Vitamin D, Metals and Preterm Birth

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PREFACE TO THESIS

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Ethical Considerations

The Maternal-Infant Research on Environmental Chemicals (MIREC) Study and its follow-up, MIREC-Endocrine and Metabolic Function (MIREC-ENDO) received research ethics board approval at the MIREC study coordinating center (CHU Ste-Justine in Montreal) and Health Canada , along with the other 10 study sites across Canada including: Children's and Women's Health (Vancouver), University of Alberta (Edmonton), Health Sciences Center and St-Boniface Hospital (Winnipeg), Mount Sinai Hospital (Toronto), McMaster University Medical Center (Hamilton), Sudbury Regional Hospital (Sudbury), Kingston General Hospital (Kingston), The Ottawa Hospital (Ottawa), CHU Ste-Justine (Montreal), Jewish General Hospital (Montreal), IWK Health Centre (Halifax). All participants provided informed consent for the study, which included statements covering analyses on environmental chemicals, nutritional factors, and pregnancy outcomes. The author of this dissertation, Mandy Fisher, is a co-investigator on the MIREC study and an employee at Health Canada.

Author Contributions

With the supervision of my thesis supervisors Dr. Beth Potter and Dr. Tye Arbuckle, I, Mandy Fisher (the PhD Candidate) am the guarantor of this project. I have led every aspect of the thesis,

including research question formulation, study design, data cleaning, data analysis, interpretation of the results, writing of the manuscripts and writing the thesis. For all three papers, I am the lead and corresponding author.

The Thesis Advisory Committee (Dr. William Fraser, Dr. Hope Weiler, Dr. Anne-Sophie Morisset) was assembled by Mandy in collaboration with her supervisors. They were selected for their expertise in MIREC, nutrition, or perinatal epidemiology and they reviewed all aspects of the thesis. Additional coauthors were invited to collaborate on the work for each paper as appropriate. Throughout this thesis project, Mandy also sought guidance from experts, including attending a course on Structural Equation Modeling by Paul D. Allison (Statistical Horizons™) for the first paper, and working closely with Leonora Marro (Health Canada Statistician) to learn Discrete Time Survival Analysis for the 2nd paper. The Supervisors, TAC and invited coauthors have been involved in the review of each paper and have provided constructive feedback.

Figures and Tables

All figures and tables in this thesis were created by the author, Mandy Fisher.

ABSTRACT

Background: Environmental chemicals may interrupt physiological adaptations necessary in pregnancy, contributing to pregnancy complications with health implications for the mother and child. Nutrients may modify the impact of chemical exposures by blocking their absorption or facilitating their excretion. However, the nature, directionality, and implications of these relationships remain unclear.

Objectives: We sought to understand: 1) the potential bidirectional nature of the relationship between vitamin D (25-hydroxyvitamin D, 25OHD) and the toxic metals cadmium (Cd) and lead (Pb) in pregnancy; 2) the association between metals (Cd, Pb, arsenic (As), mercury) and preterm birth and the potential modification of that association by 25OHD; and 3) the long-term association of pregnancy complications with maternal cardiometabolic health.

Methods: We used data from the Maternal-Infant Research on Environmental Chemicals Study (n=1983) pregnancy cohort, including long-term follow-up approximately 9 years post-pregnancy. We used cross-lagged panel models to determine the direction of the relationship of 25OHD with Cd and Pb in pregnancy, discrete-time survival analysis to examine the association between metals in pregnancy and preterm birth, and multivariable linear regression to investigate the association of pregnancy complications with long-term maternal outcomes.

Results: Each doubling in first trimester 25OHD concentrations was associated with 9% (95% CI: -15%, -3%) lower 3rd trimester Cd and 3% (-7, 0.1%) lower Pb. One-unit increases in Pb (μg/dL) and arsenic (μg/L) concentrations in pregnancy were associated with an increased relative risk (RR) of preterm birth (RR_{Pb}: 1.48, 95% CI: 1.00, 2.20; RR_{As}: 1.10, 95% CI 1.02,

1.19); the association with Pb was stronger among those with lower 25OHD. Finally, relative to uncomplicated pregnancy, experiencing a pregnancy complication was positively associated with body fat percentage (β=2.6, 95% CI: 0.3, 4.8) and systolic (average increase of 9.0 mm Hg, 95% CI 5.1, 12.8) and diastolic (average increase of 5.5 mm Hg, 95% CI: 2.6, 8.4) blood pressure 9 years later.

Conclusions: Nutrient status during pregnancy may affect and interact with environmental chemicals to impact pregnancy outcomes. Future studies should continue to use methods that elucidate the causal direction of associations and evaluate interactions. Chemicals associated with pregnancy complications could have lasting impacts on maternal health.

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I'm grateful for my first job as a nurse working on a cancer ward (D6) at the Health Sciences Center in Winnipeg. This experience shaped me for the rest of my life and pushed me to take an internship in Geneva, Switzerland where I learned about epidemiology.

I'd like to thank my son, Merrick, for wanting to play the guitar. This is how I met Beth Potter, one summer day at a guitar picnic leading to many future coffee conversations about the idea of a PhD. That was back in 2012! I'm grateful for your patience with me Beth. You have been an extraordinary mentor.

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Finally, I'd like to dedicate this thesis to my boys! You have been my inspiration. I hope I have taught you to never give up. Your goals and dreams are important and keep believing in them no matter how many bumps, big and small, are along the road. (I sincerely hope this is what you will take away from my thesis and not just think of it as something that stressed me out and made me work too much. If so, I apologize for this!)

I would like to acknowledge that although gendered terms, such as woman, maternal, and mother, are used within the context of the MIREC study and this thesis, pregnancy can be experienced by people of all gender identities. I am moving towards integrating more gender inclusive language like 'pregnant people', 'birthing parent' and 'participant'. I regret if my wording has offended any readers.

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CHAPTER 1: INTRODUCTION

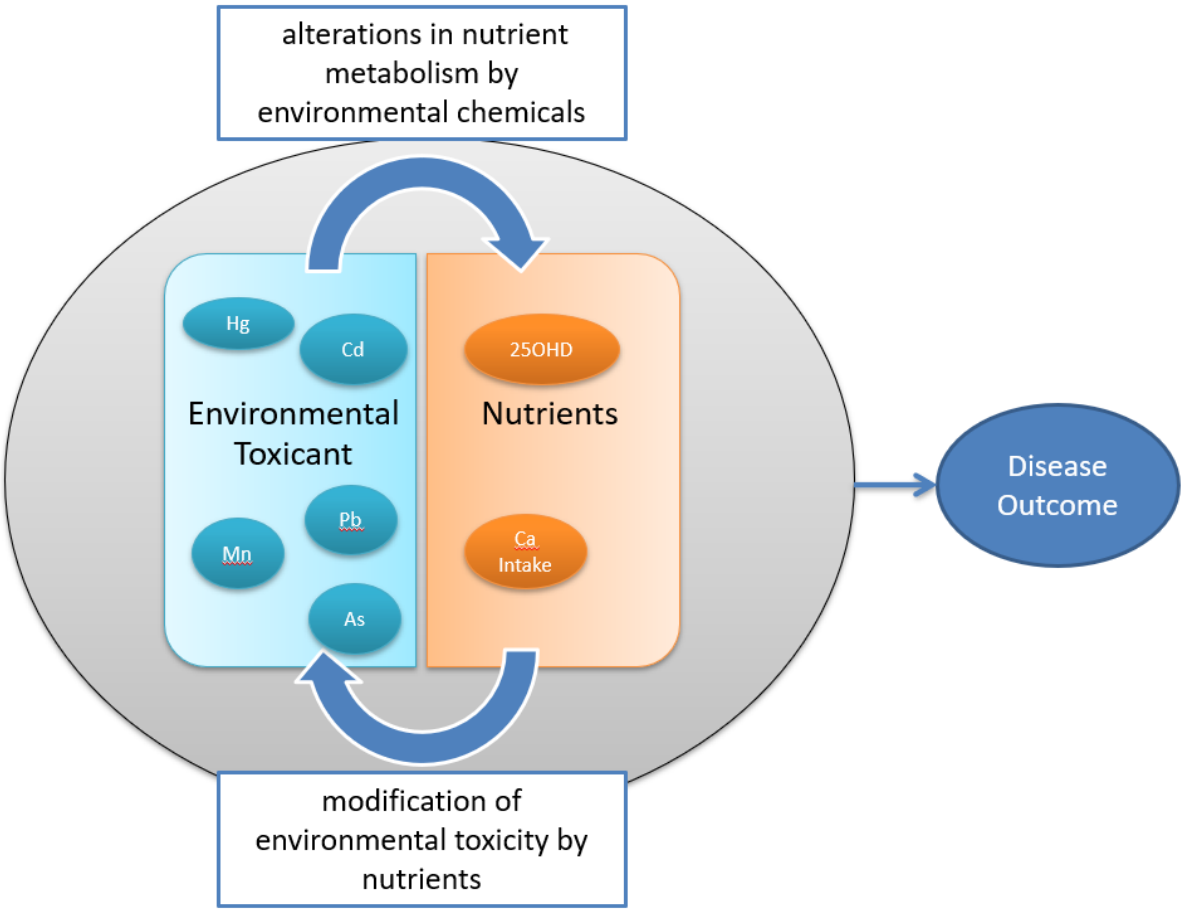
1.1 OVERVIEW OF THESIS AND RATIONALE

A growing body of evidence suggests that all age-related or chronic diseases have multifactorial causes that include dietary deficiencies, exposure to environmental agents and genetic susceptibility [1,2]. In pregnancy, deviations from optimal micronutrient levels have been associated with both fetal and maternal morbidity [3]. Low vitamin D concentrations have been associated with an increased risk of preterm birth [4], and trials suggest that vitamin D supplementation may reduce this risk [5]. The Public Health Agency of Canada recommends that during pregnancy, individuals should use Canada's Food Guide and take a daily multivitamin containing 400µg of folic acid; but no specific recommendations for vitamin D are given [6]. At the same time, studies have shown exposure to environmental chemical exposures throughout the life course to be associated with adverse outcomes in the pregnant mother and her fetus(es)[7], as well as childhood [8,9] and adult disease [10,11]. Such exposures are thus of major environmental and public health concern. Reports on the impact of environmental chemicals on health have been released by the endocrine society [12], the International Federation of Obstetrics and Gynecology [13], and the World Health Organization [14]. In addition, several countries have implemented population-based biomonitoring programs to monitor chemical levels in their populations [15–17].

Experts of nutritional sciences, medicine and environmental toxicology stress the growing need to further understand the complexity of exposure mixtures [18] and the interplay between environmental exposures, nutrition and disease risk [1,2,19–23]. There are questions related to the potential bidirectional association between environmental contaminants and nutrients. For

example: 1) is environmental chemical absorption, distribution or metabolism modified by nutrients and/or; 2) are there alterations in nutrient metabolism by environmental chemicals (See Figure 1). There are also questions about how environmental contaminants and nutrients, individually or in interaction with one another, affect shorter- and longer-term health risks.

Figure 1: Nutrient and Toxicant Interplay



Pregnancy is a sensitive time period with physiologic changes that may make the pregnant person more susceptible to the harmful effects of environmental contaminants such as heavy metals, and to inadequate nutritional status, such as low vitamin D. Understanding how environmental exposures and nutrients relate to pregnancy outcomes is thus important for the health both of the fetus/child and of the mother over the longer-term. In addition, investigating

these relationships during the sensitive period of pregnancy may help us to more broadly understand the impacts of environmental exposures and nutrition and their interactions on human health.

In this thesis, I investigated the potential relationships between metal exposures and vitamin D in pregnancy, and their relationship with preterm birth and specific preterm birth phenotypes (early vs late; spontaneous vs. medically induced). I then investigated the potential long-term impact of pregnancy complications on maternal health approximately 9 years postpartum.

1.2 VITAMIN D BACKROUND

Vitamin D is one of the primary biological regulators of calcium homeostasis [24] and is important for bone mineralization and neuromuscular function [25]. Vitamin D status is influenced by many factors including variation in sun exposure and intensity (e.g. clothing, air pollution, sunscreen use, latitude, time of day, skin pigmentation, season), age, obesity and several chronic diseases as well as dietary and supplement intake [26,27].

Vitamin D Status

The most widely accepted biomarker of vitamin D status is serum 25OHD given that this is the key substrate required conversion to the biologically active form, 1,25-hydroxyvitamin D ($1,25(OH)_2D$, also known as calcitriol) [24]. The half-life of 25OHD is 2-3 weeks in circulation in contrast to calcitriol, which has a half-life of only 4-6 h. In addition calcitriol is present in very low concentrations (1000 fold less than 25OHD) and is variable through the day with multiple peaks and nadirs making it a difficult biomarker to interpret [28] . It is estimated that 85-90% of

25OHD is bound to vitamin D binding protein (DBP), 10-15% to albumin and less than 1% is free or unbound. The biomarker 25OHD is based on the sum of bound and free 25OHD [$25OHD_2$ and $25OHD_3$][29] (See Appendix A: Vitamin D Backgrounder, Figure 2).

In common with other nutrient trials and distinct from many pharmaceutical trials, a difficulty in vitamin D supplementation trials is that participants have differing baseline circulating levels of 25OHD as well as differing activity levels of the enzyme, cytochrome P450. For example, when vitamin D status is inadequate, CYP24A1 enzyme activity (hydroxylates both 25OHD and $1,25(OH)_2D$) is lower so the response to intakes will be increased. The nutrient-response curve is an inverted U-shape, where initially the risk of adverse effects from deficiency decline as intake increases up to what is defined as an adequate range. Above this range, the risk of adverse effects may increase. Thus, the response by an individual to increased intake depends on their starting point on this curve [30]. Above this range, the risk of adverse effects may increase.

There is great heterogeneity in how studies define vitamin D deficiency and sufficiency. There is limited consensus on the criteria for insufficient/sufficient concentrations of vitamin D in pregnant women. Health Canada and the Institute of Medicine recommend 600 IU/d intake of vitamin D during pregnancy [31,32]. The Institute of Medicine has no specific recommendation for pregnant women but suggests that 25OHD concentrations >50 nmol/L are sufficient to prevent bone manifestations of vitamin D deficiency in practically all people [32]. The Canadian Paediatric Society defines optimal 25OHD concentrations at 75 to <225 nmol/L for pregnant and lactating women [33]. This level is incongruent with the IOM which considers concentrations above >125 as having the potential to cause adverse advents.

Health Canada's Recommended Dietary Allowance (RDA) is 600 IU for adults; set to ensure adequate vitamin D status without sun exposure given public concerns over skin cancer [31] (Table 1). Experts agree that concentrations of 25OHD <30 nmol/L put people at risk for deficiency (i.e. two metabolic diseases - rickets in growing children or osteomalacia in older children or adults) [31,34]. Both the Institute of Medicine and Health Canada agree that serum 25OHD concentrations ≥50 nmol/L are sufficient for bone health in most people while serum concentrations >125 nmol/L may be of concern [31]. Excessive intake of vitamin D can cause severe hypercalcemia leading to clinical symptoms of confusion, apathy, recurrent vomiting, abdominal pain, excessive urination (polyuria), thirst (polydipsia) and dehydration [35].

Table 1: Dietary Reference Intakes (DRIs)

	Recommended Dietary Allowance (RDA) per day	Tolerable Upper Intake Level (UL) per day
Infants (0-6 mo.)	400 IU	1000 IU
Infants (7-12 mo.)	400 IU	1500 IU
Children (1-3 y)	600 IU	2500 IU
Children (4-8 y)	600 IU	3000 IU
Children & Adults (9-70 y)	600 IU	4000 IU
Adults (>70y)	800 IU	4000 IU
Pregnancy & Lactation	600 IU	4000 IU

Table 2: Vitamin D Status[31]

	25OHD concentration	
	nmol/L	ng/mL
Deficient	<30	<12
Inadequate: for bone and overall health (some people potentially at risk for inadequacy)	30-50	12 to <20
Sufficient: adequate for bone and overall health for practically all people	>50 to ≤125	≥20 to <50
Of potential concern	>125	>50

Inadequate (defined as levels ranging between 30-50 nmol/L) or deficient vitamin D status has been associated with increases in risk of cardiovascular disease, cancers, poorer immune function regulation and

adverse pregnancy outcomes [4,36–40]. However, there are important controversies surrounding vitamin D research including the definitions of insufficiency and inadequacy, and the role of free versus total 25OHD measurement [41].

The next sections will introduce the toxic metals we examined in this thesis and the potential for interaction with vitamin D. Vitamin D is a probable antioxidant [42] and may help to counteract the effects of toxic metals. On the other hand, metals are known to cause nephrotoxicity [43–45] and act as endocrine disruptor [46], potentially disrupting vitamin D metabolism.

1.3 TOXIC METALS

There is no known safe level of exposure to toxic heavy metals. The half-life of metals ranges from hours to decades depending on the biological matrix (See Table 3).

Table 3: Heavy Metal half-lives in various biological matrixes

Metal	Blood	Urine	Bone	Kidney	Liver	References
Lead	20-40 d		10-30 y	20-40 d	20-40 d	[47]
Mercury (total)	42-70 d					[48,49]
Cadmium	3-4 mo			6-38 y	4-19 y	[50,51].
Arsenic	20 h	4 d				[52]

Heavy metals are naturally occurring elements that have high atomic weight and density (≥5 times that of water) and include lead (Pb), mercury (Hg), cadmium (Cd), arsenic (As) and manganese (Mn)[53]. The main source of exposure to heavy metals for most people is food (e.g. arsenic in rice, mercury in wild fish) but other sources include water, soil, dust and air (industrial and mining emissions or automobile exhaust). In the case of Pb, common exposure sources include paint chips from older houses, some imported costume jewellery, improperly glazed

pottery or ceramic dishes and leaded crystal glassware (ATSDR, 1999, 2007a, 2007b, 2012). The main source of non-occupational Cd exposure is smoking [58].

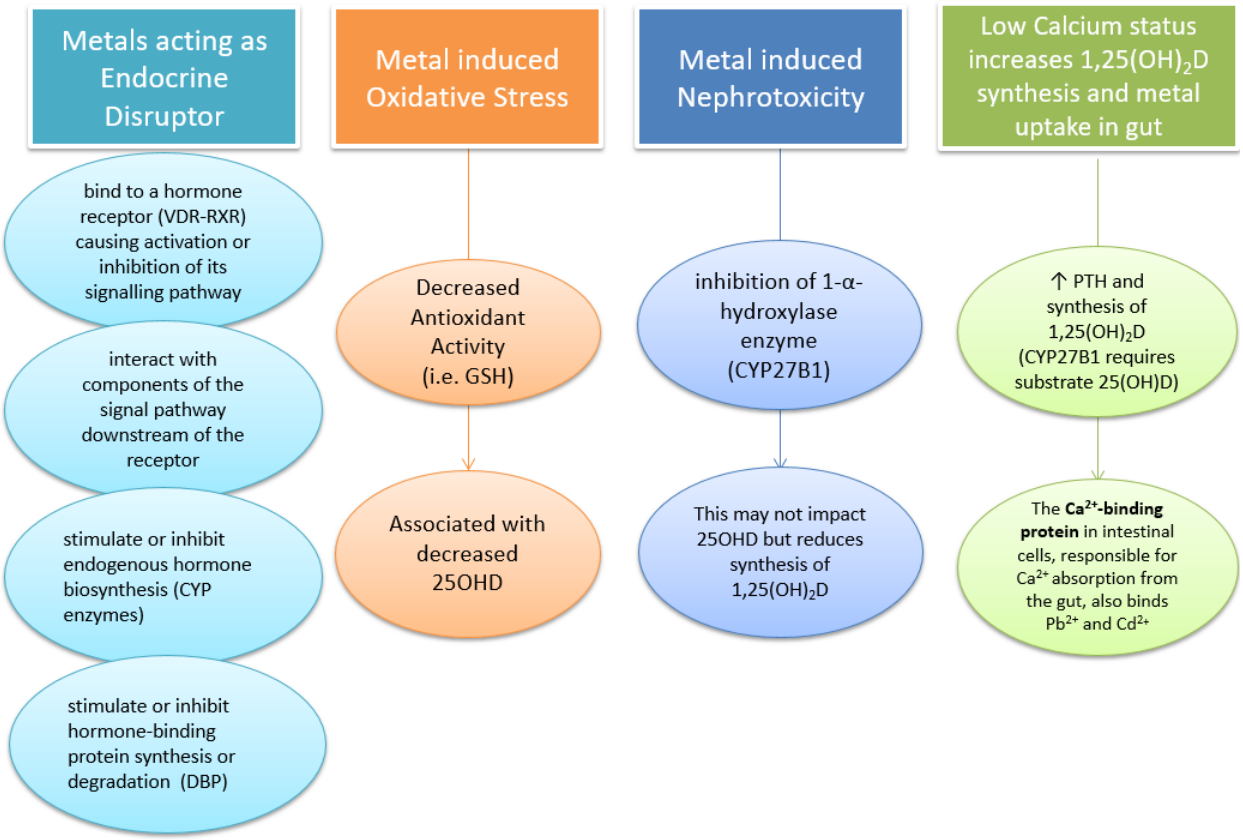
Arsenic exposure consists of both organic and inorganic arsenic (InAs). Organic arsenic is mainly found in seafood, is largely comprised of non-toxic arsenosugars, arsenolipids and arsenobetaine (AsB) and excreted essentially unchanged in urine [59]. In contrast, InAs is mainly stored in the liver over the long-term and is a known human carcinogen [60]. Long-term exposure to arsenic can increase the risk of skin, bladder and lung cancer [61]. Occupational exposure to InAs is mainly through inhalation of industrial emissions such as non-ferrous smelting, arsenic production, wood preservation, glass manufacturing and the production and application of arsenic-based pesticides and electronics. Non-occupational exposure to inorganic arsenic can occur through food (e.g. rice, apple juice, red wine) or water, in regions of high levels of naturally occurring arsenic (Taiwan, Bangladesh, West Bengal (India), northern Chile and Cordoba Province, Argentina) [61].

Mercury exists as elemental Hg (metallic Hg), inorganic Hg, and organic Hg (methylmercury, meHg). In the general population, the main sources of Hg exposure are organic Hg from seafood (tuna, marlin, swordfish, and shark [62]), inorganic Hg from emissions (coal-fired power plants, municipal and medical waste burning[63]) and skin-lightening products in some countries [63], and elemental Hg (Hg vapour) from dental amalgams [56].

1.4 TOXIC METALS AND VITMAIN D

While the exact mechanism by which heavy metals and vitamin D interact is not completely understood, but at least four plausible mechanisms have been identified (See Figure 2). Firstly, metals such as Pb and Cd are known to cause nephrotoxicity and affect vitamin D metabolism through the inhibition of the 1-α-hydroxylase enzyme in the proximal renal tubules [43–45] therefore affecting renal hydroxylation of 25OHD to $1,25(OH)_2D$. The resulting reduction in $1,25(OH)_2D$ in turn results in impaired calcium absorption across the GI tract and proximal renal tubules [43]. For example, the most severe form of chronic high dose Cd exposure is itai-itai disease, characterized by proximal tubular dysfunction in the kidneys [64] and multiple bone fractures due to osteomalacia, identified in the Jinzu River basin of Toyama Prefecture, Japan. The river had been polluted with heavy metals by slag from an upstream mine polluting the soil in rice paddies through irrigation. Patients with itai-itai disease, and Cd-exposed people with renal damage were found to have lower concentrations of the biologically active form of vitamin D ($1,25(OH)_2D_3$) than non-exposed people.[44,65] Other studies of individuals exposed to high Cd levels through pollution similarly found associations with vitamin D binding protein production[44,66,67]. Vitamin D binding protein is important for the transport of 25OHD from the liver to the kidneys so it can be hydroxylated to its active form, $1,25(OH)_2D$.

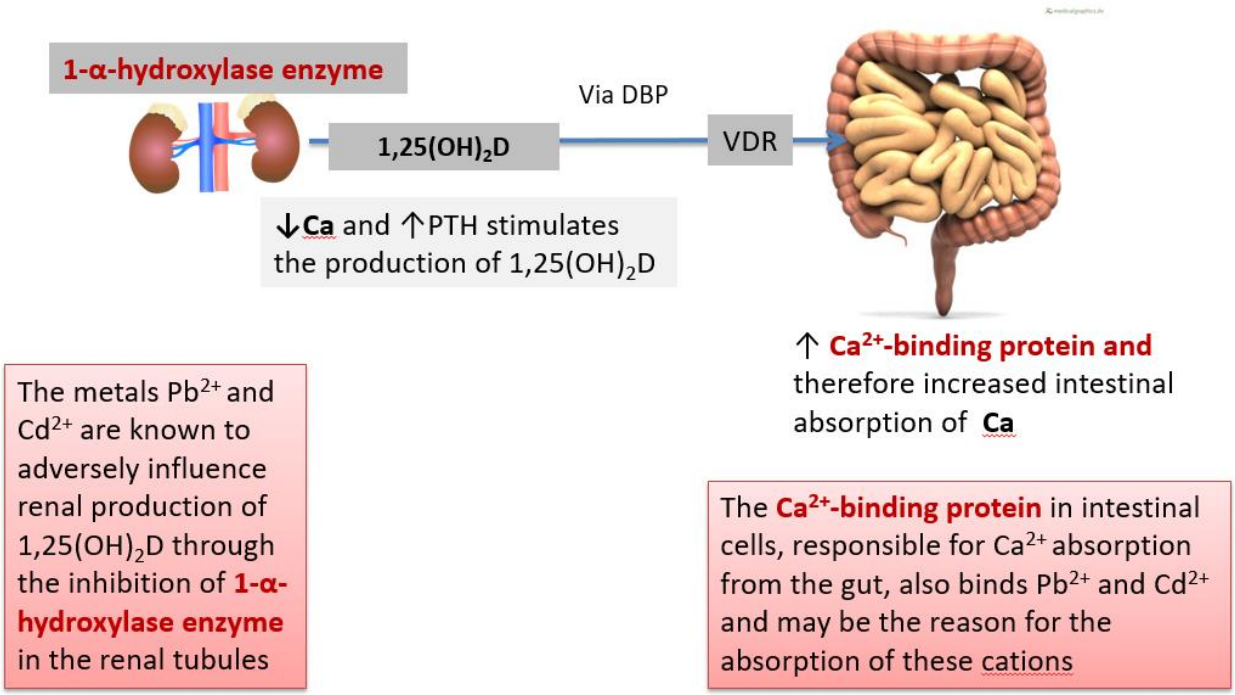
Figure 2: Potential Mechanisms that Metals are associated with Vitamin D concentrations



A second mechanism many relate to intestinal absorption of metals. $1,25(OH)_2D$ production may stimulate intestinal metal absorption through the Ca^{2+}-binding protein (See Figure 3) [45]. Therefore, calcium (Ca) status may play an important role. Low Ca and high parathyroid hormone (PTH) stimulate the production of the biologically active form of vitamin D, $1,25(OH)_2D$, which in turn binds with the Vitamin D receptor retinoid X receptors VDR-RXR heterodimer to stimulate intestinal absorption of Ca. The Ca^{2+}-binding protein in intestinal cells, responsible for Ca^{2+} absorption from the small intestine [68], also binds Pb^{2+} and Cd^{2+} and may be the mechanism for the absorption of these divalent cations. However, experimental studies

suggest that other mucosal proteins play an important role in the absorption of lead [69]. Prior to the discovery of vitamin D in the early 1930s [70], summer outbreaks of pediatric Pb poisoning were reported. Later it was thought that increased summer solar exposure and vitamin D production in the skin resulted in an increase in Ca^{2+}-binding protein, in turn causing the increased absorption of lead [45]. Corroborating these possible mechanisms, calcium supplementation may also reduce blood lead levels [71].

Figure 3: Vitamin D, Calcium and Metals



Reference: Moon, J. (1994) 'The role of vitamin D in toxic metal absorption: A review', Journal of the American College of Nutrition, 13(6), pp. 559–564. doi: 10.1080/07315724.1994.10718447.

VDR=vitamin D receptor; Ca=Calcium; PTH= Parathyroid Hormone; $1,25(OH)_2D$= 1-25hydroxyvitamin D (also known as calcitriol)

A third mechanism may be endocrine disruption. $1,25(OH)_2D$ is a steroid hormone which functions like other steroid hormones (estradiol, cortisol and aldosterone) by interacting with the VDR-RXR heterodimer [24]. Therefore, most tissues that possess the enzyme 1α-hydroxylase could be considered endocrine tissues that converts 25OHD to the hormonal form $1,25(OH)_2D$. An endocrine disrupting chemical is defined as a chemical or mixture of chemicals that interferes with normal hormone biosynthesis, signalling, or metabolism in the human body [72,73]. Specifically, this means that an endocrine disrupting chemical can:

1) bind to a hormone receptor causing activation or inhibition of its signalling pathway,
2) interact with components of the signal pathway downstream of the receptor,
3) stimulate or inhibit endogenous hormone biosynthesis,
4) bind to circulating hormone-binding protein (i.e. vitamin D binding protein),
5) stimulate or inhibit hormone-binding protein synthesis or degradation (i.e. vitamin D binding protein), and/or
6) stimulate or inhibit hormone receptor expression [74].

Close to 1000 chemicals, including metals [46], have been identified as potential endocrine disrupting chemicals yet many chemicals in commercial use have not been tested, leaving uncertainty surrounding the health risks of these chemicals [75,76]. For vitamin D, endocrine disrupting chemicals could cause induction or inhibition of the cytochrome P450 enzymes, vitamin D binding protein[77] or VDR-RXR heterodimer [78].

Finally, the fourth mechanism stems from studies that suggest that metals induce oxidative stress [48,79] which may disrupt vitamin D metabolism through an effect on the CYP2R1 enzyme [80]. Increasing concentrations of blood lead have been associated with decreased antioxidant activity,

including the antioxidant, glutathione [81]. Moreover, studies show a positive association of glutathione and one of its building blocks (L-cysteine), with 25OHD serum concentrations in people who are obese and have type II diabetes [82,83]. Supplementation with L-cysteine was shown to elevate glutathione, up-regulate 25-hydroxylase activity in the liver, and improve serum 25OHD [83].

Studies of examining the association between metals and vitamin D

Several occupational studies have identified associations between high levels of exposure to heavy metals and vitamin D concentrations. For example, battery manufacturer workers exposed to Pb in India had lower levels of serum 25OHD compared to controls [84,85]. Among male smelter works from Israel, a study also identified variations in $1,25(OH)_2D_3$ based on groupings by Cd and Pb concentrations [86]. Those with high Cd and low Pb showed the lowest concentrations of serum $1,25(OH)_2D_3$, compared to others with low levels of both contaminants or high levels of both contaminants. In Bangladesh, jewellery workers exposed to lead fumes and dust for the last 10-25 years had significantly higher blood lead levels (69 vs 15 µg/dL) and lower 25OHD concentrations (13 vs 33 ng/mL) compared to controls who were office workers not directly exposed to fumes but working in the same facility[43].

Studies of children and pregnant women have also identified relationships between heavy metals and vitamin D. For example, cross-sectional studies in the U.K. [87] and south east China [88] found inverse correlations between blood lead levels and serum 25OHD in children. Chang et al. [84] found that a greater proportion of children with 25OHD concentrations ≥39 ng/mL had low blood lead levels (61.4%) compared to children with insufficient (24.4%) or deficient (14.1%) 25OHD. In an intervention study to reduce blood lead levels in children [89], mean serum 25OHD

was significantly lower in children ages 1-5 years with higher blood lead levels (among children with Pb concentrations in blood ≥60 ug/dL, mean 25OHD was 20 ng/ml) compared to children with intermediate blood lead levels (among those with Pb 30-59 ug/dL, mean 25OHD was 28 ng/mL) and lower blood lead levels (with Pb ≤29 ug/dL, mean 25OHD was 27 ng/mL). A recent study from the U.S. LIFECODES pregnancy cohort (n=381), reported that higher concentrations of 25OHD (>50 nmol/L) were associated with lower levels of urinary Pb, but not Cd, in the 2nd trimester [90]. Just 34% of women had 25OHD concentrations that were considered low (below 50 nmol/L). Those participants with low 1st trimester 25OHD concentrations (<50 nmol/L) had 47% higher urinary lead concentrations in the 2nd trimester. In Canada, in the MIREC cohort study, investigators found significant inverse associations between estimated vitamin D intake in pregnant women (as estimated from a food frequency questionnaire) and maternal Cd, Pb, Mn and Hg, as well as cord blood Pb [91]. Finally, a recent randomized controlled trial in Bangladesh [92] investigated whether vitamin D supplementation increased the absorption of Cd and Pb. Pregnant participants in their 2nd trimester were randomized to receive weekly doses of 4,200, 16,800 or 28,000 IU of vitamin D_3 throughout pregnancy. The results did not show any association of vitamin D3 dose with metal levels during pregnancy, but there was a significant increase in cord blood Pb and Cd among higher dosed groups compared to placebo. Noteworthy, this population had much higher metal levels than found in Canada.

Summary: Association between Vitamin D and Metals

There is a body of published evidence indicating that long-term, high levels of exposure to heavy metals is associated with lower vitamin D status. However, less is known about lower doses of heavy metals that also appear to disrupt vitamin D metabolism through endocrine disruption, nephrotoxicity and/or the inhibition of 1-α-hydroxylase enzyme in the renal tubules.

In addition, although vitamin D is considered a probable antioxidant [42], metals have been shown to induce oxidative stress and reduce important antioxidants in the body, and this may in turn be associated with lower serum 25OHD [81]. Existing occupational studies in smelter workers [86], lead-acid battery manufacturers [84], and jewellery workers [43] are focused on high levels of exposure. The majority of reported studies (occupational and general population) investigating the impact of environmental exposures in the population have used cross-sectional designs, leaving uncertainty with respect to the temporal direction of associations between metals and vitamin D. Finally, few studies have looked at the relation between metals and vitamin D in the pregnant population.

A number of studies suggest that vitamin D status and toxic metals are associated with preterm birth. The next sections go on to explore this literature.

1.5 PRETERM BIRTH

Direct complications of preterm birth are the leading cause of death among children under 5 years of age globally [93]. Preterm birth is believed to be causally related to neurodevelopmental disorders [94] and is associated with an increased risk of childhood asthma [95], and cardiovascular diseases in young adulthood [96]. Having a preterm baby has also been identified as associated with approximately a doubling of the risk of a future cardiovascular disease in women [97,98].

Table 4: Risk Factors for Preterm Birth

Potentially Modifiable	Non-modifiable
BMI (maternal obesity)	
Cigarette smoking	Prior preterm birth
Drug and alcohol use	Age <18 or >40 years
Absent prenatal care	Low SES
Short inter-pregnancy interval	Cervical injury
Anemia	Uterine anomaly or fibroid
Bacterial urinary tract infection	Multiple pregnancy
Genital infection	Vaginal bleeding
High personal stress or strenuous work	Excessive uterine activity
High Temperatures	Pregnancy complications (i.e. preeclampsia)
Maternal zinc deficiency	
References: [99,100]	

Global estimates suggest that the rate of preterm birth, defined as birth <37 wk gestational age (GA), is approximately 10.6% of all live births [101] and the rates appear to be increasing in nearly all countries. In Canada the rate of preterm birth increased from 7.21% in the year 2000 to 8.15% in 2014 [102]. The majority of preterm births occur in the late preterm period (34^{+0} to 36^{+6} weeks) [103].

Although several factors associated with preterm birth (Table 4) have been identified, the precise etiology for preterm birth remains elusive [100]. McElrath et al. (2008) describes 6 pregnancy conditions that lead to preterm delivery: 'spontaneous' preterm labour, fetal indication/IUGR, preterm premature rupture of membrane (pPROM), preeclampsia, placental abruption, and cervical insufficiency. The authors suggest that these can be further classified into 2 broad categories:

1) The "inflammatory" group characterized by evidence of inflammation and infection (without indicators of impaired placentation), which includes spontaneous preterm labour, pPROM, placental abruption and cervical insufficiency.

2) The "placental dysfunction" group characterized by the relative absence of inflammation but evidence of abnormal placentation (infarcts and syncytial knots in the placenta). It includes medically indicated preterm deliveries, subsequent to conditions such as preeclampsia or intrauterine growth restriction. On average, women in this group delivered at older gestational ages [104].

1.6 PRETERM BIRTH, METALS and VITAMIN D

Hypothesized mechanism: Inflammation and Oxidative stress

Heavy metals may be associated with preterm birth through oxidative stress and inflammation [79]. "Oxidative stress" is defined as an imbalance between cellular reactive oxygen species (ROS) and antioxidants [105] and has been associated with a number of pregnancy outcomes including preterm birth [106,107]. ROS are "molecules containing at least one atom of oxygen and have the potential to generate free radicals" [106]. ROS are important in cellular function however their excess can cause DNA, lipid and protein damage. Infection and metals such as Pb are known to up-regulate ROS [79] while micronutrients are known to down-regulate ROS [108]. ROS can also stimulate pro-inflammatory enzymes [109]. Studies have shown associations between a group of intracellular proteinases called matrix metalloproteinases (MMPs) and metals [110,111]. MMPs act broadly to regulate the inflammatory process [112] and, importantly in the context of pregnancy,

they are involved in embryo implantation, placentation, and cervical dilation [113,114]. In a pregnancy cohort, metal-specific changes in MMP profiles were observed with trimester-specific differences in MMP responses [111]. Elevated MMP-1 and MMP-2 have shown borderline statistical associations with premature rupture of membranes (PROM) while MMP-9, which has known associations with cervical ripening and preterm birth [115], was associated with elevated mercury and lead exposure in the first trimester.

Studies suggest that vitamin D may also play a role in antioxidant activity [42]. In a double blind RCT of 48 pregnant women who were randomized to receive 400 IU/d of cholecalciferol (D_3) supplements or placebo for 9 weeks, investigators found significant decreases in inflammatory markers (C-Reactive Protein) and significant increases in plasma total antioxidant capacity and total glutathione concentrations, a known antioxidant, among those who received the vitamin D supplements [116]. In another RCT of 60 women with gestational diabetes, participants had reduced concentrations of biomarkers of inflammation and oxidative stress after co-supplementation with magnesium, zinc, calcium and vitamin D [117].

Vitamin D and Preterm Birth: Epidemiologic Evidence

In a meta-analysis of ten observational studies, including over 10,000 participants, investigators estimated an increased risk of preterm birth for participants with 25OHD concentrations <20 ng/mL (OR=1.29, 95% CI: 1.16, 1.45) and <30 ng/mL (OR=1.25, 95% CI: 1.11, 1.40) compared to participants with concentrations above those levels. The sensitivity analysis showed that the removal of any single study yielded similar results to the combined effect [4]. In addition, a recent meta-analysis of 6 intervention studies [3,116,118–121] found that vitamin D supplementation alone during pregnancy was associated with a reduced risk of preterm birth (pooled RR=0.57, 95% CI:

0.36-0.91) [5]. However, the synthesized trials varied in quality, supplementation dose and design.

Metals and Preterm Birth: Epidemiologic Evidence

Studies examining the association between heavy metals and preterm birth have yielded inconsistent results with most studies focusing on Pb and Cd. Many studies have limited power and/or focus on highly exposed populations. A few studies have examined the effect of metals based on the timing of the preterm birth (early, moderate, or late during the preterm period of <37 weeks); however inconsistency exists in these definitions across studies [122–124]. Timing of exposure during pregnancy may also be important but has been compared in just two studies [123,125]. In a paper by Ferguson and Chin[126] it was recommended that to understand the mechanisms behind chemical exposures and preterm birth and to strengthen our ability to make causal inferences, it is important to separate preterm birth phenotypes in the analysis and to measure the association of exposures with biomarkers of metabolic pathways. No study has looked at the potential interaction between vitamin D and metal concentrations in association with preterm birth.

Lead and Preterm Birth: Pregnancy cohort studies from China ($mean_{Pb}$ 1.5 µg/dL) [100,123], Iran (GM_{Pb} 3.8 µg/dL) [127], Australia ($mean_{Pb}$ 10.6 µg/dL)[128] and the United Kingdom ($mean_{Pb}$ 3.7 µg/dL)[129] have found elevated odds of preterm birth with increasing maternal blood Pb concentrations. In addition, studies from India [130], Indonesia [108], Australia [131] and Spain [132] have shown elevated placental Pb levels in preterm versus term deliveries. In contrast, other studies that measured maternal blood Pb exposure during pregnancy[124,133,134], at birth [108] or at the time of follow-up[135], did not report an association with preterm birth.

