.9 (0.5–1.5)	0.8 (0.5–1.3)	0.8 (0.5–1.3)
1.14 to ≤ 1.72	0.8 (0.5–1.4)	0.8 (0.5–1.4)	0.8 (0.5–1.3)	1.0 (0.6–1.6)	1.0 (0.6–1.6)	1.0 (0.6–1.7)
> 1.72	0.9 (0.5–1.5)	1.0 (0.6–1.6)	0.9 (0.6–1.6)	0.9 (0.5–1.5)	0.9 (0.5–1.5)	0.9 (0.5–1.6)
Mercury (μg/L)						
≤ 0.46	1.0	1.0	1.0	1.0	1.0	1.0
0.47 to ≤ 0.99	0.9 (0.5–1.5)	0.9 (0.5–1.6)	0.9 (0.5–1.6)	1.1 (0.6–1.9)	1.0 (0.6–1.7)	1.0 (0.6–1.7)
1.0 to ≤ 1.86	1.1 (0.7–1.9)	1.3 (0.8–2.1)	1.2 (0.7–2.1)	1.2 (0.7–2.1)	1.1 (0.6–1.8)	1.1 (0.6–1.9)
> 1.86	0.9 (0.5–1.5)	1.0 (0.6–1.7)	0.9 (0.5–1.6)	1.2 (0.7–2.1)	1.1 (0.6–1.8)	1.2 (0.7–2.0)
Cadmium (μg/L)						
≤ 0.20	1.0	1.0	1.0	1.0	1.0	1.0
0.21 to ≤ 0.29	1.0 (0.6–1.7)	1.0 (0.6–1.7)	1.0 (0.6–1.7)	0.8 (0.5–1.4)	0.7 (0.4–1.2)	0.7 (0.4–1.2)
0.30 to ≤ 0.44	0.9 (0.5–1.5)	0.9 (0.5–1.5)	0.9 (0.5–1.5)	1.0 (0.6–1.6)	0.8 (0.5–1.4)	0.8 (0.5–1.4)
> 0.44	0.8 (0.5–1.3)	0.8 (0.4–1.3)	0.8 (0.4–1.3)	1.0 (0.6–1.6)	0.9 (0.6–1.6)	1.0 (0.6–1.6)

[a] Adjusted for maternal age, education.
[b] Adjusted for maternal age, education, birth weight z-score.

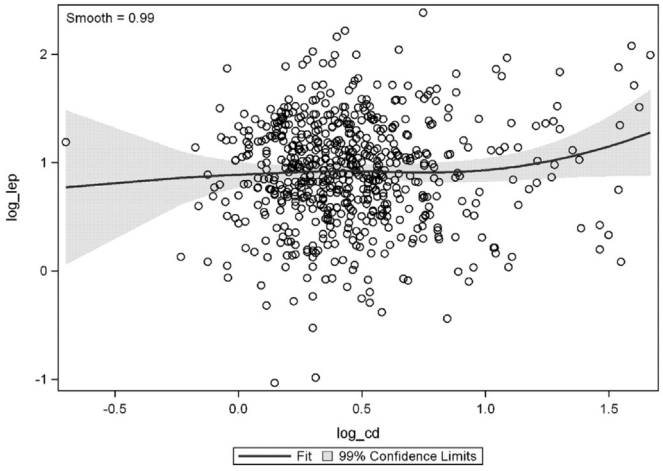

Fig. 1. LOESS plot of association between $\log_{10}$ maternal cadmium and $\log_{10}$ cord blood leptin.

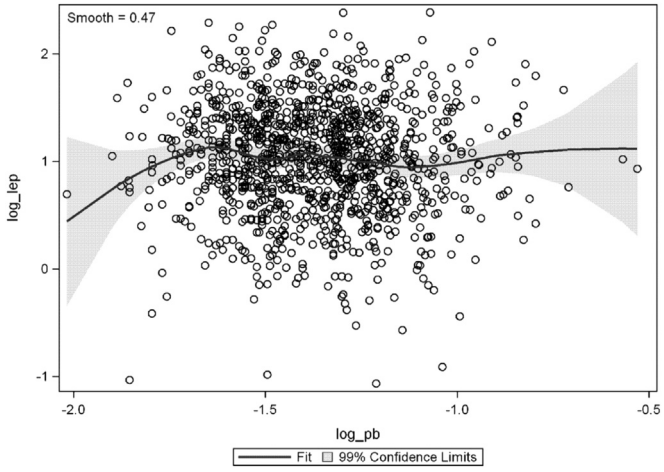

Fig. 2. LOESS plot of association between $\log_{10}$ maternal lead and $\log_{10}$ cord blood leptin.