Cadmium and Preterm Birth: The Japan Environment and Children's Study, a cohort of nearly 15,000 women and their children, found an association between higher whole blood Cd

concentrations measured during pregnancy (14-39 wk) and early preterm birth (22 to <34 wk) compared to term birth (≥37 wk) [124]. Those pregnancies in the highest quartile of Cd exposure were almost twice as likely to have an early preterm vs. term birth (OR:1.91, 95% CI: 1.12-3.27). In a Chinese cohort study, high Cd concentrations (3rd tertile) in both the 1st (OR:4.51; 95% CI: 2.02, 10.06) and 2nd (OR:2.68; 95% CI: 1.68, 4.27) trimester were associated with an elevated risk of preterm birth compared to the 1st tertile of exposure [100].

Mercury and Preterm Birth: In a US study [122], high hair Hg (≥90th percentile), collected between the 15th and 27th week of pregnancy, was associated with elevated odds of preterm delivery at <37 wk (OR:1.55, 95% CI: 0.7, 29) and <35 wk (OR: 3.0, 95% CI: 1.3, 6.7) compared to term births. Placental Hg levels were increased in preterm vs. term deliveries in a cross-sectional study of 51 pregnant women from Indonesia [108]. However, no association was seen with maternal serum or cord blood Hg levels. Other studies have found no association between maternal blood Hg collected during pregnancy [124] or at birth [136] and preterm birth. The inconsistencies across these studies may be due to the different in biological matrixes used for the biomarker analysis of total Hg (hair, placenta, maternal serum, cord blood) which may reflect different exposure windows and measurement error.

Arsenic and Preterm Birth: Fewer studies have explored the association between blood As and preterm birth [137]. In the China-Anhui Birth Cohort, higher preterm birth rates were observed among participants with As concentrations above the 75th percentile compared to those below this cut-point (OR=1.5, 95% CI: 1.08, 2.09). Highly exposed populations in Bangladesh and Taiwan, have shown elevated rates of preterm birth based on drinking water [138–140] and toenail As levels [140]. In the US, preterm birth was associated with higher estimates of As in water [141,142].

1.7 PREGNANCY AS A SENSITIVE WINDOW

Understanding the associations of metals, vitamin D, and their interactions with pregnancy complications such as preterm birth has important implications for offspring health, during infancy and beyond. In addition, pregnancy is increasingly being recognized as a window of time during which we can identify women with higher future cardiovascular disease risk. Pregnancy is a natural 'physiological stress test' to the heart, causing structural and hemodynamic changes due to increased blood volume and cardiac output [97,143,144]. A number of reviews have found that women who experience a pregnancy complication, including preterm birth, gestational diabetes and hypertensive disorders in pregnancy, have a higher risk of cardiovascular morbidity and mortality [98,145,146]. Many of these studies are based on administrative databases, with a focus on clinical endpoints, and there is great heterogeneity in follow-up time within and across studies. Fewer studies have explored the long-term impact of pregnancy complications on markers of inflammation and insulin resistance. For example, studies suggest dysregulation of adipokines in women experiencing hypertensive disorders in pregnancy [147] and gestational diabetes [148] compared to uncomplicated pregnancies but few studies have explored the long-term effect of pregnancy complications on these markers in the postpartum period [149,150].

1.8 OBJECTIVES OF DISSERTATION

The objectives of this thesis are to understand the associations between metal exposures and 25OHD in pregnancy, their relationship with preterm birth, and the potential long-term health consequences for maternal cardiometabolic health. I pursued three specific research questions, each of which is addressed in a manuscript within this dissertation:

Paper #1: Are increasing blood metal exposures in pregnancy (Pb, Cd), associated with maternal plasma 25OHD concentrations concurrently and longitudinally? Is there a bidirectional association between metals and 25OHD?

Paper #2: Are metal concentrations associated with preterm birth and spontaneous preterm birth, and is this association modified vitamin D status?

Paper #3: Are pregnancy complications, such as preterm birth, associated with long-term markers of maternal cardiometabolic health? This includes percent body fat, systolic and diastolic blood pressure, blood lipids, glucose and markers of inflammation and insulin resistance.

1.9 THE MATERNAL-INFANT RESEARCH ON ENVIRONMENTAL CHEMICALS (MIREC) STUDY

This thesis relied on secondary analyses of data collected from the Maternal-Infant Research on Environmental Chemicals (MIREC) study. The MIREC Study [151] is a pregnancy cohort of approximately 2000 women recruited from 10 cities across Canada. The MIREC study recruited participants through medical clinics (ultrasound, midwife and/or doctor's clinics) during the first trimester of pregnancy (6 to <14 weeks) between 2008 and 2011. Participants who gave informed consent participated in scheduled visits in each trimester, at delivery and in the postpartum period (up to 8-10 weeks). At each pregnancy visit women completed questionnaires and provided both blood and urine samples.

The MIREC study has had a number of follow-up studies since 2011, mainly focusing on the health of the child [152,153]. However, most recently in Phase I of the MIREC-Endocrine and

Metabolic Function study (MIREC-ENDO) mothers were invited back into the study to measure cardiometabolic health outcomes. With consent, mothers attended a clinic visit and completed a questionnaire that asked about their obstetrical history, smoking status, sociodemographics and stressful life events. They had their height, weight and body fat percentage and blood pressure measured. Participants also provided a fasting blood sample, which was subsequently analyzed for concentrations of glucose, cholesterol (low density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (TC), triglycerides (TG), high-sensitivity C-reactive protein (hs-CRP), leptin and adiponectin.

1.10 ETHICAL CONSIDERATIONS

The MIREC study received research ethics board approval at the MIREC study coordinating center (CHU Ste-Justine in Montreal) and Health Canada, along with the other 10 study sites across Canada including: Children's and Women's Health (Vancouver), University of Alberta (Edmonton), Health Sciences Center and St-Boniface Hospital (Winnipeg), Mount Sinai Hospital (Toronto), McMaster University Medical Center (Hamilton), Sudbury Regional Hospital (Sudbury), Kingston General Hospital (Kingston), The Ottawa Hospital (Ottawa), CHU Ste-Justine (Montreal), Jewish General Hospital (Montreal), IWK Health Centre (Halifax).

All women provided informed consent for the study.

1.11 RELEVANCE AND IMPLICATIONS

This research provides further understanding of the nutrient-environment interplay between metals and vitamin D status, provides important insight into the relationships of these exposures to preterm birth risk, and identifies the potential impacts for long-term maternal health. Nutrition

could be a practical means for intervention and prevention of adverse outcomes associated with metal exposures, given the ubiquitous nature of heavy metals [2,15]. The prevalence of vitamin D deficiency (<30 nmol/L) is estimated at 7.4% in Canada while levels of <50nmol/L are estimated at 37% [154]. Many countries report vitamin D deficiency among >20% of their population including India, Tunisia, Pakistan and Afghanistan [155]. The health and economic costs of preterm birth are enormous at $26.2 billion in the U.S. [156] and close to $600 million in Canada [157]. Being born preterm is the number one cause of neonatal death, and greatly increases the risk of childhood neurodevelopment disorders and asthma and cardiovascular disease later in life [93,94,158]. Mothers who have a preterm baby are also more likely to develop cardiovascular disease later in life [159] and this research contributes to understanding the role of the association of complications with markers of cardiometabolic health. It is also evident that metal exposure is persistent. Lithium and cobalt production will undergo exponential growth over the coming decades due to the demand for lithium-ion batteries in electric vehicles [160] . Metal exposure is increasing through solar panel production, which includes heavy metals such as lead [161], and electronic waste [162]**.** Moreover, climate change is projected to cause increased soil leaching and runoff of metals such as Cd [163].

1.12 SUMMARY

This thesis sheds light on the association between heavy metals and vitamin D status in pregnancy, their association with preterm birth, and the potential longer-term impacts for maternal health. I first sought to understand the relationships between metals and vitamin D using data from a maternal-child cohort with multiple measures of both 25OHD and heavy metals in pregnancy, specifically investigating the potential bidirectional nature of these relationships. In the same cohort, we then examined the associations between metals and preterm

birth and the possible modification of these associations by vitamin D status. Finally, we investigated the impact of pregnancy complications on long-term cardiometabolic health among mothers in this cohort. This thesis used a variety of statistical techniques to investigate bidirectional associaitons and time-to-event outcomes. The impact of heavy metal exposure, vitamin D deficiency (<50 nmol/L) and preterm birth on maternal and child health is substantial, underscoring the importance of this study.

1.13 THESIS ORGANIZATION

This thesis is organized in article format in accordance with the School of Epidemiology and Public Health (SEPH), University of Ottawa, Guidance for Thesis by Article. A short summary is provided below:

Chapter 1: is the introduction and provides background and rationale for the thesis and outlines the objectives;

Chapter 2: is the first article of the thesis, titled "Blood Metals and Vitamin D Status in a Pregnancy Cohort: A Bidirectional Biomarker Analysis". This article was published in ***Environmental Research.***

> Fisher M, Potter B, Little J, Oulhote Y, Weiler HA, Fraser W, Morisset AS, Braun J, Ashley-Martin J, Borghese MM, Shutt R, Kumarathasan P, Lanphear B, Walker M, Arbuckle TE. Blood metals and vitamin D status in a pregnancy cohort: A bidirectional biomarker analysis. Environ Res. 2022 Aug;211:113034. doi: 10.1016/j.envres.2022.113034. Epub 2022 Feb 28. PMID: 35240110.

Chapter 3: representst the second article of the thesis, titled "Association between toxic metals, vitamin D and Preterm Birth in the Maternal-Infant Research on Environmental Chemicals Study". This article has been accepted for publication in ***Paediatric and Perinatal Epidemiology.***

> Fisher, M., Marro, L., Arbuckle, T.E., Potter, B.K., Little, J., Weiler, H., Morisset, A.S., Lanphear, B., Oulhote, Y., Braun, J.M., Kumarathasan, P., Walker, M., Borghese, M.M.,

Ashley-Martin, J., Shutt, R., Fraser, W.D. Association between toxic metals, vitamin D and Preterm Birth in the Maternal-Infant Research on Environmental Chemicals Study. Paediatric and Perinatal Epidemiology (accepted January 27, 2023).

Chapter 4: is the third article of the thesis, titled "The Association between Pregnancy Complications and Long-Term Maternal Cardiometabolic Health in the MIREC Cohort Study". This article is in preparation for submission to a peer reviewed journal.

Chapter 5: is the integrated discussion and provides a summary of chapters 2-4 and gives some insight into the main points of integration across the studies and lessons learned that could be applied to the field of environmental epidemiology. We used the integrated discussion framework proposed by Lewis et al. [164] to help guide the writing of this chapter.

Appendix A: provides a thorough background of vitamin D metabolism, titled "Vitamin D Backgrounder".

REFERENCES

1. Hennig, B. *et al.* Using nutrition for intervention and prevention against environmental chemical toxicity and associated diseases. *Environ. Health Perspect.* **115**, 493–495 (2007).

2. Hennig, B. *et al.* The role of nutrition in influencing mechanisms involved in environmentally mediated diseases. *Reviews on Environmental Health* at https://doi.org/10.1515/reveh-2017-0038 (2018).

3. Asemi, Z. & Esmaillzadeh, A. Effects of high-dose Vitamin D supplementation on metabolic status and pregnancy outcomes in pregnant women at risk for pre-eclampsia. *Int. J. Prev. Med.* **6**, 62 (2015).

4. Qin, L. L., Lu, F. G., Yang, S. H., Xu, H. L. & Luo, B. A. Does maternal Vitamin D deficiency increase the risk of preterm birth: A meta-analysis of observational studies. *Nutrients* **8**, (2016).

5. Zhou, S. S., Tao, Y. H., Huang, K., Zhu, B. B. & Tao, F. B. Vitamin D and risk of preterm birth: Up-to-date meta-analysis of randomized controlled trials and observational studies. *J. Obstet. Gynaecol. Res.* **43**, 247–256 (2017).

6. Public Health Agency of Canada; Government of Canada. Prenatal Nutriton Guidelines for Health Professionals: Gestational Weight Gain. https://www.canada.ca/en/health-canada/services/food-nutrition/healthy-eating/healthy-weights/prenatal-guidelines-professionals-gestational-weight-gain.html (2014).

7. Al-Gubory, K. H. Environmental pollutants and lifestyle factors induce oxidative stress and poor prenatal development. *Reprod. Biomed. Online* **29**, 17–31 (2014).

8. Renz, H. *et al.* An exposome perspective: Early-life events and immune development in a changing world. *J. Allergy Clin. Immunol.* **140**, 24–40 (2017).

9. Shafei, A. E. S. *et al.* Prenatal Exposure to Endocrine Disruptors and Reprogramming of Adipogenesis: An Early-Life Risk Factor for Childhood Obesity. *Child. Obes.* (2018) doi:10.1089/chi.2017.0180.

10. Heindel, J. J., Skalla, L. A., Joubert, B. R., Dilworth, C. H. & Gray, K. A. Review of developmental origins of health and disease publications in environmental epidemiology. *Reprod. Toxicol.* **68**, 34–48 (2017).

11. Schrott, R., Song, A. & Ladd-Acosta, C. Epigenetics as a Biomarker for Early-Life Environmental Exposure. *Curr. Environ. Heal. Reports* 604–624 (2022) doi:10.1007/s40572-022-00373-5.

12. Gore, A. C. *et al.* EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. *Endocrine Reviews* at https://doi.org/10.1210/er.2015-1010 (2015).

13. Di Renzo, G. C. *et al.* International Federation of Gynecology and Obstetrics opinion on

reproductive health impacts of exposure to toxic environmental chemicals. *Int. J. Gynecol. Obstet.* (2015) doi:10.1016/j.ijgo.2015.09.002.

14. WHO-UNEP. *State of the Science on Endocrine Disrupting Chemicals 2012. Summary for Decision Makers.* (2013).

15. Health Canada. *Fourth Report on Human Biomonitoring and Environmental Chemicals in Canada: Results of the Canadian Health Measures Survey*. (2017).

16. CDC. *Fourth National Report on Human Exposure to Environmental Chemicals: National Health and Nutrition Examination Survey (NHANES)*. (2015).

17. The German Environmental Agency. *German Environmental Survey, GerES 2014-2017*. https://www.umweltbundesamt.de/en/topics/health/assessing-environmentally-related-health-risks/german-environmental-surveys/german-environmental-survey-2014-2017-geres-v#textpart-1 (2017).

18. Escher, B. I., Stapleton, H. M. & Schymanski, E. L. Tacking complex mixtures of chemicals in Our Changing Environment. *Science (80-.).* **392**, 388–392 (2020).

19. Hennig, B., Oesterling, E. & Toborek, M. Environmental toxicity, nutrition, and gene interactions in the development of atherosclerosis. *Nutrition, Metabolism and Cardiovascular Diseases* at https://doi.org/10.1016/j.numecd.2006.01.003 (2007).

20. Hennig, B. *et al.* Introductory comments: nutrition, environmental toxins and implications in prevention and intervention of human diseases. *J. Nutr. Biochem.* (2007) doi:10.1016/j.jnutbio.2006.12.004.

21. Hennig, B. *et al.* Modification of environmental toxicity by nutrients: Implications in atherosclerosis. *Cardiovascular Toxicology* at https://doi.org/10.1385/CT:5:2:153 (2005).

22. Hennig, B., Toborek, M., Bachas, L. G. & Suk, W. A. Emerging issues: Nutritional awareness in environmental toxicology. *J. Nutr. Biochem.* (2004) doi:10.1016/j.jnutbio.2004.01.002.

23. Hu, H., Kotha, S. & Brennan, T. The role of nutrition in mitigating environmental insults: Policy and ethical issues. in *Environmental Health Perspectives* (1995). doi:10.1289/ehp.95103s6185.

24. Norman, A. W. From vitamin D to hormone D: Fundamentals of the vitamin D endocrine system essential for good health. *Am. J. Clin. Nutr.* **88**, (2008).

25. Wilson, L. R., Tripkovic, L., Hart, K. H. & Lanham-new, S. A. Conference on ' New technology in nutrition research and practice ' Postgraduate Symposium Vitamin D de fi ciency as a public health issue : using vitamin D 2 or vitamin D 3 in future forti fi cation strategies Proceedings of the Nutrition Society. **76**, 392–399 (2017).

26. Tsiaras, W. G. & Weinstock, M. A. Factors influencing vitamin d status. *Acta Derm. Venereol.* **91**, 115–124 (2011).

27. Mousavi, S. E., Amini, H., Heydarpour, P., Amini Chermahini, F. & Godderis, L. Air pollution, environmental chemicals, and smoking may trigger vitamin D deficiency: Evidence and potential mechanisms. *Environ. Int.* **122**, 67–90 (2019).

28. Holick, M. F. Vitamin D Status: Measurement, Interpretation, and Clinical Application. *Ann. Epidemiol.* **19**, 73–78 (2009).

29. Pilz, S. *et al.* The role of vitamin D in fertility and during pregnancy and lactation: A review of clinical data. *Int. J. Environ. Res. Public Health* **15**, (2018).

30. Heaney, R. P. Guidelines for optimizing design and analysis of clinical studies of nutrient effects. *Nutr. Rev.* **72**, 48–54 (2014).

31. Health Canada. Vitamin D and Calcium: Updated Dietary Reference Intakes. https://www.canada.ca/en/health-canada/services/food-nutrition/healthy-eating/vitamins-minerals/vitamin-calcium-updated-dietary-reference-intakes-nutrition.html (2019).

32. Institute of Medicine. *Dietary Reference Intakes for Calcium and Vitamin D.* (2011).

33. Canadian Paediatric Society. Vitamin D supplementation: Recommendations for Canadian mothers and infants (Paediatrics and Child Health (2007) 12:7 (583-589)). *Paediatr. Child Health (Oxford).* **12**, 858 (2007).

34. Munns, C. F. *et al.* Global Consensus Recommendations on Prevention. *J Clin Endocrinol Metab.* **101**, 394–415 (2016).

35. Marcinowska-Suchowierska, E., Kupisz-Urbanska, M., Lukaszkiewicz, J., Pludowski, P. & Jones, G. Vitamin D Toxicity a clinical perspective. *Front. Endocrinol. (Lausanne).* **9**, 1–7 (2018).

36. Brandenburg, V. M., Vervloet, M. G. & Marx, N. The role of vitamin D in cardiovascular disease: From present evidence to future perspectives. *Atherosclerosis* **225**, 253–263 (2012).

37. Muscogiuri, G. *et al.* Can vitamin D deficiency cause diabetes and cardiovascular diseases? Present evidence and future perspectives. *Nutrition, Metabolism and Cardiovascular Diseases* at https://doi.org/10.1016/j.numecd.2011.11.001 (2012).

38. Woloszynska-Read, A., Johnson, C. S. & Trump, D. L. Vitamin D and cancer: Clinical aspects. *Best Practice and Research: Clinical Endocrinology and Metabolism* at https://doi.org/10.1016/j.beem.2011.06.006 (2011).

39. Baeke, F., Takiishi, T., Korf, H., Gysemans, C. & Mathieu, C. Vitamin D: Modulator of the immune system. *Current Opinion in Pharmacology* at https://doi.org/10.1016/j.coph.2010.04.001 (2010).

40. Wei, S. Q., Qi, H. P., Luo, Z. C. & Fraser, W. D. Maternal vitamin D status and adverse pregnancy outcomes: A systematic review and meta-analysis. *J. Matern. Neonatal Med.* (2013) doi:10.3109/14767058.2013.765849.

41. Minisola, S. *et al.* Controversies Surrounding Vitamin D : Focus on Supplementation and Cancer. 1–10 (2019) doi:10.3390/ijerph16020189.

42. Akbari, M. *et al.* The Effects of Vitamin D Supplementation on Biomarkers of Inflammation and Oxidative Stress among Women with Polycystic Ovary Syndrome: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Horm. Metab. Res.* (2018) doi:10.1055/s-0044-101355.

43. Mazumdar, I., Goswami, K. & Ali, M. S. Status of Serum Calcium, Vitamin D and Parathyroid Hormone and Hematological Indices Among Lead Exposed Jewelry Workers in Dhaka, Bangladesh. *Indian J. Clin. Biochem.* **32**, 110–116 (2017).

44. Nogawa, K. *et al.* Serum vitamin D metabolites in cadmium-exposed persons with renal damage. *Int. Arch. Occup. Environ. Health* **62**, 189–193 (1990).

45. Moon, J. The role of vitamin D in toxic metal absorption: A review. *J. Am. Coll. Nutr.* **13**, 559–564 (1994).

46. Iavicoli, I., Fontana, L. & Bergamaschi, A. The effects of metals as endocrine disruptors. *J. Toxicol. Environ. Heal. - Part B Crit. Rev.* **12**, 206–223 (2009).

47. EFSA. Scientific Opinion on Lead in Food: EFSA Panel on Contaminants in the Food Chain (CONTAM). *EFSA (European Food Saf. Authority) J.* **8**, 1–151 (2010).

48. Jan, A. T., Azam, M., Siddiqui, K., Ali, A. & Choi, I. Heavy Metals and Human Health : Mechanistic Insight into Toxicity and Counter Defense System of Antioxidants. 29592–29630 (2015) doi:10.3390/ijms161226183.

49. Health Canada. *Fifth Report on Human Biomonitoring if Environmental Chemicals in Canada: Results of the Canadian Health Measures Survey Cycle 5 (2016-2017)*. (2019).

50. Kjellström, T. & Nordberg, G. F. A kinetic model of cadmium metabolism in the human being. *Environ. Res.* (1978) doi:10.1016/0013-9351(78)90160-3.

51. Rahimzadeh, M. R., Rahimzadeh, M., Kazemi, S. & Moghadamnia, A. Cadmium toxicity and treatment: An update. **8**, 135–145 (2017).

52. Hughes, M. F. Biomarkers of Exposure : A Case Study with Inorganic Arsenic. **1790**, 1790–1796 (2006).

53. Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K. & Sutton, D. J. Heavy Metals Toxicity and the Environment. *Mol. Clin. Environ. Toxicol.* **101**, 133–164 (2012).

54. ATSDR. Public Health Statement for Cadmium. *Public Heal. Statement* 1–10 (2012).

55. ATSDR. Public Health Statement Lead CAS#: 7439-92-1. *Div. Toxicol. Environ. Med.* 1–13 (2007).

56. ATSDR. Public Health Statement Mercury Public Health Statement Mercury. *Agency Toxic Subst. Dis. Regist.* 20 (1999).

57. ATSDR. Public Health Statement Arsenic. *U.S Public Heal. Serv. Agency Toxic Subst. Dis. Regist.* 1–10 (2007) doi:10.1038/npg.els.0000869.

58. Ganguly, K., Levänen, B., Palmberg, L., Åkesson, A. & Lindén, A. Cadmium in tobacco smokers: A neglected link to lung disease? *Eur. Respir. Rev.* **27**, 1–8 (2018).

59. Bozack, A. K. *et al.* Folic acid supplementation enhances arsenic methylation: Results from a folic acid and creatine supplementation randomized controlled trial in Bangladesh. *Am. J. Clin. Nutr.* **108**, 1–12 (2018).

60. International Agency for Research on Cancer(IARC). Arsenic and Arsenic Compounds Monograph. *IARC Monogr. Eval. Carcinog. Risks Humans* **100C**, 41–93 (2012).

61. Straif, K. *et al.* A review of human carcinogens--part C: metals, arsenic, dusts, and fibres. *Lancet Oncol.* **10**, 453–454 (2009).

62. Toronto Public Health. A Guide to Eating Fish. https://www.toronto.ca/wp-content/uploads/2017/12/8d3f-tph-91f6-guide-eat-fish.pdf.

63. Park, J. D. & Zheng, W. Human exposure and health effects of inorganic and elemental mercury. *J. Prev. Med. Public Heal.* **45**, 344–352 (2012).

64. Kido, T. *et al.* alpha1-Microglobulin determination in urine for the early detection of renal tubular dysfunctions caused by exposure to cadmium. **24**, 195–201 (1985).

65. Nogawa, K. *et al.* Mechanism for bone disease found in inhabitants environmentally exposed to cadmium: decreased serum 1α, 25-dihydroxyvitamin D level. *Int. Arch. Occup. Environ. Health* (1987) doi:10.1007/BF00377675.

66. Uchida, M. *et al.* Elevated urinary levels of vitamin D-binding protein in the inhabitants of a cadmium polluted area, Jinzu River basin, Japan. *Tohoku J. Exp. Med.* **211**, 269–274 (2007).

67. Aoshima, K. & Kasuya, M. Preliminary study on serum levels of 1, 25-dihydroxyvitamin D and 25-hydroxyvitamin D in cadmium-induced renal tubular dysfunction. *Toxicol. Lett.* **57**, 91–99 (1991).

68. Bronner, F. Mechanisms of intestinal calcium absorption. *J. Cell. Biochem.* **88**, 387–393 (2003).

69. Barton, J. C., Conrad, M. E., Harrison, L. & Nuby, S. Effect of vitamin D on lead absorption and retention. *Am. J. Physiol.* **238**, G124-30 (1980).

70. DeLuca, H. F. History of the discovery of vitamin D and its active metabolites. *Bonekey Rep.* **3**, 1–8 (2014).

71. Ettinger, A. S. *et al.* Effect of Calcium Supplementation on Blood Lead Leves in Pregnancy: A Randomized Placebo-Cotnrolled Trial. *Environ. Health Perspect.* **117**, 26–41 (2009).

72. Colborn, T., vom Saal, F. S. & Soto, A. M. Developmental effects of endocrine-disrupting

chemicals in wildlife and humans. *Environ. Impact Assess. Rev.* **14**, 469–489 (1994).

73. Shanle, E. K. & Xu, W. Endocrine disrupting chemicals targeting estrogen receptor signaling: Identificaiton and mechanisms of action. **24**, 6–19 (2011).

74. Combarnous, Y. Comparative Overview of the Mechanisms of Action of Hormones and Endocrine Disruptor Compounds. 1–11 (2019) doi:10.3390/toxics7010005.

75. Bergman, Å. *et al.* The impact of endocrine disruption: A consensus statement on the state of the science. *Environ. Health Perspect.* **121**, 104–106 (2013).

76. Street, M. E. *et al.* Current knowledge on endocrine disrupting chemicals (EDCs) from animal biology to humans, from pregnancy to adulthood: Highlights from a national italian meeting. *Int. J. Mol. Sci.* **19**, (2018).

77. Pavlíková, N. *et al.* Upregulation of vitamin D-binding protein is associated with changes in insulin production in pancreatic beta-cells exposed to p , p ′ -DDT and p , p ′ -DDE. 1–11 (2019) doi:10.1038/s41598-019-54579-z.

78. Balaguer, P., Delfosse, V. & Grimaldi, M. Structural and functional evidences for the interactions between nuclear hormone receptors and endocrine disruptors at low doses. *Comptes rendus - Biol.* **340**, 414–420 (2017).

79. Jomova, K. & Valko, M. Advances in metal-induced oxidative stress and human disease. *Toxicology* **283**, 65–87 (2011).

80. Ma, R. *et al.* Expressions of vitamin D metabolic components VDBP, CYP2R1, CYP27B1, CYP24A1, and VDR in placentas from normal and preeclamptic pregnancies. *Am. J. Physiol. - Endocrinol. Metab.* **303**, 928–935 (2012).

81. Jangid, A. *et al.* Effect of Lead on Human Blood Antioxidant Enzymes and Glutathione. *Int. J. Biochem. Res. Rev.* **13**, 1–9 (2016).

82. Jain, S. K., Kahlon, G., Bass, P., Levine, S. N. & Warden, C. Can l-cysteine and Vitamin D rescue Vitamin D and Vitamin D binding protein levels in blood Plasma of African American type 2 diabetic patients? *Antioxidants Redox Signal.* **23**, 688–693 (2015).

83. Jain, S. K., Parsanathan, R., Achari, A. E., Kanikarla-Marie, P. & Bocchini, J. A. Glutathione Stimulates Vitamin D Regulatory and Glucose-Metabolism Genes, Lowers Oxidative Stress and Inflammation, and Increases 25-Hydroxy-Vitamin D Levels in Blood: A Novel Approach to Treat 25-Hydroxyvitamin D Deficiency. *Antioxidants Redox Signal.* **29**, 1792–1807 (2018).

84. Dongre, N. N., Suryakar, A. N., Patil, A. J., Hundekari, I. A. & Devarnavadagi, B. B. Biochemical effects of lead exposure on battery manufacture workers with reference to blood pressure, calcium metabolism and bone mineral density. *Indian J. Clin. Biochem.* **28**, 65–70 (2013).

85. Datta, S. K. *et al.* Association of high blood lead levels with FOK1, APA1 and BSM1 Vitamin D Receptor gene polymorphism in occupationally exposed battery workers. *Drug*

Metab. Pharmacokinet. **34**, S28–S29 (2019).

86. Chalkley, S., Richmond, J. & Barltrop, D. Measurement of vitamin D3 metabolites in smelter workers exposed to lead and cadmium (multiple letters) [1]. *Occup. Environ. Med.* **55**, 446–452 (1998).

87. Box, V. *et al.* Plasma Vitamin D and Blood Lead concentrations in Asian Children. *Lancet* **Aug 15**; **2**, 373 (1981).

88. Chang, L. *et al.* Association of 25-hydroxyvitamin D with Hb and lead in children: A Chinese population-based study. *Public Health Nutr.* **17**, 827–832 (2014).

89. Rosen, J. F. *et al.* Reduction in 1,25-Dihydroxyvitamin D in Children with Increased Lead Absorption. *N. Engl. J. Med.* (1980) doi:10.1056/NEJM198005153022006.

90. Jukic, M. A. Z. *et al.* A prospective study of maternal 25-hydroxyvitamin D (25OHD) in the first trimester of pregnancy and second trimester heavy metal levels. *Environ. Res.* **199**, 111351 (2021).

91. Arbuckle, T. E. *et al.* Maternal and fetal exposure to cadmium, lead, manganese and mercury: The MIREC study. *Chemosphere* **163**, 270–282 (2016).

92. Jukic, A. M. Z. *et al.* Vitamin D treatment during pregnancy and maternal and neonatal cord blood metal concentrations at delivery: Results of a randomized controlled trial in Bangladesh. *Environ. Health Perspect.* **128**, 1–11 (2020).

93. Blencowe, H. *et al.* Born Too Soon: The global epidemiology of 15 million preterm births. *Reprod. Health* (2013) doi:10.1186/1742-4755-10-S1-S2.

94. Sutton, P. S. & Darmstadt, G. L. Preterm birth and neurodevelopment: A review of outcomes and recommendations for early identification and cost-effective interventions. *Journal of Tropical Pediatrics* at https://doi.org/10.1093/tropej/fmt012 (2013).

95. Sonnenschein-Van Der Voort, A. M. M. *et al.* Preterm birth, infant weight gain, and childhood asthma risk: A meta-analysis of 147,000 European children. *J. Allergy Clin. Immunol.* (2014) doi:10.1016/j.jaci.2013.12.1082.

96. Abitbol, C. L. & Rodriguez, M. M. The long-term renal and cardiovascular consequences of prematurity. *Nature Reviews Nephrology* at https://doi.org/10.1038/nrneph.2012.38 (2012).

97. Minissian, M. B. *et al.* Association of spontaneous preterm delivery and future maternal cardiovascular disease. *Circulation* **137**, 865–871 (2018).

98. Grandi, S. M. *et al.* Cardiovascular Disease-Related Morbidity and Mortality in Women with a History of Pregnancy Complications: Systematic Review and Meta-Analysis. *Circulation* **139**, 1069–1079 (2019).

99. Singh, L., Anand, M., Singh, S. & Taneja, A. Environmental toxic metals in placenta and their effects on preterm delivery-current opinion. *Drug Chem. Toxicol.* **0**, 1–8 (2018).

100. Wang, H. *et al.* Association of maternal serum cadmium level during pregnancy with risk of preterm birth in a Chinese population. *Environ. Pollut.* **216**, 851–857 (2016).

101. WHO. Preterm Birth. *World Health Organization: Health Topics* https://www.who.int/reproductivehealth/global-estimates-preterm-birth/en/ (2018).

102. WHO. Global Preterm Birth Estimates. *World Health Organization* http://ptb.srhr.org/ (2018).

103. Frey, H. A. & Klebanoff, M. A. The epidemiology, etiology, and costs of preterm birth. *Semin. Fetal Neonatal Med.* **21**, 68–73 (2016).

104. McElrath, T. F. *et al.* Pregnancy disorders that lead to delivery before the 28th week of gestation: An epidemiologic approach to classification. *Am. J. Epidemiol.* **168**, 980–989 (2008).

105. Shachar, B. Z., Carmichael, S. L., Stevenson, D. K. & Shaw, G. M. Could genetic polymorphisms related to oxidative stress modulate effects of heavy metals for risk of human preterm birth? *Reprod. Toxicol.* **42**, 24–26 (2013).

106. Duhig, K., Chappell, L. C. & Shennan, A. H. Oxidative stress in pregnancy and reproduction. *Obstet. Med.* **9**, 113–116 (2016).

107. Moore, T. A., Ahmad, I. M. & Zimmerman, M. C. Oxidative Stress and Preterm Birth: An Integrative Review. *Biol. Res. Nurs.* **20**, 497–512 (2018).

108. Irwinda, R., Wibowo, N. & Putri, A. S. The Concentration of Micronutrients and Heavy Metals in Maternal Serum, Placenta, and Cord Blood: A Cross-Sectional Study in Preterm Birth. *J. Pregnancy* **2019**, (2019).

109. Morgan, M. J. & Liu, Z. G. Crosstalk of reactive oxygen species and NF-κB signaling. *Cell Res.* **21**, 103–115 (2011).

110. Dagouassat, M., Lanone, S. & Boczkowski, J. Interaction of matrix metalloproteinases with pulmonary pollutants. **39**, 1021–1032 (2012).

111. Au, F. *et al.* Blood metal levels and third trimester maternal plasma matrix metalloproteinases (MMPs). *Chemosphere* **159**, 506–515 (2016).

112. Manicone, A. M. & McGuire, J. K. Matrix Metalloproteinases as Modulators of Inflammation. **19**, 34–41 (2008).

113. Tu, F. F. *et al.* Prenatal Plasma Matrix Metalloproteinase-9 Levels to Predict Spontaneous Preterm Birth. *Obstet. Gynecol.* **92**, 446–449 (1999).

114. Lee, S. M., Park, K. H., Jung, E. Y., Cho, S. & Ryu, A. Prediction of spontaneous preterm birth in women with cervical insuf fi ciency : Comprehensive analysis of multiple proteins in amniotic fl uid. **42**, 776–783 (2016).

115. Vadillo-ortega, F. & Estrada-gutie, G. Role of matrix metalloproteinases in preterm labour. **112**, 19–22 (2005).

116. Asemi, Z., Samimi, M., Tabassi, Z., Shakeri, H. & Esmaillzadeh, A. Vitamin D Supplementation Affects Serum High-Sensitivity C-Reactive Protein, Insulin Resistance, and Biomarkers of Oxidative Stress in Pregnant Women. *J. Nutr.* **143**, 1432–1438 (2013).

117. Jamilian, M. *et al.* The effects of magnesium-zinc-calcium-vitamin D co-supplementation on biomarkers of inflammation, oxidative stress and pregnancy outcomes in gestational diabetes. *BMC Pregnancy Childbirth* **19**, 1–8 (2019).

118. Cooper, C. *et al.* Maternal gestational vitamin D supplementation and offspring bone health (MAVIDOS): A multicentre, double-blind, randomised placebo-controlled trial. *Lancet Diabetes Endocrinol.* **4**, 393–402 (2016).

119. Grant, C. C. *et al.* Vitamin D during pregnancy and infancy and infant serum 25-hydroxyvitamin D concentration. *Pediatrics* **133**, (2014).

120. Hossain, N. *et al.* Obstetric and neonatal outcomes of maternal vitamin D supplementation: Results of an open-label, randomized controlled trial of antenatal vitamin D supplementation in Pakistani women. *J. Clin. Endocrinol. Metab.* **99**, 2448–2455 (2014).

121. Sablok, A. *et al.* Supplementation of Vitamin D in pregnancy and its correlation with feto-maternal outcome. *Clin. Endocrinol. (Oxf).* **83**, 536–541 (2015).

122. Xue, F., Holzman, C., Rahbar, M. H., Trosko, K. & Fischer, L. Maternal fish consumption, mercury levels, and risk of preterm delivery. *Environ. Health Perspect.* **115**, 42–47 (2007).

123. Li, J. *et al.* Maternal serum lead level during pregnancy is positively correlated with risk of preterm birth in a Chinese population. *Environ. Pollut.* **227**, 484–489 (2017).

124. Tsuji, M. *et al.* The association between whole blood concentrations of heavy metals in pregnant women and premature births: The Japan Environment and Children's Study (JECS). *Environ. Res.* **166**, 562–569 (2018).

125. Jelliffe-Pawlowski, L. L., Miles, S. Q., Courtney, J. G., Materna, B. & Charlton, V. Effect of magnitude and timing of maternal pregnancy blood lead (Pb) levels on birth outcomes. **26**, 154–162 (2006).

126. Ferguson, K. K. & Chin, H. B. Environmental Chemicals and Preterm Birth: Biological Mechanisms and the State of the Science. *Curr. Epidemiol. Reports* **4**, 56–71 (2017).

127. Vigeh, M. *et al.* Blood lead at currently acceptable levels may cause preterm labour. *Occup. Environ. Med.* **68**, 231–234 (2011).

128. McMichael, A. J., Vimpani, G. V, Robertson, E. F., Baghurst, P. A. & Clark, P. D. The Port Pirie cohort study : maternal blood lead and pregnancy outcome. **40**, 18–25 (1986).

129. Taylor, C. M., Golding, J. & Emond, A. M. Adverse effects of maternal lead levels on birth outcomes in the ALSPAC study: A prospective birth cohort study. *BJOG An Int. J. Obstet. Gynaecol.* **122**, 322–328 (2015).

130. Ahamed, M., Mehrotra, P. K., Kumar, P. & Siddiqui, M. K. J. Placental lead-induced oxidative stress and preterm delivery. *Environ. Toxicol. Pharmacol.* **27**, 70–74 (2009).

131. Baghurst, P. A. *et al.* Lead in the Placenta , Membranes , and Umbilical Cord in Relation to Pregnancy Outcome in a Lead-Smelter Community. **90**, 315–320 (1991).

132. Falcón, M., Viñas, P. & Luna, A. Placental lead and outcome of pregnancy. *Toxicology* **185**, 59–66 (2003).

133. Sowers, M. *et al.* Blood Lead Concentrations and Pregnancy Outcomes. *Arch. Environ. Health* **57**, 489–495 (2002).

134. Factor-Litvak, P. *et al.* A prospective study of birthweight and length of gestation in a population surrounding a lead smelter in Kosovo, Yugoslavia. *Int. J. Epidemiol.* **20**, 722–728 (1991).

135. Laudanski, T. *et al.* Influence of high lead and cadmium soil content on human reproductive outcome. *Int. J. Gynecol. Obstet.* **36**, 309–315 (1991).

136. Yıldırım, E. *et al.* Is the Concentration of Cadmium, Lead, Mercury, and Selenium Related to Preterm Birth? *Biol. Trace Elem. Res.* **191**, 306–312 (2019).

137. Milton, A. H. *et al.* A review of the effects of chronic arsenic exposure on adverse pregnancy outcomes. *Int. J. Environ. Res. Public Health* **14**, (2017).

138. Ahmad, S. A. *et al.* Arsenic in Drinking Water and Pregnancy Outcomes. **109**, 2000–2002 (2001).

139. Yang, C. Y. *et al.* Arsenic in drinking water and adverse pregnancy outcome in an arseniasis-endemic area in northeastern Taiwan. *Environ. Res.* **91**, 29–34 (2003).

140. Rahman, M. L. *et al.* Prenatal arsenic exposure , child marriage , and pregnancy weight gain : Associations with preterm birth in Bangladesh. *Environ. Int.* **112**, 23–32 (2018).

141. Shi, X. *et al.* Geospatial association between adverse birth outcomes and arsenic in groundwater in New Hampshire , USA. 333–351 (2015) doi:10.1007/s10653-014-9651-2.

142. Almberg, K. S. *et al.* Arsenic in drinking water and adverse birth outcomes in Ohio. *Environ. Res.* **157**, 52–59 (2017).

143. Wu, P., Park, K. & Gulati, M. Pregnancy as a Predictor of Cardiovascular Disease The Fourth Trimester: *Eur. Card. Rev.* **16**, (2021).

144. Norris, C. M. *et al.* State of the Science in Women's Cardiovascular Disease: A Canadian Perspective on the Influence of Sex and Gender. *J. Am. Heart Assoc.* **9**, (2020).

145. Bellamy, L., Casas, J. P., Hingorani, A. D. & Williams, D. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. *Lancet (London, England)* **373**, 1773–1779 (2009).

146. Bellamy, L., Casas, J., Hingorani, A. & Williams, D. Pre-eclampsia and risk of

cardiovascular disease and cancer in later life: systematic review and meta-analysis. *BMJ* **335**, (2007).

147. Liu, Y. *et al.* Hypertension in Pregnancy Adiponectin Levels in Circulation and Breast Milk and mRNA Expression in Adipose Tissue of Preeclampsia Women Adiponectin Levels in Circulation and Breast Milk and mRNA Expression in Adipose Tissue of Preeclampsia Women. **31**, 40–49 (2012).

148. Miehle, K., Stepan, H. & Fasshauer, M. Leptin, adiponectin and other adipokines in gestational diabetes mellitus and pre-eclampsia. *Clin. Endocrinol. (Oxf).* **76**, 2–11 (2012).

149. Boyadzhieva, M., Atanasova, I., Zacharieva, S. & Kedikova, S. Adipocytokines during pregnancy and postpartum in women with gestational diabetes and healthy controls. *J. Endocrinol. Invest.* **36**, 944–949 (2013).

150. Girouard, J., Giguère, Y., Moutquin, J. M. & Forest, J. C. Previous hypertensive disease of pregnancy is associated with alterations of markers of insulin resistance. *Hypertension* **49**, 1056–1062 (2007).

151. Arbuckle, T. E. *et al.* Cohort profile: the maternal-infant research on environmental chemicals research platform. *Paediatr. Perinat. Epidemiol.* **27**, 415–425 (2013).

152. Ashley-Martin, J. *et al.* Blood metal levels and early childhood anthropometric measures in a cohort of Canadian children. *Environ. Res.* **179**, 108736 (2019).

153. Braun, J. M. *et al.* Associations of prenatal urinary bisphenol a concentrations with child behaviors and cognitive abilities. *Environ. Health Perspect.* **125**, 1–9 (2017).

154. Sarafin, K. *et al.* Standardizing 25-hydroxyVitamin D values from the Canadian Health Measures Survey. *Am. J. Clin. Nutr.* **102**, 1044–1050 (2015).

155. Amrein, K. *et al.* Vitamin D deficiency 2.0: an update on the current status worldwide. *Eur. J. Clin. Nutr.* (2020) doi:10.1038/s41430-020-0558-y.

156. Behrman, R. E., Butler, A. S., Outcomes, I. of M. (US) C. on U. & Healthy, P. B. and A. Societal Costs of Preterm Birth. in *Preterm birth: causes, consequences, and prevention* (2007).

157. Johnston, K. M. *et al.* The economic burden of prematurity in Canada. *BMC Pediatr.* **14**, (2014).

158. Bavineni, M. *et al.* Mechanisms linking preterm birth to onset of cardiovascular disease later in adulthood. *Eur. Heart J.* **40**, 1107–1112 (2019).

159. Wu, P. *et al.* Preterm delivery and future risk of maternal cardiovascular disease: A systematic review and meta-analysis. *J. Am. Heart Assoc.* **7**, (2018).

160. Husseini, T. The future of mining: eight bold industry predictions. *Mining Technology* https://www.mining-technology.com/mining-safety/future-of-mining-industry-predictions (2018).

161. Shellenberger, M. If Solar Panels Are So Clean, Why Do They Produce So Much Toxic Waste? *Forbes Magazine* https://www.forbes.com/sites/michaelshellenberger/2018/05/23/if-solar-panels-are-so-clean-why-do-they-produce-so-much-toxic-waste/#d9918d3121cc (2018).

162. Guo, Y., Huang, C., Zhang, H. & Dong, Q. Heavy Metal Contamination from Electronic Waste Recycling at Guiyu, Southeastern China. *J. Environ. Qual.* **38**, 1617–1626 (2009).

163. Wijngaard, R. R., van der Perk, M., van der Grift, B., de Nijs, T. C. M. & Bierkens, M. F. P. The Impact of Climate Change on Metal Transport in a Lowland Catchment. *Water. Air. Soil Pollut.* **228**, (2017).

164. Lewis, K. B., Graham, I. D., Boland, L. & Stacey, D. Writing a compelling integrated discussion: A guide for integrated discussions in article-based theses and dissertations. *Int. J. Nurs. Educ. Scholarsh.* **18**, (2021).

CHAPTER 2

Blood Metals and Vitamin D Status in a Pregnancy Cohort: A Bidirectional Biomarker Analysis

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Preface to Chapter 2

This chapter explains how we examined the relationship between vitamin D and metals in pregnancy in the Maternal-Infant Research on Environmental Chemicals (MIREC) cohort study. Our dataset had repeated measures of these biomarkers so a variety of statistical techniques were used to explore the relationship. We utilized a cross-lagged panel model to determine causal direction. Elsevier allows us to include the final draft form of the paper in the thesis and the coauthors have also agreed. Author contributions are listed below.

Authorship credits:

Mandy Fisher was involved in the design and implementation of the study, data analysis, writing and interpretation of the study. **Beth Potter** was involved in the analysis of the study, and review of the article and revisions. **Julian Little** was involved in the analysis of the study, review of the article and revisions. **Youssef Oulhote** was involved in the analysis of the study and the review of the article and revisions. **Hope Weiler** was involved in the design of the study and the review of the article and revisions. **William Fraser** was involved in the design and implementation of the study and the review of the article and revisions. **Anne-Sophie Morisset** was involved in the design and implementation of the study and the review of the article and revisions. **Joseph Braun** was involved in the analysis of the study and the review of the article and revisions. **Jillian Ashley-Martin** was involved in the analysis of the study and the review and revisions of the article. **Michael Borghese** was involved in the review and revisions of the article. **Robin Shutt** was involved in the review and revisions of the article. **Premkumari Kumarathasan** was involved in the review and revisions of the article. **Bruce Lanphear** was involved in the review and revisions of the article. **Mark Walker** was involved in the design and implementation of the study, review and revisions of the article. **Tye Arbuckle** was involved in the design and implementation of the study, review and revisions of the article

ABSTRACT

Low 25-hydroxyvitamin D (25OHD), a biomarker of vitamin D status, is associated with reduced immune function and adverse pregnancy outcomes, such as preterm birth. Observational studies indicate that long-term, high level exposure to metals such as cadmium (Cd) and lead (Pb) can impact a person's vitamin D status. However, the directionality of the association is uncertain, particularly for low-level exposures. We used three distinct longitudinal data analysis methods to investigate cross-sectional, longitudinal and bidirectional relationships of Cd and Pb biomarkers with 25-hydroxyvitamin D (25OHD) in a Canadian pregnancy cohort. Maternal whole blood Cd and Pb and plasma 25OHD concentrations were measured in the 1st (n=1905) and 3rd (n=1649) trimester and at delivery (25OHD only, n=1542). Our multivariable linear regression analysis showed weak inverse associations between Cd and 25OHD concentrations cross-sectionally and longitudinally while the latent growth curve models showed weak associations with Pb on the 25OHD intercept. In the bidirectional analysis, using cross lagged panel models, we found no association between 1st trimester metals and 3rd trimester 25OHD. Instead, 1st trimester 25OHD was associated with 9% (-15%, -3%) lower 3rd trimester Cd and 3% (-7, 0.1%) lower Pb. These findings suggest the 25OHD may modify metal concentrations in pregnancy and demonstrates the value of controlling for contemporaneous effects and the persistence of a biomarker over time, in order to rule out reverse causation.

KEYWORDS: lead, cadmium, pregnancy, cross lagged panel model

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RESEARCH ETHICS REVIEW

Research Ethics Board approval was obtained at Health Canada and all study sites including: British Columbia's Children's & Women's Health (Vancouver); University of Alberta (Edmonton); Health Sciences & St-Boniface Hospital (Winnipeg); Mount Sinai Hospital (Toronto); McMaster University Medical Center (Hamilton); Sudbury Regional Hospital (Sudbury); Kingston General Hospital (Kingston); The Ottawa Hospital (Ottawa); CHU Ste-Justine (Montreal); Jewish General Hospital (Montreal); IWK Health Centre (Halifax)

ABBREVIATIONS

25OHD: 25-hydroxyvitamin D
BMI: Body Mass Index
Cd: Cadmium
CFI: Comparative Fit Index
CHMS: Canadian Health Measures Survey
FIML: Full Information Maxiumum Likelihood
GFI: Goodness of Fit Index
ICC: Intraclass Correlation Coefficient
IOM: Institute of Medicine
LGCM: Latent Growth Curve Models
LOD: Limit of Detection
MIREC: Maternal-Infant Research on Environmental Chemicals
NHANES: National Health and Nutrition Survey
Pb: Lead
RMSE: Root Mean Square Error
SEM: Structural Equation Modelling
SGA: Small for gestational age

INTRODUCTION

Vitamin D, conventionally associated with calcium homeostasis and bone mineralization, appears to play an important role in suppressing the maternal immune response and inflammatory reactions in the placenta [1–3]. During pregnancy, the immune system must balance maternal tolerance of foreign proteins and protection from pathogens [4]. Imbalances in this process are suspected to cause pregnancy complications [5]. The cut-point for vitamin D deficiency was set by the Institute of Medicine (IOM) at <30 nmol/L of serum 25-hydroxyvitamin D (25OHD), whereas the cut-point for sufficiency was set at ≥50 nmol/L in support of bone health [6]. While the IOM considered cut-points associated with pregnancy outcomes, at the time data was not sufficient to guide dietary recommendations. Since then, studies have observed associations between serum 25OHD <50 nmol/L and adverse pregnancy conditions related to the immune system, including recurrent pregnancy loss, preterm birth, and preeclampsia [1,7]. Globally it is estimated that 54% of pregnant women have serum 25OHD concentrations below 50 nmol/L, with the prevalence greatest (>80%) in South-East Asian and Western Pacific World Health Organization regions [8]. In North America, national surveys suggest that 41% of pregnant women and adults of reproductive age 20-29 have levels below 50 nmol/L [9,10].

Evidence from observational studies indicate that long-term, high level exposure to metals such as cadmium (Cd) can cause renal damage, and impact a person's vitamin metabolism [11–13]. Workers with higher lead (Pb) exposure show lower concentrations of serum 25OHD compared to controls [14–16]. However, the ability of lower exposure levels to disrupt vitamin D metabolism remains unclear. Some observational studies bring into question the directionality of these associations, particularly at low levels; several studies suggest that higher vitamin D concentrations or intake

are associated with lower blood metals in pregnant women and children [17–19]. A recent vitamin D trial showed maternal vitamin D_3 supplementation was associated with increases in cord blood Pb and Cd; however this trial was conducted in Bangladesh where maternal blood metals are considerably higher than those observed in Canada [20]. To our knowledge no study has investigated the potential bidirectional relationship of low level metal exposures and 25OHD concentrations.

We used data from the Maternal-Infant Research on Environmental Chemicals Study (MIREC) to examine the cross-sectional, prospective and bidirectional relationships between metals (Cd, Pb) and 25OHD during pregnancy. Specifically our three objectives were to determine: 1) whether Cd and Pb are associated with 25OHD concentrations during pregnancy; 2) whether blood Cd and Pb are associated with the 25OHD trajectory across pregnancy; and finally 3) whether there is a bidirectional association between these metals and 25OHD.

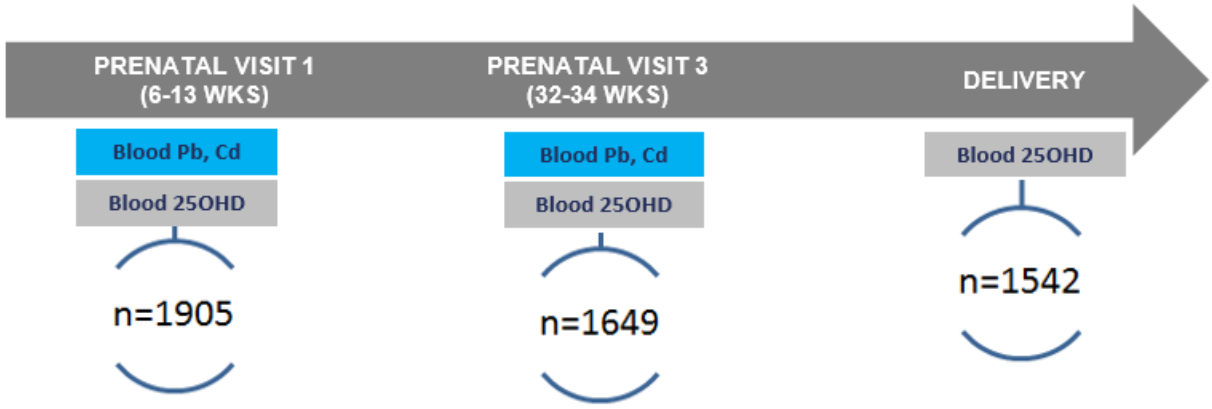
METHODS

Study Design

The MIREC Study [21] is a national-level pregnancy cohort of 2001 women recruited from 10 cities across Canada (Vancouver, Edmonton, Winnipeg, Sudbury, Toronto, Hamilton, Kingston, Ottawa, Montreal, Halifax). Research Ethics Board approval was obtained at Health Canada and all study sites. MIREC participants were recruited through medical clinics (ultrasound, midwife and/or doctor's clinics) during the first trimester of pregnancy (6 to <14 weeks) between 2008 and 2011. We excluded women if they had known fetal abnormalities or medical complications such as renal, heart, liver, pulmonary or a collagen disease, epilepsy, threatened spontaneous abortion, illicit drug use, or a haematological disorder. All participants provided informed consent and provided blood

samples in each trimester and at delivery. Pb, Cd and 25OHD were measured in blood samples collected between 6 and 13 completed weeks gestation (median 12 weeks), between 32 and 33 completed weeks (median 33 weeks) and at delivery. Eighteen women withdrew from the study leaving 1983 participants. Among these 1905 had Pb, Cd and 25OHD measurements available in the 1st trimester and 1649 in the 3rd trimester. There were a total of 1542 25OHD measures at delivery while Cd and Pb were not measured at this time point (See Figure 1).

Figure 1: MIREC Study Pregnancy Visits and Maternal Biomarker Sample Sizes



Questionnaire Data: We collected sociodemographic data in the 1st trimester including maternal age, pre-pregnancy body mass index (BMI), education, household income, and maternal ethnicity. We collected information during both the 1st and 3rd trimester visits about fish consumption in the past 3 months, smoking status, and how many days, in the last 4 weeks, were spent outside for at least 30 mins between 9:00-16:00.

Specimen collection and laboratory analysis: Maternal whole blood specimens were analysed at the Centre de Toxicologie du Québec (CTQ), Institut national de Santé Publique du Québec (INSPQ) for Pb and Cd concentrations [19]. Maternal blood plasma collected in the 1st and 3rd trimester and at delivery was analyzed for 25-hydroxyvitamin D_3 and 25-hydroxyvitamin D_2 at Health Canada's Foods Directorate Laboratory [22]. Briefly, two methods were used to quantify maternal plasma 25OHD; 1) total 25OHD immunoassay kits and the LIAISON autoanalyzer and 2) liquid chromatography-mass spectrometry (LC-MS/MS) that produces individual values for $25OHD_2$ and $25OHD_3$, the sum of which gives total 25OHD. Quality control for the LIAISON using Bio-Rad as an external control had CV% of <10% [22]. The immunoassay results were standardized to the LC-MS/MS using a calibration equation (standardized y= 5.36 + 0.96 (original immunoassay))[23]. Therefore all the samples were standardized to the international reference measurement procedures [24], producing uniformity across the 25OHD results.

Covariates: We selected confounders using a Directed Acyclic Graphs (DAGs) [25,26]. We consider variables as potential confounders if they could be associated with both 25OHD levels and toxic metal exposure but were not conceptualized as intermediate variables (along the causal pathway) or colliders (common consequences) (See Supplemental Information (SI) Figure S1). We included the following covariates in the final models: smoking status (Never, Former, Quit during Pregnancy, Current), maternal education (Some college, High School or Less than High School; College/Trade Diploma; Undergraduate degree; Graduate degree), household income (< = to $50 000; between 50,001-100 000; >100 000; Don't know/refuse to answer), maternal age, pre-pregnancy BMI, maternal ethnicity (White, Black, Aboriginal, Chinese, Latin American, Other), general fish consumption per week in past 3 months prior to visit (0 to 4+ servings), study site,

days spent outside in the past 4 weeks between 9 a.m. and 4 p.m. (quartiles: 0-4; 5-10 ; 11-20; > 20 days), season at date of visit.

Statistical Analysis: We conducted univariate analysis on Cd, Pb and 25OHD. Next, we examined bivariate associations for 25OHD and metals with maternal characteristics. We log2 transformed Cd and Pb because they were right skewed. The 25OHD concentrations were approximately normally distributed and were not transformed initially. We used chi-square statistics to determine differences across categories; we used t-tests and ANOVA to compare the means between groups. We calculated pairwise correlations (Pearson) for concentrations of 25OHD and log2-transformed Cd and Pb across visits and estimated intraclass correlation coefficients (ICC) across time points for all the biomarkers. We estimated the increase in 25OHD by gestational week of visit using a mixed effects models with a random subject effect and unstructured covariance matrix to determine the potential trajectory of 25OHD across pregnancy. We also adjusted for other fixed effects measured in the 1st trimester including study site, maternal education, parity, income, maternal age, pre-pregnancy BMI, maternal ethnicity, smoking status, season, time spent outside, general fish consumption in the past 3 months. We substituted values below the limit of detection (LOD) with LOD/2 because the proportion below the LOD was low (<5%).

For our first objective, we used multivariable linear regression to determine if Cd and Pb are cross-sectionally and longitudinally associated with 25OHD concentrations in pregnancy. In this analysis we used separate models for each metal and for each visit. Given that smoking is a major source of Cd [27] and smokers show lower 25OHD [28], we examined effect modification by smoking status by introducing a cross-product term in the models. In addition, to descriptively investigate

heterogeneity of the association between Cd or Pb and 25OHD according to smoking status, we also stratified by smoking (Never and Former vs. Current and Quit during pregnancy). Assumptions of homoskedasticity and normality were assessed using residual plots.

For our second objective, we used latent growth curve models (LGCM) to determine whether 1st trimester Cd and Pb (log_2 transformed) are associated with the 25OHD trajectory across pregnancy. We modeled the linear change in 25OHD concentrations over time using a structural equation modelling (SEM) framework. LGCM estimate a latent intercept and latent slope based on the individual trajectories. The slope provides the average trajectory and the individual variation around that trajectory over time [29]. The use of LGCM allows us to adjust for time-varying covariates [30]. Therefore we considered both observed fixed (sociodemographics, parity, pre-pregnancy BMI, study site) and time-varying (season, smoking status, time spent outside, fish consumption) covariates in these models. We examined model fit for LGCM by evaluating goodness of fit tests including the goodness of fit index (GFI) where 1 indicates perfect fit; the root mean square error of approximation (RMSEA) where values less than 0.05 indicate the model's good fit; the comparative fit index (CFI) in which values greater than 0.9 are expected; and the chi-square test where a statistically non-significant result indicates that the sample's covariance matrix and covariance matrix estimated by the model are comparable and not statistically different [31].

Finally, to investigate whether there is a bidirectional association between these metals (Cd, Pb) and 25OHD we used cross lagged panel models (CLPM) which use SEM to allow for simultaneous

regressions and uniquely, make it possible to specify reciprocal associations by allowing for a variable to be a response variable in one equation and a predictor variable in another [32,33]. The cross lagged panel model controls for both contemporaneous effects (or the correlations between variables within the same time point) and the stability or persistence of a variable over time (autoregressive effects) [34]. This helps to rule out the possibility that the effect of X_1 on Y_2 is driven by the correlation between X_1 and Y_1 [35]. See equation below :

$$X_2 = \beta_1 X_1 + \beta_2 Y_1 + \boldsymbol{\beta_n X_n} + \mu^x$$

$$Y_2 = \beta_3 Y_1 + \beta_4 X_1 + \boldsymbol{\beta_n X_n} + \mu^y$$

Where X_1, X_2, Y_1, Y_2 represent a metal (X) and 25OHD (Y) concentration at the two time points in pregnancy (1st and 3rd trimester), β_1 and β_3 represent autoregressive effects or the degree to which a variable is unchanging or stable over time, β_2 and β_4 represent the cross lagged effects and $\boldsymbol{\beta_n X_n}$ represents a matrix of confounders. The residual covariance (r) at the same cross-sectional time point represents the leftover error (μ^x and μ^y) [36]. Thus, r_{x2y2} represents the unexplained covariance after controlling for both the autoregressive (β_1, β_3) and cross-lagged (β_2, β_4) effects. Because there were only 2 waves of data (1st and 3rd trimester) the CLPM is *just identified* [37]. Thus, the model allows for estimation of parameters and standard errors but no interpretation of model fit can be made because it inherently gives perfect model fit [38].

All variables in our cross-lagged models were observed (i.e. no latent variables) and we included other confounding variables, as described above. The CLPM uses maximum likelihood estimation and assumes a multivariate normal distribution. Given metals and 25OHD are both a predictor and outcome in the cross-lagged model, we also $\log_2$ transformed 25OHD, to be consistent with

the metals, so the β estimates represent the change in $\log_2(Y)$ per doubling in the predictor variable. Percent change in the outcome was calcuated using the formula: % change = $(2^{\beta}-1)*100$ (See SI Table S1). We also present standardized coefficients to compare causal predominance [34].

For both the CLPM and LGCM, we present models with and without the use of the Full Information Maximum Likelihood (FIML) method where all cases, irrespective of missing covariate data, are retained in the analysis [31]. FIML assumes that the data are missing at random and the missing data have a multivariate normal distribution. All analyses were conducted using R Version 4.0.2 lavaan package[39] and SAS Enterprise Guide 7.1[40].

RESULTS

A total of 1905 participants at the 1st trimester visit and 1649 at the 3rd trimester visit, respectively, had measures of Cd, Pb and 25OHD; at delivery 1542 participants had plasma 25OHD concentrations assessed. Nearly three quarters (73%) of the participants had all 3 measures of plasma 25OHD (1st and 3rd trimester and delivery). These participants (n=1387) were comparable to the full sample at visit 1 (n=1905) with respect to sociodemographic (See SI Table S2). Most participating pregnant women were married, close to 40% had a household income above $100,000 CAD and were highly educated, with over 80% had a college or university degree (See Table 1). Over eighty percent of the women self-identified as white and the mean maternal age was 32 years (range 17-48). Nearly half (44%) of the women were nulliparous and the majority of women had pre-pregnancy BMI in the normal range (60%). Only 6% reported being current smokers while an additional 6% reported quitting smoking during pregnancy.

Table 1: Participant Characteristics								
Factor	1st trimester		Maternal blood lead (µg/dL)		Maternal blood cadmium (µg/L)		Maternal serum 25-hydroxyvitamin D (nmol/L)	
			1st trimester	3rd trimester	1st trimester	3rd trimester	1st trimester	3rd trimester
Mother's education level	Frequency	%	GM	GM	GM	GM	mean	mean
Some college, High School or < High School	278	14.19	0.62	0.56	0.33	0.25	64.07	72.96
College/Trade Diploma	463	23.63	0.57	0.52	0.23	0.19	69.27	77.21
Undergrad degree	713	36.40	0.61	0.57	0.20	0.19	71.08	80.17
Graduate degree	505	25.78	0.69	0.64	0.18	0.19	71.68	80.39
Household income								
≤ to $50 000	444	22.64	0.64	0.61	0.27	0.22	64.36	74.29
between 50,001-100 000	675	34.42	0.60	0.55	0.21	0.19	70.21	79.24
>100 000	750	38.25	0.63	0.57	0.19	0.19	73.31	81.39
Don't know/refuse to answer	92	4.69	0.63	0.59	0.29	0.26	64.42	69.07
Marital Status								
Married	1406	71.7	0.60	0.54	0.20	0.19	70.69	79.21
Same partner for 1+ years	461	23.51	0.68	0.64	0.27	0.22	68.53	78.03
Divorced, separated, single or other	94	4.79	0.66	0.69	0.33	0.27	62.62	70.84
Maternal age group								
<20	13	0.66	0.57	0.51	0.30	0.26	57.04	70.39
20-24	125	6.37	0.58	0.49	0.29	0.20	62.20	74.33
25-29	455	23.2	0.60	0.51	0.21	0.19	68.46	76.57
30-34	702	35.8	0.69	0.56	0.21	0.19	70.92	80.07
35+	666	33.96	0.61	0.66	0.22	0.21	71.19	79.28
Parity								
0	865	44.11	0.64	0.60	0.22	0.21	69.70	80.83

1	792	40.39	0.61	0.54	0.21	0.18	70.30	77.41
2	228	11.63	0.60	0.58	0.22	0.21	68.33	74.84
3+	76	3.88	0.63	0.57	0.23	0.20	69.80	75.17
Pre-pregnancy BMI								
Underweight (BMI < 18.5)	52	2.87	0.64	0.60	0.25	0.27	71.79	82.73
Normal (18.5 ≤ BMI < 25)	1096	60.39	0.61	0.54	0.22	0.20	72.28	80.75
Overweight (25 ≤ BMI < 30)	397	21.87	0.60	0.58	0.21	0.19	68.82	76.63
Obese (BMI ≥30)	270	14.88	0.63	0.57	0.21	0.18	62.21	73.09
Smoking status at time of visit								
Current	115	5.87	0.68	0.61	1.27	0.85	61.59	71.25
Former	534	27.26	0.66	0.59	0.21	0.20	71.04	78.47
Never	1190	60.75	0.59	0.56	0.17	0.17	70.11	78.97
Quit during Pregnancy	120	6.13	0.66	0.55	0.47	0.21	69.05	80.86
Maternal ethnicity								
White	1643	83.78	0.61	0.56	0.21	0.19	71.86	80.74
Black	59	3.01	0.71	0.80	0.27	0.22	53.68	61.61
Aboriginal	44	2.24	0.55	0.53	0.36	0.35	53.89	66.95
Chinese	45	2.29	0.81	0.74	0.34	0.32	63.15	64.14
Latin American	47	2.40	0.66	0.60	0.19	0.15	62.08	70.24
Other	123	6.27	0.73	0.68	0.28	0.26	60.39	69.76
Season at time of blood sampling								
fall	572	29.41	0.62	0.61	0.22	0.19	68.96	74.73
spring	447	22.98	0.62	0.55	0.19	0.19	69.55	80.32
summer	461	23.7	0.63	0.61	0.23	0.21	74.19	77.02
winter	465	23.91	0.62	0.53	0.23	0.21	66.71	82.97
Fish Consumption over the past 3 months								
None	279	14.27	0.58	0.50	0.25	0.19	64.49	80.39
>0 to ≤1/wk	840	42.97	0.61	0.55	0.21	0.20	70.23	76.68

1 to ≤2/wk	433	22.15	0.62	0.60	0.21	0.19	71.34	79.43
2 to ≤3/wk	212	10.84	0.68	0.62	0.22	0.19	71.37	81.32
>3 to ≤4/wk	107	5.47	0.67	0.69	0.21	0.23	72.87	80.22
>4/wk	84	4.30	0.70	0.65	0.25	0.24	67.03	77.61
GM=geometric mean								

The majority of participants had 25OHD concentrations above 50 nmol/L throughout pregnancy: in 1st trimester (85%), 3rd trimester (87%) and at delivery (84%). Less than 4% of participants were considered deficient at <30 nmol/L [6,41] at each visit (1st trimester: 2.5%, 3rd trimester: 2.5%, Delivery: 3.6%). On average, 25OHD concentrations increased across pregnancy. Based on the adjusted mixed models with a random subject effect we observed a 0.27 nmol/L (95% CI: 0.23, 0.30) increase in 25OHD concentration with each gestational week of pregnancy (See SI Figures S2). 25OHD concentration was moderately correlated (r >0.50) across visits with an ICC of 0.57 (See SI Table S3-S4).

Blood Pb and Cd were nearly 100% detected in the 1st and 3rd trimester visits (See Table 2). We found similar geometric mean concentrations in the 1st and 3rd trimester for blood Pb (0.62 vs 0.57 µg/dl) and Cd (0.22 vs 0.20 µg/L). First and 3rd trimester concentrations were highly correlated within participants for each of $\log_2$ Cd (r=0.60) and Pb (r=0.73) with strong ICCs (Cd ICC=0.77 and Pb ICC=0.73) (See SI Tables S4-S5).

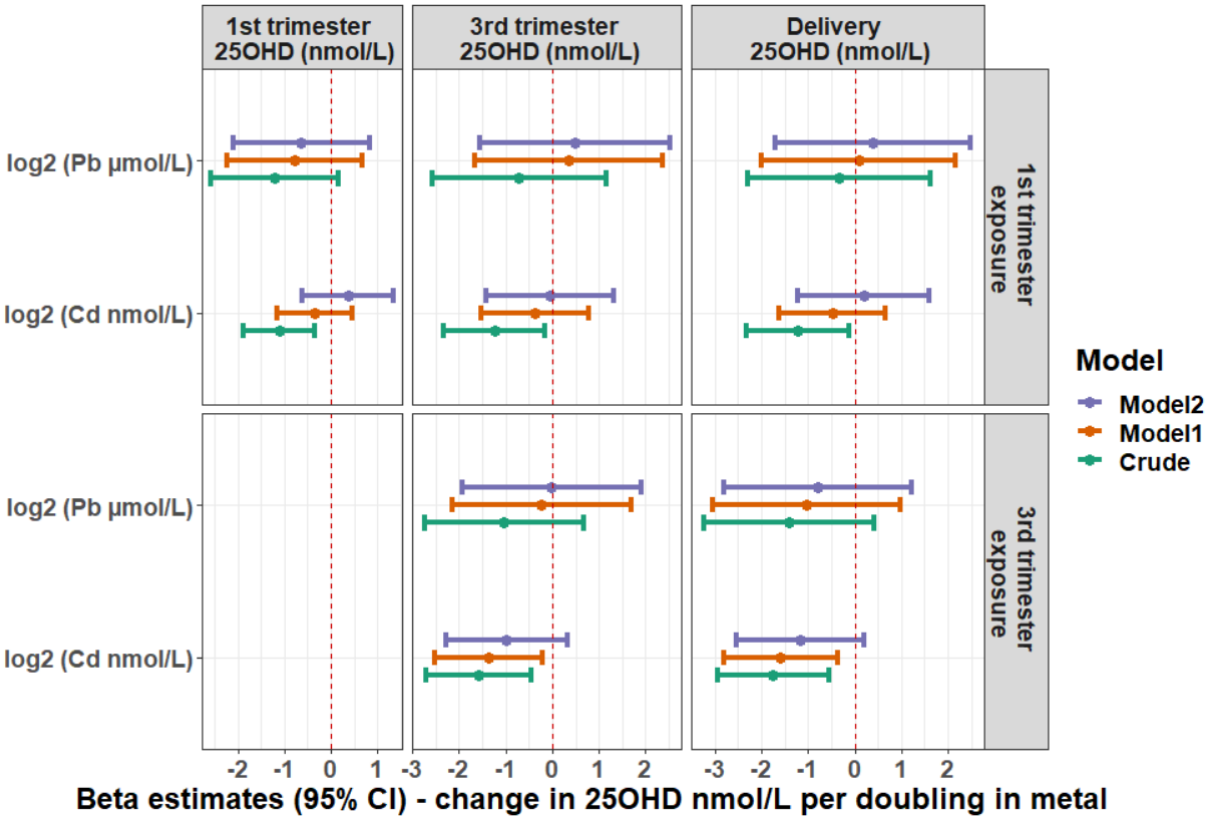
Table 2: Summary statistics for concentrations of lead (Pb), cadmium (Cd) and 25-hydroxyvitamin D (25OHD), by time point in pregnancy												
Label	**Units**	**N**	**LOD***	**% below LOD**	**Min**	**25th Pctl**	**50th Pctl**	**Mean**	**75th Pctl**	**95th Pctl**	**Max**	**GM**
Metals												
1st trimester												
Pb	µmol/L	1932	<0.005	0%	0.01	0.02	0.03	0.03	0.04	0.07	0.25	0.03
	µg/dL	1932	<0.10	0%	0.16	0.44	0.60	0.70	0.85	1.41	5.18	0.62
Cd	nmol/L	1932	<0.4	2.6%	0.20	1.20	1.80	2.95	2.80	10.00	50.00	1.93
	µg/L		<0.04	2.6%	0.02	0.13	0.20	0.33	0.31	1.12	5.61	0.22
3rd trimester												
Pb	µmol/L	1673	<0.005	0.2%	0.00	0.02	0.03	0.03	0.04	0.07	0.20	0.03
	µg/dL	1673	<0.10	0%	0.05	0.41	0.56	0.66	0.79	1.39	4.14	0.57
Cd	nmol/L	1673	<0.4	3.9%	0.20	1.20	1.80	2.46	2.60	6.60	38.00	1.76
	µg/L		<0.04	3.9%	0.02	0.13	0.20	0.28	0.29	0.74	4.27	0.20
25OHD												
1st trimester	nmol/L	1905	<10	0.3%	5.00	56.24	68.91	69.78	81.50	106.16	194.80	66.23
3rd trimester	nmol/L	1649	<3, <10	0.1%	1.50	60.50	77.36	78.56	95.02	121.52	293.40	73.62
Delivery	nmol/L	1542	<3, <10	0.1%	1.50	57.90	74.70	75.71	91.20	121.00	245.20	70.54
LOD=limit of detection; GM=geometric mean; Pctl=percentile												

Are Cd and Pb associated with 25OHD during pregnancy?

We found that 1st and 3rd trimester Cd concentrations were inversely associated with 25OHD concentrations cross-sectionally and longitudinally, in the unadjusted linear regression models or prior to smoking adjustment (see Figure 2, SI Table S6). The strongest association was between 3rd trimester Cd and 25OHD at delivery. For each doubling in 3rd trimester Cd concentrations, there was 1.75 nmol/L decrease in 25OHD concentrations at delivery (β_{crude}=-1.75; 95% CI: -2.95, -0.56). The strength of the associations was substantially reduced after controlling for covariates, especially smoking status ($\beta_{adjusted}$ for 3rd trimester Cd and 25OHD at delivery = -1.17; 95% CI: -

2.54, 0.20). While we did not identify evidence supporting a statistical interaction between smoking status and Cd concentrations in the interaction models ($p>0.20$, see SI Figure S3), the stratified analysis showed an inverse but imprecise association between Cd and 25OHD concentrations in smokers (n=235) while non-smokers showed mainly null results (See SI Figure S4). For Pb, there was a weak inverse association with 25OHD both cross-sectionally and longitudinally, but the confidence intervals crossed the null value for both the crude and adjusted models.

Figure 2: Linear Regression Models looking at visit specific associations between 1st and 3rd trimester metals (exposure) on 25OHD concentrations (outcome) in the 1st and 3rd trimester and at delivery



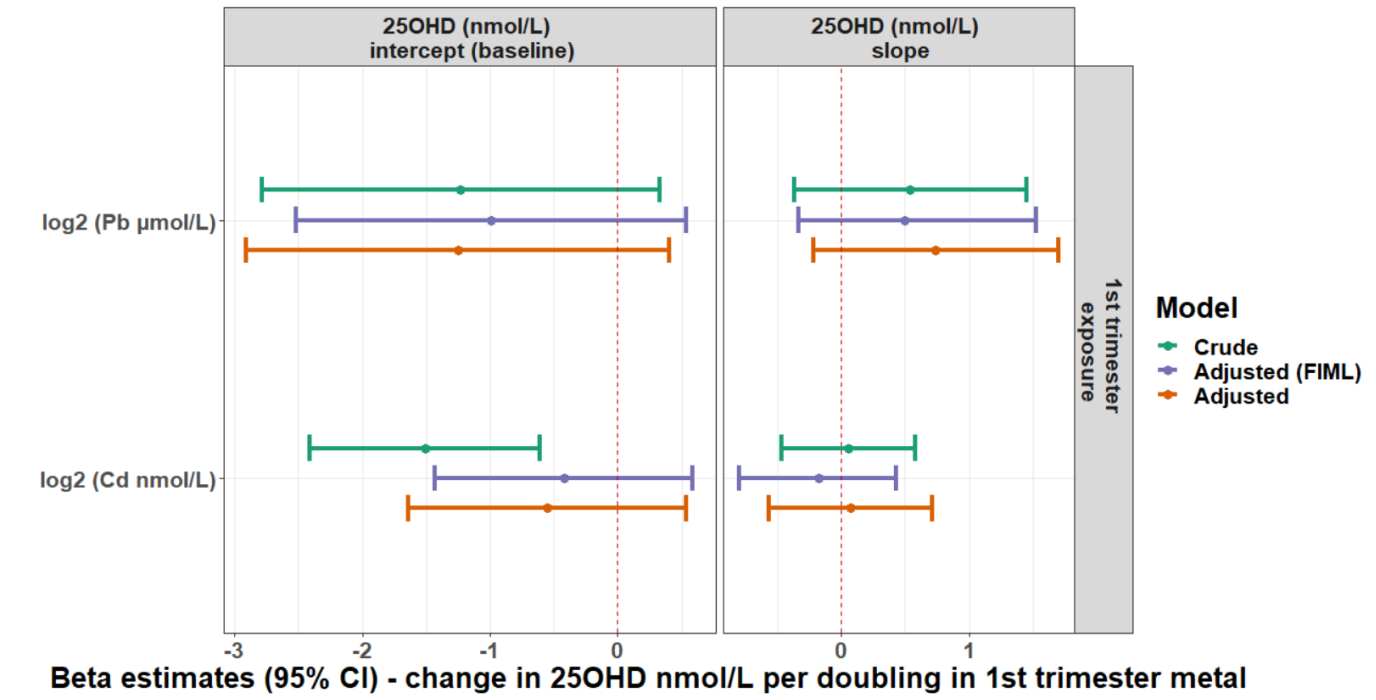
MODEL 1: adjusted for maternal education, income, maternal age, pre-pregnancy BMI, maternal race, general fish consumption per week (in past 3 months of exposure measure), study site, time spent outdoors, blood collection season at time of exposure measure

MODEL 2: model 1 plus smoking status at time of exposure measure

Are Cd and Pb associated with the 25OHD Trajectory?

We used LGCMs to analyze the effect of 1st trimester Cd and Pb on the 25OHD intercept and slope across pregnancy (see Figure 3 and SI Table S7). In the adjusted models, we saw no evidence of an association between 1st trimester Cd and Pb concentrations on the 25OHD slope (or change in 25OHD across pregnancy). For Cd, there was some evidence of an inverse association with the 25OHD intercept (baseline average 25OHD concentrations) in the crude models; each doubling in Cd exposure was associated with a 1.51 nmol/L decrease in 25OHD concentrations (β= -1.51, 95% CI: -2.41, -0.61). However, this estimate was attenuated in the adjusted models (β_{FIML}= -0.42, 95% CI: -1.43, 0.59). For 1st trimester Pb concentrations, we see a small but consistent inverse association with the 25OHD intercept across all models, but the estimates are imprecise (β_{FIML}= -0.99, 95% CI: -2.52, 0.54). The adjusted models showed good model fit for both Cd and Pb based on the CFI, RMSEA, and TLI but not the chi-squared statistic.