skeletal growth. On the other hand, the observed finding in the present study suggests that lead may be positively associated with leptin, a surrogate of fat mass. Thus, it is possible that lead impacts fetal growth through two different and potentially opposing pathways. Further work is necessary to clarify and confirm the biological mechanisms underlying these potential pathways. Adjustment for birth weight was the best available measure in the present study to determine whether the effect of lead on leptin levels was independent of skeletal growth. This approach, however, is limited by the fact that birth weight encompasses both fat mass and skeletal growth. Future investigations that can adjust solely for skeletal growth will offer an even further refined ability to disentangle the relationships between lead and fetal fat mass. Moreover, considering that the median levels (0.87 μg/dL) of lead in the MIREC study are notably lower than the Health Canada blood intervention level of 10 μg/dL (Health Canada, 2013), any lead-related effects on fetal growth and development may be more pronounced in populations with high levels of exposure.

We did not identify any association between either arsenic or mercury and the adipokines. Though comparisons are difficult due to the difference in exposure medium, a Bangladesh birth cohort study reported an inverse association between prenatal urinary arsenic levels and birth weight, chest and head circumferences at maternal urinary arsenic levels below 100 μg/L. Exposure levels greater than this threshold, however, were not associated with any effect (Rahman et al., 2009). The literature on prenatal mercury exposure and fetal growth is limited and inconsistent. A French birth cohort study (EDEN) reported no association between prenatal mercury and fetal growth in the entire study population though a positive association between mercury levels and birth weight were seen among overweight women (Drouillet-Pinard et al., 2010). A Viennese study similarly identified no association between maternal mercury levels and birth weight yet was limited

by low sample size (Gundacker et al., 2010). And, a Spanish birth cohort reported both positive and inverse associations between mercury and fish consumption and SGA depending on the type and amount of fish consumed (Ramon et al., 2009).

In addition to the differences in metal measurement techniques and study populations, the discrepancy between our findings and previous birth cohort studies may be explained by the difference in outcome measures. The present examination of cord blood adipokine levels has facilitated examination of the effects of maternal blood metal levels on neonatal endocrine and metabolic development. Further investigation of this cohort is necessary to understand how fetal leptin and adiponectin translate into childhood growth trajectories. Leptin levels at birth have been found to be correlated with anthropometric measures in childhood (Karakosta and Chatzi, 2011; Mantzoros et al., 2009); the relationship between adiponectin and BMI is more complicated (Mazaki-Tovi et al., 2005; Volberg et al., 2013). Though birth weight is positively correlated with cord blood adiponectin, adiponectin levels are low in obese adults (Mazaki-Tovi et al., 2005).

This longitudinal cohort study was able to assess the relationship between maternal metal concentrations and cord blood levels of leptin and adiponectin using biomonitoring data from a trans-Canada cohort of study participants. The sample size in this study exceeds many of the previous analyses of the potential health effects of metals in birth cohort studies. Blood metal concentrations were analyzed in the first and third trimesters thus allowing us to calculate an average exposure index over the duration of pregnancy with a minimal amount of missing data. The comprehensive questionnaire data available in the MIREC study allowed us to control for key confounders such as smoking, maternal pre-pregnancy BMI, and parity.

Despite these strengths, our study was subject to limitations common to observational studies. First, though the MIREC study had a rich set of covariate data, the role of residual confounding cannot be ruled out. For instance, nutritional and iron status was not available. Second, due to the number of chemicals and outcomes tested, the possibility of identifying a significant association by chance cannot be dismissed. Lastly, the MIREC study population is on average from a higher socioeconomic status, lower BMI, and less likely to smoke than the general population. Thus, caution is warranted in generalizing these findings to the population at large.