Figure 3: Latent Growth Curve and Mixed Models to examine the effect of 1st trimester lead (Pb) and cadmium (Cd) on the 25-hydroxyvitamin D (25OHD) trajectory in pregnancy

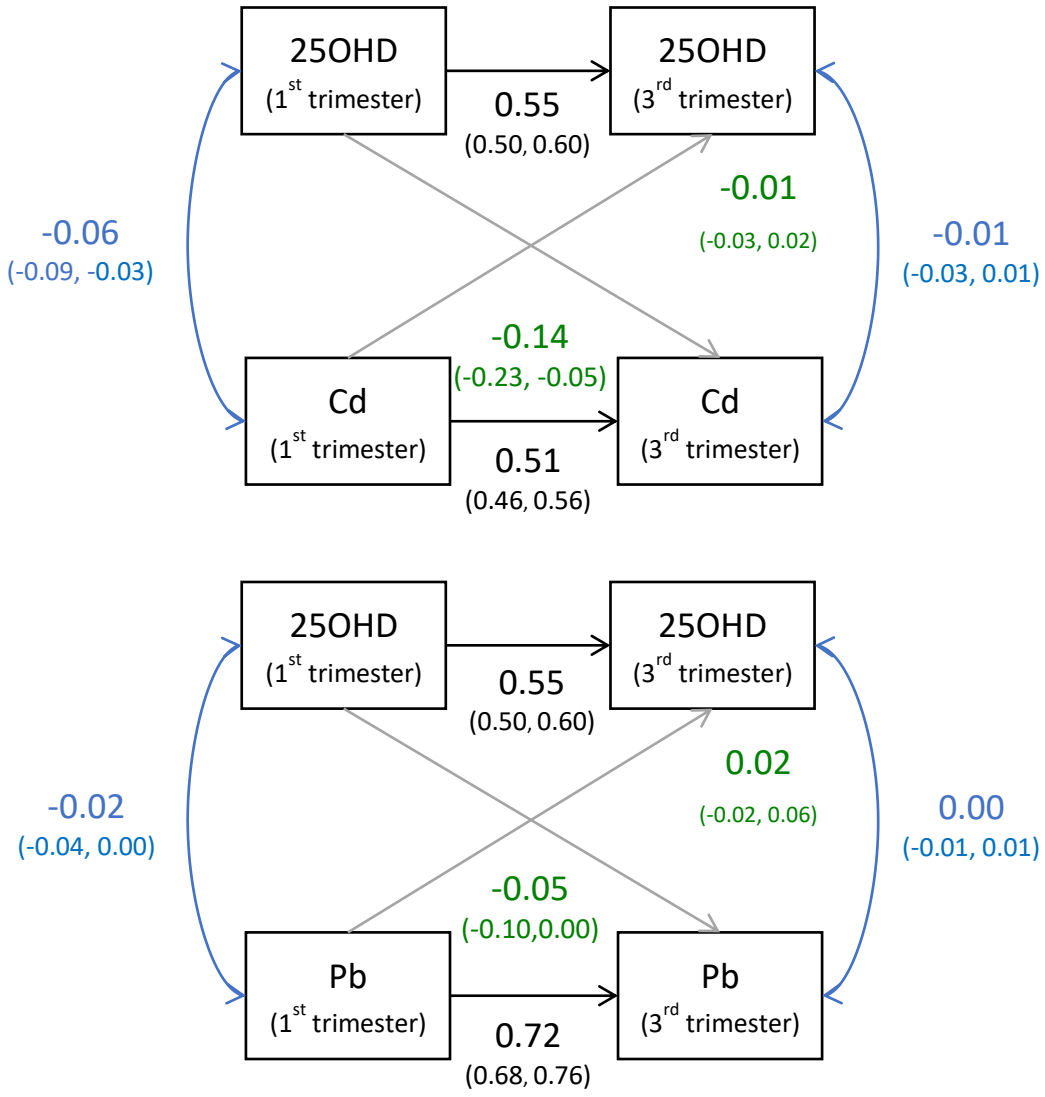


Note: Estimates (β and 95% CI) for the effect of first trimester Pb and Cd on the 25OHD latent intercept and slope. Other fixed effects include maternal education, parity, income, maternal age, pre-pregnancy BMI, maternal ethnicity and study site. Time varying covariates measured in 1st and 3rd trimester: smoking status, season, time spent outside, general fish consumption in the past 3 months.
Model Fit Statistics: All models had good model fit based on the CFI (>0.90), RMSEA (<0.05), TLI(>0.90). See Supplemental Table S7.

Is there a Bidirectional Association?

In the cross lag panel models (See Figure 4) all of the biomarkers (Cd, Pb and 25OHD) show large autoregressive effects (i.e. 0.51-0.72) suggesting little variation of these biomarkers over time, and therefore a need to consider influence from the previous time point in interpreting longitudinal associations [34]. In both models, we saw a weak association between 1st trimester metals and 3rd trimester 25OHD. The strongest cross-lag association appears to be the association between 1st trimester 25OHD and 3rd trimester metal concentrations in both the unstandardized (See Figure 4) and standardized beta estimates (SI Table S8). Each doubling in 1st trimester 25OHD was associated with a -0.14 (-0.23, -0.05) reduction in 3rd trimester $\log_2$ Cd, or a -9% change in Cd. The unexplained covariance after controlling for both the autoregressive and cross-lagged association (i.e. the residual association) between 3rd trimester Cd and 3rd trimester 25OHD was close to zero ($r_{Cd,25OHD}$ -0.01, 95% CI: -0.03, 0.01). We saw similar but weaker association in the Pb model. Each doubling in 1st trimester 25OHD was associated with a -0.05 reduction in 3rd trimester $\log_2$ Pb, which is a -3.3% change. Again, the residual covariance, reflecting the remaining unexplained association between 3rd trimester 25OHD and 3rd trimester Pb was not different from zero ($r_{Pb,25OHD}$=0.00, 95% CI: -0.01, 0.01) after controlling for the autoregressive and cross lagged association.

Figure 4: Cross Lagged Panel Model for Bi-directional Analysis of Lead (Pb) and Cadmium (Cd) and 25-hydroxyvitamin D (25OHD)



Cross Lagged Effects (crossed arrows); **Autoregressive Effects (effect of variable on itself)**; **Covariance (double headedarrow)**; 25OHD, Cd and Pb log2 transformed: Log2 change in outcome per doubling in exposure

DISCUSSION

In this study we investigated the relationship of low-level maternal blood concentrations of Cd and Pb on 25OHD concentrations in pregnancy and examined whether there is a bidirectional relationship between these metals and 25OHD. We saw some evidence of an inverse association between metals, especially for Cd, and 25OHD at different time points but these associations were attenuated in the fully adjusted models. In addition, the latent growth curve models suggested that 1st trimester Cd and Pb were not associated with the 25OHD trajectory (slope) in pregnancy. When we examined bidirectional associations, we found no association between 1st trimester metals and 3rd trimester 25OHD. Instead, each doubling in 1st trimester 25OHD was associated with 9% lower 3rd trimester Cd and 3% lower Pb on average. These findings suggest that vitamin D status in early pregnancy may moderate Cd and Pb concentrations in later pregnancy.

The blood Cd and Pb concentrations measured in our study are lower than those measured in females aged 20-39 in the Cycle 1 Canadian Health Measures Survey (CHMS) (2007-2009), which was conducted around the same time period as MIREC (2008 and 2011) (geometric mean for MIREC 1st trimester samples vs CHMS: Pb 0.6 vs 0.9 μg/dL; Cd 0.2 vs 0.4 μg/L) [42]. The 25OHD concentrations in MIREC suggest that the majority of women (~85%) have concentrations >50 nmol/L at each visit. Both the Institute of Medicine (IOM) and Health Canada agree that 25OHD serum concentrations ≥50 nmol/L is sufficient for most people while serum concentrations >125 nmol/L may be of concern [41]. 25OHD concentrations were first measured in cycle 2 (2009-2011) of the CHMS with females having average blood concentrations (67 nmol/L) similar to that of MIREC mothers in the 1st trimester (69 nmol/L) [43].

In our initial trimester-specific analyses we looked at the effect of metals on 25OHD concentrations in pregnancy and saw a weak inverse association, especially between 3rd trimester Cd and 25OHD concentrations in the 3rd trimester and at delivery (See Figure 2), particularly in smokers (See SI Figure S4). Studies of male smelter workers [44], lead exposed battery manufacture workers [14,15], and lead-exposed jewellery workers [16] have reported inverse associations between workers exposed to Cd and/or Pb and biomarkers of vitamin D. Cross-sectional and case studies of men and women highly exposed to Cd [12,13,45–47] also show inverse associations with serum 25OHD and vitamin D's hormonally active form, calcitriol. Cross-sectionally, lower 25OHD concentrations were seen among children with higher (≥60 ug/dl) compared to lower blood Pb (≤29 ug/dl) concentrations prior to chelation therapy [18]. In contrast, no association was observed in a cross-sectional study between blood concentrations of Pb and 25OHD in Mexican children (12-15 yrs) [48].

Previous studies have found that the vitamin D status trajectory during pregnancy (defined as the measured change in 25OHD from the 1st trimester to delivery) is inversely associated with small for gestational age (SGA), preterm birth, low birth weight and preeclampsia [49,50]. We are not aware of any other studies that have examined the association of metals with the 25OHD trajectory in pregnancy. We did not identify a strong association of Pb and Cd with the 25OHD trajectory during pregnancy. The latent growth curve models suggest a small inverse association between 1st trimester Pb on the 25OHD intercept, reflecting the average 1st trimester 25OHD, but not on the 25OHD slope in pregnancy (See Figure 3 and SI Table S7).

Our study examined the bidirectional relationship between toxic metals and 25OHD concentrations. The main objective of cross lagged panel models is to examine causal influences between variables [34]. The cross lagged panel model controls for both contemporaneous effects and the stability of a variable over time, thus obviating the potential for reverse causation. Controlling for the stability of a variable over time gives us more confidence that the change in 3rd trimester Cd predicted by 1st trimester 25OHD is the left over change, after controlling for previous levels of Cd (1st trimester Cd). This rules out the possibility that the cross lagged association was simply due to the correlation of Cd and 25OHD in the 1st trimester [35]. Our results show that the inverse association between 1st trimester 25OHD concentrations and 3rd trimester metals is the strongest cross lagged effect (See Figure 4 and SI Table S8). Only a few studies have examined the modifying effect of 25OHD on metal concentrations in pregnancy. A recent study from the LIFECODES pregnancy cohort (n=381), reported that higher concentrations of 25OHD (>50 nmol/L) were associated with lower levels of urinary Pb, but not Cd, in the 2nd trimester [51]. Women with low 1st trimester 25OHD concentrations (<50 nmol/L) had 47% higher urinary lead concentrations in the 2nd trimester. A previous analysis of the MIREC study [19] found a significant inverse association between estimated vitamin D intake, derived from a 2nd trimester food frequency questionnaire and supplement form, and maternal Cd, Pb, as well as cord blood Pb. Nonetheless, an NHANES study found no association between the use of vitamin D supplements in the past month and blood Pb concentrations in pre-menopausal women [52]. Cross-sectional studies among children in the U.K. [53] and south east China [17] show inverse correlations between serum 25OHD and blood Pb levels. Chang et al. (2014) found that a greater proportion of children

with 25OHD concentrations ≥39 ng/ml had lower blood lead concentrations (61.4%) than children with insufficient (24.4%) or deficient (14.1%) 25OHD concentrations.

The mechanism by which higher early pregnancy 25OHD concentrations might be related to lower concentrations of Cd and Pb later in pregnancy is unknown. In our study, higher 25OHD concentrations may be an indicator of sufficient mineral status. It is suggested that sufficient vitamin D status along with essential mineral sufficiency is required to prevent increased uptake of toxic metals [54]. Adequate dietary intake of calcium and phosphate are essential for bone mass and bone quality because they constitute the mineral component of bone. Intestinal absorption of both is partly regulated by the hormonally active form of vitamin D (calcitriol). The Ca^{2+}-binding protein in intestinal cells, responsible for Ca^{2+} absorption from the small intestine, also binds Pb^{2+} and Cd^{2+} and may be the reason for the absorption of these divalent cations [55]. Prior to the discovery of vitamin D in the early 1930s, summer outbreaks of pediatric Pb poisoning were reported by physicians in the United Kingdom. Later it was thought that increased summer solar exposure and vitamin D production in the skin resulted in an increase in Ca^{2+}-binding protein, in turn causing the increased absorption of lead [56]. A recent randomized controlled trial of vitamin D supplementation in Bangladesh [20] investigated whether vitamin D supplementation increased the absorption of Cd and Pb. In the trial, pregnant women in their 2nd trimester randomized to receive weekly doses of 4200, 16 800 or 28 000 IU of vitamin D_3 throughout pregnancy did not show any association of vitamin D3 dose with metal levels during pregnancy, but there was a significant increase in cord blood Pb and Cd among higher dose groups compared to placebo. It is also possible that vitamin D can increase metallothionein levels [57] which could promote

clearance of Cd [58]. Finally, a recent study showed that vitamin D supplementation increased gut microbial diversity [59] which could potentially modify the metal uptake pattern in the gut [60].

Strengths and Limitations

A strength of this study is the prospective nature of the MIREC cohort and repeated measurements of both metals and 25OHD, thus allowing us to examine the association of first trimester metals on 25OHD concentrations later in pregnancy but also to investigate the reverse association. Our study employed a variety of statistical techniques to study repeated measures of exposures and outcomes. Given we only had 2 time points for the cross lag panel analysis we were not able to control for all unobserved time invariant confounders with a fixed effect. Therefore, there is a potential for unmeasured confounding in our cross lagged models. The cross lagged model also assumes synchronicity, which assumes that the measurements at each time point occurred at the exact same time [34]. In MIREC participants were given time-windows to schedule visits (See Figure 1) so participants were not seen on the exact same gestational day in pregnancy. We also lacked sufficient measures of other nutrients such as iron and calcium which could be confounding this relationship. We also did not control for air pollution which has shown some association with metals and vitamin D status [61]. We cannot rule out false positive findings given the number of models applied in this study. Finally, the MIREC study is comprised of mainly high income, highly educated, white women so is not representative of the Canadian population giving birth during this time frame [21].

CONCLUSIONS

In this analysis of the MIREC study we investigated the prospective and reciprocal relationship between blood concentrations of Cd or Pb and 25OHD. Although our initial linear regression

analysis showed some evidence that increasing metals may be associated with lower 25OHD concentrations, the bidirectional analysis, which controlled for both contemporaneous effects and the persistence of a biomarker over time, suggest that vitamin D status may modify toxic metals in pregnancy. First trimester 25OHD concentrations were associated with lower 3rd trimester blood metals, whereas no association was seen between 1st trimester metals and 3rd trimester 25OHD. This research demonstrates the value of controlling for the persistence of a biomarker over time and contemporaneous effects. Replication of the cross lagged method in both vitamin D sufficient and deficient populations is warranted. In addition, given the potential health consequences of low level Cd and Pb exposure in pregnancy to both the mother and developing fetus [62,63], further research into the possible modifying effects of early pregnancy vitamin D status on Cd and Pb concentrations is warranted.

REFERENCES

1. Schröder-Heurich, B., Springer, C. J. P. & von Versen-Höynck, F. Vitamin D effects on the immune system from periconception through pregnancy. *Nutrients* **12**, 21–30 (2020).

2. Baeke, F., Takiishi, T., Korf, H., Gysemans, C. & Mathieu, C. Vitamin D: Modulator of the immune system. *Current Opinion in Pharmacology* at https://doi.org/10.1016/j.coph.2010.04.001 (2010).

3. Norman, A. W. From vitamin D to hormone D: Fundamentals of the vitamin D endocrine system essential for good health. *Am. J. Clin. Nutr.* **88**, (2008).

4. Abu-Raya, B., Michalski, C., Sadarangani, M. & Lavoie, P. M. Maternal Immunological Adaptation During Normal Pregnancy. *Front. Immunol.* **11**, 1–18 (2020).

5. Wagner, C. L. & Hollis, B. W. The implications of vitamin D status during pregnancy on mother and her developing child. *Front. Endocrinol. (Lausanne).* **9**, 1–11 (2018).

6. Institute of Medicine (IOM). *DRI, dietary reference intakes : calcium, vitamin D*. *Dietary reference intakes : calcium, vitamin D* (National Academies Press, 2011).

7. Qin, L. L., Lu, F. G., Yang, S. H., Xu, H. L. & Luo, B. A. Does maternal Vitamin D deficiency increase the risk of preterm birth: A meta-analysis of observational studies. *Nutrients* **8**, (2016).

8. Saraf, R., Morton, S. M. B., Camargo, C. A. & Grant, C. C. Global summary of maternal and newborn vitamin D status – a systematic review. *Matern. Child Nutr.* **12**, 647–668 (2016).

9. Statistics Canada. Vitamin D levels of Canadians. *Health at a Glance* https://www150.statcan.gc.ca/n1/pub/82-624-x/2013001/article/11727-eng.htm.

10. Ginde, A. A., Sullivan, A. F., Mansbach, J. M. & Camargo, C. A. Vitamin D insufficiency in pregnant and non-pregnant women of childbearing age in the United States. *Am J Obs. Gynecol* **202**, 436.e1-436.e8 (2010).

11. Kido, T. *et al.* alpha1-Microglobulin determination in urine for the early detection of renal tubular dysfunctions caused by exposure to cadmium. **24**, 195–201 (1985).

12. Nogawa, K. *et al.* Mechanism for bone disease found in inhabitants environmentally exposed to cadmium: decreased serum 1α, 25-dihydroxyvitamin D level. *Int. Arch. Occup. Environ. Health* (1987) doi:10.1007/BF00377675.

13. Nogawa, K. *et al.* Serum vitamin D metabolites in cadmium-exposed persons with renal damage. *Int. Arch. Occup. Environ. Health* **62**, 189–193 (1990).

14. Dongre, N. N., Suryakar, A. N., Patil, A. J., Hundekari, I. A. & Devarnavadagi, B. B. Biochemical effects of lead exposure on battery manufacture workers with reference to blood pressure, calcium metabolism and bone mineral density. *Indian J. Clin. Biochem.* **28**, 65–70 (2013).

15. Datta, S. K. *et al.* Association of high blood lead levels with FOK1, APA1 and BSM1 Vitamin D Receptor gene polymorphism in occupationally exposed battery workers. *Drug Metab. Pharmacokinet.* **34**, S28–S29 (2019).

16. Mazumdar, I., Goswami, K. & Ali, M. S. Status of Serum Calcium, Vitamin D and Parathyroid Hormone and Hematological Indices Among Lead Exposed Jewelry Workers in Dhaka,

Bangladesh. *Indian J. Clin. Biochem.* **32**, 110–116 (2017).

17. Chang, L. *et al.* Association of 25-hydroxyvitamin D with Hb and lead in children: A Chinese population-based study. *Public Health Nutr.* **17**, 827–832 (2014).

18. Rosen, J. F. *et al.* Reduction in 1,25-Dihydroxyvitamin D in Children with Increased Lead Absorption. *N. Engl. J. Med.* (1980) doi:10.1056/NEJM198005153022006.

19. Arbuckle, T. E. *et al.* Maternal and fetal exposure to cadmium, lead, manganese and mercury: The MIREC study. *Chemosphere* **163**, 270–282 (2016).

20. Jukic, A. M. Z. *et al.* Vitamin D treatment during pregnancy and maternal and neonatal cord blood metal concentrations at delivery: Results of a randomized controlled trial in Bangladesh. *Environ. Health Perspect.* **128**, 1–11 (2020).

21. Arbuckle, T. E. *et al.* Cohort profile: The maternal-infant research on environmental chemicals research platform. *Paediatr. Perinat. Epidemiol.* (2013) doi:10.1111/ppe.12061.

22. Weiler, H. A. *et al.* Early prenatal use of a multivitamin diminishes the risk for inadequate vitamin D status in pregnant women : results from the Maternal-Infant Research on Environmental Chemicals (MIREC) cohort study. *Am. J. Clin. Nutr.* **114**, 1238–1250 (2021).

23. Sarafin, K. *et al.* Standardizing 25-hydroxyVitamin D values from the Canadian Health Measures Survey. *Am. J. Clin. Nutr.* **102**, 1044–1050 (2015).

24. Durazo-Arvizu, R. *et al.* The Vitamin D Standardization Program (VDSP) Manual for Retrospective Laboratory Standardization of Serum 25-Hydroxyvitamin D Data. **25**, 1234–1243 (2017).

25. Textor, J. & Hardt, J. DAGitty: A graphical tool for analyzing causal diagrams. *Epidemiology* at https://doi.org/10.1097/EDE.0b013e318225c2be (2011).

26. Hernán, M. A., Hernández-Díaz, S. & Robins, J. M. A structural approach to selection bias. *Epidemiology* (2004) doi:10.1097/01.ede.0000135174.63482.43.

27. Ganguly, K., Levänen, B., Palmberg, L., Åkesson, A. & Lindén, A. Cadmium in tobacco smokers: A neglected link to lung disease? *Eur. Respir. Rev.* **27**, 1–8 (2018).

28. Banihosseini, S. Z., Baheiraei, A., Shirzad, N., Heshmat, R. & Mohsenifar, A. The effect of cigarette smoke exposure on vitamin D level and biochemical parameters of mothers and neonates. *J. Diabetes Metab. Disord.* **12**, 1 (2013).

29. Felt, J. M., Depaoli, S. & Tiemensma, J. Latent growth curve models for biomarkers of the stress response. *Front. Neurosci.* **11**, 1–17 (2017).

30. Kiang, T. SAS Global Forum 2010 Statistics and Data Analysis Building Latent Growth Models Using PROC CALIS : A Structural Equation Modeling Approach. 1–19 (2010).

31. Kline, R. B. *Principles and Practice of Structural Equation Modeling*. (The Guilford Press, 2016).

32. Allison, P. D., Williams, R. & Moral-Benito, E. Maximum Likelihood for Cross-lagged Panel Models with Fixed Effects. *Socius Sociol. Res. a Dyn. World* **3**, 237802311771057 (2017).

33. Amorim, L. D. A. F. *et al.* Structural equation modeling in epidemiology. *Cad. Saude Publica* **26**,

2251–2262 (2010).

34. Kearney, M. Cross-lagged panel analysis. *SAGE Encycl. Commun. Res. Methods* **355**, 165–174 (2017).

35. Selig, J. P. & Little, T. D. Autoregressive and Cross- - Lagged Panel Analysis for Longitudinal Data. *Handb. Dev. Res. Methods.* (2012).

36. Kumar, R. G. *et al.* Temporal Acute Serum Estradiol and Tumor Necrosis Factor-α Associations and Risk of Death after Severe Traumatic Brain Injury. *J. Neurotrauma* **37**, 2198–2210 (2020).

37. Raykov, T., Marcoulides, G. A. & Patelis, T. Saturated Versus Just Identified Models: A Note on Their Distinction. *Educ. Psychol. Meas.* **73**, 162–168 (2013).

38. Hamaker, E. L., Kuiper, R. M. & Grasman, R. P. P. P. A critique of the cross-lagged panel model. *Psychol. Methods* (2015) doi:10.1037/a0038889.

39. Alkarkhi, A. F. M. & Alqaraghuli, W. A. A. R Statistical Software. in *Applied Statistics for Environmental Science with R* (2020). doi:10.1016/b978-0-12-818622-0.00002-2.

40. SAS Enterprise Guide. https://support.sas.com/en/software/enterprise-guide-support.html#8ab798a7-2f78-4e1b-a869-d0dbf323212c.

41. Health Canada. Vitamin D and Calcium: Updated Dietary Reference Intakes. https://www.canada.ca/en/health-canada/services/food-nutrition/healthy-eating/vitamins-minerals/vitamin-calcium-updated-dietary-reference-intakes-nutrition.html (2019).

42. Health Canada. *Report on Human Biomonitoring of Environmental Chemicals in Canada*. vol. 1 (Health Canada, 2010).

43. Janz, T. & Pearson, C. *Vitamin D Status of Canadians, 2007 to 2009*. *Statistics Canada Catalogue no. 82-624-X* https://www150.statcan.gc.ca/n1/pub/82-624-x/2013001/article/11727-eng.htm (2013).

44. Chalkley, S., Richmond, J. & Barltrop, D. Measurement of vitamin D3 metabolites in smelter workers exposed to lead and cadmium (multiple letters) [1]. *Occup. Environ. Med.* **55**, 446–452 (1998).

45. Uchida, M. *et al.* Elevated urinary levels of vitamin D-binding protein in the inhabitants of a cadmium polluted area, Jinzu River basin, Japan. *Tohoku J. Exp. Med.* **211**, 269–274 (2007).

46. Aoshima, K. [Itai-itai disease: cadmium-induced renal tubular osteomalacia]. *Nihon eiseigaku zasshi. Japanese journal of hygiene* at https://doi.org/10.1265/jjh.67.455 (2012).

47. Aoshima, K. & Kasuya, M. Preliminary study on serum levels of 1, 25-dihydroxyvitamin D and 25-hydroxyvitamin D in cadmium-induced renal tubular dysfunction. *Toxicol. Lett.* **57**, 91–99 (1991).

48. Zamoiski, R. D. *et al.* Association of arsenic and metals with concentrations of 25-hydroxyvitamin d and 1,25-dihydroxyvitamin D among adolescents in Torreón, Mexico. *Environ. Health Perspect.* **122**, 1233–1238 (2014).

49. Bärebring, L. *et al.* Trajectory of vitamin D status during pregnancy in relation to neonatal birth size and fetal survival: A prospective cohort study. *BMC Pregnancy Childbirth* **18**, 1–7 (2018).

50. Bärebring, L. *et al.* Preeclampsia and blood pressure trajectory during pregnancy in relation to Vitamin D status. *PLoS One* (2016) doi:10.1371/journal.pone.0152198.

51. Jukic, M. A. Z. *et al.* A prospective study of maternal 25-hydroxyvitamin D (25OHD) in the first trimester of pregnancy and second trimester heavy metal levels. *Environ. Res.* **199**, 111351 (2021).

52. Jackson, L. W., Cromer, B. A. & Panneerselvamm, A. Association between bone turnover, micronutrient intake, and blood lead levels in pre- and postmenopausal women, NHANES 1999-2002. *Environ. Health Perspect.* **118**, 1590–1596 (2010).

53. Box, V. *et al.* Plasma Vitamin D and Blood Lead concentrations in Asian Children. *Lancet* **Aug 15**; **2**, 373 (1981).

54. Schwalfenberg, G. K. & Genuis, S. J. Vitamin D, essential minerals, and toxic elements: Exploring interactions between nutrients and toxicants in clinical medicine. *Sci. World J.* (2015) doi:10.1155/2015/318595.

55. Bronner, F. Mechanisms of intestinal calcium absorption. *J. Cell. Biochem.* **88**, 387–393 (2003).

56. Moon, J. The role of vitamin D in toxic metal absorption: A review. *J. Am. Coll. Nutr.* **13**, 559–564 (1994).

57. Karasawa, M. *et al.* Regulation of metallothionein gene expression by 1α,25-dihydroxyvitamin D3 in cultured cells and in mice. *Proc. Natl. Acad. Sci. U. S. A.* **84**, 8810–8813 (1987).

58. Klaassen, C. D., Liu, J. & Choudhuri, S. Metallothionein: An intracellular protein to protect against cadmium toxicity. *Annu. Rev. Pharmacol. Toxicol.* **39**, 267–294 (1999).

59. Singh, P., Rawat, A., Alwakeel, M., Sharif, E. & Al Khodor, S. The potential role of vitamin D supplementation as a gut microbiota modifier in healthy individuals. *Sci. Rep.* **10**, 1–14 (2020).

60. Duan, H. *et al.* Gut microbiota: A target for heavy metal toxicity and a probiotic protective strategy. *Sci. Total Environ.* **742**, 140429 (2020).

61. Hosseinpanah, F. *et al.* The effects of air pollution on vitamin D status in healthy women: A cross sectional study. *BMC Public Health* **10**, (2010).

62. Kumar, S. & Sharma, A. Cadmium toxicity: Effects on human reproduction and fertility. *Rev. Environ. Health* **34**, 327–338 (2019).

63. Dórea, J. G. Environmental exposure to low-level lead (Pb) co-occurring with other neurotoxicants in early life and neurodevelopment of children. *Environ. Res.* **177**, 108641 (2019).

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Supplemental Information

Blood Metals and Vitamin D Status in a Pregnancy Cohort: A Bidirectional Biomarker Analysis

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Tables

Figures

Table S1: Formula for predicted percent change in the outcome per doubling of the exposure concentration (based on linear regression model where both the outcome and the predictor are $\log_2$-transformed)

For a linear regression model $\log_2 Y = \beta^* \log_2 X + e$, where Y is an outcome variable, X is a single predictor variable, and e is a random error term, predicted exposure concentrations y_1 and y_2 corresponding to exposure concentrations $X= x_1$ and $X= x_2$ satisfy the following relations:

$\log_2 y_1 = \beta^* \log_2 x_1$ (1)

$\log_2 y_2 = \beta^* \log_2 x_2$ (2)

If we subtract (1) from (2) and consider doubling of X (i.e. $x_2=2x_1$), we get:

$\log_2(y_2/y_1) = \beta$

and, respectively:

$y_2/y_1=2^{\beta}$ (3)

The predicted % change in the outcome is as follows:

%change in $Y=((Y_2-Y_1)/Y_1)*100=(Y_2/Y_1-1)*100$ (4)

Substituting (3) in (4), we get:

%change in $Y = \mathbf{(2^{\beta}-1)*100}$ ++

++For a multivariable model, the formula represents a predicted percent change in the outcome per unit change in $\log_2$ of the predictor of interest (or doubling of the predictor of interest), keeping all other covariates constant.

Note: thank you to Orly Brion for this formula

Table S2: Comparison of those who had all three visit 25OHD blood measures verses the whole sample

	Full sample at visit 1 (n=1905)		Participants with all three 25OHD measures (n=1387)	
Factor	Freq	%	Freq	%
Mother's education level				
Some college, High School or Less than High School	278	14.19	189	13.65
College/Trade Diploma	463	23.63	323	23.32
Undergrad degree	713	36.40	516	37.26
Graduate degree	505	25.78	357	25.78
Household income				
< = to $50 000	444	22.64	312	22.49
between 50,001-100 000	675	34.42	487	35.11
>100 000	750	38.25	529	38.14
Don't know/refuse to answer	92	4.69	59	4.25
Marital Status				
Married	1406	71.70	995	71.74
Same partner for 1+ years	461	23.51	327	23.58
Divorced, separated, single or other (no widowers)	94	4.79	65	4.69
maternal age group, yrs				
<20	13	0.66	10	0.72
20-24	125	6.37	76	5.48
25-29	455	23.2	342	24.66
30-34	702	35.8	492	35.47
35+	666	33.96	467	33.67
Parity				
0	865	44.11	626	45.13
1	792	40.39	548	39.51
2	228	11.63	159	11.46
3+	76	3.88	54	3.89
Pre-pregnancy BMI				
Underweight (BMI < 18.5)	52	2.87	36	2.79
Normal (18.5 <= BMI < 25)	1096	60.39	783	60.74
Overweight (25 <= BMI < 30)	397	21.87	275	21.33
Obese (BMI >=30)	270	14.88	195	15.13
Smoking status (1st trimester)				
Current	115	5.87	76	5.48

Former	534	27.26	362	26.1
Never	1190	60.75	862	62.15
Quit during Pregnancy	120	6.13	87	6.27
Maternal ethnicity				
0 = "White"	1643	83.78	1181	85.15
1 = "Black"	59	3.01	42	3.03
2 = "Aboriginal"	44	2.24	22	1.59
3 = "Chinese"	45	2.29	21	1.51
4 = "Latin American"	47	2.40	38	2.74
5 = "Other"	123	6.27	83	5.98
Season (1st trimester)				
fall	572	29.41	411	29.63
winter	465	23.91	331	23.86
spring	447	22.98	322	23.22
summer	461	23.7	323	23.29

Table S3: Pearson Correlations for plasma 25OHD, nmol/L

	First trimester (Visit 1)	Third Trimester (Visit 3)	Delivery (Visit 4)
First trimester (Visit 1)	1	0.55	0.51
Third Trimester (Visit 3)		1	0.71
Delivery (Visit 4)			1

Table S4: Intraclass Correlation Coefficients (ICCs)

Intraclass Correlation Co-efficients (ICCs)			
Variable	time points	No. Subjects with non-missing data	ICC
Cadmium	1st, 3rd	1648	0.77
Lead	1st, 3rd	1648	0.73
25OHD	1st, 3rd, delivery	1387	0.57

Table S5: Pearson Correlation for log_2 Metals and log_2 25OHD

Pearson Correlation Coefficients						
Prob > |r| under H0: Rho=0						
	Pb_1st	Pb_3rd	Cd_1st	Cd_3rd	25OHD_1st	25OHD_3rd
Pb_1st	1	0.73 (<.0001)	0.22 (<.0001)	0.15 (<.0001)	-0.06 (0.0071)	-0.03 (0.3077)
Pb_3rd		1	0.13 (<.0001)	0.26 (<.0001)	-0.09 (0.0004)	-0.02 (0.3502)
CD_1st			1	0.60 (<.0001)	-0.08 (0.0005)	-0.057 (0.0224)
CD_3rd				1	-0.12 (<.0001)	-0.07 (0.006)
25OHD_1st					1	0.50 (<.0001)

Table S6: Linear Regression Models looking at trimester specific associations between metals (exposure) on 25OHD (outcome) concentrations

EXPOSURE	OUTCOME (25OHD nmol/L)								
	Linear Regression by Visit								
	1st trimester			3rd trimester			delivery		
	β estimate	UCI	LCI	β estimate	UCI	LCI	β estimate	UCI	LCI
CADMIUM (log2)									
1st trimester (crude)	**-1.108**	**-1.875**	**-0.341**	**-1.241**	**-2.333**	**-0.148**	**-1.222**	**-2.326**	**-0.119**
1st trimester (Model 1)	-0.346	-1.148	0.457	-0.387	-1.541	0.767	-0.484	-1.634	0.667
1st trimester (Model 2)	0.365	-0.616	1.345	-0.048	-1.419	1.323	0.187	-1.212	1.586
3rd trimester (crude)				**-1.592**	**-2.717**	**-0.466**	**-1.754**	**-2.945**	**-0.564**
3rd trimester (Model 1)				**-1.367**	**-2.528**	**-0.206**	**-1.589**	**-2.803**	**-0.375**
3rd trimester (Model 2)				-0.989	-2.291	0.313	-1.172	-2.542	0.198
LEAD (log2)									
1st trimester (crude)	-1.207	-2.571	0.157	-0.720	-2.586	1.145	-0.343	-2.294	1.609
1st trimester (Model 1)	-0.794	-2.244	0.656	0.351	-1.672	2.374	0.087	-1.989	2.163
1st trimester (Model 2)	-0.640	-2.104	0.823	0.474	-1.565	2.513	0.384	-1.712	2.479
3rd trimester (crude)				-1.038	-2.749	0.672	-1.413	-3.228	0.402
3rd trimester (Model 1)				-0.230	-2.141	1.681	-1.031	-3.036	0.975
3rd trimester (Model 2)				-0.014	-1.930	1.902	-0.804	-2.816	1.207

MODEL 1: adjusted for maternal education, income, maternal age, pre-pregnancy BMI, maternal race, general fish consumption per week (in past 3 months of exposure measure), study site, time spent outdoors, blood collection season at time of exposure measure
MODEL 2: model 1 plus smoking status at time of exposure measure

Table S7: Latent Growth Curve Models Parameter Estimates and Fit Statistics.

Latent Growth Curve Models Parameter Estimate							**Model Fit Statistics** (criteria for goodness of fit)			
	β intercept	**LCI**	**UCI**	**β slope**	**LCI**	**UCI**	**χ2** p>0.05	**CFI** >0.90	**RMSEA** <0.05	**TLI** >0.90
Effect of 1st trimester blood Cd on 25OHD intercept and slope										
Crude Model	**-1.51**	**-2.41**	**-0.61**	0.06	-0.47	0.58	139, df 2, p<0.001	0.91	0.22	0.73
Adjusted Model	-0.55	-1.64	0.54	0.07	-0.57	0.71	136, df 43, p<0.001	0.95	0.04	0.87
Adjusted Model (with FIML)	-0.42	-1.43	0.59	-0.18	-0.80	0.43	135, df 43, p<0.001	0.95	0.04	0.88
Effect of 1st trimester blood Pb on 25OHD intercept and slope										
Crude Model	-1.23	-2.79	0.33	0.54	-0.37	1.45	140, df 2, p<0.001	0.91	0.22	0.73
Adjusted Model	-1.25	-2.91	0.40	0.74	-0.22	1.70	110, df 43, p<0.001	0.97	0.04	0.92
Adjusted Model (with FIML)	-0.99	-2.52	0.54	0.50	-0.33	1.53	108, df 43, p<0.001	0.97	0.03	0.93

Adjusted Model: Other fixed effects include maternal education, parity, income, maternal age, pre-pregnancy BMI, maternal race, and study site. Time varying covariates measured in 1st and 3rd trimester: smoking status, season, time spent outside, general fish consumption in the past 3 months.

Table S8: Estimates from Cross Lagged Models (Unstandardized and Standardized)

		Unstandardized β Estimates							Standardized β Estimates				Type of effect
Outcome*	Exposure*	β	SE	LCI	UCI	% change	LCI	UCI	β	SE	LCI	UCI	
CADMIUM													
Regressions													
3rd 25OHD	1st 25OHD	0.547	0.025	0.498	0.597	46.13	41.20	51.22	0.503	0.021	0.462	0.543	AR
	1st Cd	-0.009	0.013	-0.034	0.017	-0.60	-2.34	1.17	-0.018	0.027	-0.071	0.035	CL
3rd Cd	1st Cd	0.510	0.024	0.463	0.556	42.38	37.87	47.04	0.530	0.023	0.486	0.574	AR
	1st 25OHD	-0.136	0.046	-0.226	-0.046	-8.99	-14.50	-3.12	-0.062	0.021	-0.103	-0.021	CL
Covariance													
1st 25OHD	1st Cd	-0.056	0.016	-0.087	-0.026				-0.094	0.026	-0.145	-0.043	
3rd Cd	3rd 25OHD	-0.006	0.010	-0.027	0.014				-0.010	0.017	-0.043	0.022	
LEAD													
Regressions													
3rd 25OHD	1st 25OHD	0.550	0.025	0.500	0.599	46.38	41.44	51.48	0.505	0.021	0.465	0.545	AR
	1st Pb	0.018	0.020	-0.020	0.056	0.92	-1.72	3.63	0.023	0.024	-0.025	0.070	CL
3rd Pb	1st Pb	0.722	0.020	0.683	0.760	64.89	60.50	69.40	0.667	0.015	0.637	0.697	AR
	1st 25OHD	-0.049	0.026	-0.099	0.002	-3.31	-6.63	0.12	-0.033	0.018	-0.068	0.001	CL
Covariance													
1st 25OHD	1st Pb	-0.019	0.009	-0.037	-0.001				-0.053	0.026	-0.104	-0.002	
3rd Pb	3rd 25OHD	0.003	0.006	-0.009	0.014				0.007	0.014	-0.021	0.034	

*****log2 transformed

1st =1st trimester; 3rd =3rd trimester; β =beta estimate; LCI=lower 95% Confidence Interval; UCI=Upper 95% Confidence Interval; SE=Standard Error; AR=autoregressive; CL = cross lag. Other covariates (exogenous variables): smoking status at visit 1, maternal education, income, maternal age, pre-pregnancy BMI, maternal race, general fish consumption per week (in past 3 months of exposure measure), study site, time spent outdoors at visit 1, blood collection season at time of exposure measure (visit 1) included as exogenous variables in the model.