5. Conclusions

In conclusion, the observed relationships between lead and cadmium and fetal leptin levels suggest that prenatal exposure to elevated levels of these metals may impact fetal metabolic development. These findings contribute to the growing body of evidence demonstrating the susceptibility of fetal development to exogenous chemical exposures and the multifactorial etiology of childhood growth trajectories (Keith et al., 2006; Cunningham et al., 2014; Warrington et al., 2013). Future follow up in this cohort will facilitate examination of the relationship between prenatal metal exposure, fetal adipokine levels, and anthropometric measures in childhood.

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References

Ananth, C.V., Kleinbaum, D.G., 1997. Regression models for ordinal responses: a review of methods and applications. Int. J. Epidemiol. 26, 1323–1333.
Arbuckle, T.E., Fraser, W.D., Fisher, M., Davis, K., Liang, C.L., Lupien, N., et al., 2013. Cohort profile: the maternal–infant research on environmental chemicals research platform. Paediatr. Perinat. Epidemiol. 27, 415–425. http://dx.doi.org/10.1111/ppe.12061.
Barr, D.B., Bishop, A., Needham, L.L., 2007. Concentrations of xenobiotic chemicals in the maternal–fetal unit. Reprod. Toxicol. 23, 260–266. http://dx.doi.org/10.1016/j.reprotox.2007.03.003.
Bellinger, D.C., 2005. Teratogen update: lead and pregnancy. Birth Defects Res. A Clin. Mol Teratol. 73, 409–420. http://dx.doi.org/10.1002/bdra.20127.
Cunningham, S., Kramer, M., Venkat Narayan, K.M., 2014. Incidence of childhood obesity in the United States. N. Engl. J. Med. 370, 403–411. http://dx.doi.org/10.1056/NEJMoa1309753.
Drouillet-Pinard, P., Huel, G., Slama, R., Forhan, A., Sahuquillo, J., Goua, V., et al., 2010. Prenatal mercury contamination: relationship with maternal seafood consumption during pregnancy and fetal growth in the "EDEN mother–child" cohort. Br. J. Nutr. 104, 1096–1100. http://dx.doi.org/10.1017/S0007114510001947.
Garcia-Morales, P., Saceda, M., Kenney, N., Kim, N., Salomon, D.S., Gottardisnll, M.M., et al., 1994. Effect of cadmium on estrogen receptor levels and estrogen-induced responses in human breast cancer cells. J. Biol. Chem. 269, 16896–16901.
Gluckman, P.D., Hanson, M.A., Cooper, C., Thornburg, K.L., 2008. Effect of in utero and early-life conditions on adult health and disease. N. Engl. J. Med. 359, 61–73. http://dx.doi.org/10.1056/NEJMra0708473.
Gonzalez-Cossio, T., Peterson, K.E., Sanin, L.-H., Fishbein, E., Palazuelos, E., Aro, A., et al., 1997. Decrease in birth weight in relation to maternal bone-lead burden. Pediatrics 100, 856–862. http://dx.doi.org/10.1542/peds.100.5.856.
Gundacker, C., Fröhlich, S., Graf-Rohrmeister, K., Eibenberger, B., Jessenig, V., Gicic, D., et al., 2010. Perinatal lead and mercury exposure in Austria. Sci. Total Environ. 408, 5744–5749. http://dx.doi.org/10.1016/j.scitotenv.2010.07.079.
Health Canada, 2013. What is Lead. ⟨http://www.hc-sc.gc.ca/ewh-semt/contaminants/lead-plomb/index-eng.php⟩ (retrieved 09.07.14).