Supplemental Figures

Figure S1: Directed Acyclic Graph (DAG)

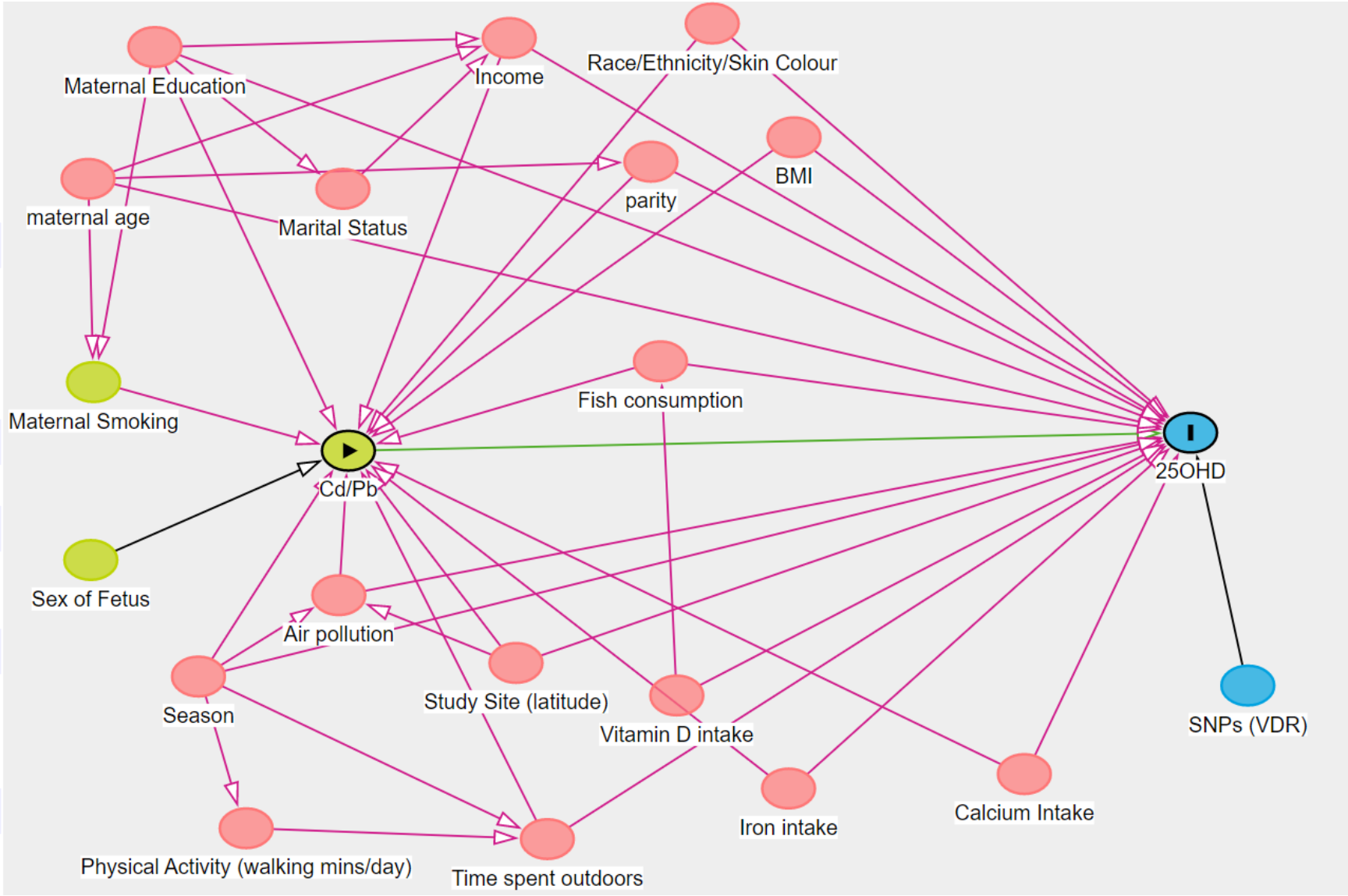
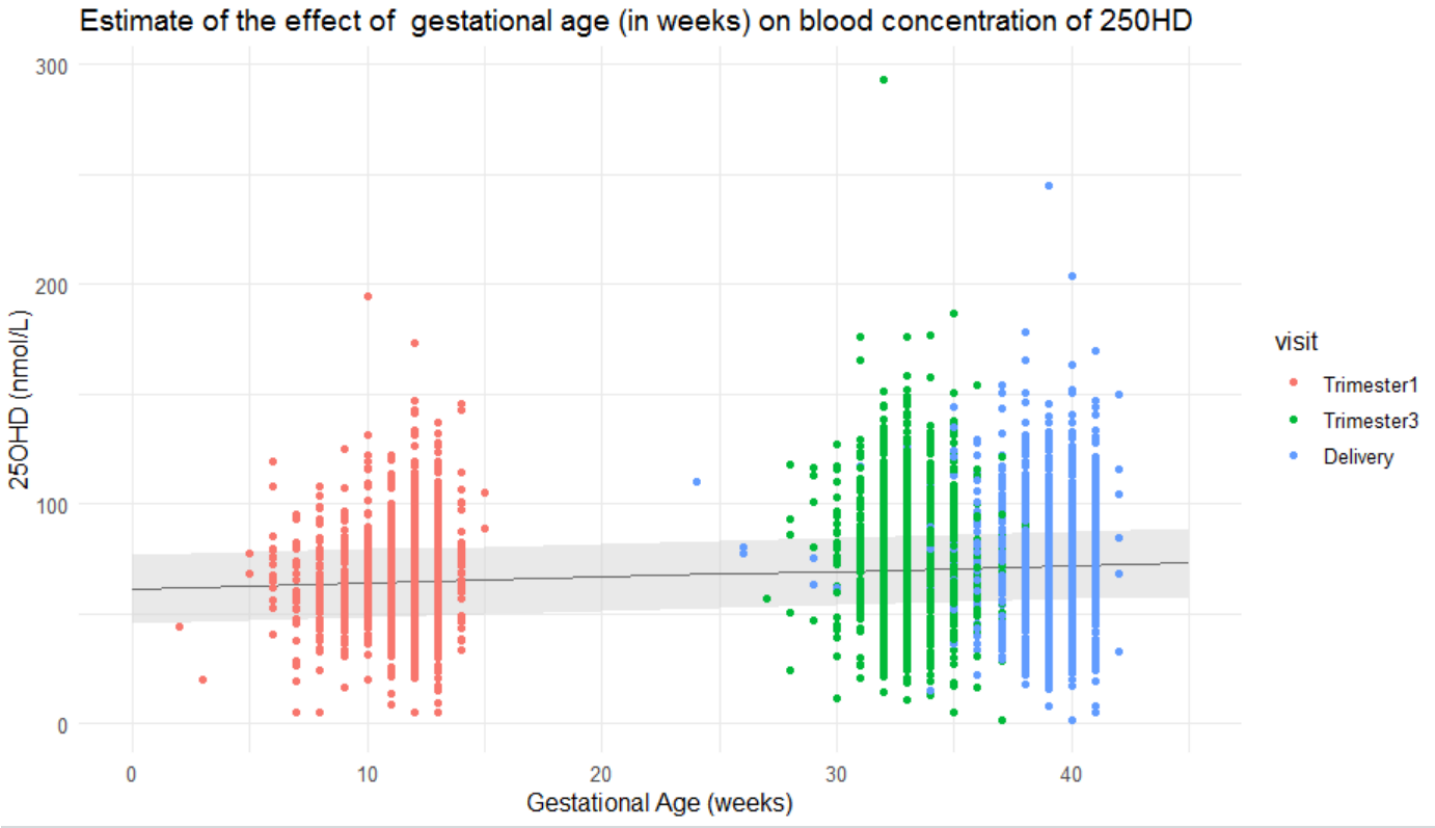


Figure S2: Gestational Week and 25OHD concentrations in pregnancy



Gestational Age estimate : 0.27(0.23, 0.30)
This suggests that for each unit change in gestational age (week) there is a 0.27 nmol/L increase in 25OHD
Adjusted Mixed model: random subject and other fixed effects (study site, Pre-pregnancy BMI, parity, income, maternal education, ethnicity, fish consumption in past 3 months, smoking status, days outside in last 30 days).

Figure S3: Analysis of Covariance model: relation between Cadmium and Total 25OHD, by smoking status.

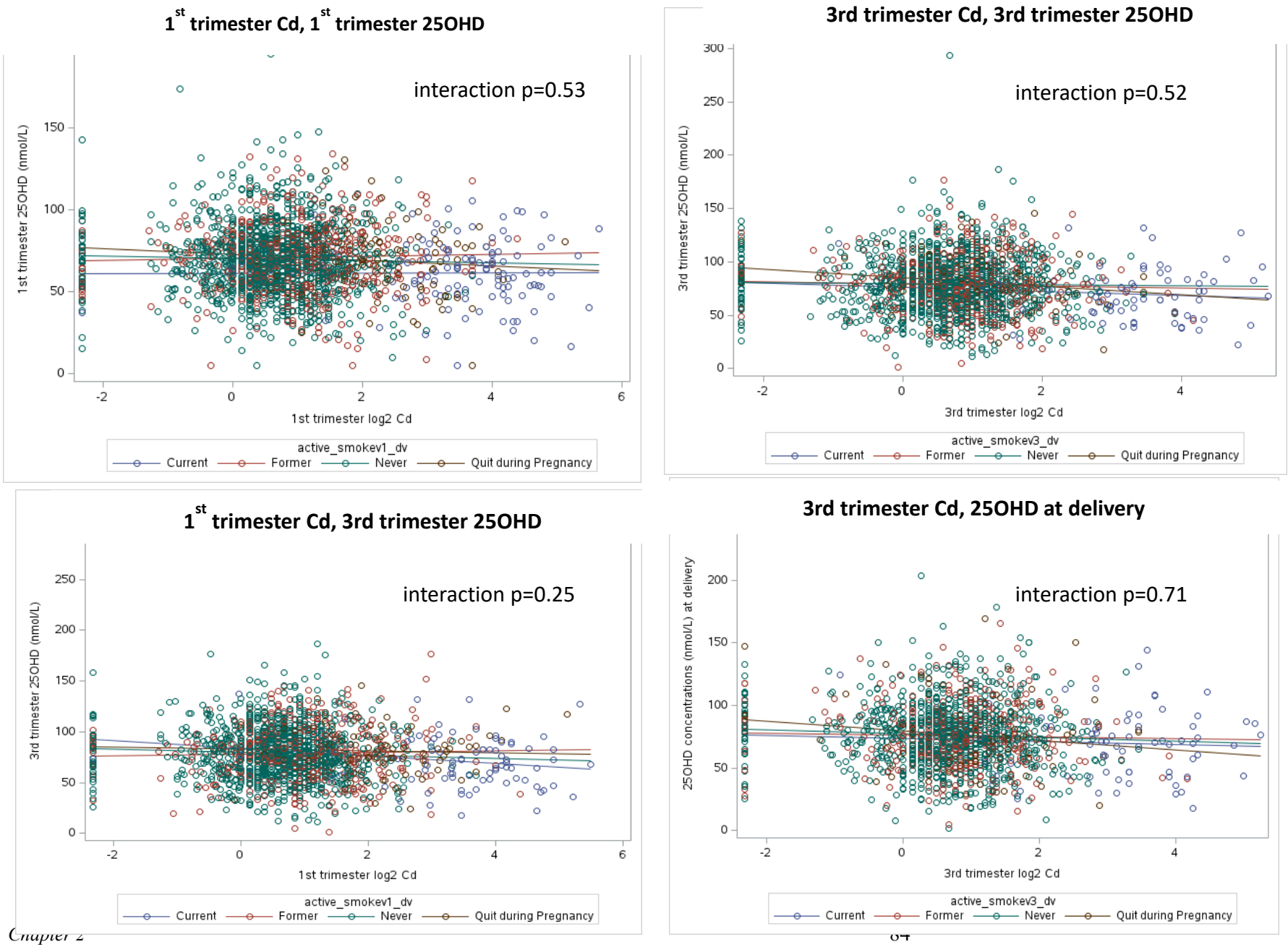
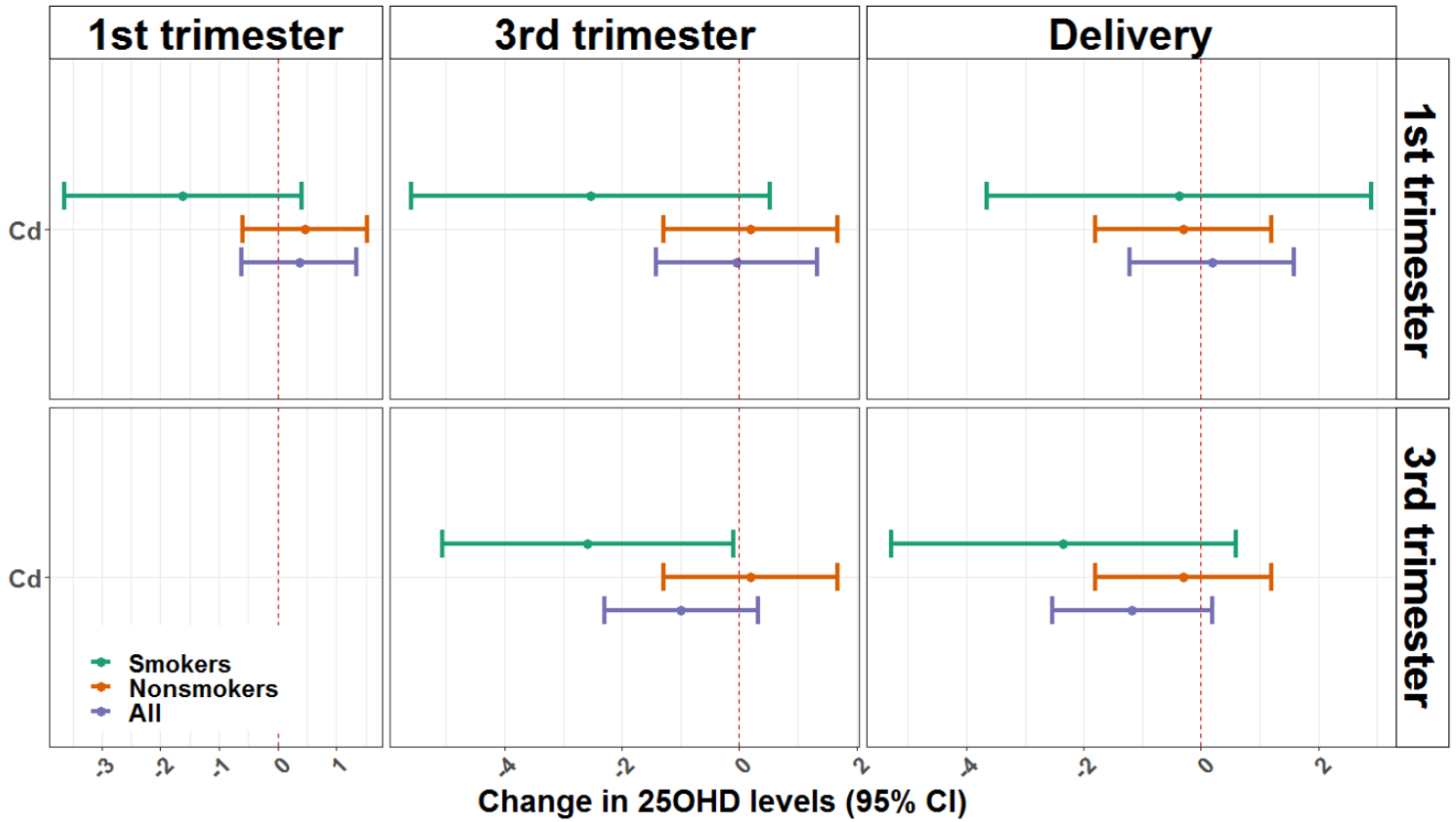


Figure S4: Multivariable Linear Regression by visit, and smoking status



Results (β and 95% CI) from several linear regression models looking at the effect of log2 Cd concentrations measured in the 1st and 3rd trimester on 25OHD measured in the 1st and 3rd trimester and at delivery by all participants, smokers (n=235) and non-Smokers (n=1724). Models include: smoking status at time of exposure measure (only ALL), maternal education, income, maternal age, pre-pregnancy BMI, maternal race, general fish consumption per week (in past 3 months of exposure measure), study site, time spent outdoors, blood collection season at time of exposure measure

CHAPTER 3

Association between Toxic Metals, Vitamin D and Preterm Birth in the Maternal-Infant Research on Environmental Chemicals Study

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Preface to Chapter 3

Our first thesis article, presented in chapter 2, provided insight into the direction of the association between vitamin D and metals. Given our results suggested that early vitamin D concentrations may modify toxic metal exposures later in pregnancy, in Paper 2 we carry on to examine vitamin D as a potential modifier in the association between toxic metals and preterm birth. The author contributions are listed below.

Author Contributions:

Mandy Fisher was involved in the design and implementation of the study, statistical analysis, writing and interpretation of the study. **Leonora Marro** was involved in statistical analysis. **Tye Arbuckle** was involved in the design and implementation of the study, review and revisions of the article. **Beth Potter** was involved in the analysis of the study, and review of the article and revisions. **Julian Little** was involved in the analysis of the study, review of the article and revisions. **Hope Weiler** was involved in the design of the study and the review of the article and revisions. **Anne-Sophie Morisset** was involved in the design and implementation of the study and the review of the article and revisions. **Bruce Lanphear** was involved in the review and revisions of the article. **Joseph Braun** was involved in the analysis of the study and the review of the article and revisions. **Youssef Oulhote** was involved in the analysis of the study and the review of the article and revisions. **Premkumari Kumarathasan** was involved in the review and revisions of the article. **Mark Walker** was involved in the design and implementation of the study, review and revisions of the article. **Michael Borghese** was involved in the review and revisions of the article. **Jillian Ashley-Martin** was involved in the analysis of the study and the review and revisions of the article. **Robin Shutt** was involved in the review and revisions of the article. **William Fraser** was involved in the design and implementation of the study and the review of the article and revisions

ABSTRACT

BACKGROUND

Toxic metals, like lead, are risk factors for preterm birth (PTB), but few studies have examined low levels found in most Canadians. Vitamin D, which may have antioxidant activity, protects against PTB.

OBJECTIVES

In this study, we investigated the impact of toxic metals (lead, mercury, cadmium and arsenic) on PTB and examined if maternal plasma vitamin D concentrations modify these associations.

METHODS

We investigated whether concentrations of metals in whole blood measured in early and late pregnancy were associated with PTB (<37 weeks) and spontaneous PTB in 1851 live births from the Maternal-Infant Research on Environmental Chemicals Study using discrete time survival analysis. We also investigated whether the risk of PTB was modified by 1st trimester plasma 25-hydroxyvitamin D (25OHD) concentrations.

RESULTS

Of 1851 live births, 6.1% (n=113) were PTBs and 4.9% (n=89) were spontaneous PTB. A 1 μg/dL increase in blood lead concentrations during pregnancy was associated with an increased risk of PTB (relative risk [RR] 1.48, 95% confidence interval [CI] 1.00, 2.20) and spontaneous PTB (RR 1.71, 95% CI 1.13, 2.60). The risk was higher in women with insufficient vitamin D concentrations (25OHD <50 nmol/L) for both PTB (RR 2.42, 95% CI 1.01, 5.79) and spontaneous PTB (RR 3.04, 95% CI 1.15, 8.04). However, an interaction on the additive scale was not present. Arsenic was associated with a higher risk of PTB (RR 1.10, 95% CI 1.02, 1.19) and spontaneous PTB (RR 1.11, 95% CI 1.03, 1.20) per 1 μg/L.

CONCLUSIONS

Gestational exposure to low levels of lead and arsenic may increase the risk of PTB and spontaneous PTB; individuals with insufficient vitamin D may be more susceptible to the adverse effects of lead. Given our relatively small number of cases, we encourage testing of this hypothesis in other cohorts; especially those with vitamin D deficient populations.

KEYWORDS: Preterm birth, 25-hydroxyvitamin D, metals, lead, cadmium, mercury, arsenic

BACKGROUND

Preterm birth (PTB) is the leading risk factor for death in children under 5 years of age globally.[1] PTB is a risk factor for neurodevelopmental disorders[2] childhood asthma [3], and cardiovascular diseases in young adulthood. [4] Globally, 10.6% of all live births are PTB (< 37 wk gestational age); in Canada about 8% of births are preterm. [5]

Toxic metals such as lead (Pb) are associated with PTB [6–9] but few studies have explored this association at the low levels found in most Canadian women (GM_{Pb} 0.87 μg/dL, 95% CI 0.81-0.94).[10] Pb and other toxic metals are associated with higher concentrations of reactive oxygen species in the placenta [11] and inflammatory markers, such as matrix metalloproteinases (MMPs) in 3rd trimester maternal blood. [12] MMPs have been implicated in cervical ripening and premature rupture of membranes. [13] Conversely, vitamin D, which may have antioxidant activity [14] protects against PTB. [15] Further, vitamin D may modify toxic metal exposures in pregnancy. [16] We found that increasing 25-hydroxyvitamin D (25OHD) concentrations during early pregnancy were associated with lower cadmium (Cd) and Pb concentrations in the 3rd trimester. [17]

We sought to investigate the individual and joint association between the metals arsenic (As), Cd, Pb, and mercury (Hg), and vitamin D (25OHD) with PTB. In addition, PTB has a multifactorial aetiology and more focus is needed to understand the mechanisms behind chemical exposures and PTB by separating PTB phenotypes. [18] To address this, we also investigated a sub-phenotype of PTB, thought to be prompted by inflammation or infection, by examining a

subgroup of PTBs with spontaneous preterm labour and/or preterm premature rupture of membranes (pPROM).[19]

METHODS

The Maternal-Infant Research on Environmental Chemicals Study (MIREC) is a prospective cohort study that recruited individuals in their 1st trimester of pregnancy (<14 weeks gestation) between 2008-2011 from medical clinics (ultrasound, midwife and/or doctor's clinics) in 10 Canadian cities (Vancouver, Edmonton, Winnipeg, Sudbury, Hamilton, Toronto, Kingston, Ottawa, Montreal, and Halifax).[20] Participants were eligible for the MIREC study if they were 18 years or older, < 14 weeks pregnant, willing to provide a cord blood sample and planning on delivering at a local hospital.[20] We excluded pregnant individuals if they had known fetal abnormalities or medical complications such as renal, heart, liver, pulmonary or a collagen disease, epilepsy, threatened spontaneous abortion, illicit drug use, or a haematological disorder (See (SI) eFigure1). Participants provided blood and urine samples and completed questionnaires in the 1st and 3rd trimesters of pregnancy. At delivery, a chart review was completed in order to obtain information on the delivery and neonatal outcomes. Research Ethics Board approval was obtained at Health Canada and all study sites; all study participants provided informed consent.

Specimen collection and laboratory analysis

We analyzed maternal whole blood specimens from the first and third trimester at the Centre de Toxicologie du Québec (CTQ), Institut national de Santé Publique du Québec (INSPQ) for total blood arsenic, Cd, Pb, and Hg concentrations.[21] At Health Canada's Foods Directorate

Laboratory, we analyzed maternal blood plasma collected in the 1st and 3rd trimesters for total 25OHD, as previously reported in detail;[22] this laboratory participates in the Vitamin D Standardization Certification Program (VDSCP). For values below the limit of detection (LOD), we imputed LOD/2.

Exposures

Time-varying exposures (arsenic, Cd, Pb and Hg and 25OHD) were analyzed both continuously and categorically for 25OHD (< 50 or ≥50 nmol/L) basing our cut points on those proposed by the Institute of Medicine [23] for plasma 25OHD, the widely accepted biomarker of vitamin D status.[24] No specific guidelines exist for vitamin D in pregnancy but a concentration of 50 nmol/L (sufficient vitamin D status) would meet the needs of at least 97.5% of the population (Recommended Dietary Allowance; RDA). In addition, as a sensitivity analysis, we considered the population recommendation for adequate vitamin D status ≥40 nmol/L [23] as the lower cut point in our categorical analysis for 25OHD.²²¹⁷²¹⁷²¹⁷²¹⁷²

Outcomes

We defined PTB as live births occurring between 20 and <37 weeks as indicated by chart review at delivery. Consistent with work done by Ferguson and Chin [25], we defined spontaneous PTB as PTB with spontaneous labour and/or pPROM as noted in their medical chart at delivery. We excluded medically indicated (e.g., preeclampsia, gestational diabetes) PTB from this subgroup analysis (**eFigure1**).

Covariates

We used Directed Acyclic Graphs (DAGs) to conceptualize potential covariate relationships and identify confounders, not on the causal pathway between each exposure and PTB (**eFigure 2-6**). We considered both continuous (maternal age, pre-pregnancy BMI) and categorical time-invariant covariates (mother's birthplace, maternal education, gross annual household income, marital status, parity and sex of baby), and time-varying confounders (season of blood collection, fish consumption, smoking status, and walking duration on a typical day as a proxy measure for physical activity) assessed in the 1st and 3rd trimester (See Table 1). We considered all covariates in our analysis but for our final models we prioritized model parsimony due to the modest sample size, 17 time-indicator variables (see below), and relative rarity of the outcome. We included pre-pregnancy BMI, maternal age, season, smoking status, fish consumption, and self-reported walking in the final models; these covariates were most strongly associated with PTB in our data and/or changed a metal effect estimate by 10% or more.

Statistical analysis

We used discrete-time survival analysis with logistic regression[26–28] to characterise the hazard probability of PTB between 20 and 36 wks +6 days of gestation. Given that we obtained the exposure data in discrete time intervals during pregnancy but over a range of gestational ages, we modeled time using 17 indicator variables for each gestational week (20 to 36 completed weeks) at risk of PTB (the event). Births prior to 20 weeks were considered miscarriages and births that occurred at 37 weeks or later were censored because they were no longer at risk of the event (PTB). This method produces an overall odds ratio for the effect of a predictor on PTB and a hazard function. We plotted this function at different values of a predictor (e.g., 95th percentile

of Pb), holding all other variables constant for each of the 17-time points. Given the prevalence of PTB is <10%, the odds ratios are a valid approximation to the relative risk (RR) and therefore are referred to as RR in this paper.

We fit discrete-time survival analysis with logistic regression following the sequential methods proposed by Singer and Willet [27] beginning with Model 1 (Base Model), where the number of PTBs is considered to only depend on time (17 gestational weeks at risk). Model 2 builds on the Base Model where we examined the effect of each covariate selected in the DAG and described above (time-varying and time-invariant) on the model, the exposures, along with an interaction term between covariate or exposures and time variables to determine the validity of the proportionality assumption.[27] For our final model, we included pre-pregnancy BMI, maternal age, season, smoking status, fish consumption, and self-reported walking.

We examined time-varying (taking into account both 1st and 3rd trimester measures) exposures for each metal in separate models. We did not consider exposure time points separately given the high correlation between 1st and 3rd trimester metals and 25OHD concentrations (**eTable 1**) making it difficult to consider one trimester without the other. To determine if an interaction was present, we examined the log odds of the interaction term between vitamin D (<50 vs ≥ 50 nmol/L) and metals with PTB. We also conducted stratified analyses to estimate the association between metals and PTB in women with insufficient (<50 nmol/L) and sufficient vitamin D status (≥50 nmol/L) separately. Finally, to determine departure from additivity, we estimated the crude and adjusted relative excess risk due to interaction (RERI) using the method proposed by Knol et al. [29] for one continuous and one dichotomous determinant and dichotomous outcome, bootstrapping 1000 times. As a sensitivity analysis, we re-ran all models with continuous

exposure estimated for a change in interquartile range (**eTables 2 and 3**). We assumed that the predictors are linearly associated with the logistic transformation of hazard (logit hazard). To test for non-linearity between the metal exposures and the outcome we tested a quadratic term (e.g., $Pb + Pb^2$) in the model and compared model fit (deviance/AIC) with and without the term.

Missing Data

Discrete-time survival analysis requires a person-period format; therefore, the number of records for each participant may vary by the duration at risk. When gestational age was missing for 3rd trimester exposure measurements (n=3, 1.8% of participants) we imputed the average gestational age (32 weeks) for all third trimester measurements in the cohort. We had complete exposure, outcome and covariate data for 77% of our participants. For missing time-varying covariates (smoking, fish consumption and walking duration per day) for which we had information from one time point (1st trimester) for that participant, we carried this information forward. Other exposures or covariates with >5% missing included pre-pregnancy BMI (7.5%), 3rd trimester metals (12%), and 25OHD (13%). We assumed that missing data were missing at random (MAR) and did not find any major differences across groups based on missingness (**eTable 4-5** and **eFigure 7-8**). We used Multiple Imputation using Chained Equations (MICE) with predicted mean matching (PMM) [30] to impute the remaining missing exposure and covariates information (m = 50 datasets) using SAS PROC MI. To consider unmeasured confounding, we estimated the E-value for those estimates with non-overlapping confidence intervals. [31]

All statistical analyses were performed using SAS Enterprise Guide 7.15 (SAS Institute Inc., Cary, NC 2017).

RESULTS

The MIREC study initially recruited 2001 pregnant women between the years 2008-2011. Eighteen participants subsequently withdrew from the study and 1851 (93%) participants had a live singleton birth with one or more metal or 25OHD concentration measurements (See SI eFigure1). Of these, 113 (6.1%) were PTBs and 89 (4.9%) were spontaneous PTBs, which is lower than the Canadian PTB rate during this time period (range 7.6-8.0% of live births).[32] The characteristics of the MIREC cohort are shown in Table 1). The prevalence of PTB was higher in older and younger participants and with increasing pre-pregnancy BMI levels. The prevalence of PTB was somewhat lower in participants who were higher fish consumers and self-reported that they walked more. A higher prevalence of PTB (9.8%) and spontaneous PTB (8.1%) was observed among participants whose 3rd trimester visit (mean 33 weeks' gestation) occurred in the summer. First and third trimester metal and 25OHD concentrations were moderately to highly correlated between trimesters (See SI eTable1).

First trimester geometric means for total blood arsenic, Cd , Pb and Hg were slightly higher than 3rd trimester concentrations while the opposite pattern emerged for 25OHD (See Table 2). Mean 25OHD concentrations were lower in the 1st trimester (69.7 nmol/L) compared to the 3rd (78.4 nmol/L).

In adjusted models, we observed an increased risk of PTB per unit increase in Pb exposure (**Table 2**). A 1 µg/dL increase in Pb exposure during pregnancy was associated with an increased risk of any PTB and a spontaneous PTB. A 1 µg/L increase in arsenic was also associated with

an elevated risk of preterm and spontaneous PTB. These associations were constant over the 17 time periods (20-36 wks) given we did not see an interaction between any of the metals and time in our dataset. Because we also modelled time discretely as a predictor, we can show the hazard of PTB at each time point (20-36 wks) given it did not previously occur (conditional probability). **Figures 1 and 2** provide the hazard profile at each time point for lead and arsenic at the median, 5th and 95th percentile of exposure. Cadmium and mercury concentrations were not associated with odds of PTB in this cohort (**Table 3**). We tested for non-linearity in the exposures (Pb and arsenic) by fitting a quadratic term in the model and found no evidence of nonlinearity. In addition, the deviance and AIC results were comparable to those from the linear model. We found consistent but somewhat attenuated results per IQR increase in Pb (0.39 μg/dL) with PTB and spontaneous PTB. For arsenic, an IQR increase (0.63 μg/L) was associated with a 6% (RR 1.06, 95% CI 1.01, 1.11) and 7% (RR 1.07, 95% CI 1.02, 1.12) higher risk of PTB and spontaneous PTB respectively. As a continuous variable, 25OHD showed no evidence of an association with PTB (**Table 3**). Similarly, no association was seen in our categorical analysis (<50 vs ≥50 nmol/L) with PTB (**Table 3, eFigure 9**); in a sensitivity analysis, however, participants with 25OHD concentrations below 40 nmol/L had an increased, but imprecise, risk of PTB and spontaneous PTB (**eFigure 10 and eTable 6**).

Next, we examined whether vitamin D status modified the association between Pb and the risk of PTB. We observed a weak interaction between categorical 25OHD (<50, ≥50 nmol/L) and continuous Pb with PTB (log odds 0.98, 95% CI -0.17, 2.13) and spontaneous PTB (log odds: 1.13, 95% CI -0.07, 2.33), that we further investigated in a stratified analysis with 25OHD concentrations <50 and ≥50 nmol/L [23]. Participants with insufficient vitamin D status (<50

nmol/L) had an elevated risk of PTB (RR 2.42, 95% CI 1.01, 5.79) and spontaneous PTB (RR 3.04, 95% CI 1.15, 8.04) for each unit increase in Pb. By contrast, for participants with 25OHD concentrations ≥50 nmol/L, the risk of PTB (RR 1.29, 95% CI 0.80, 2.07) and spontaneous PTB (RR 1.50, 95% CI 0.91, 2.45) were attenuated. However, we did not see evidence of an additive interaction in our adjusted models (**Table 4**).

COMMENT

Principal Findings

Although our pregnant population had low blood Pb levels (95^{th} percentile < 1.5 ug/dL), we observed an elevated risk of PTB with increasing blood Pb exposure. Our results show that vitamin D status may modify the adverse effect of blood Pb on PTB risk. The risk of spontaneous PTB was 3-fold higher for each 1 µg/dL increase in blood Pb in participants with 25OHD concentrations below 50 nmol/L. The association was attenuated among participants with 25OHD concentrations ≥50 nmol/L. However, we did not see evidence of an interaction on the additive scale. Finally, we observed an elevation in the odds of PTB and spontaneous PTB for a 1-unit increase in arsenic concentrations however no modification was seen by vitamin D status.

Strengths of the Study

A strength of this study is the prospective nature of the MIREC cohort and measurements at two time points during pregnancy of the metals (arsenic, Cd, Pb, Hg), 25OHD, and several covariates. The discrete-time survival analysis allowed us to use all of our data by accounting for several time-varying covariates and exposures, and to include time as a predictor of PTB. In

addition, the time-varying method allowed us to take into account the change in metal levels between trimesters 1 and 3 instead of a single time point. Unlike most previous studies, we conducted analyses by sub-phenotype of PTB.[18]

Limitations of the Data

We did not distinguish between inorganic and organic arsenic, but we did control for fish consumption, the main source of organic arsenic. Although we considered covariates known to be associated with vitamin D status (maternal age, socioeconomic status, ethnicity, physical activity and higher BMI) [22], measurement error is a ubiquitous problem. Still, we suspect that this would have been non-differential and biased our results towards the null. According to the E-value calculation[31] of the effect estimates, a strong unmeasured confounder would be needed to explain away the association between continuous lead and spontaneous preterm birth and for this same association in those with <50 nmol/L 25OHD. Finally, the MIREC study had a relatively small number of preterm births and limited power for examining those with low vitamin D. The MIREC study also consists of mainly white women of higher socioeconomic status. De facto restrictions of this nature minimized unmeasured confounding, but it limited exposure variability and generalisability.

Interpretation

Pregnancy cohort studies from China ($mean_{Pb}$ 1.5 µg/dL) [33,34], Iran (GM_{Pb} 3.8 µg/dL) [9], Australia ($mean_{Pb}$ 10.6 µg/dL)[35] and the United Kingdom ($mean_{Pb}$ 3.7 µg/dL)[6] also found elevated odds of PTB with increasing maternal blood Pb concentrations. These studies had higher Pb levels and more variability than the MIREC cohort. A California-based study (1995-

2002) that linked birth records with blood Pb testing during pregnancy (anytime between day 1 and delivery) of 262 mother-infant pairs found a negative association between blood Pb levels and gestational length, among those whose blood Pb levels were ≥10 μg/dL [8]. In addition, studies from India [36], Indonesia [37], Australia [38] and Spain [39] have shown elevated placental Pb levels in preterm versus term deliveries. In contrast, other studies that measured maternal blood Pb exposure during pregnancy[40–42], at birth [37] or currently, in a retrospective study [43], did not report an association with PTB. The lack of association in some previous studies may be due to low power [41–43], study design (cross-sectional[37], retrospective [43]), very low blood Pb levels,[40] differing timings of exposure measures, statistical methods and distribution of effect modifiers, such as serum vitamin D concentrations.

Few studies have explored the association between blood arsenic and PTB.[44] In the China-Anhui Birth Cohort, where the median serum total arsenic concentrations collected between 4-17 weeks' gestation was 4.87 μg/L, higher PTB rates were observed among participants with As concentrations above the 75th percentile compared to those below this cut-point (OR=1.5, 95% CI: 1.08, 2.09). Highly exposed populations in Bangladesh and Taiwan, have shown elevated rates of PTB based on drinking water [45–47] and toenail arsenic levels.[47] In the United States, PTB was spatially associated with higher estimated levels of arsenic in groundwater from New Hampshire.[48] In Ohio, among individuals living in counties where fewer than 10 or 20% of households used private wells as their drinking water source, those counties with higher arsenic levels in their water supply showed a significant increase in the odds of PTB relative to those who lived in counties with lower arsenic.[49]

In a previous analysis of the MIREC cohort, increasing vitamin D intake, measured by a food frequency questionnaire in the 2nd trimester, was associated with reduced blood Pb and Cd in the 3rd trimester and in cord blood.[50] In addition, we previously reported that increasing 25OHD concentrations in the 1st trimester of pregnancy was associated with reduced Pb and Cd concentrations in the 3rd trimester.[17] A study from the LIFECODES pregnancy cohort [16] reported that women with low 1st trimester 25OHD concentrations (<50 nmol/L) had 47% higher urinary Pb concentrations in the 2nd trimester.

25OHD concentrations may modify the association between Pb and PTB via vitamin D's role as a probable antioxidant.[51] Oxidative stress, defined as an imbalance between cellular reactive oxygen species (ROS) and antioxidants [52] has been associated with a number of pregnancy outcomes including PTB.[53] Pb has been shown to up-regulate ROS [57] which can stimulate pro-inflammatory enzymes.[54] Associations have been observed between blood Pb and elevated inflammatory biomarkers such as IL-1β, IL-6 and TNF-α [55] and MMPs.[12,56] MMP-9, which has known associations with cervical ripening and PTB [57], was associated with elevated Pb exposure in the first trimester of pregnancy in another analysis of the MIREC cohort.[12] On the other hand, micronutrients are known to down-regulate ROS.[37] Reports show an inverse relationship between 25OHD concentrations and serum MMP-9 levels in vascular diseases.[58] A double blind randomized controlled trial of 48 pregnant women who were randomized to receive 400 IU/d of cholecalciferol (D_3) supplements or placebo for 9 weeks, showed significant decreases in inflammatory markers and significant increases in plasma concentrations of total antioxidant capacity and the major antioxidant glutathione.[59] It may be that vitamin D helps to counteract increased systemic inflammation induced by lead by acting as a free radical scavenger and

preventing and repairing damages caused by ROS;[60] however, whether vitamin D acts as an antioxidant remains controversial.[61]

Conclusions

Lead and arsenic may increase the risk of PTB and spontaneous PTB, even at the lower levels found in this Canadian pregnancy and birth cohort. For lead, this association was stronger in participants with insufficient 25OHD concentrations (<50 nmol/L) suggesting that these women may be more vulnerable to the potential adverse effects of Pb. However, we did not see an interaction on the additive scale and given our lack of power, we encourage testing of this hypothesis in other pregnancy cohorts with vitamin D deficient populations.

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REFERENCES

1. Blencowe, H. *et al.* Born Too Soon: The global epidemiology of 15 million preterm births. *Reprod. Health* (2013) doi:10.1186/1742-4755-10-S1-S2.

2. Sutton, P. S. & Darmstadt, G. L. Preterm birth and neurodevelopment: A review of outcomes and recommendations for early identification and cost-effective interventions. *Journal of Tropical Pediatrics* at https://doi.org/10.1093/tropej/fmt012 (2013).

3. Sonnenschein-Van Der Voort, A. M. M. *et al.* Preterm birth, infant weight gain, and childhood asthma risk: A meta-analysis of 147,000 European children. *J. Allergy Clin. Immunol.* (2014) doi:10.1016/j.jaci.2013.12.1082.

4. Abitbol, C. L. & Rodriguez, M. M. The long-term renal and cardiovascular consequences of prematurity. *Nature Reviews Nephrology* at https://doi.org/10.1038/nrneph.2012.38 (2012).

5. Centre for Surveillance and Applied Research. Perinatal Health Indicators Quick Stats , 2020 Edition. at (2020).

6. Taylor, C. M., Golding, J. & Emond, A. M. Adverse effects of maternal lead levels on birth outcomes in the ALSPAC study: A prospective birth cohort study. *BJOG An Int. J. Obstet. Gynaecol.* **122**, 322–328 (2015).

7. Baghurst, P. A. *et al.* Lead in the placenta, membranes, and umbilical cord in relation to pregnancy outcome in a lead-smelter community. *Environ. Health Perspect.* **90**, 315–320 (1991).

8. Jelliffe-Pawlowski, L. L., Miles, S. Q., Courtney, J. G., Materna, B. & Charlton, V. Effect of magnitude and timing of maternal pregnancy blood lead (Pb) levels on birth outcomes. **26**, 154–162 (2006).

9. Vigeh, M. *et al.* Blood lead at currently acceptable levels may cause preterm labour. *Occup. Environ. Med.* **68**, 231–234 (2011).

10. Health Canada. *Fifth Report on Human Biomonitoring of Environmental Chemicals in Canada. Results of the Canadian Health Measures Survey Cycle 5 (2016-2017).* (2019).

11. Singh, L., Agarwal, P., Anand, M. & Taneja, A. Toxic and essential metals in placenta and its relation with lipid peroxides/glutathione status in pre-term and full-term deliveries. *Asian J. Med. Sci.* **7**, 34–39 (2015).

12. Au, F. *et al.* Blood metal levels and third trimester maternal plasma matrix metalloproteinases (MMPs). *Chemosphere* **159**, 506–515 (2016).

13. Sukhikh, G. . *et al.* The role of extracellular inducer of matrix metalloproteinases in premature rupture of membranes. *J Matern Fetal Neonatal Med* **29**, 656–659 (2016).

14. Akbari, M. *et al.* The Effects of Vitamin D Supplementation on Biomarkers of Inflammation and Oxidative Stress among Women with Polycystic Ovary Syndrome: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Horm. Metab. Res.* (2018) doi:10.1055/s-0044-101355.