Health Canada, 2010. Report on Human Biomonitoring of Environmental Chemicals in Canada. ⟨http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/chms-ecms/index-eng.php#n1⟩ (retrieved 08.05.12).
Henson, M.C., Chedrese, P.J., 2004. Endocrine disruption by cadmium, a common environmental toxicant with paradoxical effects on reproduction. Exp. Biol. Med. 229, 383–392.
Johnson, M.D., Kenney, N., Stoica, A., Hilakivi-Clarke, L., Singh, B., Chepko, G., et al., 2003. Cadmium mimics the in vivo effects of estrogen in the uterus and mammary gland. Nat. Med. 9, 1081–1084. http://dx.doi.org/10.1038/nm902.
Karakosta, P., Chatzi, L., 2011. Leptin levels in cord blood and anthropometric measures at birth: a systematic review and meta-analysis. Paediatr. Perinat. Epidemiol. 25, 150–163. http://dx.doi.org/10.1111/j.1365-3016.2010.01163.x.
Kawakami, T., Nishiyama, K., Kadota, Y., Sato, M., Inoue, M., Suzuki, S., 2013. Cadmium modulates adipocyte functions in metallothionein-null mice. Toxicol. Appl. Pharmacol. 272, 625–636. http://dx.doi.org/10.1016/j.taap.2013.07.015.
Keith, S., Redden, D., Katzmarzyk, Boggiano, M., Hanlon, E., Benca, R., Ruden, R., 2006. Putative contributors to the secular increase in obesity: exploring the roads less traveled. Int. J. Obes. 30, 1585–1594. http://dx.doi.org/10.1038/sj.ijo.0803326.
Kippler, M., Engström, K., Mlakar, S.J., Bottai, M., Ahmed, S., Hossain, M.B., et al., 2013. Sex-specific effects of early life cadmium exposure on DNA methylation and implications for birth weight. Epigenetics 8, 494–503. http://dx.doi.org/10.4161/epi.24401.
Kippler, M., Hoque, a M.W., Raqib, R., Ohrvik, H., Ekström, E.-C., Vahter, M., 2010. Accumulation of cadmium in human placenta interacts with the transport of micronutrients to the fetus. Toxicol. Lett. 192, 162–168. http://dx.doi.org/10.1016/j.toxlet.2009.10.018.
Kippler, M., Tofail, F., Gardner, R., Rahman, A., Hamadani, J., Bottai, M., 2012. Cadmium exposure during pregnancy and size at birth: a prospective cohort study. Environ. Health Perspect. 284, 284–289.
Klaasen, C., 2010. Casarett and Doull's Essentials of Toxicology, 2nd ed. McGraw-Hill Medical, New York.
Kuhnert, P., Kuhnert, B., Bottoms, S., Erhard, P., 1982. Cadmium levels in maternal blood, fetal cord blood, and placental tissues of pregnant women who smoke. Am. J. Obstet. Gynecol. 142, 1021–1025.
Lin, C.-M., Doyle, P., Wang, D., Hwang, Y.-H., Chen, P.-C., 2011. Does prenatal cadmium exposure affect fetal and child growth? Occup. Environ. Med. 68, 641–646. http://dx.doi.org/10.1136/oem.2010.059758.
Mantzoros, C.S., Rifas-shiman, S.L., Williams, C.J., Jessica, L., Kelesidis, T., Gillman, M. W., 2009. Cord blood leptin and adiponectin as predictors of adiposity in children at 3 years of age: a prospective cohort study. Pediatrics 123, 682–689. http://dx.doi.org/10.1542/peds.2008-0343.
Mazaki-Tovi, S., Kanety, H., Sivan, E., 2005. Adiponectin and human pregnancy. Curr. Diabet. Rep. 5, 278–281.