15. Zhou, S. S., Tao, Y. H., Huang, K., Zhu, B. B. & Tao, F. B. Vitamin D and risk of preterm birth: Up-to-date meta-analysis of randomized controlled trials and observational studies. *J. Obstet. Gynaecol. Res.* **43**, 247–256 (2017).

16. Jukic, M. A. Z. *et al.* A prospective study of maternal 25-hydroxyvitamin D (25OHD) in the first trimester of pregnancy and second trimester heavy metal levels. *Environ. Res.* **199**, 111351 (2021).

17. Fisher, M. *et al.* Blood Metals and Vitamin D Status in a Pregnancy Cohort: A Bidirectional Biomarker Analysis. *Environ. Res.* (2022).

18. Ferguson, K. K. & Chin, H. B. Environmental Chemicals and Preterm Birth: Biological Mechanisms and the State of the Science. *Curr. Epidemiol. Reports* **4**, 56–71 (2017).

19. McElrath, T. F. *et al.* Pregnancy disorders that lead to delivery before the 28th week of gestation: An epidemiologic approach to classification. *Am. J. Epidemiol.* **168**, 980–989 (2008).

20. Arbuckle, T. E. *et al.* Cohort profile: the maternal-infant research on environmental chemicals research platform. *Paediatr. Perinat. Epidemiol.* **27**, 415–425 (2013).

21. Arbuckle, T. E. *et al.* Maternal and fetal exposure to cadmium, lead, manganese and mercury: The MIREC study. *Chemosphere* **163**, 270–282 (2016).

22. Weiler, H. A. *et al.* Early prenatal use of a multivitamin diminishes the risk for inadequate vitamin D status in pregnant women : results from the Maternal-Infant Research on Environmental Chemicals (MIREC) cohort study. *Am. J. Clin. Nutr.* **114**, 1238–1250 (2021).

23. Institute of Medicine (IOM). *DRI, dietary reference intakes : calcium, vitamin D*. *Dietary reference intakes : calcium, vitamin D* (National Academies Press, 2011).

24. Norman, A. W. From vitamin D to hormone D: Fundamentals of the vitamin D endocrine system essential for good health. *Am. J. Clin. Nutr.* **88**, (2008).

25. Ferguson, K. K. *et al.* Prenatal and peripubertal phthalates and bisphenol A in relation to sex hormones and puberty in boys. *Reprod. Toxicol.* **47**, 70–76 (2014).

26. Cox, D. R. Regression Models and Life-Tables. *J. fo R. Stat. Soc. Ser. B* **34**, 187–220 (1972).

27. Singer, J. D. & Willett, J. B. It's About Time: Using Discrete-Time Survival Analysis to Study Duration and Timing of Events. *J. Educ. Stat.* **18**, 155–195 (1993).

28. Xie, H., McHugo, G., Drake, R. & Sengupta, A. Using discrete-time survival analysis to examine patterns of remission from substance use disorder among persons with severe mental illness. *Ment. Health Serv. Res.* **5**, 55–64 (2003).

29. Knol, M. J., van der tweel, I., Grobbee, D. E., Numans, M. E. & Geerlings, M. I. Estimating interaction on an additive scale between continuous determinants in a logistic regression model. *Int. J. Epidemiol.* **36**, 1111–1118 (2007).

30. Yuan, Y. Multiple Imputation for Missing Data: Concepts and New Development (Version 9.0). *J. Stat. Softw.* **45**, 1–25 (2011).

31. VanderWeele, T. J. & Ding, P. Sensitivity analysis in observational research: Introducing the E-Value. *Ann. Intern. Med.* **167**, 268–274 (2017).

32. Statistics Canada. Preterm live births in Canada, 2000-2013. https://www150.statcan.gc.ca/n1/pub/82-625-x/2016001/article/14675-eng.htm (2016).

33. Li, J. *et al.* Maternal serum lead level during pregnancy is positively correlated with risk of

preterm birth in a Chinese population. *Environ. Pollut.* **227**, 484–489 (2017).

34. Wang, H. *et al.* Association of maternal serum cadmium level during pregnancy with risk of preterm birth in a Chinese population. *Environ. Pollut.* **216**, 851–857 (2016).

35. McMichael, A. J., Vimpani, G. V, Robertson, E. F., Baghurst, P. A. & Clark, P. D. The Port Pirie cohort study : maternal blood lead and pregnancy outcome. **40**, 18–25 (1986).

36. Ahamed, M., Mehrotra, P. K., Kumar, P. & Siddiqui, M. K. J. Placental lead-induced oxidative stress and preterm delivery. *Environ. Toxicol. Pharmacol.* **27**, 70–74 (2009).

37. Irwinda, R., Wibowo, N. & Putri, A. S. The Concentration of Micronutrients and Heavy Metals in Maternal Serum, Placenta, and Cord Blood: A Cross-Sectional Study in Preterm Birth. *J. Pregnancy* **2019**, (2019).

38. Baghurst, P. A. *et al.* Lead in the Placenta , Membranes , and Umbilical Cord in Relation to Pregnancy Outcome in a Lead-Smelter Community. **90**, 315–320 (1991).

39. Falcón, M., Viñas, P. & Luna, A. Placental lead and outcome of pregnancy. *Toxicology* **185**, 59–66 (2003).

40. Tsuji, M. *et al.* The association between whole blood concentrations of heavy metals in pregnant women and premature births: The Japan Environment and Children's Study (JECS). *Environ. Res.* **166**, 562–569 (2018).

41. Sowers, M. *et al.* Blood Lead Concentrations and Pregnancy Outcomes. *Arch. Environ. Health* **57**, 489–495 (2002).

42. Factor-Litvak, P. *et al.* A prospective study of birthweight and length of gestation in a population surrounding a lead smelter in Kosovo, Yugoslavia. *Int. J. Epidemiol.* **20**, 722–728 (1991).

43. Laudanski, T. *et al.* Influence of high lead and cadmium soil content on human reproductive outcome. *Int. J. Gynecol. Obstet.* **36**, 309–315 (1991).

44. Milton, A. H. *et al.* A review of the effects of chronic arsenic exposure on adverse pregnancy outcomes. *Int. J. Environ. Res. Public Health* **14**, (2017).

45. Ahmad, S. A. *et al.* Arsenic in Drinking Water and Pregnancy Outcomes. **109**, 2000–2002 (2001).

46. Yang, C. Y. *et al.* Arsenic in drinking water and adverse pregnancy outcome in an arseniasis-endemic area in northeastern Taiwan. *Environ. Res.* **91**, 29–34 (2003).

47. Rahman, M. L. *et al.* Prenatal arsenic exposure , child marriage , and pregnancy weight gain : Associations with preterm birth in Bangladesh. *Environ. Int.* **112**, 23–32 (2018).

48. Shi, X. *et al.* Geospatial association between adverse birth outcomes and arsenic in groundwater in New Hampshire , USA. 333–351 (2015) doi:10.1007/s10653-014-9651-2.

49. Almberg, K. S. *et al.* Arsenic in drinking water and adverse birth outcomes in Ohio. *Environ. Res.* **157**, 52–59 (2017).

50. Arbuckle, T. E. *et al.* Maternal and fetal exposure to cadmium, lead, manganese and mercury: The MIREC study. *Chemosphere* **163**, 270–282 (2016).

51. Tagliaferri, S. *et al.* Controversial role of vit D as antixoidant: results from randomised controlled trials. *Nutr. Res. Rev.* **32**, 99–105 (2019).

52. Shachar, B. Z., Carmichael, S. L., Stevenson, D. K. & Shaw, G. M. Could genetic polymorphisms related to oxidative stress modulate effects of heavy metals for risk of human preterm birth? *Reprod. Toxicol.* **42**, 24–26 (2013).

53. Moore, T. A., Ahmad, I. M. & Zimmerman, M. C. Oxidative Stress and Preterm Birth: An Integrative Review. *Biol. Res. Nurs.* **20**, 497–512 (2018).

54. Morgan, M. J. & Liu, Z. G. Crosstalk of reactive oxygen species and NF-κB signaling. *Cell Res.* **21**, 103–115 (2011).

55. Brucker, N. *et al.* Relationship between blood metals and inflammation in taxi drivers. *Clin. Chim. Acta* **444**, 176–181 (2015).

56. Dagouassat, M., Lanone, S. & Boczkowski, J. Interaction of matrix metalloproteinases with pulmonary pollutants. **39**, 1021–1032 (2012).

57. Vadillo-ortega, F. & Estrada-gutie, G. Role of matrix metalloproteinases in preterm labour. **112**, 19–22 (2005).

58. Wasse, H. *et al.* 25-hydroxyvitamin D concentration is inversely associated with serum MMP-9 in a cross-sectional study of African American ESRD patients. *BMC Nephrol.* **12**, (2011).

59. Asemi, Z., Samimi, M., Tabassi, Z., Shakeri, H. & Esmaillzadeh, A. Vitamin D Supplementation Affects Serum High-Sensitivity C-Reactive Protein, Insulin Resistance, and Biomarkers of Oxidative Stress in Pregnant Women. *J. Nutr.* **143**, 1432–1438 (2013).

60. Pham-Huy, L. A., He, H. & Pham-Huy, C. Free radicals, antioxidants in disease and health. *Int. J. Biomed. Sci.* **4**, 89–96 (2008).

61. Tagliaferri, S. *et al.* The controversial role of Vitamin D as an antioxidant: results from randomised controlled trials. *Nutr. Res. Rev.* **32**, 99–105 (2019).

Table 1: Population characteristics by category of Preterm Births (PTBs) - number of PTBs by category, % of total births in category

Covariates	All preterm birth (n=113, 6.1% of births)	Spontaneous preterm birth (n=89, 4.9% of births)
	Number (%)	Number (%)
Maternal age[a] (years)		
<25	9 (7.0)	4 (3.3)
25-29	18 (4.1)	14 (3.2)
30-34	38 (5.8)	33 (5.1)
35+	48 (7.7)	38 (6.2)
Parity[a]		
0	50 (6.2)	37 (4.6)
1	47 (6.3)	39 (5.3)
2+	16 (5.8)	13 (4.5)
Sex of baby[b]		
Male	62 (6.3)	50 (5.2)
Female	51 (5.8)	39 (4.5)
Pre-pregnancy BMI[c] (kg/m^2)		
Underweight (<18.5)	54 (5.0)	48 (4.4)
Normal (<25)		
Overweight (25-29)	20 (5.4)	15 (4.1)
Obese (BMI ≥30)	31 (12.2)	20 (8.2)
Education [b]		
Some college, High School or Less than High School	18 (6.3)	11 (4.4)
College/Trade Diploma	32 (6.6)	28 (6.5)
Undergraduate degree	36 (5.3)	28 (4.2)
Graduate degree	27 (5.7)	22 (4.7)
Household Income ($, CAD) [a]		
<50,000	26 (6.6)	16 (4.0)
50,000-100,000	42 (6.7)	34 (5.4)
>100,000	28 (5.6)	32 (4.5)
Don't know/refused to answer	7 (8.1)	7 (8.1)
Canadian Born [a]		
No	24 (6.8)	21 (6.1)
Yes	89 (5.9)	68 (4.6)
Marital status [a]		
Married	77 (5.8)	64 (4.9)
Same partner for > 1 year	29 (6.7)	19 (4.5)
Divorced/separated/single/other	7 (8.0)	6 (6.9)
Season at sample collection (trimester 1) [b]		
Fall (Sept 21-Dec 20)	27 (5.0)	19 (3.6)
Winter (Dec 21- March 20)	37 (8.4)	28 (6.5)
Spring (Mar 21 – June 20)	24 (5.6)	21 (5.0)
Spring (Mar 21 – June 20)	25 (5.7)	21 (4.9)
Season at sample collection (trimester 3)		
Fall (Sept 21-Dec 20) [b]	23 (5.1)	18 (4.0)

Winter (Dec 21- March 20)	22 (5.3)	17 (4.2)
Spring (Mar 21 – June 20)	27 (4.9)	20 (3.7)
Spring (Mar 21 – June 20)	41 (9.6)	34 (8.1)
Smoking status at trimester 1[b]		
Never/former smoker	99 (6.1)	78 (4.9)
Smoker/quit during pregnancy	14 (6.4)	11 (5.1)
Smoking status at trimester 3 [b]		
Never/former smoker	99 (6.1)	78 (4.9)
Smoker/quit during pregnancy	14 (6.2)	11 (4.9)
Fish consumption at trimester 1 [b]		
No fish	14 (5.4)	13 (5.0)
up to 1 time per week	55 (6.9)	47 (6.0)
>1 but ≤2 times per week	22 (5.3)	14 (3.5)
>2 times per week	22 (5.8)	15 (4.0)
Fish consumption at trimester 3 [b]		
No fish	22 (8.1)	19 (7.0)
up to 1 time per week	48 (6.1)	38 (4.9)
>1 but ≤2 times per week	21 (4.9)	16 (3.8)
>2 times per week	22 (6.2)	16 (4.6)
Walking duration at trimester 1 (minutes) [b]		
<30 minutes per day	13 (7.3)	9 (5.2)
between 30 and <60 minutes per day	23 (5.8)	17 (4.4)
between 60 and <120 minutes per day	34 (7.2)	31 (6.7)
≥120 minutes per day	43 (5.3)	32 (4.0)
Walking duration at trimester 3 (minutes) [b]		
<30 minutes per day	15 (8.8)	10 (6.1)
between 30 and <60 minutes per day	29 (7.9)	24 (6.7)
between 60 and <120 minutes per day	24 (4.9)	21 (4.3)
≥120 minutes per day	45 (5.5)	34 (4.2)
1st trimester Vitamin D (nmol/L)[d]		
<50 nmol/L 25OHD	20 (7.2)	14 (5.2)
≥50 nmol/L 25OHD	90 (5.9)	72 (4.8)

Missing covariate information for sample (n_{total}=1851); a: 0% b: <1%; c: 7.5%; d: 3.3%. See Supporting Information eFigure7-8 for further information on missingness.
n=number of preterm births within category
%=the percent of births that are preterm in that category
25OHD=25-hydroxyvitamin D

Table 2: Distribution of metals and 25hydroxyvitamin D (25OHD) in the MIREC Cohort Study

Chemical	units	n	Trimester	LOD	% > LOD[c]	Missing (%) (total n=1851)	Min	5th Pctl	25th Pctl	50th Pctl	75th Pctl	95th Pctl	Max	Geometric Mean[a] (95% CI)
Arsenic	µg/L	1826	1	0.22	92.7%	1.5%	0.11	0.11	0.52	0.75	1.12	2.32	34.46	0.73 (0.71, 0.76)
Arsenic	µg/L	1631	3	0.22	87.3%	11.9%	0.11	0.11	0.42	0.69	1.12	2.77	32.96	0.64 (0.61, 0.67)
Cadmium (Cd)	µg/L	1826	1	0.04	97.2%	1.5%	0.02	0.07	0.13	0.20	0.31	1.12	5.06	0.22 (0.21, 0.22)
Cadmium (Cd)	µg/L	1631	3	0.04	96.1%	11.9%	0.02	0.06	0.13	0.20	0.29	0.75	4.27	0.20 (0.19, 0.20)
Lead (Pb)	µg/dL	1826	1	0.10	100%	1.5%	0.16	0.29	0.44	0.61	0.85	1.41	4.14	0.62 (0.61, 0.63)
Lead (Pb)	µg/dL	1631	3	0.10	99.8%	11.9%	0.05	0.27	0.41	0.56	0.79	1.35	4.14	0.57 (0.56, 0.58)
Mercury (Hg)	µg/L	1826	1	0.10 0.12	90.5%	1.5%	0.05	0.06	0.32	0.70	1.36	2.81	10.03	0.60 (0.57, 0.63)
Mercury (Hg)	µg/L	1631	3	0.10 0.12	88.8%	11.9%	0.05	0.06	0.26	0.56	1.00	2.21	12.44	0.48 (0.46, 0.51)
			Trimester			% missing	Min	5th Pctl	25th Pctl	50th Pctl	75th Pctl	95th Pctl	Max	Mean[b] (95% CI)
25OHD	nmol/ L	1796	1	10	99.8%	3.10%	5.00	37.23	56.24	68.91	81.50	106.10	194.80	69.7 (68.7, 70.7)
25OHD	nmol/ L	1608	3	3, 10	99.9%	13.20%	1.50	37.50	60.45	77.22	94.72	121.52	293.40	78.4 (77.1, 79.7)

a. The metals were not normally distributed, so we calculated the geometric mean
b. 25OHD is normally distributed so we calculated the mean.
c. For values below the limit of detection (LOD) we imputed LOD/2

Table 3: Relative Risk (95% CI) of preterm birth per unit increase in exposure

Exposure (units)	Relative risk (95% confidence interval)	
	All Preterm Births	Spontaneous Preterm Births
Arsenic (µg/L)	1.10 (1.02, 1.19)	1.11 (1.03, 1.20)
Cadmium (µg/L)	0.70 (0.32, 1.52	0.68 (0.28, 1.63)
Lead (µg/dL)	1.48 (1.00, 2.20)	1.71 (1.13, 2.60)
Mercury (µg/L)	0.83 (0.59, 1.17)	0.85 (0.59, 1.21)
25OHD (nmol/L)	1.00 (0.99, 1.01)	1.00 (1.00, 1.01)
250HD (<50 nmol/L vs ≥50 nmol/L (reference)	0.89 (0.45, 1.76)	0.93 (0.44, 1.99)

Imputed Models (m = 50 imputations) adjusted for maternal age, pre-pregnancy BMI, season, fish consumption, self-reported walking and smoking. All models contain time varying exposures (arsenic, Cd, Pb, Hg, 25OHD continuous and then dichotomous with a cut point at 50 nmol/L) and covariates (season, fish consumption, self-reported walking and smoking) and time fixed covariates (maternal age, pre-pregnancy BMI).

Table 4: Associations of in metal exposure (per unit increase) by Vitamin D Status and Preterm Birth – Stratified analysis by 25OHD concentrations <50 and ≥50 nmol/L

	Participants with 25OHD < 50 nmol/L, (n=20 PTB,n=357 term births)	Participants with 25OHD ≥ 50 nmol/L, (n=90 PTB, n=1426 term births)
All preterm Birth		
Arsenic (µg/L)	1.18 (0.86, 1.61)	1.01 (0.80, 2.28)
Cadmium (µg/L)	0.97 (0.33, 2.89)	0.53 (0.17, 1.68)
Lead (µg/dL)	2.42 (1.01, 5.79)	1.29 (0.80, 2.07)
Mercury (µg/L)	0.72 (0.29, 1.76)	0.86 (0.60, 1.23)
Spontaneous preterm Birth	25OHD < 50 nmol/L (n=14 PTB n=257 term births)	25OHD ≥ 50 nmol/L (n=72 PTB, n=1426 term births)
	RR (95% CI)	RR (95% CI)
Arsenic (µg/L)	1.20 (0.80, 1.80)	1.01 (0.80, 1.28)
Cadmium (µg/L)	0.86 (0.27, 2.70)	0.50 (0.13, 1.92)
Lead (µg/dL)	3.04 (1.15, 8.04)	1.50 (0.91, 2.45)
Mercury (µg/L)	0.64 (0.21, 2.00)	0.88 (0.60, 1.28)

Relative risks were adjusted for maternal age, pre-pregnancy BMI, season, fish consumption, self-reported walking and smoking
Exposure is continuous and time varying.
Relative Excess Risk due to Interaction spontaneous PTB bootstrapped 1000 times ($RERI_{unadjusted}$) 0.46 (95% CI -1.66, 1.81); $RERI_{adjusted}$: 0.52(-95% CI 2.91, 1.68)

Figure 1: Adjusted hazard probability of preterm birth with respect to blood lead concentration at the median, 5th and 95th percentile.

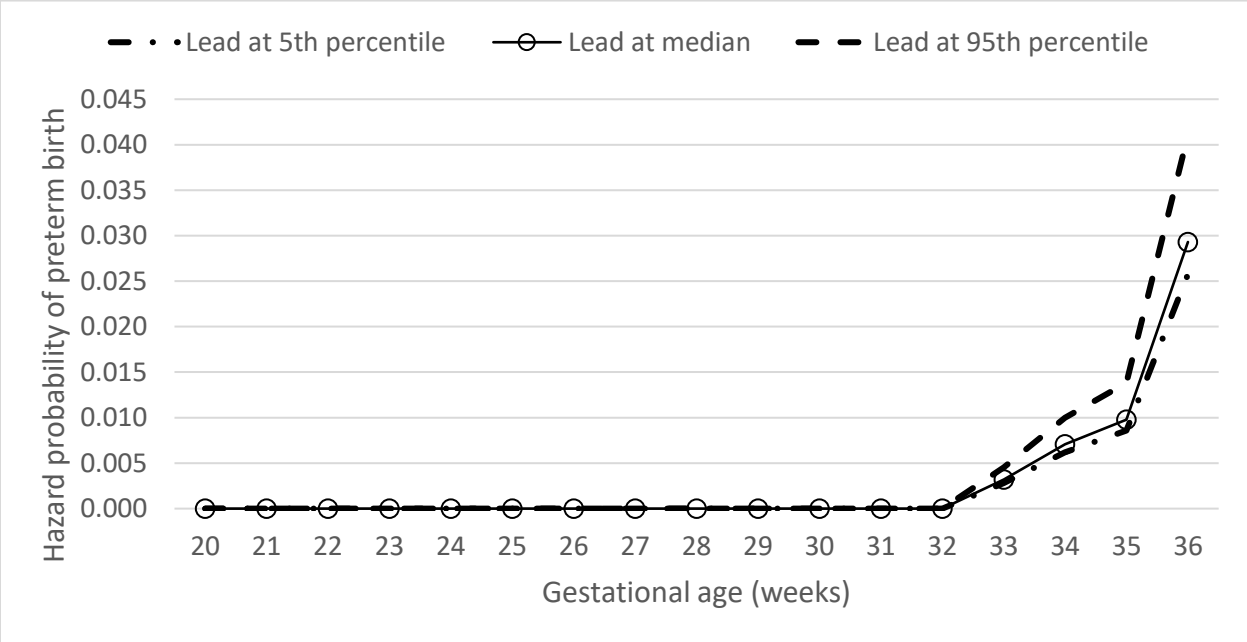
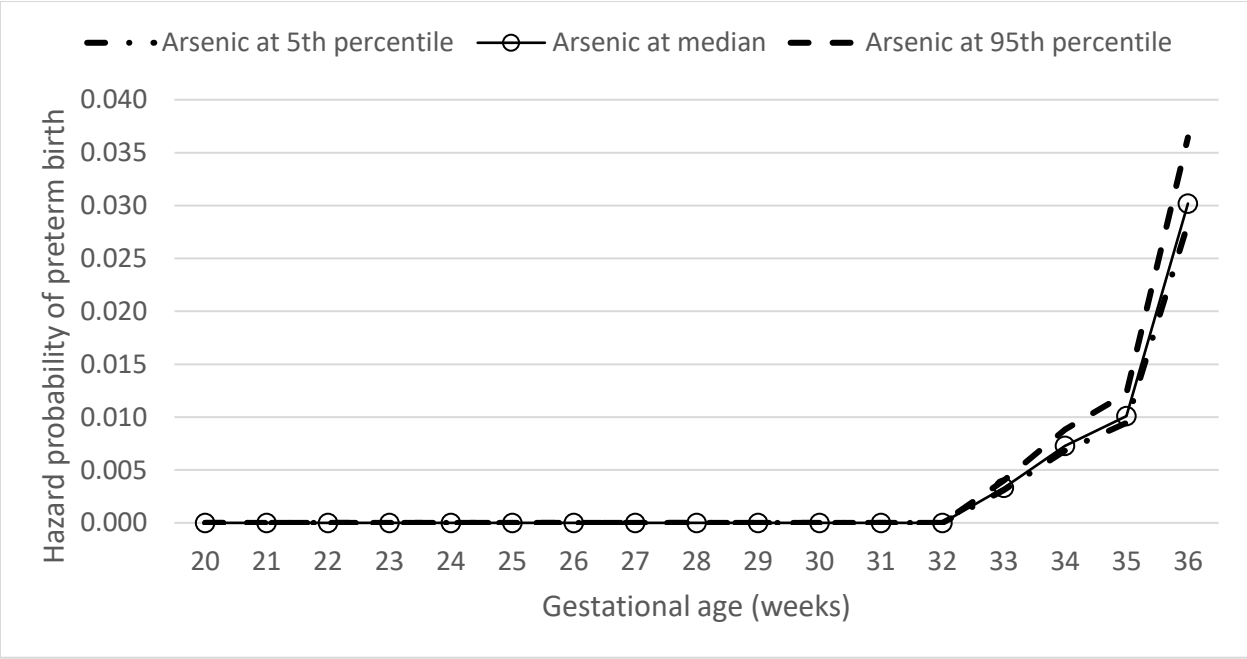


Figure 2: Adjusted hazard probability of preterm birth with respect to blood arsenic concentration at the median, 5th and 95th percentile.



Supplemental Tables and Figures

Association between toxic metals, vitamin D and Preterm Birth in the Maternal-Infant Research on Environmental Chemicals Study

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eTable S1: Correlation coefficients between blood metal and 25OHD concentrations

Metals (Spearman)								
	PB_1st	**PB_3rd**	**CD_1st**	**CD_3rd**	**HG_1st**	**HG_3rd**	**AS_1st**	**AS_3rd**
PB_1st	1	**0.76**	0.23	0.18	0.26	0.24	0.17	0.13
PB_3rd		1	0.15	0.27	0.26	0.28	0.20	0.17
CD_1st			1	**0.64**	0.05	0.00	-0.04	-0.01
CD_3rd				1	0.08	0.10	0.04	0.03
HG_1st					1	**0.75**	0.38	0.33
HG_3rd						1	0.36	0.38
AS_1st							1	**0.41**
AS_3rd								1

Vitamin D	**Spearman**	**Pearson**
	25OHD 3rd trimester	**25OHD 3rd trimester**
25OHD 1st trimester	0.55	0.56

eTable S2: Relative Risk of Preterm Birth per IQR increase in exposure

Exposure	**IQR**	**All PTBs**		**Spontaneous PTB**	
		RR	95% CI	RR	95% CI
Arsenic (µg/L)	0.63	1.06	1.01, 1.11	1.07	1.02, 1.12
Cadmium (µg/L)	0.17	0.94	0.82, 1.07	0.94	0.81, 1.09
Lead (µg/dL)	0.39	1.16	1.00, 1.36	1.23	1.05, 1.45
Mercury (µg/L)	0.96	0.84	0.61, 1.16	0.85	0.61, 1.20
25OHD (nmol/L)	27.3	1.07	0.85, 1.34	1.13	0.89, 1.45

Imputed Models (m=50 imputations) adjusted for maternal age, pre-pregnancy BMI, season, fish consumption, self-reported walking and smoking. All models contain time varying exposures (As, Cd, Pb, Hg, 25OHD) and covariates (season, fish consumption, self-reported walking and smoking) and time fixed covariates (maternal age, pre-pregnancy BMI).

eTable S3: Relative Risk of Preterm Birth per IQR increased in exposure by Vitamin D Status – Stratified analysis by 25OHD concentrations <50 and ≥50 nmol/L

ALL Preterm Births	IQR	Participants with 25OHD < 50 nmol/L, (n=20 PTB,n=357 term births)	Participants with 25OHD ≥ 50 nmol/L, (n=90 PTB, n=1426 term births)
		OR (95% CI)	OR (95% CI)
Arsenic (µg/L)	0.63	1.11 (0.91, 1.35)	1.01 (0.87, 1.17)
Cadmium (µg/L)	0.17	1.00 (0.83, 1.20)	0.90 (0.74, 1.09)
Lead (µg/dL)	0.39	1.41 (1.00, 1.98)	1.10 (0.92, 1.33)
Mercury (µg/L)	0.96	0.73 (0.31, 1.72)	0.86 (0.61, 1.22)
Spontaneous Preterm Births			
Exposure*		25OHD < 50 nmol/L (n=14 PTB n=257 term births)	25OHD ≥ 50 nmol/L (n=72 PTB, n=1426 term births)
		OR (95% CI)	OR (95% CI)
Arsenic (µg/L)	0.63	1.12 (0.87, 1.45)	1.00 (0.83, 1.19)
Cadmium (µg/L)	0.17	0.97 (0.80, 1.18)	0.89 (0.71, 1.12)
Lead (µg/dL)	0.39	1.54 (1.05, 2.25)	1.17 (0.96, 1.42
Mercury (µg/L)	0.96	0.66 (0.22,1.95)	0.88 (0.61, 1.27)

Model: adjusted for maternal age, pre-pregnancy BMI, season, fish consumption, self-reported walking and smoking

Exposure is continuous and time varying. The odds per IQR increase in exposure.

$p_{interaction}$=<0.10 (continuous Pb and 25OHD category (<50, ≥50 nmol/L).

Relative Excess Risk due to Interaction for spontaneous PTB: bootstrapped 1000 times ($RERI_{crude}$): 0.46 (-1.66, 1.81); $RERI_{adjusted}$:-0.13 (-0.94, 2.02)

eTable S4: Characteristics of Participants with Missingness – categorical variables

		Major Missing Covariates*	Any Missing Covariates**	All Data	No Missing
		Percent	Percent	Percent	Percent
Household income					
1	$50K or less	28	27	22	21
2	between $50K to $100K	30	32	35	35
3	greater than $100K	35	36	39	39
4	Don't know/refuse to answer	7	6	5	4
Marital status					
1	Married	69	70	72	73
2	Same partner for > 1 year	26	25	23	23
3	Divorced/separated/single/other	6	5	5	5
parity					
1	0	41	42	44	44
2	1	41	41	40	40
3	2+	18	17	16	15
Maternal education					
1	Some college, High School or Less than High School	18	17	14	13
2	College/Trade Diploma	26	25	24	23
3	Undergrad degree	33	35	37	37
4	Graduate degree	22	23	26	26
Born in Canada					
1	No	18	18	19	19
2	Yes	82	82	81	81
Fetal Sex					
1	Male	55	55	53	52
2	Female	45	45	47	48
Fish Consumption in 1st trimester					
1	No fish	20	18	14	13
2	up to 1 time per week	42	41	43	43
3	>1 but ≤2 times per week	18	20	22	23
4	>2 times per week	21	20	21	21
Fish Consumption in 3rd trimester					
1	No fish	22	20	15	13
2	up to 1 time per week	39	40	43	43
3	>1 but ≤2 times per week	19	19	23	24
4	>2 times per week	20	21	19	19
Smoking Status 1st Trimester					

1	Never/former smoker	84	85	88	89
2	Smoker/quit during pregnancy	16	16	12	11
Smoking Status 3rd trimester					
1	Never/former smoker	84	84	88	89
2	Smoker/quit during pregnancy	16	16	12	11
Walking duration at 1st trimester					
1	<30 minutes per day	12	12	10	9
2	between 30 and <60 minutes per day	20	20	21	22
3	between 60 and <120 minutes per day	26	26	25	25
4	≥120 minutes per day	42	42	44	44
Walking duration at 3rd trimester					
1	<30 minutes per day	8	8	9	10
2	between 30 and <60 minutes per day	20	20	20	20
3	between 60 and <120 minutes per day	27	28	27	26
4	≥120 minutes per day	45	45	44	44
Season at 1st trimester visit					
1	Fall (Sept 21-Dec 20)	27	28	29	30
2	Winter (Dec 21- March 20)	23	23	24	24
3	Spring (Mar 21 – June 20)	21	21	23	24
4	Summer (June 21 – Sept 20)	28	28	24	22
Season at 3rd trimester Visit					
1	Fall (Sept 21-Dec 20)	23	23	25	25
2	Winter (Dec 21- March 20)	28	27	22	21
3	Spring (Mar 21 – June 20)	27	28	30	30
4	Summer (June 21 – Sept 20)	23	22	23	24

* Covariates with >5% missing (3rd trimester metals, vitamin D and Pre-pregnancy BMI)

** Any of 1st or 3rd trimester measures (metals, vitamin D, season, walking, smoking) plus baseline measures of parity, income, maternal age, education, marital status and country of birth

eTable S5: Characteristics of Participants with Missingness – continuous covariates/exposures

	Any miss	major	no miss	Any miss	major	no miss	Any miss	major	no miss	Any miss	major	no miss	Any miss	major	no miss
Variable	**25th Pctl**			**50th Pctl**			**75th Pctl**			**95th Pctl**			**Mean**		
Arsenic 1st trimester (µg/L)	0.5	0.5	0.5	0.8	0.7	0.8	1.1	1.0	1.2	2.1	2.0	2.3			
Cadmium 1st trimester (µg/L)	0.1	0.1	0.1	0.2	0.2	0.2	0.3	0.4	0.3	1.7	1.8	0.9			
Lead 1st trimester (µg/dL)	0.5	0.5	0.4	0.6	0.6	0.6	0.8	0.8	0.8	1.4	1.4	1.4			
Mercury 1st trimester (µg/L)	0.2	0.2	0.3	0.6	0.6	0.7	1.3	1.3	1.4	2.8	2.6	2.8			
25OHD 1st trimester (nmol/L)	53	53	57	66	66	70	79	79	82	108	108	106	68	68	70
Pre-pregnancy BMI (kg/m2)	21	22	21	24	24	23	27	27	27	37	37	36	25	25	25
Maternal age (yrs)	28	28	29	31	32	32	36	36	36	40	40	40	32	32	32

Any miss: participants with any of 1st or 3rd trimester measures (metals, vitamin D, season, walking, smoking) plus baseline measures of parity, income, maternal age, education, marital status and country of birth missing
Major: Those participants with missing covariates with >5% missing (3rd trimester metals, vitamin D and Pre-pregnancy BMI)
No miss: Participants with no missing covariates

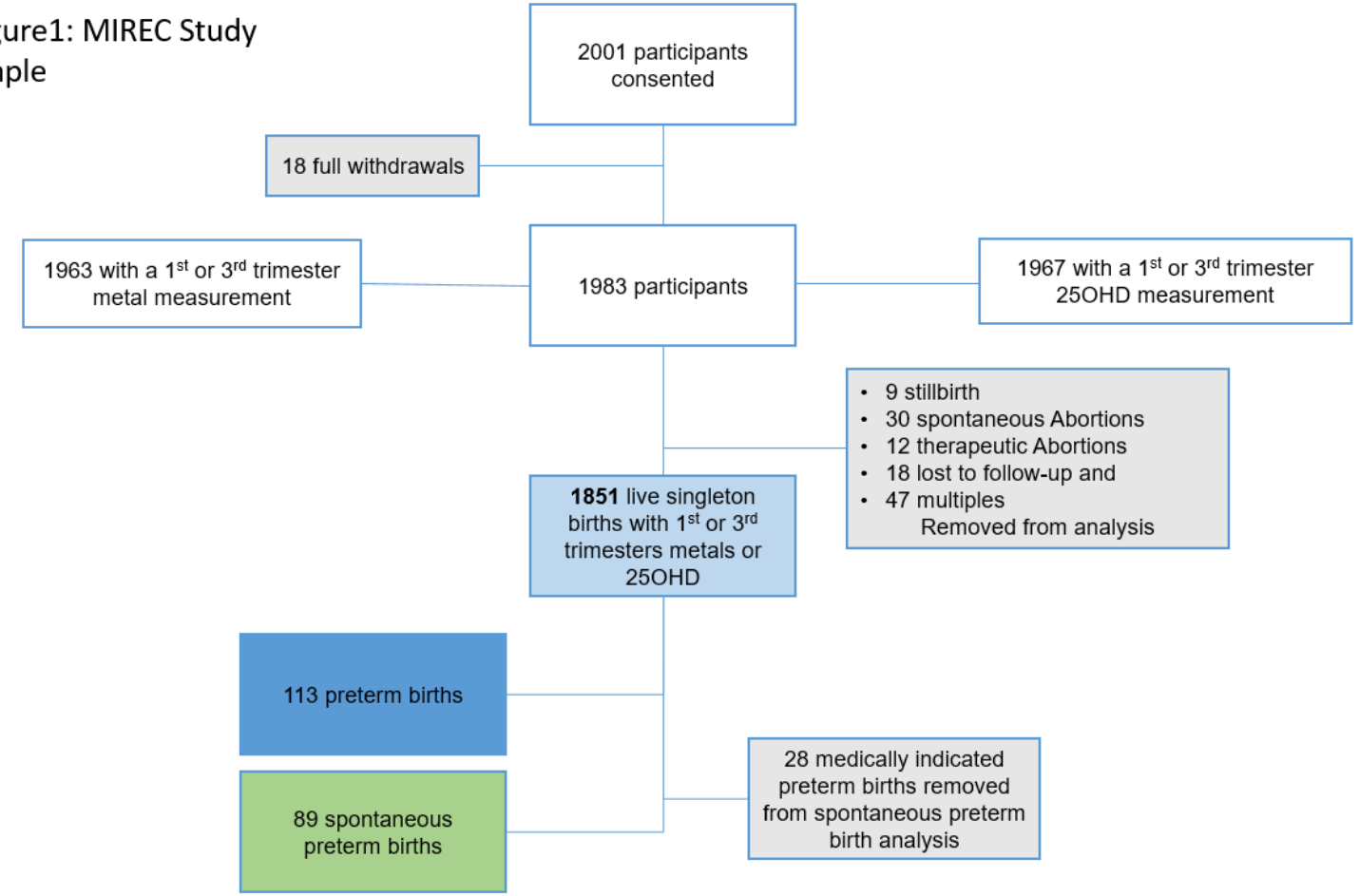
eTable S6: Secondary Categorical Analysis of the association between Vitamin D and Preterm Birth.

	All Preterm Births		**Spontaneous Preterm Birth**	
Exposure Category	OR	95% CI	OR	95% CI
Vitamin D (25OHD)				
<40 nmol/L vs >=40 nmol/L	1.22	0.56, 2.67	1.19	0.48, 2.99

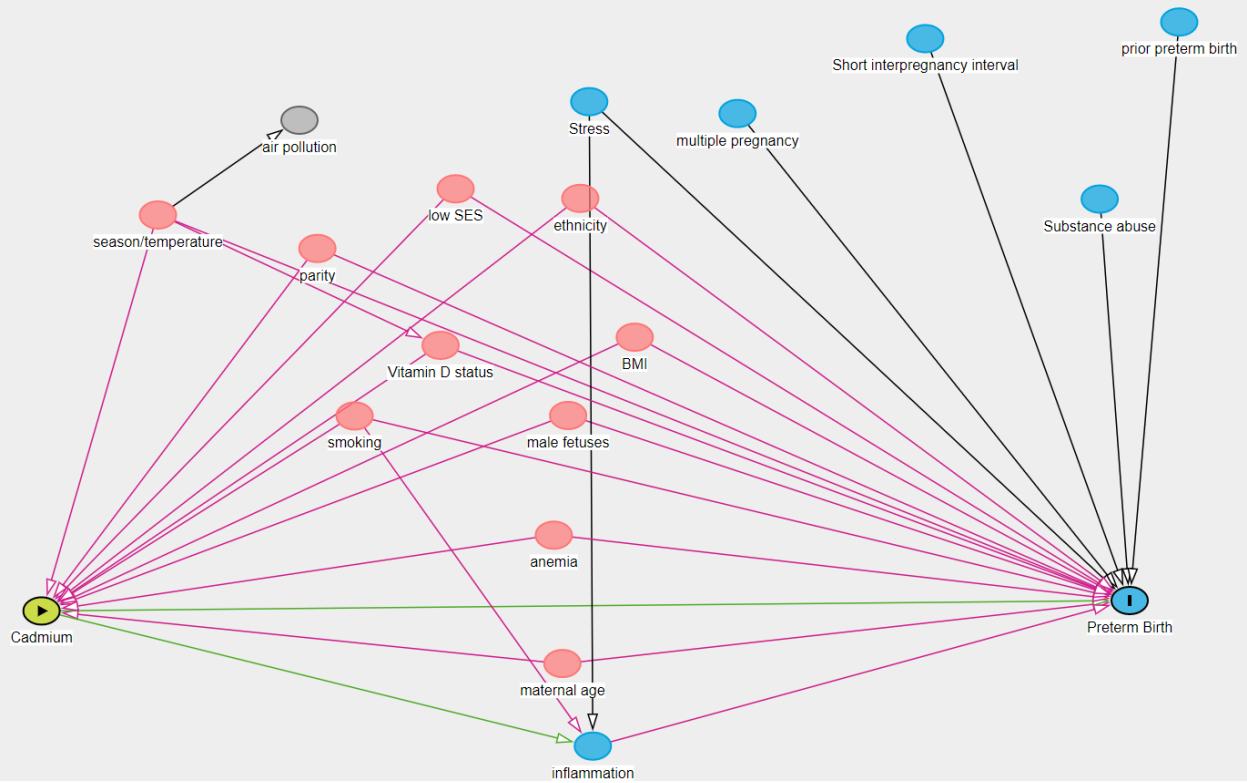
Models adjusted for maternal age, pre-pregnancy BMI, season, fish consumption, self-reported walking and smoking

Also see Figure S9 for Hazard Profile.

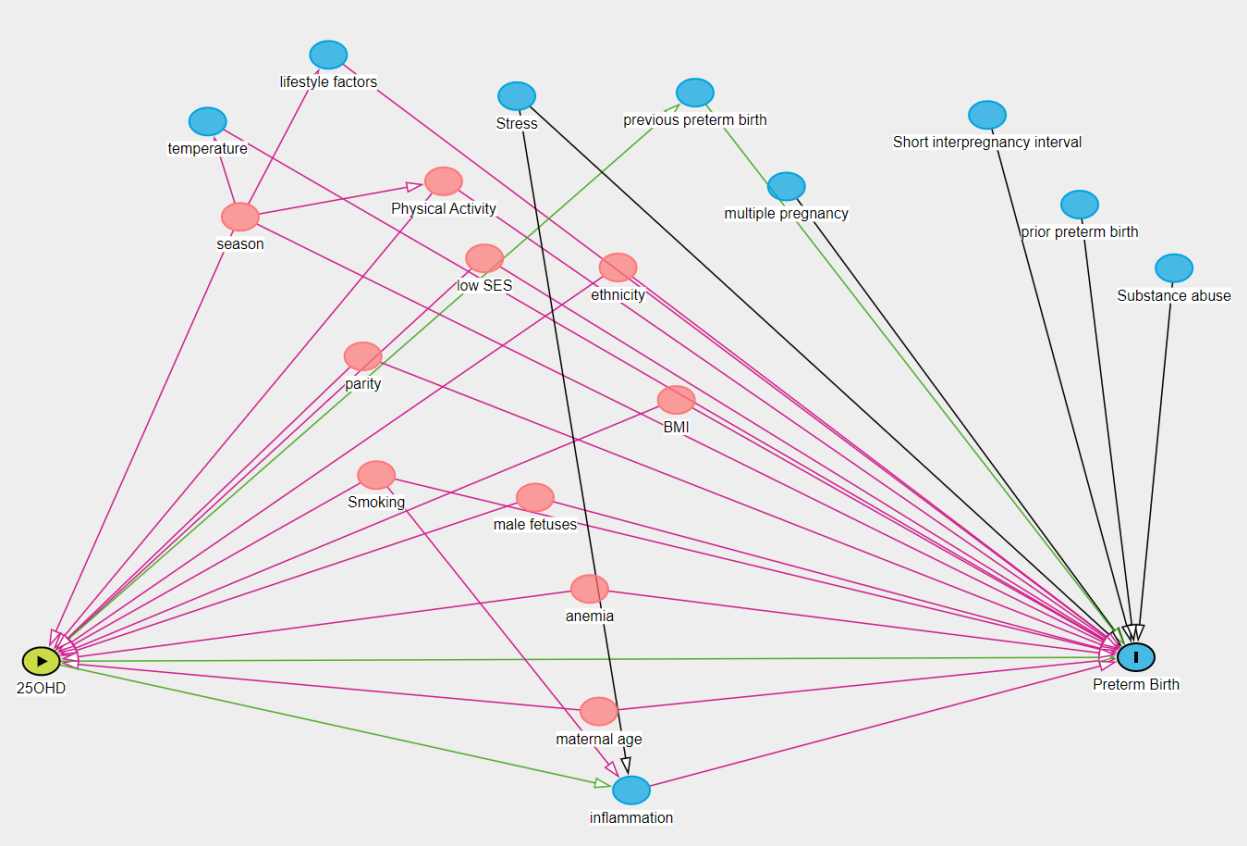
eFigure1: MIREC Study Sample



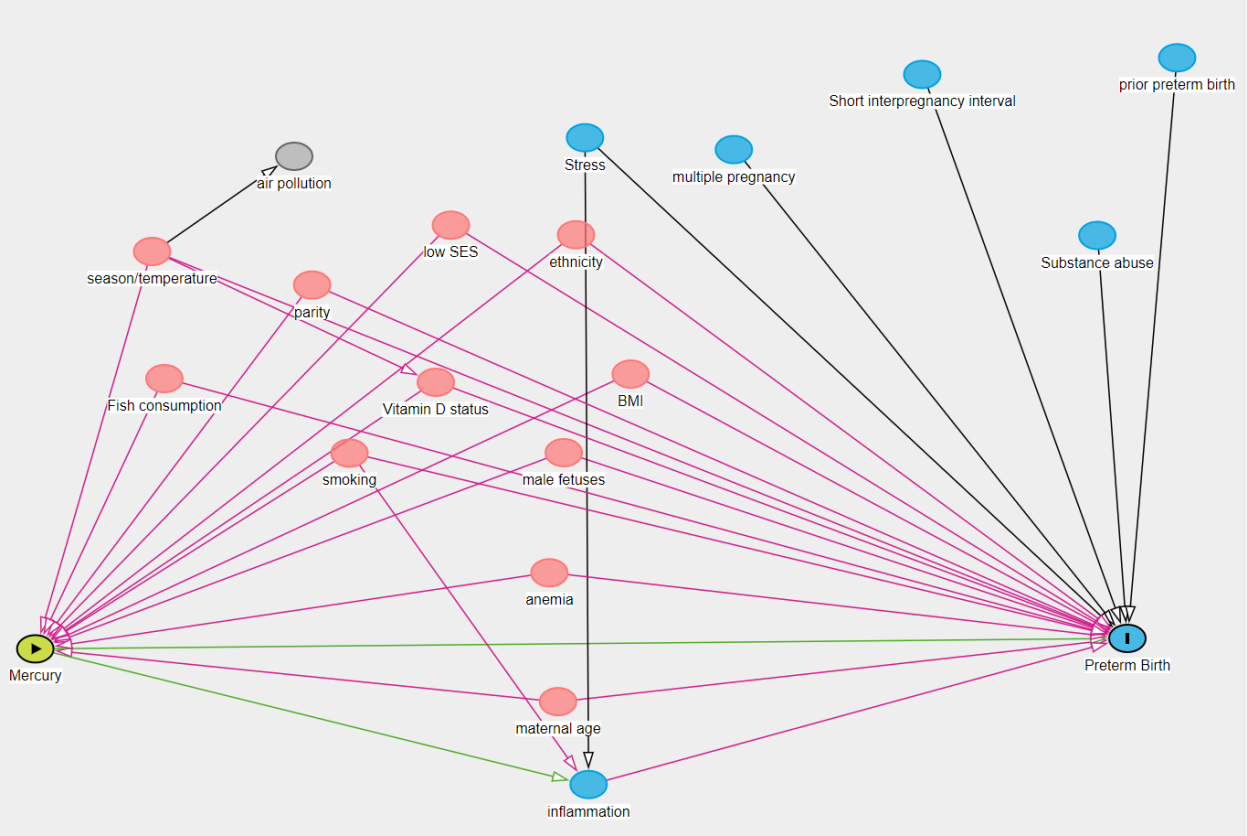
eFigure2: DAG – Cd and Preterm



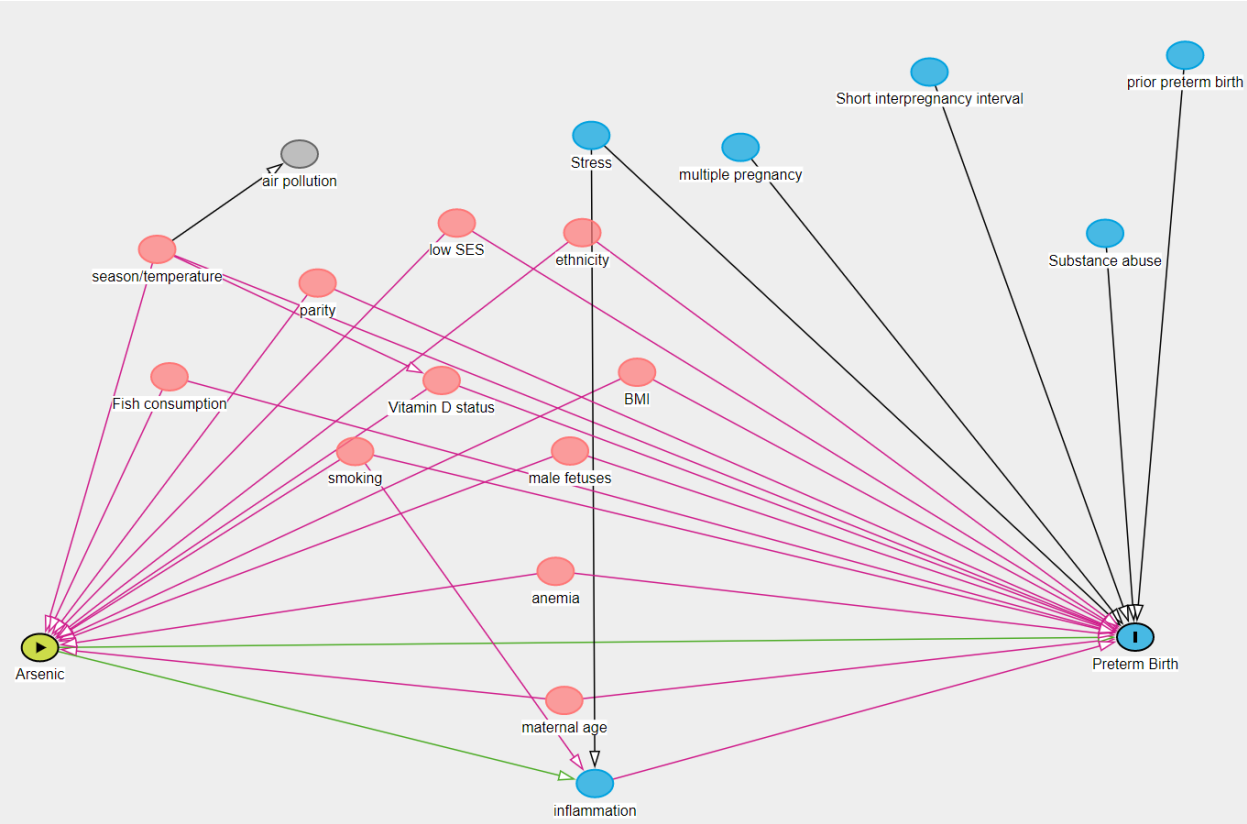
eFigure3: DAG - 25OHD and Preterm



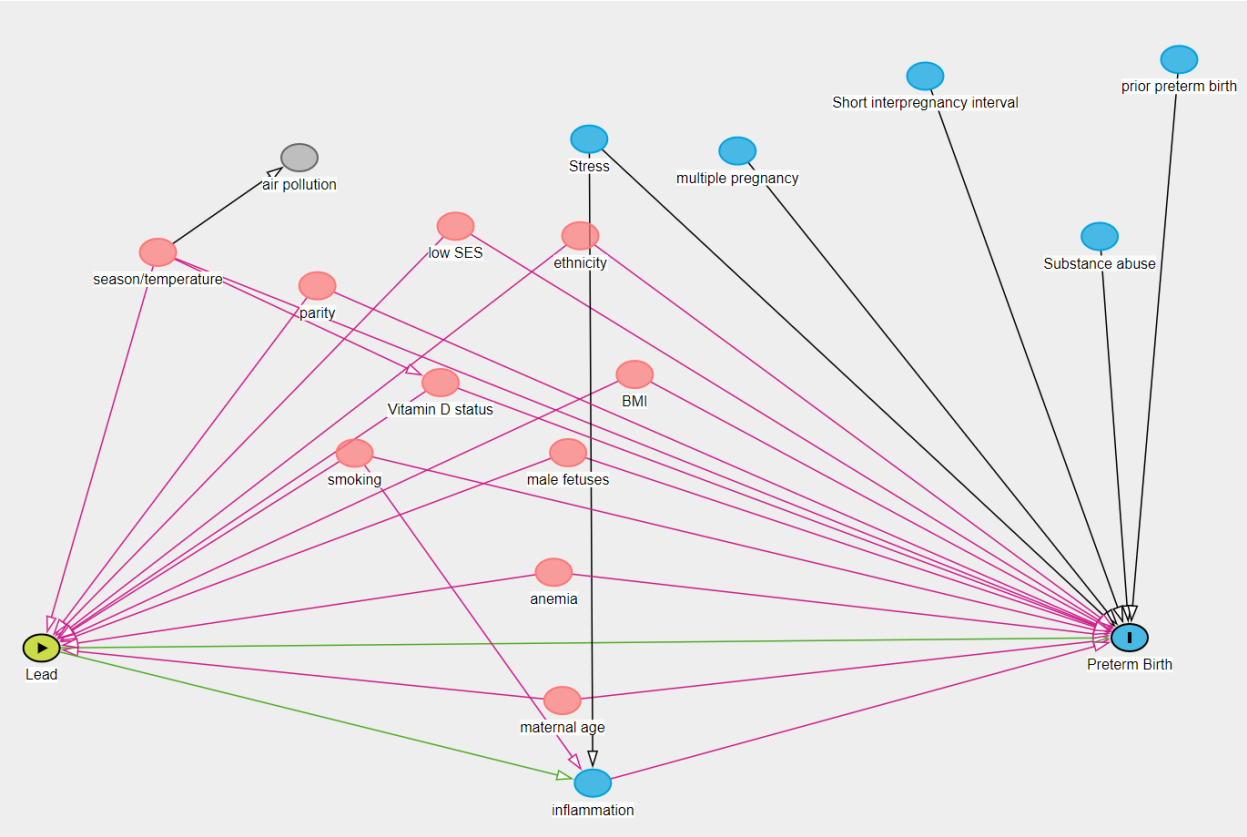
eFigure4: DAG – Hg and Preterm



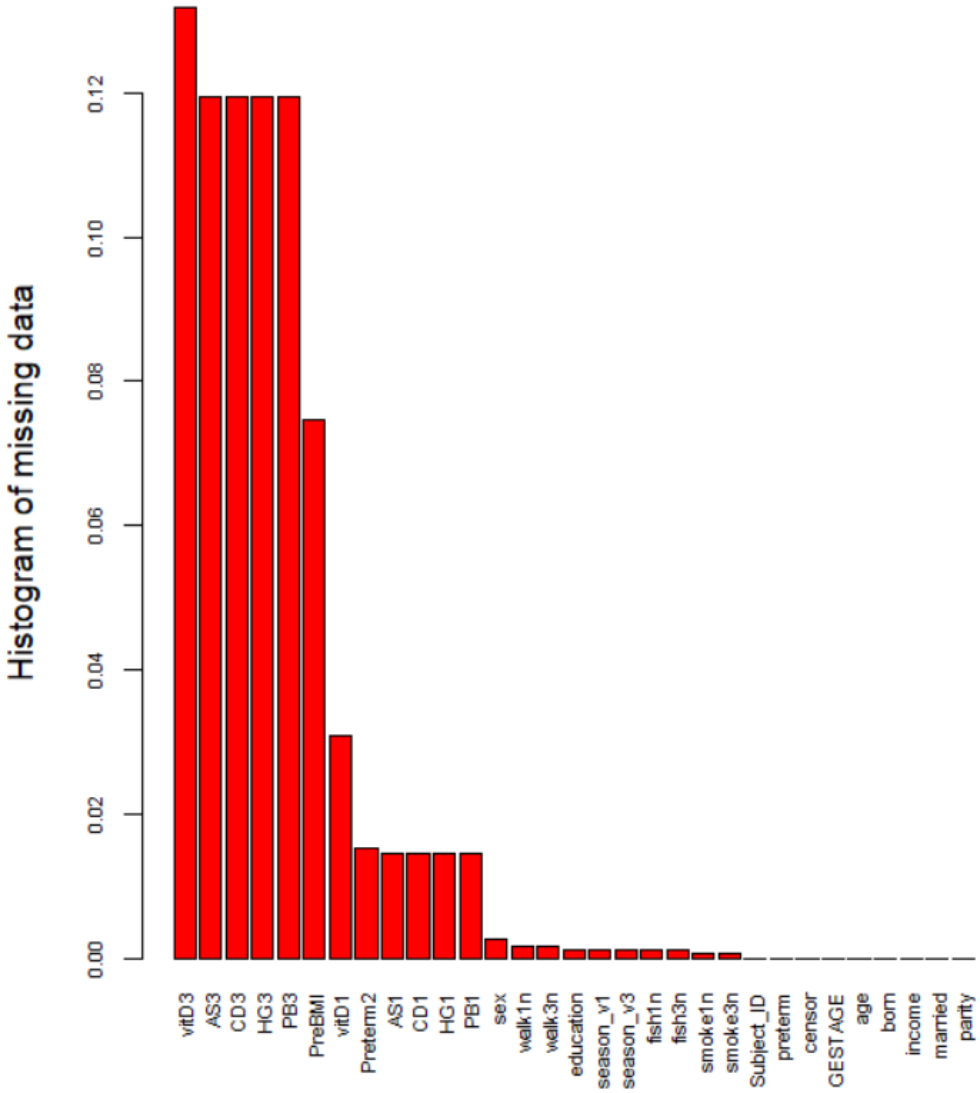
eFigure5: DAG – As and Preterm



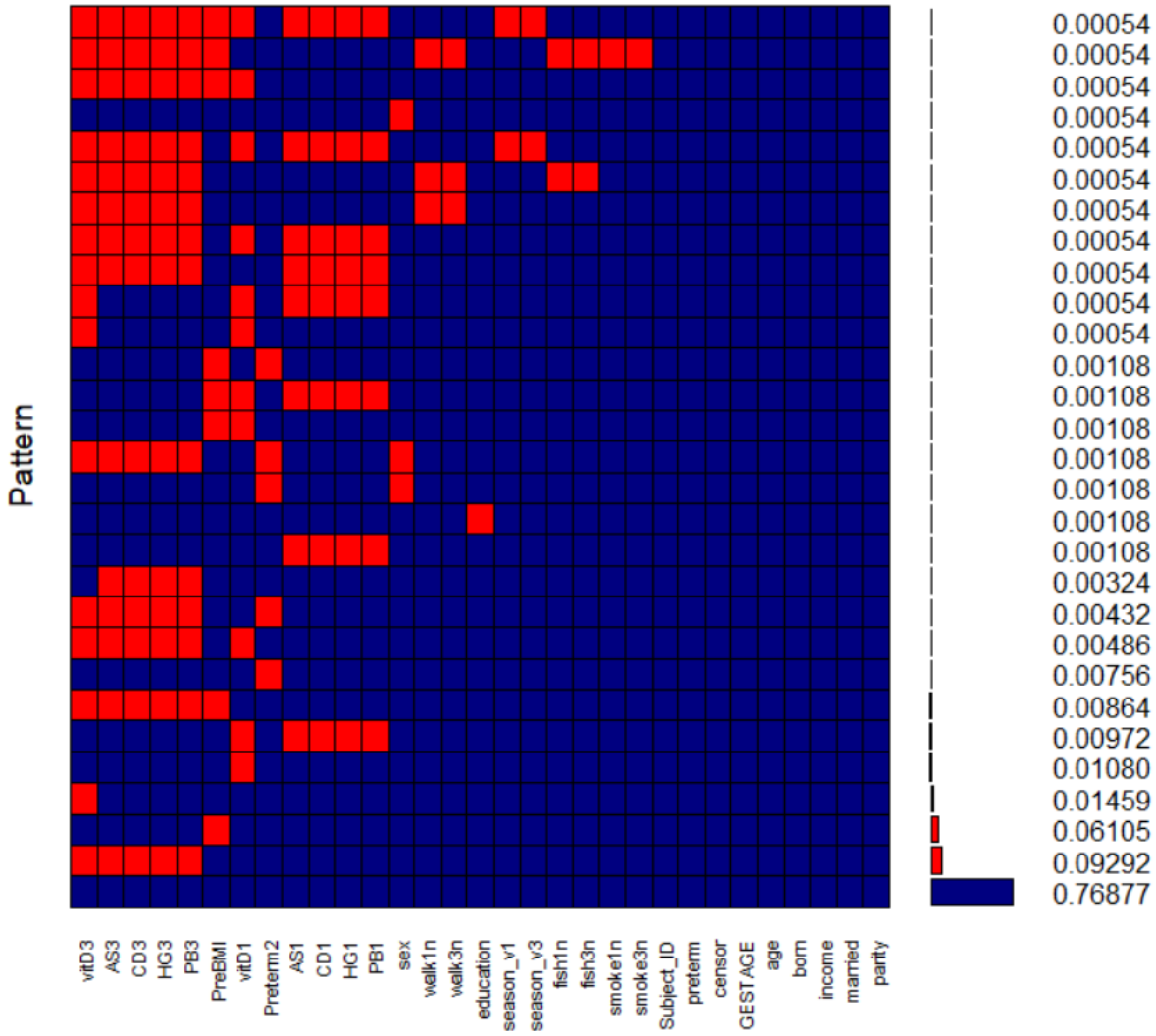
eFigure6: DAG – Lead and Preterm



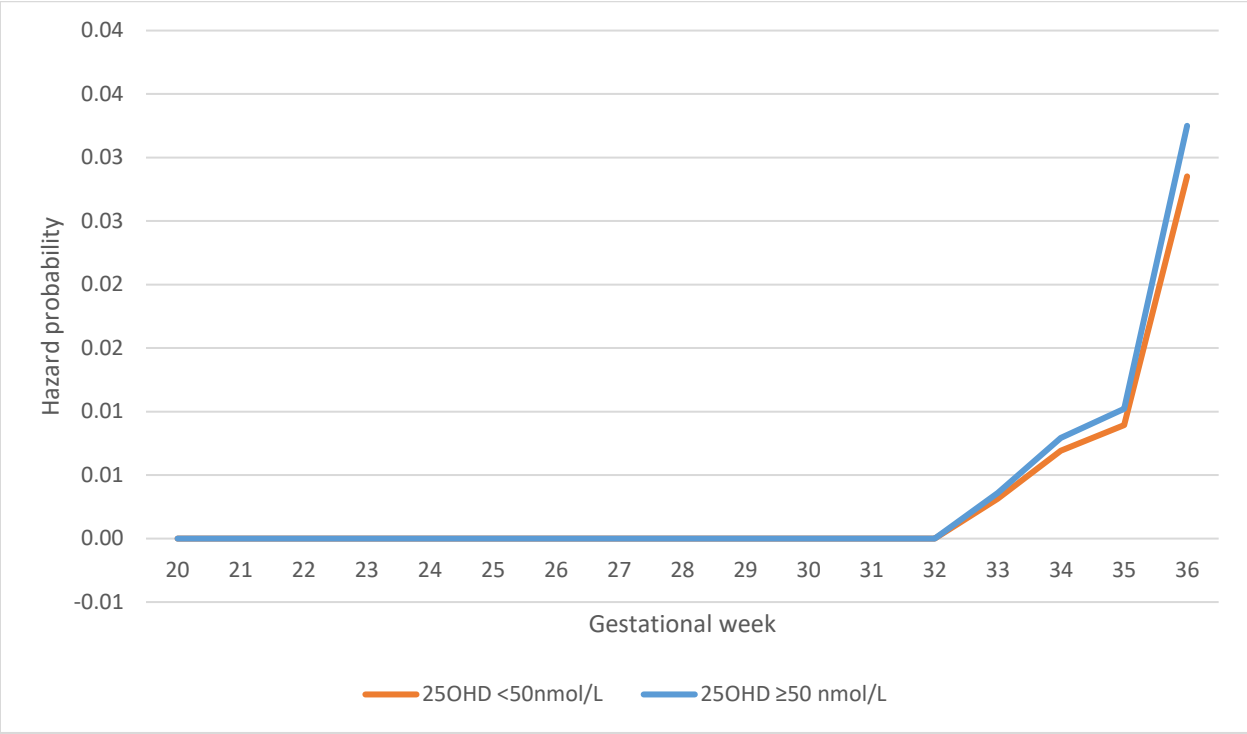
eFigure7: Pattern of Missingness (Histogram)



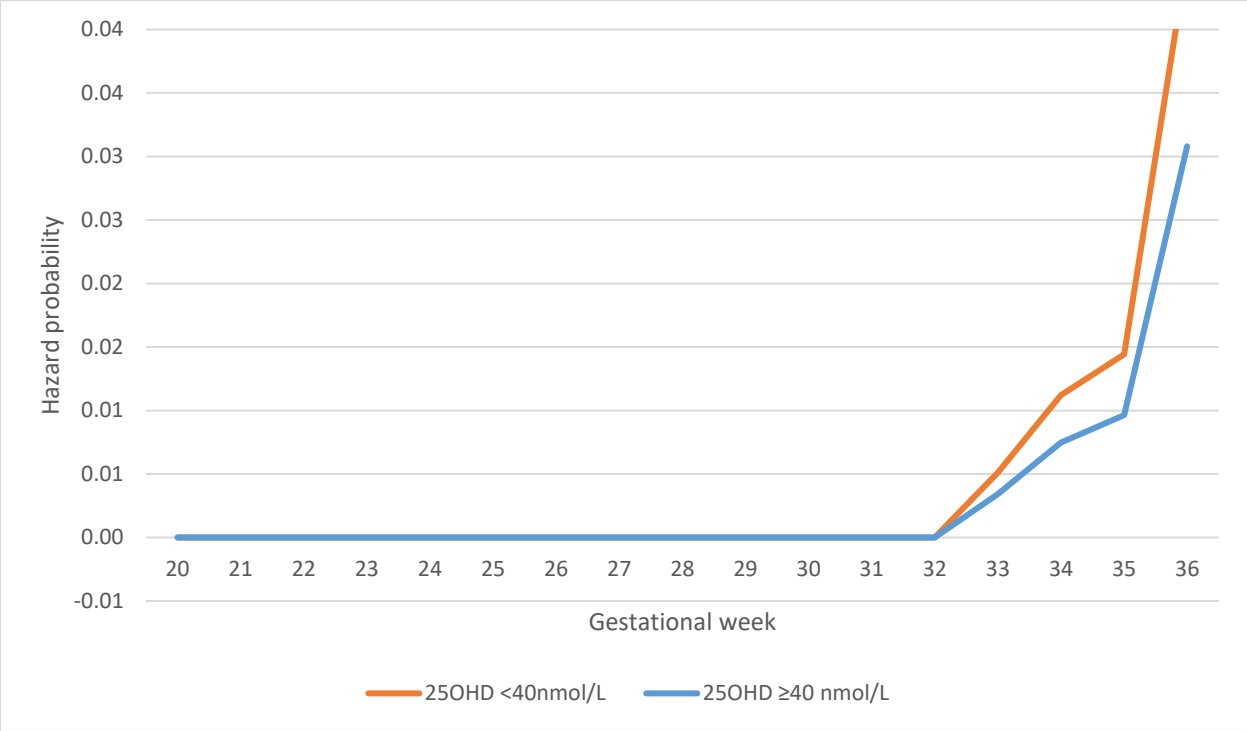
eFigure8: Pattern of Missingness



eFigure9: Adjusted Hazard Ratio for 25OHD (<50 and ≥50 nmol/L)



eFigure10: Adjusted Hazard Ratio for 25OHD (<40 and ≥40 nmol/L)



CHAPTER 4

The Association between Pregnancy Complications and Long-Term Maternal Cardiometabolic Health in the MIREC Cohort Study.

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Preface to Chapter 4

In our second thesis article (Chapter 3) we observed an association between the toxic metals lead and arsenic with preterm birth. Research suggests that pregnancy complications including preterm birth may have long-term implications for maternal health. In the most recent follow-up of the MIREC, Endocrine and Metabolic Function (MIREC-ENDO), we invited mothers back into the study and collected information on cardiometabolic health approximately 9 years postpartum. This third article examines the association between pregnancy complications and these cardiometabolic endpoints. The author contributions are presented below.

Authorship credits:

Mandy Fisher was involved in the design and implementation of the study, statistical analysis, writing and interpretation of the study. **Graeme Smith** is a content expert and was involved in the review of the article and revisions. **Beth Potter** was involved in the analysis of the study, and review of the article and revisions. **Tye Arbuckle** was involved in the design and implementation of the study, review and revisions of the article. **Julian Little** was involved in the analysis of the study, review of the article and revisions. **Hope Weiler** was involved in the design of the study and the review of the article and revisions. **Anne-Sophie Morisset** was involved in the design and implementation of the study and the review of the article and revisions. **Bruce Lanphear** was involved in the review and revisions of the article. **Joseph Braun** was involved in the analysis of the study and the review of the article and revisions. **Premkumari Kumarathasan** was involved in the review and revisions of the article. **Mark Walker** was involved in the design and implementation of the study, review and revisions of the article. **Michael Borghese** was involved in the review and revisions of the article. **Jillian Ashley-Martin** was involved in the analysis of the study and the review and revisions of the article. **Robin Shutt** was involved in the review and revisions of the article. **Linda Dodds** was involved in the review and revisions of the article. **Isabelle Massarelli** was involved in the design of the study, review and revisions of the article. **Mark Palmert** was involved in the design and implementation of the study, review and revisions of the article. **William Fraser** was involved in the design and implementation of the study and the review of the article and revisions.

ABSTRACT

Background: Pregnancy is increasingly recognized as a window to identify women at risk for cardiovascular disease. Women with pregnancy complications have elevations in biomarkers of inflammation and insulin resistance in pregnancy, but studies of longer term effects of these complications are limited.

Methods: Using data from the Maternal-Infant Research on Environmental Chemicals (MIREC) pregnancy cohort we investigated the association between hypertensive disorders in pregnancy (HDP) (n=36), or any pregnancy complication (n=56, preterm birth, HDP, impaired glucose tolerance or gestational diabetes mellitus), on maternal cardiometabolic health indicators 9 years postpartum compared to women who had uncomplicated pregnancies (n=187). We used multivariable linear regression in the analysis.

Results: From our adjusted linear regression results, participants with HDP during pregnancy had significantly higher systolic (mean difference 11.9 mm Hg, 95% CI 7.1, 16.7) and diastolic (6.0 mm Hg, 95% CI: 2.4, 9.6) blood pressure, and 32% (-14%, 104%) and 18% (-15%, 65%) higher high sensitivity-C-reactive protein (hsCRP) and leptin respectively, compared to participants with uncomplicated pregnancies. We also observed higher % body fat (mean difference 2.6%, 95% CI: 0.3%, 4.8%) and fasting plasma glucose (3.4 mg/dL 95% CI: 0.4, 6.4) in those who had at least one pregnancy complication, compared to those who had uncomplicated pregnancies.

Conclusion: In this prospective study of mainly healthy and premenopausal participants who were 9 years post index pregnancy, those who experienced a pregnancy complication, or experienced HDP, had significant higher blood pressure values, while those who experienced

pregnancy complications also had significant elevations in percent body fat and plasma glucose compared to women who had uncomplicated pregnancies.

Data Availability Statement: Due to data privacy issues, we are not able to make these data publicly available. Individuals may apply to access the data through the MIREC Biobank (www.mirec-canada.ca/en/research)

Acknowledgements

We would like to thank the participants of the MIREC study, study coordinators, nurses, and research assistants who made this work possible. This work was supported by Health Canada.

BACKGROUND

Cardiovascular disease (CVD) is the leading cause of premature death in Canadian women [1]. Women, especially younger (≤55 y) women, are more likely than men to die within the first year after an acute myocardial infarction [2]. Pregnancy is increasingly recognized as a window to identify women with future CVD risk as it is a natural equivalent to a physiological stress test causing structural and hemodynamic changes due to increased blood volume and cardiac output [1,3,4]. Cardiac output increases by 20-50% starting as early as 5 weeks gestation and peaking in mid to late pregnancy [4]. These pregnancy changes along with the pattern of recovery approximately 2 weeks postpartum have been compared to the acute response and recovery observed during an exercise stress test [4]. If a woman has a pregnancy complication, such as a hypertensive disorder in pregnancy (HDP), preterm birth or gestational diabetes mellitus (GDM), but is otherwise considered healthy, she has 'failed' this 'stress test'. Each of these complications has been associated with nearly a two-fold increase in risk of CVD events later in life, including myocardial infarction, stroke, and cardiovascular death [5–8]. The risk is even higher among those with recurrence (recurring HDP) [9] or co-occurrence (e.g., preeclampsia and preterm birth) of these conditions [7].

It has been hypothesized that these pregnancy complications may have a shared underlying mechanism; specifically, they are suspected to be a result of placental dysfunction due to increased inflammation followed by oxidative stress and mitochondrial dysfunction [3,8]. Inflammation may also be a common mechanism underlying both HDP and CVD [10]. Several studies suggest that women who experience HDP experience more risk factors for CVD in the

following years and decades, such as elevated blood pressure and dyslipidemia [11–13]. High sensitivity C-reactive protein (hsCRP) is a systemic inflammatory marker and multiple studies support hsCRP as an independent predictor for incident Type II diabetes [14] and CVD [15,16]. In addition, incorporating information about elevated hsCRP plus metabolic syndrome (a cluster of cardiovascular risk factors) improves the predictability of cardiovascular events by nearly 2-fold, compared to just metabolic syndrome alone [17,18]. Women who experience a HDP have been found to have elevated hsCRP eighteen [11], twenty [10] and thirty-two years [19] later, compared to women who experienced normotensive pregnancies.

Insulin resistance progressively increases as pregnancy advances (peaking in the 3rd trimester) providing metabolic adaptation to the increasing glucose demands of the growing fetus [20]. Adipocyte derived hormones such as adiponectin, leptin, tumour necrosis factor alpha (TNFα), interleukin-6 [21] along with CRP [22,23] are thought to play a role in the development of insulin resistance in pregnancy [24] which can become exaggerated in women with HDP [25] and GDM [20]. Studies suggest dysregulation of adipokines in women experiencing HDP [26] and GDM [27] compared to women with uncomplicated pregnancies. However, few studies have explored the long-term effect of pregnancy complications on these markers in the postpartum period [28,29].

Thus, while it is established that women with pregnancy complications have a higher risk of CVD and type 2 diabetes later in life, few studies have investigated the effects of pregnancy complications on multiple biomarkers of cardiometabolic health in the same longer-term follow-up period.

The objectives of this study are to examine the association between HDP (gestational hypertension or preeclampsia) and pregnancy complications combined (HDP, gestational diabetes or impaired glucose tolerance, and preterm birth) and cardiometabolic risk factors approximately 9 years. The cardiometabolic risk factors include body fat percentage, weight gain, blood pressure, and concentrations of blood lipids, fasting glucose, CRP, leptin, and adiponectin.

METHODS

Study Population

The Maternal-Infant Research on Environmental Chemicals Study (MIREC) [30] is a cohort study that recruited 2001 pregnant women from ten geographically dispersed cities in Canada between 2008 and 2011. Women were recruited through medical clinics (ultrasound, midwife and/or doctor's clinics) during the first trimester of pregnancy (6 to <14 weeks). Participants who gave informed consent scheduled visits in each trimester, at delivery and in the postpartum period (up to 10 weeks post delivery).

Pregnancy Complications

Participants completed questionnaires about their obstetrical history, smoking status, sociodemographic factors, diet, health history and evolution of their index pregnancy. In each trimester (6^0-13^{+6}, 16^0-21^{+6}, and 32^0-34^{+6} weeks) participants had their blood pressure measured using a sphygmomanometer, a urine sample tested for proteinuria and provided urine and blood specimens. Two systolic (SBP) and diastolic (DBP) measurements were taken, 1 minute apart, and if they differed by more than 10 mmHg, a 3rd measurement was taken. Medical chart

information was extracted throughout pregnancy and at delivery. Gestational age was based on the date of last menstrual period or ultrasound if they differed by >7 days. Gestational hypertension was defined as new onset hypertension (SBP ≥140 mmHg and/or DBP ≥90 mmHg) that develops after 20 weeks of pregnancy without proteinuria. Preeclampsia was defined as new hypertension that develops after week 20 of pregnancy with proteinuria (protein dipstick test ≥1+ or proteinuria ≥300 mg/24h or ≥0.3 g/L) or evidence of adverse conditions (abdominal right upper quadrant pain, headaches, visual disturbance, shortness of breath with $Sp0_2$ <95%, elevated creatinine) or complications (abruption placenta, disseminate intravascular coagulation, pulmonary edema, convulsions/eclampsia, stroke or coma, blood transfusion, elevated liver enzyme levels and or platelet count <50 x 10^9/L) [31]. Information on GDM and impaired glucose tolerance (IGT) were obtained through chart review of the results of the glucose challenge test and oral glucose tolerance test according to Canadian guidelines [32,33] and described in detail elsewhere [34] and in Supplemental (Table S1). Preterm birth was defined as birth at <37 weeks gestation based on chart review [35].

Cardiometabolic Health Measures

The MIREC parent-child cohort study has had a number of waves of follow-up since 2011, mainly focusing on the health of the child [36,37]. However, most recently in Phase I of our MIREC-Endocrine and Metabolic Function study (MIREC-ENDO) we invited mothers to a follow-up study to measure maternal cardiometabolic health outcomes (Supplemental Figure S1). Consenting participants attended a clinic visit and completed a questionnaire that assessed their obstetrical history, smoking status, sociodemographics and stressful life events. Height (Seca 217™ Stadiometer), weight and body fat percentage were measured (Tanita™ SC-240 Scale). Weight was measured twice and if the results differed by > 0.1 kg a 3rd measured was

obtained. Body fat percentage was measured using the validated bioelectrical impedance analysis method [38,39] which measures body composition based on the rate at which a safe low-level electrical current travels through the body – adipose tissues slows the rate of the electrical current given its greater resistance (impedance) than lean mass or water [40]. These measurements were most often made in the morning given women were also giving a fasting blood sample at the clinic visit. Healthy body fat percentage ranges from 23-33% [41]. Blood pressure (OMRON, intelli sense HEM 742) was measured twice in the sitting position, 1 minute apart. If the measures differed by > 10 mm/Hg a 3rd measure was done. Participants also provided a fasting blood sample which was subsequently measured for glucose, cholesterol (low density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (TC), triglycerides (TG), high-sensitivity C-reactive protein (hsCRP), leptin and adiponectin.

Covariates

We selected potential confounders using a Directed Acyclic Graph (DAG) [43,44] for those factors potentially associated with pregnancy complications and cardiometabolic risk factors but that were not conceptualized as intermediate variables (along the causal pathway) or colliders (common consequences) (Supplemental Figure S2). This included maternal age and pre-pregnancy BMI as continuous variables and the following categorical variables: maternal income, education, parity, smoking status (ever vs. never), marital status (married vs. other - which included common-law, divorced, separated or single), education level (University degree vs less than this), household income (< or ≥ $100,000 CDN), and time spent walking in pregnancy in minutes/day (quartiles) and fasting status. The sample was not sufficiently diverse to consider ethnicity as a covariate. Excess wight gain and breastfeeding were included in the descriptive analysis. Excess gestational weight gain (yes/no) was defined as weight gain greater

than the recommendation by the Institute of Medicine (IOM), which considers the pre-pregnancy BMI [45,46]. Information on breastfeeding history was obtained at follow-up from the participants.

Research Ethics

The MIREC study and its follow-up study, MIREC-ENDO, received research ethics board approval at the MIREC study coordinating center (CHU Ste-Justine in Montreal) and Health Canada, along with the other 10 study sites across Canada including: Children's and Women's Health (Vancouver), University of Alberta (Edmonton), Health Sciences Center and St-Boniface Hospital (Winnipeg), Mount Sinai Hospital (Toronto), McMaster University Medical Center (Hamilton), Sudbury Regional Hospital (Sudbury), Kingston General Hospital (Kingston), The Ottawa Hospital (Ottawa), CHU Ste-Justine (Montreal), Jewish General Hospital (Montreal), IWK Health Centre (Halifax). All participants provided informed consent for the study.