Menai, M., Heude, B., Slama, R., Forhan, A., Sahuquillo, J., Charles, M.-A., Yazbeck, C., 2012. Association between maternal blood cadmium during pregnancy and birth weight and the risk of fetal growth restriction: the EDEN mother–child cohort study. Reprod. Toxicol. 34, 622–627. http://dx.doi.org/10.1016/j.reprotox.2012.09.002.

Needham, L.L., Grandjean, P., Heinzow, B., Jørgensen, P.J., Nielsen, F., Patterson, D.G., et al., 2011. Partition of environmental chemicals between maternal and fetal blood and tissues. Environ. Sci. Technol. 45, 1121–1126. http://dx.doi.org/10.1021/es1019614.

NHANES, 2013. Fourth National Report on Human Exposure to Environmental Chemicals. ⟨http://www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Sep2013.pdf⟩ (retrieved 20.05.14).

OSHA, (2004). Cadmium. ⟨https://www.osha.gov/Publications/osha3136.pdf⟩ (retrieved 04.09.14).

Pounds, J., 1984. Effect of lead intoxication on calcium homeostatis and calcium-mediated cell function: a review. Neurotoxicology 5, 295–332.

Rahman, A., Vahter, M., Smith, A.H., Nermell, B., Yunus, M., El Arifeen, S., et al., 2009. Arsenic exposure during pregnancy and size at birth: a prospective cohort study in Bangladesh. Am. J. Epidemiol. 169, 304–312. http://dx.doi.org/10.1093/aje/kwn332.

Ramon, R., Ballester, F., Aguinagalder, X., Amurrio, A., Vioque, J., Lacasana, M., et al., 2009. Fish consumption during pregnancy, prenatal mercury exposure, and anthropometric measures at birth in a prospective mother–infant cohort. Am. J. Clin. Nutr. 90, 1047–1055. http://dx.doi.org/10.3945/ajcn.2009.27944.1.

Retnakaran, R., Ye, C., Hanley, A., 2012. Effect of maternal weight, adipokines, glucose intolerance and lipids on infant birth weight among women without gestational diabetes mellitus. Can. Med. Assoc. J. 184, 1353–1360. http://dx.doi.org/10.1503/cmaj.120682.

Roels, H., Hubermont, G., Buchet, J., Lauwerys, R., 1978. Placental transfer of lead, mercury, cadmium and cardbon monoxide in women. Environ. Res. 16, 236–247.

Schell, L.M., Denham, M., Stark, A.D., Parsons, P.J., Schulte, E.E., 2009. Growth of infants' length, weight, head and arm circumferences in relation to low levels of blood lead measured serially. Am. J. Hum. Biol. 21, 180–187. http://dx.doi.org/10.1002/ajhb.20842.

Selevan, S., Kimmel, C., Mendola, P., 2000. Identifying critical windows of exposure for children's health. Environ. Health Perspect. 108 (Suppl. 3), 451–455.

Stasenko, S., Bradford, E.M., Piasek, M., Henson, M.C., Varnai, V.M., Jurasović, J., Kusec, V., 2010. Metals in human placenta: focus on the effects of cadmium on steroid hormones and leptin. J. Appl. Toxicol. 30, 242–253. http://dx.doi.org/10.1002/jat.1490.

Trujillo, M.E., Scherer, P.E., 2005. Adiponectin—journey from an adipocyte secretory protein to biomarker of the metabolic syndrome. J. Intern. Med. 257, 167–175. http://dx.doi.org/10.1111/j.1365-2796.2004.01426.x.

Volberg, V., Heggeseth, B., Harley, K., Huen, K., Yousefi, P., Davé, V., et al., 2013. Adiponectin and leptin trajectories in mexican-american children from birth to 9 years of age. PloS One 8, e77964. http://dx.doi.org/10.1371/journal.pone.0077964.

Walsh, J.M., Byrne, J., Mahony, R.M., Foley, M.E., McAuliffe, F.M., 2014. Leptin, fetal growth and insulin resistance in non-diabetic pregnancies. Early Hum. Dev. 90, 271–274. http://dx.doi.org/10.1016/j.earlhumdev.2014.03.007.

Warrington, N.M., Howe, L.D., Wu, Y.Y., Timpson, N.J., Tilling, K., Pennell, C.E., et al., 2013. Association of a body mass index genetic risk score with growth throughout childhood and adolescence. PloS One 8, e79547. http://dx.doi.org/10.1371/journal.pone.0079547.

World Health Organization, 2006. WHO BMI Classification. ⟨http://apps.who.int/bmi/index.jsp?introPage=intro_3.html⟩ (retrieved 08.09.14).

Xie, X., Ding, G., Cui, C., Chen, L., Gao, Y., Zhou, Y., et al., 2013. The effects of low-level prenatal lead exposure on birth outcomes. Environ. Pollut. 175, 30–34. http://dx.doi.org/10.1016/j.envpol.2012.12.013.

Zhu, M., Fitzgerald, E.F., Gelberg, K.H., Lin, S., Druschel, C.M., 2010. Maternal low-level lead exposure and fetal growth. Environ. Health Perspect. 118, 1471–1475. http://dx.doi.org/10.1289/ehp.0901561.