Laboratory Analysis

Fasting glucose, lipids and hsCRP were measured at the Advanced Research and Diagnostics Laboratory (ARDL) at the University of Minnesota using the Roche Cobas 6000 Chemistry analyzer (Roche Diagnostics, Indianapolis, IN 46250). Glucose was measured in serum by the Roche hexokinase method (04404483 190 Glucose Reagent Pack, Roche Diagnostics, Indianapolis, IN 46250). The lower limit of detection is 2 mg/dL. The normal sample volume is 2 μL. Total cholesterol was measured in serum using a cholesterol oxidase method (03039773 190 Cholesterol Reagent Pack, Roche Diagnostics, Indianapolis, IN 46250) on a Roche Cobas 6000 Chemistry Analyzer (Roche Diagnostics Corporation). This method incorporates cholesterol esterase and perioxidase in the reagent and monitors cholesterol oxidation at 500 nm upon conversion of 4-aminoantipyrine to quinoneimine. The lower limit of detection is 3.86

mg/dL. The normal sample volume is 2 µL. HDL-cholesterol was measured directly in serum using the Roche HDL-Cholesterol 3rd generation direct method (07528566 190 HDL Reagent Pack, Roche Diagnostics, Indianapolis, IN 46250) on a Roche Cobas 6000 Chemistry Analyzer. In this method, a magnesium/dextran sulfate solution is first added to the specimen to form water-soluble complexes with non-HDL cholesterol fractions. The lower limit of detection is 3 mg/dL. The normal sample volume is 2.4 µL. Triglyceride (TG) were measured in serum using Triglyceride L reagent (20767107 322 Triglycerides Reagent Pack, Roche Diagnostics, Indianapolis, IN 46250) on the Roche cobas c502 chemistry analyzer. The lower limit of detection is 9 mg/dL. The normal sample volume is 2 µL. LDL-cholesterol was calculated in plasma specimens having a triglyceride value <400 mg/dL using the formula of Friedewald LDL = TC - HDL - TG/5.0 (mg/dL) [42]. hsCRP was measured in serum using latex-particle enhanced immunotubidimetric assay kit (04628919 190 CRPhs Reagent Pack, Roche Diagnostics, Indianapolis, IN 46250) and read on the Roche Cobas 6000 Chemistry analyzer. The normal sample volume is 6 µL. A Bio-Rad® Bio-plex® Luminex® Human Premixed Multi-Analyte Kit (Catalog No. LXSAHM) was used to measure Leptin and adiponectin at the CHUQ lab in Quebec at the University of Laval with a Luminex® MAGPIX® Analyzer.

Statistical Analysis

We created a dichotomous composite risk factor variable called "pregnancy complications" and included those with a HDP (preeclampsia or gestational hypertension), preterm birth or GDM or IGT in the MIREC Index Pregnancy (Figure 1). These specific complications were selected based on previous evidence indicating that preeclampsia, hypertension in pregnancy, gestational diabetes and preterm birth are associated with future cardiovascular disease [7,8]; for other pregnancy complications and birth outcomes the evidence is less established [47].

We used multivariable linear regression to determine if pregnancy complications (all, and HDP only) were associated with each of the selected cardiometabolic outcomes measured at approximately nine years postpartum: body fat percentage, weight retention, SBP, DBP, fasting glucose, cholesterol (TC, LDL, HDL, TG), hsCRP, leptin and adiponectin. The comparison group was uncomplicated pregnancies. This included those participants who had no history of pregnancy complications (GDM, HDP or preterm birth) in the MIREC index pregnancy, pregnancies prior to MIREC (past pregnancies) or pregnancies after the MIREC index pregnancies (future pregnancies) (Supplemental Figure S3). We took the average of the two closest measures for blood pressure, weight, height, and body fat percentage. We assessed the pattern of missing covariate information by examining the distribution of our outcomes in those missing key covariate information and those with complete information (See Supplemental Tables S2). We removed outliers in our adjusted models (3*IQR). Outliers included weight retention lost > -23 kg (n=1); glucose >136 mg/dL (n=2); LDL >216 mg/dL (n=1); TG >259 mg/dL (n=2); CRP >11 mg/L (n=5). We assumed this information was missing at random and used Multiple Imputation using Chained Equations (MICE) [48] to impute missing covariate information (m=50 datasets) using SAS PROC MI. We log transformed those outcomes with highly skewed distributions (TC, LDL, HDL, TG, CRP, leptin and adiponectin) upon visual inspection to approximate normality. For those outcomes, we expressed the beta estimates as a percent change $[(e^{\beta} - 1) \times 100]$ for those with a pregnancy complication verses uncomplicated pregnancies. In sensitivity analyses we examined the effect of additional adjustment for excess weight gain in the multivariable models, and we examined the association between recurrent pregnancy complications (Supplemental Figure S3) and cardiometabolic health. Assumptions of

homoskedasticity and normality were assessed using residual plots. All statistical analyses were performed using SAS Enterprise Guide 7.15 (SAS Institute Inc., Cary, NC 2017).

RESULTS

Study Population

Of 2001 participants who consented to the original MIREC study, 77% (n=1539) provided consent to be contacted for future research, 1103 (72% of 1539) were contacted, and just over half (53%) of this group (n=590) agreed to participate in the MIREC-ENDO study. This follow-up study consisted of a Clinic Visit or an at Home Adaptation, due to Covid-19 restrictions (See Supplemental Figure S1). A total of 310 mothers completed a MIREC ENDO Clinic Visit of which three did not provide blood samples, three were currently pregnant, four had a history of chronic hypertension (HTN), and another seven had a diagnosis of diabetes prior to enrollment in the MIREC study leaving a total of 293 participants. There were 56 participants who experienced a pregnancy complication (HDP, preterm birth, GDM/IGT), 36 who had a HDP in the MIREC index pregnancy and 184 participants who had uncomplicated pregnancies (Figure 1).

Participant Characteristics

The participants in this study reported that they were mostly pre-menopausal (~60%) at the time of the MIREC-ENDO follow-up with a mean age of close to 42 years (Tables 1, Table 2, Supplemental Table S3). Participants with a pregnancy complication or with HDP tended to be of lower income, were less educated, and more likely to be overweight or obese prior to the MIREC birth and gained excess weight during the MIREC index pregnancy. 44% of women who developed a HDP were obese versus only 8% of those with uncomplicated pregnancies

according to BMI. A large portion of participants who developed a pregnancy complication or HDP (68% and 76% respectively) gained excess weight [49] during the MIREC index pregnancy compared to only 51% of those with an uncomplicated pregnancy. Women with a pregnancy complication (57%) or HDP (53%) tended to be pregnant with a male baby more often during the MIREC index pregnancy versus those who had uncomplicated pregnancies (40% male).

Descriptive Analysis

At follow-up, those who developed HDP during the MIREC index pregnancy had a body fat percentage in the obese range (>39%), on average, while those with uncomplicated pregnancies were in the "healthy range" between 23-33% (Table 2). At follow-up, average and median fasting blood glucose, blood pressure and lipid measures all fell within the normal ranges. However, mean values were higher (or lower in the case of HDL cholesterol) among those with HDP or pregnancy complications. Median hsCRP concentrations at follow-up were close to the recommended cut-off of 3 mg/L in those with pregnancy complications or HDP in the MIREC index pregnancy whereas participants with no history of pregnancy complications had median hsCRP concentrations below 1 mg/L. Leptin concentrations tended to be higher among those with pregnancy complications however adiponectin levels appeared similar across groups.

Multivariate Analysis

Systolic and Diastolic Blood Pressure

In the multivariable analysis, for women with a HDP in the MIREC Index pregnancy, both the mean SBP and mean DBP were higher at follow-up than among those with uncomplicated pregnancies (Mean Differences: SBP 11.92 mmHg, 95% CI: 7.14, 16.70; DBP: 6.03 mmHg, 95% CI:2.42, 9.63) (Table 3). When examining the dichotomous composite exposure variable - 'any pregnancy complication', the results were somewhat attenuated but mean blood pressure

measures remained higher in the exposed group (Mean Differences: SBP 8.96 mmHg, 95% CI: 5.13, 12.80; DBP 5.47 mmHg, 95% CI: 2.57, 8.36) (Table 3).

Body Fat Percentage and Weight Retention

Body fat percentage was an average of 2.5% (95% CI: -0.4, 5.38) and 2.6% (95% CI: 0.28, 4.82) higher in those who had a HDP or any pregnancy complication in the MIREC Index pregnancy respectively, relative to those with no history of pregnancy complications. Body fat percentage was even more elevated (3.6%, 95% CI: 0.2%, 7.0%) in those participants reporting a recurrent pregnancy complication compared to uncomplicated pregnancies (Supplemental Table S4). We did not see any evidence for an association between pregnancy complications and weight retention, except for a borderline association with recurrent pregnancy complications (Supplemental Table S4). Those who reported a recurrent pregnancy complication were on average 2.8 kg (95% CI: -0.8 kg, 6.3 kg) heavier than their pre-pregnancy weight 9 years earlier compared to those participants who reported uncomplicated pregnancies.

Plasma Glucose

Fasting plasma glucose at follow-up was elevated among those with any pregnancy complication at baseline (3.40 mg/dL 95% CI: 0.38, 6.41) or with recurrent pregnancy complications (Supplemental Table S4) compared to those who had uncomplicated pregnancies.

Lipids

The point estimates for TC, LDL and TG were elevated but statistically insignificant in women with HDP or a pregnancy complication in the MIREC index pregnancy compared to those with uncomplicated pregnancies.

C-Reactive Protein

The mean hsCRP value was higher among women with HDP (32% higher), pregnancy complications (26% higher) or recurrent pregnancy complications (41% higher) compared to women with uncomplicated pregnancies however these estimates had wide confidence intervals ranging from a small negative percent change to a large positive percent change.

Leptin and adiponectin

Leptin levels were 18% and 21% higher in participants with HDP and pregnancy complications, respectively, compared to participants who had uncomplicated pregnancies, but the estimated associations were similarly imprecise (Table 3). Those with recurrent pregnancy complication showed even higher leptin concentrations (53%, 95% CI: 2.7%, 129%) compared to uncomplicated pregnancies and lower adiponectin (-12%, 95% CI: -22.7%, 0.1%) (Supplemental Table S4).

Sensitivity Analysis

Additional adjustment for excess weight gain, or the removal of those on hypertensive medication or taking hypoglycemic agents did not appreciably change the results (Supplemental Table S5-S6).

DISCUSSION

In this cohort of mainly pre-menopausal women, we found that women who had a HDP or any pregnancy complication (preterm birth, HDP and/or GDM or IGT) had, on average, higher levels of systolic and diastolic blood pressure 9 years later compared to women who had uncomplicated pregnancies (Table 3). We also observed that body fat percentage was on average higher in women with a history of a HDP, any pregnancy complication (preterm birth, GDM/IGT, or

HDP) or a recurrent pregnancy complication compared to women who had uncomplicated pregnancies. These associations were substantially unchanged after controlling for important potential confounding variables, including pre-pregnancy BMI. We also observed elevations in fasting glucose and markers of inflammation and insulin resistance at follow-up among participants with baseline pregnancy complications.

Blood pressure has been shown to be one of the most modifiable risk factors for CVD [50]. Similar to other authors [11,12,51] we found elevations in both systolic and diastolic blood pressure among women with a history any pregnancy complication (HDP, GDM/IGT, and/or preterm birth) or specifically HDP. Several studies have reported a higher risk of hypertension or cardiovascular-related morbidity in the years following a HDP, gestational diabetes or preterm birth [6–8,11]. A recent meta-analysis of 38 studies [13] found a combined elevation of 8.3 mmHg (95%: 6.8, 9.7) and 6.8 mmHg (95% CI: 5.6, 8.0) in SBP and DBP respectively following a preeclampsia/eclampsia. Importantly, elevations in blood pressure including pre-hypertension or hypertension in mid-life has been shown to increase lifetime risk for CVD including heart attack or stroke [50].

We observed that women with a history of a pregnancy complication or recurrent pregnancy complications had a higher body fat percentage 9 years later. The consequences of this could be far reaching. Body fat percentage has been linked to Type II diabetes [52] and fat-to-muscle ratio with Coronary Artery Disease [53]. That we observed these changes even after controlling for pre-pregnancy BMI raises questions as to whether pregnancy complications themselves are

associated with long-term metabolic changes, independent of existing pre-pregnancy risk. In a sensitivity analysis we additionally adjusted for excess weight gain in pregnancy and found little change in the estimates (Supplemental Table S5). Few studies have examined the long-term implications of pregnancy complications on body composition. McLennon et al. [54] conducted a full metabolic study 6.7 months postpartum among 22 women who had preeclampsia and 75 normotensive women. Women with a history of preeclampsia had a higher BMI and fat mass percentage (40.7 ± 7.4) at 6 months postpartum compared to normotensive women (34.9 ± 8.1) but this study did not control for pre-pregnancy BMI. That study identified evidence of a more sedentary lifestyle in women with previous preeclampsia, indicating a role for pre-pregnancy risk.

Our point estimates suggested elevated levels of leptin and lower levels of adiponectin at follow-up among participants who experienced a HDP or a pregnancy complication during the MIREC index pregnancy versus those who had uncomplicated pregnancies, although estimated associations were imprecise. The association with adiponectin was stronger among those who had a recurrent pregnancy complication; this group had 12% lower adiponectin concentrations on average (β=-12.0%, 95% CI:-22.7%, 0.01%) compared to those who had uncomplicated pregnancies. Leptin is synthesized mainly by white adipocyte tissues and plays a large role in appetite control [21]. It is also highly expressed in the placenta [55]. Women with gestational diabetes mellitus [55,56] and preeclampsia show elevations in leptin in the 3rd trimester [57]. Adiponectin is a protein which is product of adipose tissue and has insulin-sensitizing, anti-inflammatory and antiatherogenic activity [28]. Studies suggest that lower adiponectin levels are associated with GDM and preeclampsia [24,26,58] but few studies have examined levels in the

postpartum period. Boyadzhieva et al. [28] found reduced adiponectin levels in participants who had GDM and this was maintained into the early postpartum period (8 weeks)[28]. In a prospective study, Girouard et al.[29] observed higher leptin and lower adiponectin at approximately 8 years (range 5-13 years) after the index pregnancy in participants who had a HDP. These alterations in leptin and adiponectin may contribute to future insulin resistance.

Fasting plasma glucose concentrations were somewhat elevated, with the strongest association being for those who had any pregnancy complication compared to those with uncomplicated pregnancies. The direction of this association is in agreement with a number of studies showing fasting glucose elevations in the years following preeclampsia/eclampsia [13], and GDM [59,60]. The point estimates for lipid levels were elevated for TC, LDL and TG, but the 95% confidence interval did not exclude the null effect. Elevated levels of LDL and TG are risk factors for the initiation and progression of atherosclerosis leading to hypertension and CVD [61,62]. In a meta-analysis of 29 studies, TC was higher and HDL cholesterol was lower in women with a previous history of preeclampsia/eclampsia; while 24 studies showed elevations in LDL cholesterol among these women compared to normotensive pregnancies [13].

We found elevated hsCRP approximately 9 years later among participants with HDP compared to those with uncomplicated pregnancies. However, the confidence intervals were wide and crossed the null. hsCRP has been deemed a robust biomarker of chronic systemic inflammation and a risk marker for CVD and insulin resistance [63,64]. Studies suggest that levels above 3 mg/L put people at higher risk of cardiovascular events [65]. hsCRP also has relatively high within

person reproducibility over time compared to other markers, even though it can increase acutely during an infection or ischemic stress [19]. A number of studies show elevations in hsCRP in women with pregnancy complications [58,66] however, fewer studies have explored the long-term impact of pregnancy complications on hsCRP concentrations in the following decade. A recent meta-analysis of 11 studies comparing post-pregnancy hsCRP concentrations among women who experienced preeclampsia/eclampsia relative to those with normotensive pregnancies found these women had slightly higher hsCRP levels; however the follow-up time ranged from 1 to 32 years across the studies [13]. Studies that examined women more than a decade after HDP show elevations of hsCRP at eighteen [11] and twenty [10] and thirty [19] years post index pregnancy. The Genetic Epidemiology Network of Arteriopathy followed up 405 women with a self-reported history of a hypertensive disorder in pregnancy and 1800 normotensive pregnancy women at a median age of 61 years and found that those who had a HDP had a higher odds of elevated hsCRP (>3 mg/L) compared to those with normotensive pregnancies [67]. In a nested case-control study only 8 years post-delivery, Girouard et al. [29] found higher hsCRP concentrations in 168 women who had hypertension in pregnancy and 168 controls (matched for age and year of delivery of index pregnancy). Other studies have not seen strong elevations in participants with preeclampsia within the first year [68], or decade [51,69,70] following the index pregnancy or more than 2 decades later [71]. Inconsistencies across these studies may be due to the varying follow-up times and inclusion of both pre- and post-menopausal women.

Strengths and Limitations

A strength of this study is that it is a prospective cohort study that directly measured and collected health information in pregnancy (e.g. measured blood pressure during pregnancy and proteinuria) and at follow-up. A number of key covariates including pre-pregnancy BMI were well documented. We did not have measures of body composition in the pre-pregnancy period. We did not have information on family history of CVD among 1st degree relatives in our study which is needed to calculate the Framingham Risk Score. For individuals between the ages of 30-59 (without a diagnosis of diabetes), the presence of premature CVD in a first degree relative increases that individual's Framingham Risk Score by 2 fold [61]. Finally, our study is limited by its modest sample size which affected the precision of our estimates. Also, the MIREC participants are mainly of high socioeconomic status and limited diversity with most self-identifying as 'white' ethnicity making our result non-generalizable to the Canadian population. However, de facto restrictions of this nature may reduce residual confounding. Finally, we only have 1 time point of our cardiometabolic measures post pregnancy.

Conclusions

In this prospective study of mainly healthy pre-menopausal women, we found elevations in blood pressure, body fat percentage, and markers of inflammation and insulin resistance 9 years post index pregnancy in women who experienced a pregnancy complication, or specifically a HDP compared to women who had uncomplicated pregnancies. We suggest, given the imprecision of many of our estimates, that larger studies explore the long-term impact of pregnancy complications on body composition and markers of inflammation and insulin resistance.

REFERENCES

1. Norris, C. M. *et al.* State of the Science in Women's Cardiovascular Disease: A Canadian Perspective on the Influence of Sex and Gender. *J. Am. Heart Assoc.* **9**, (2020).
2. Izadnegahdar, M. *et al.* Do younger women fare worse? Sex differences in acute myocardial infarction hospitalization and early mortality rates over ten years. *J. Women's Heal.* **23**, 10–17 (2014).
3. Wu, P., Park, K. & Gulati, M. Pregnancy as a Predictor of Cardiovascular Disease The Fourth Trimester: *Eur. Card. Rev.* **16**, (2021).
4. Minissian, M. B. *et al.* Association of spontaneous preterm delivery and future maternal cardiovascular disease. *Circulation* **137**, 865–871 (2018).
5. Rich-Edwards, J. W., Fraser, A., Lawlor, D. A. & Catov, J. M. Pregnancy characteristics and women's future cardiovascular health: An underused opportunity to improve women's health? *Epidemiol. Rev.* **36**, 57–70 (2014).
6. Brown, M. C. *et al.* Cardiovascular disease risk in women with pre-eclampsia : systematic review and meta-analysis. *Eur. J. Epidemiol.* **28**, 1–19 (2013).
7. Bellamy, L., Casas, J., Hingorani, A. & Williams, D. Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. *BMJ* **335**, (2007).
8. Grandi, S. M. *et al.* Cardiovascular Disease-Related Morbidity and Mortality in Women with a History of Pregnancy Complications: Systematic Review and Meta-Analysis. *Circulation* **139**, 1069–1079 (2019).
9. Brouwers, L. *et al.* Recurrence of pre-eclampsia and the risk of future hypertension and cardiovascular disease: a systematic review and meta-analysis. *BJOG An Int. J. Obstet. Gynaecol.* **125**, 1642–1654 (2018).
10. Freeman, D. J. *et al.* Short- and Long-Term Changes in Plasma Inflammatory Markers Associated With Preeclampsia. 708–714 (2004) doi:10.1161/01.HYP.0000143849.67254.ca.
11. Fraser, A. *et al.* Associations of pregnancy complications with calculated cardiovascular disease risk and cardiovascular risk factors in middle age: The avon longitudinal study of parents and children. *Circulation* **125**, 1367–1380 (2012).
12. Hromadnikova, I., Kotlabova, K., Dvorakova, L. & Krofta, L. Maternal cardiovascular risk assessment 3-to-11 years postpartum in relation to previous occurrence of pregnancy-related complications. *J. Clin. Med.* **8**, 1–21 (2019).
13. Alonso-Ventura, V. *et al.* Effects of preeclampsia and eclampsia on maternal metabolic and biochemical outcomes in later life: a systematic review and meta-analysis. *Metabolism.* **102**, 154012 (2020).
14. Pradhan, A. D., Manson, J. E., Rifai, N., Buring, J. E. & Ridker, P. M. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *J. Am. Med. Assoc.* **286**, 327–334 (2001).
15. Danesh, J. *et al.* Low grade inflammation and coronary heart disease: Prospective study and updated meta-analyses. *Br. Med. J.* **321**, 199–204 (2000).
16. Ridker, P. M., Hennekens, C. ., Buring, J. E. & Nader, R. C-reactive protein and other markers of

inflammation in the prediction of cardiovascular disease in diabetes. *N. Engl. J. Med.* **342**, 836–43.

17. Ridker, P. M., Buring, J. E., Cook, N. R. & Rifai, N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. *Circulation* **107**, 391–397 (2003).

18. Alberti, K. G. M. M. *et al.* Harmonizing the metabolic syndrome: A joint interim statement of the international diabetes federation task force on epidemiology and prevention; National heart, lung, and blood institute; American heart association; World heart federation; International . *Circulation* **120**, 1640–1645 (2009).

19. Hubel, C. A. *et al.* C-Reactive Protein is Elevated 30 Years After Eclamptic Pregnancy. *Hypertensoin* **51**, 1499–1505 (2008).

20. Kampmann, U., Knorr, S., Fuglsang, J. & Ovesen, P. Determinants of Maternal Insulin Resistance during Pregnancy: An Updated Overview. *J. Diabetes Res.* **2019**, (2019).

21. Rondinone, C. M. Adipocyte-derived hormones, cytokines, and mediators. *Endocrine* **29**, 81–90 (2006).

22. Tanigaki, K. *et al.* C-reactive protein causes insulin resistance in mice through fcg receptor IIB-mediated inhibition of skeletal muscle glucose delivery. *Diabetes* **62**, 721–731 (2013).

23. Tan, H. C. *et al.* Mother's pre-pregnancy BMI is an important determinant of adverse cardiometabolic risk in childhood. *Pediatr. Diabetes* **16**, 419–426 (2015).

24. Pheiffer, C., Dias, S., Jack, B., Malaza, N. & Adam, S. Adiponectin as a potential biomarker for pregnancy disorders. *Int. J. Mol. Sci.* **22**, 1–18 (2021).

25. Solomon, C. G. & Seely, E. W. Hypertension in pregnancy a manifestation of the insulin resistance syndrome? *Hypertension* **37**, 232–239 (2001).

26. Liu, Y. *et al.* Hypertension in Pregnancy Adiponectin Levels in Circulation and Breast Milk and mRNA Expression in Adipose Tissue of Preeclampsia Women Adiponectin Levels in Circulation and Breast Milk and mRNA Expression in Adipose Tissue of Preeclampsia Women. **31**, 40–49 (2012).

27. Miehle, K., Stepan, H. & Fasshauer, M. Leptin, adiponectin and other adipokines in gestational diabetes mellitus and pre-eclampsia. *Clin. Endocrinol. (Oxf).* **76**, 2–11 (2012).

28. Boyadzhieva, M., Atanasova, I., Zacharieva, S. & Kedikova, S. Adipocytokines during pregnancy and postpartum in women with gestational diabetes and healthy controls. *J. Endocrinol. Invest.* **36**, 944–949 (2013).

29. Girouard, J., Giguère, Y., Moutquin, J. M. & Forest, J. C. Previous hypertensive disease of pregnancy is associated with alterations of markers of insulin resistance. *Hypertension* **49**, 1056–1062 (2007).

30. Arbuckle, T. E. *et al.* Cohort profile: the maternal-infant research on environmental chemicals research platform. *Paediatr. Perinat. Epidemiol.* **27**, 415–425 (2013).

31. Borghese, M. M. *et al.* Individual, independent, and combined associations of toxic metals and manganese on hypertensive disorders of pregnancy: results from the MIREC Canadian pregnancy cohort. *Enviromental Heal. Perspect.*

32. Berger, H., Crane, J. & Farine, D. Screening for Gestational Diabetes Mellitus. *J. Obstet. Gynaecol. Canada* **25**, 96 (2003).

33. Canadian Diabetes Association. Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2008. *Can J. Diabetes* **32**, S1–S201 (2008).

34. Shapiro, G. D. *et al.* Exposure to phthalates, bisphenol A and metals in pregnancy and the association with impaired glucose tolerance and gestational diabetes mellitus: The MIREC study. *Environ. Int.* **83**, 63–71 (2015).

35. Fisher, M. *et al.* Association between toxic metals, vitamin D and Preterm Birth in the Maternal-Infant Research on Environmental Chemicals Study. *Paediatr. Perinat. Epidemiol.* **Under Revi**, (2022).

36. Ashley-Martin, J. *et al.* Blood metal levels and early childhood anthropometric measures in a cohort of Canadian children. *Environ. Res.* **179**, 108736 (2019).

37. Braun, J. M. *et al.* Associations of prenatal urinary bisphenol a concentrations with child behaviors and cognitive abilities. *Environ. Health Perspect.* **125**, 1–9 (2017).

38. Nuñez, C., Gallagher, D. & Heymsfield, S. B. *Bioimpedance Analysis: A New Approach to Measuring Resistance*. (1997).

39. Rubiano, F., Nuñez, C. & Heymsfield, S. B. *Validity of Consumer Model Bioimpedance Analysis System with Established Dual Energy X-Ray Absorptiometry (DXA)*. (1999).

40. Tanita. Tanita: How BIA Works. https://www.tanita.com/en/howbiaworks/.

41. Gallagher, D. *et al.* Healthy percentage body fat ranges: An approach for developing guidelines based on body mass index. *Am. J. Clin. Nutr.* **72**, 694–701 (2000).

42. Friedewald, W., Levy, R. & Fredrickson, D. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* **18**, 499–502 (1972).

43. Textor, J. & Hardt, J. DAGitty: A graphical tool for analyzing causal diagrams. *Epidemiology* at https://doi.org/10.1097/EDE.0b013e318225c2be (2011).

44. Hernán, M. A., Hernández-Díaz, S. & Robins, J. M. A structural approach to selection bias. *Epidemiology* (2004) doi:10.1097/01.ede.0000135174.63482.43.

45. National Research Council. *Guidelines on Weight Gain and Pregnancy*. *Guidelines on Weight Gain and Pregnancy* (2013). doi:10.17226/18291.

46. Gilmore, A. L., Klempel-Donchenko, M. & Redman, L. . Pregnancy as a window to future health: Excessive gstational weight gain and obesity. *Semin Perinatol* **39**, 296–303 (2015).

47. Cusimano, M. C., Pudwell, J., Roddy, M., Cho, C. K. & Smith, G. N. The maternal health clinic: an initiative for cardiovascular risk identification in women with pregnancy-related complications. *Am. J. Obstet. Gynecol.* **210**, 438.e1-438.e9 (2014).

48. Yuan, Y. Multiple Imputation for Missing Data: Concepts and New Development (Version 9.0). *J. Stat. Softw.* **45**, 1–25 (2011).

49. Widen, E. M. *et al.* Excessive gestational weight gain is associated with long-term body fat and weight retention at 7 y postpartum in African American and Dominican mothers with underweight, normal, and overweight prepregnancy BMI. *Am. J. Clin. Nutr.* **102**, 1460–1467 (2015).

50. Allen, N. *et al.* Impact of blood pressure and blood pressure change during middle age on the

remaining lifetime risk for cardiovascular disease: The cardiovascular lifetime risk pooling project. *Circulation* **125**, 37–44 (2012).

51. Portelinha, A. *et al.* Altered alanine aminotransferase and γ-glutamyl transpeptidase in women with history of preeclampsia: Association with waist-to-hip ratio and body mass index. *Eur. J. Gastroenterol. Hepatol.* **21**, 196–200 (2009).

52. Zhang, S., Jiang, H., Wang, L. & Jia, X. Longitudinal relationship between body fat percentage and risk of type diabetes in Chinese adults : Evidence from the China Health and Nutrition Survey.

53. Eun, Y. *et al.* Fat-to-muscle ratio: A new indicator for coronary artery disease in healthy adults. *Int. J. Med. Sci.* **18**, 3738–3743 (2021).

54. McLennan, S. L. *et al.* Maternal Adiposity and Energy Balance after Normotensive and Preeclamptic Pregnancies. *J. Clin. Endocrinol. Metab.* **106**, E2941–E2952 (2021).

55. Pérez-Pérez, A., Vilariño-García, T., Guadix, P., Dueñas, J. L. & Sánchez-Margalet, V. Leptin and nutrition in gestational diabetes. *Nutrients* **12**, 1–18 (2020).

56. Xu, J. *et al.* Maternal circulating concentrations of tumor necrosis factor-alpha, Leptin, and Adiponectin in gestational diabetes mellitus: A systematic review and meta-analysis. *Sci. World J.* **2014**, (2014).

57. El Shahat, A. M., Ahmed, A. B., Ahmed, M. R. & Mohamed, H. S. Maternal serum leptin as a marker of preeclampsia. *Arch. Gynecol. Obstet.* **288**, 1317–1322 (2013).

58. Retnakaran, R. *et al.* Low adiponectin concentration during pregnancy predicts postpartum insulin resistance, beta cell dysfunction and fasting glycaemia. *Diabetologia* **53**, 268–276 (2010).

59. Bellamy, L., Casas, J. P., Hingorani, A. D. & Williams, D. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. *Lancet (London, England)* **373**, 1773–1779 (2009).

60. García-Patterson, A., Balsells, M., Solà, I., Gich, I. & Corcoy, R. Impact of gestational diabetes mellitus treatment on medium/long-term outcomes after pregnancy: A systematic review and meta-analysis. *Diabet. Med.* 1–11 (2022) doi:10.1111/dme.14998.

61. Pearson, G. J. *et al.* 2021 Canadian Cardiovascular Society Guidelines for the Management of Dyslipidemia for the Prevention of Cardiovascular Disease in Adults. *Can. J. Cardiol.* **37**, 1129–1150 (2021).

62. Adank, M. C. *et al.* Is maternal lipid profile in early pregnancy associated with pregnancy complications and blood pressure in pregnancy and long term postpartum? *Am. J. Obstet. Gynecol.* **221**, 150.e1-150.e13 (2019).

63. Cozlea, D. L. *et al.* The impact of C reactive protein on global cardiovascular risk on patients with coronary artery disease. *Curr. Heal. Sci. J.* **39**, 225–31 (2013).

64. Ruiz-Ramie, J. J. *et al.* Cardiovascular health trajectories and elevated C-reactive protein: The CARDIA study. *J. Am. Heart Assoc.* **10**, (2021).

65. Pearson, T. A. *et al.* Markers of inflammation and cardiovascular disease: Application to clinical and public health practice: A statement for healthcare professionals from the centers for disease control and prevention and the American Heart Association. *Circulation* **107**, 499–511 (2003).

66. Hamadeh, R., Mehsen, A., Kobeissy, F., Karouni, A. & Akoum, H. C-Reactive Protein for Prediction or Early Detection of Pre-Eclampsia: A Systematic Review. *Gynecol. Obstet. Investig.*

86, 13–26 (2021).

67. Brown, C. M. *et al.* Hypertension in pregnancy is associated with elevated C-reactive protein levels later in life. *J Hypertens* **31**, 2213–2219 (2013).

68. Smith, G. N. *et al.* A history of preeclampsia identifies women who have underlying cardiovascular risk factors. *Am. J. Obstet. Gynecol.* **200**, 58.e1-58.e8 (2009).

69. Lampinen, K. H., Rönnback, M., Groop, P. H. & Kaaja, R. J. A relationship between insulin sensitivity and vasodilation in women with a history of preeclamptic pregnancy. *Hypertension* **52**, 394–401 (2008).

70. Drost, J. T. *et al.* Cardiovascular risk factors in women 10 years post early preeclampsia: The Preeclampsia Risk EValuation in FEMales study (PREVFEM). *Eur. J. Prev. Cardiol.* **19**, 1138–1144 (2012).

71. Spaan, J. J. *et al.* Insulin resistance relates to microvascular reactivity 23years after preeclampsia. *Microvasc. Res.* **80**, 417–421 (2010).

Table 1: Participant Characteristics and Pregnancy Complications in the MIREC Index Pregnancy vs Uncomplicated Pregnancies

Characteristic	Values	Pregnancy Complication (Preterm, GDM or IGT, or HDP) in MIREC Index (n=56)		HDP in MIREC Index (n=36)		Uncomplicated (n=187): MIREC Index, past or future	
		n	%	n	%	n	%
Household Income	< $100,000 CDN	40	73	26	**74**	96	52
	≥ $100,000 CDN	15	27	9	26	87	48
Education	Less than University Degree	23	41	16	**44**	46	25
	University Degree	33	59	20	56	141	75
Marital	Married	35	63	20	56	130	70
	Other – mainly common-law	21	38	16	44	57	30
Born in Canada	yes	48	86	34	94	163	87
	no	8	14	2	6	24	13
Ethnicity	other	4	8	2	6	6	3
	white	49	92	33	94	174	97
Smoking	never	36	64	23	64	124	66
	ever in life*	20	36	13	36	63	34
Parity	0	24	43	19	53	92	49
	1+	32	57	17	47	95	51
Sex at birth (infant)	0 - Male	32	57	19	53	75	40
	1 - Female	24	43	17	47	112	60
Walking in pregnancy	<30 minutes per day	14	25	8	22	47	25
	30 to <60 minutes per day	13	23	9	25	55	30
	60 to <120 minutes per day	12	21	9	25	30	16
	≥120 minutes per day	17	30	10	28	54	29
Pre-pregnancy BMI	underweight/normal	21	40	10	29	124	70
	overweight	14	26	9	26	38	21
	obese	18	34	15	44	15	8
Singleton Pregnancy	yes	52	93	34	94	187	100
	no	4	7	2	6	0	0
Extra weight gain in pregnancy according to the IOM guidelines	no	17	32	8	24	86	49
	Yes	36	68	26	76	91	51
	Pre	34	62	21	60	111	59

Menopausal Status at follow-up (ENDO) – self reported	Peri/Post	12	22	7	20	33	18
	Other	9	16	7	20	43	23
Breastfeeding history (total)**	< 6 months of breastfeeding	25	52	14	48	63	40
	≥ 6 months of breastfeeding	23	48	15	52	93	60

*very few current smokers so current smokers were combined with former smokers.
**collected at follow-up (MIREC-ENDO); based on all pregnancies.
Note: Only 4% of the sample reported that they were post-menopausal.

Table 2: Distribution of continuous characteristics and cardiometabolic outcomes among participants

Participant Characteristics	Units	Pregnancy Complications		Hypertensive Disorders in Pregnancy (HDP)		Uncomplicated Pregnancies (n=187)		Reference Ranges (when available)*
		mean (SD)	median (IQR)	mean (SD)	median (IQR)	mean (SD)	median (IQR)	
Age at enrollment	y	32 (4.7)	32 (7)	32 (5.2)	32 (8)	33 (4.4)	32 (7)	
Age at follow-up	y	42 (8.5)	41 (9)	43 (5.4)	41 (9)	42 (4.7)	41 (8)	
Time gap between visits	y	9.5 (0.9)	9.0 (1.0)	9.5 (0.8)	9.0 (1.0)	9.4 (0.8)	9.0 (1.0)	
Pre-pregnancy BMI MIREC (self-report)	kg/m^2	27.6 (6.6)	26.3 (9.4)	28.6 (6.5)	28.3 (9.4)	23.9 (4.1)	23.0 (4.8)	18.5–24.9 = normal[b] 25.0–29.9 = overweight/pre-obese 30.0 and over = obese
Pre-pregnancy weight (self-report)	kg	74.3 (19.2)	70 (20.4)	78.0 (19.2)	75.3 (21.8)	66.0 (12.9)	63.5 (15.0)	
Gestational weight gain (MIREC INDEX)	kg	16.1 (6.9)	15.2 (7.6)	16.9 (7.6)	15.8 (9.1)	15.4 (5.4)	15.1 (5.7)	
BMI at follow-up (measured)	kg/m^2	29.7 (6.8)	28.4 (9.4)	30.8 (6.8)	31.7 (8.4)	25.5 (5.35)	24.5 (6.4)	18.5–24.9 = normal 25.0–29.9 = Overweight/Pre-obese 30.0 and over = Obese
Weight at follow-up (measured)	kg	80.8 (21.1)	79.5 (22.1)	85.3 (22.2)	83.6 (23.8)	71.1 (15.7)	68 (18.3)	
Weight retention at follow-up	kg	5.7 (10.7)	6.3 (8.9)	4.8 (12.2)	6.6 (8.3)	4.5 (7.1)	3.0 (8.0)	
Body Fat Percentage at follow-up	%	38.2 (7.7)	39.5 (11.2)	39.8 (7.4)	40.9 (9.3)	32.8 (8.4)	32.6 (11)	For 40-59 yrs <23 “underfat” 23-33 healthy range 34-39 “overfat” >39% obese[d]
Fasting Glucose at follow-up	mg/dL	96.9 (20.4)	90.5 (17)	95.2 (19.1)	90 (18)	88.4 (8.7)	88 (11)	Fasting: 70-110 mg/dl[a]
Systolic BP at follow-up	mm/Hg	123.2 (15.8)	119 (20)	127.4 (16.3)	126.5 (22)	111.4 (11)	110.5 (11)	>135 mmHg (Stage 1 HTN)[e]
Diastolic BP at follow-up	mm/Hg	84.0 (10.4)	84 (13.5)	85.9 (10.4)	85 (14)	76.1 (9.9)	75 (11)	>85 mmHg (Stage 1 HTN) [e]
Total Cholesterol at follow-up	mg/dL	185.4 (32.7)	180 (36)	188.8 (27.3)	183 (26)	176.0 (34.6)	168.5 (43)	<200 mg/dL[a]

LDL Cholesterol at follow-up	mg/dL	105.7 (29.3)	102.7 (33.7)	107.9 (27.2)	109.5 (35.2)	96.9 (29.0)	89.4 (30.4)	<130 mg/dL[a]; <135mg/dL (3.5 mmol/L)[f]
HDL Cholesterol at follow-up	mg/dL	58.2 (15.0)	55.4 (21.7)	57.8 (16.2)	51.9 (26.2)	62.0 (13.3)	61.1 (18.7)	>35 mg/dL[a]
Triglycerides at follow-up	mg/dL	108.7 (75.6)	82 (91)	118.3 (85.0)	99.5 (92)	85.5 (42.5)	73.5 (44)	40-150 mg/dL[a]
High sensitivity C-reactive Protein (hsCRP) at follow-up	mg/L	3.9 (5.9)	2.5 (4.1)	3.6 (3.7)	2.8 (3.3)	2.1 (2.8)	0.8 (2.2)	≤ 3 mg/L
Leptin at follow-up	ng/mL	26.6 (20.5)	24.3 (23.4)	27.5 (18.8)	25.0 (25.3)	16.3 (13.2)	12.5 (15.0)	
Adiponectin at follow-up	ng/mL	12.3 (4.4)	11.5 (4.8)	12.6 (4.7)	12 (4.1)	13.0 (3.2)	12.8 (4.3)	

*References:

[a]Royal College of Physicians and Surgeons. Clinical Laboratory Tests, reference values. TMCE 07- 2017

[b] https://www.canada.ca/en/health-canada/services/food-nutrition/healthy-eating/healthy-weights/canadian-guidelines-body-weight-classification-adults/questions-answers-public.html#a2

[c] Rakhmat, I. I. *et al.* Cardiometabolic risk factors in adults with normal weight obesity: A systematic review and meta-analysis. *Clin. Obes.* **12**, (2022).

[d]Tanita Scale Manual. Body Composition Analyzer. Sc-240/SC-240IM Instruction Manual. Based on Gallagher et al. 2000. Healthy percentage body fat ranges: an approach for development guidelines based on body mass index. Am J Clin Nutr. 72: 694-701

[e] Musameh, M., Tomaszewski, M., Williams, B. Hypertension - a clinical update for physicians. Clinical Medicine Journal. April 2013. https://doi.org/10.7861/clinmedicine.13-2-182

f Pearson, G. J. *et al.* 2021 Canadian Cardiovascular Society Guidelines for the Management of Dyslipidemia for the Prevention of Cardiovascular Disease in Adults. *Can. J. Cardiol.* 37, 1129–1150 (2021).

Table 3: Multivariable Linear Regression: change in outcome in those with Hypertensive Disorders in Pregnancy (HDP, n=36) and those with pregnancy complications (n=56) vs those who had uncomplicated pregnancies (n=187)

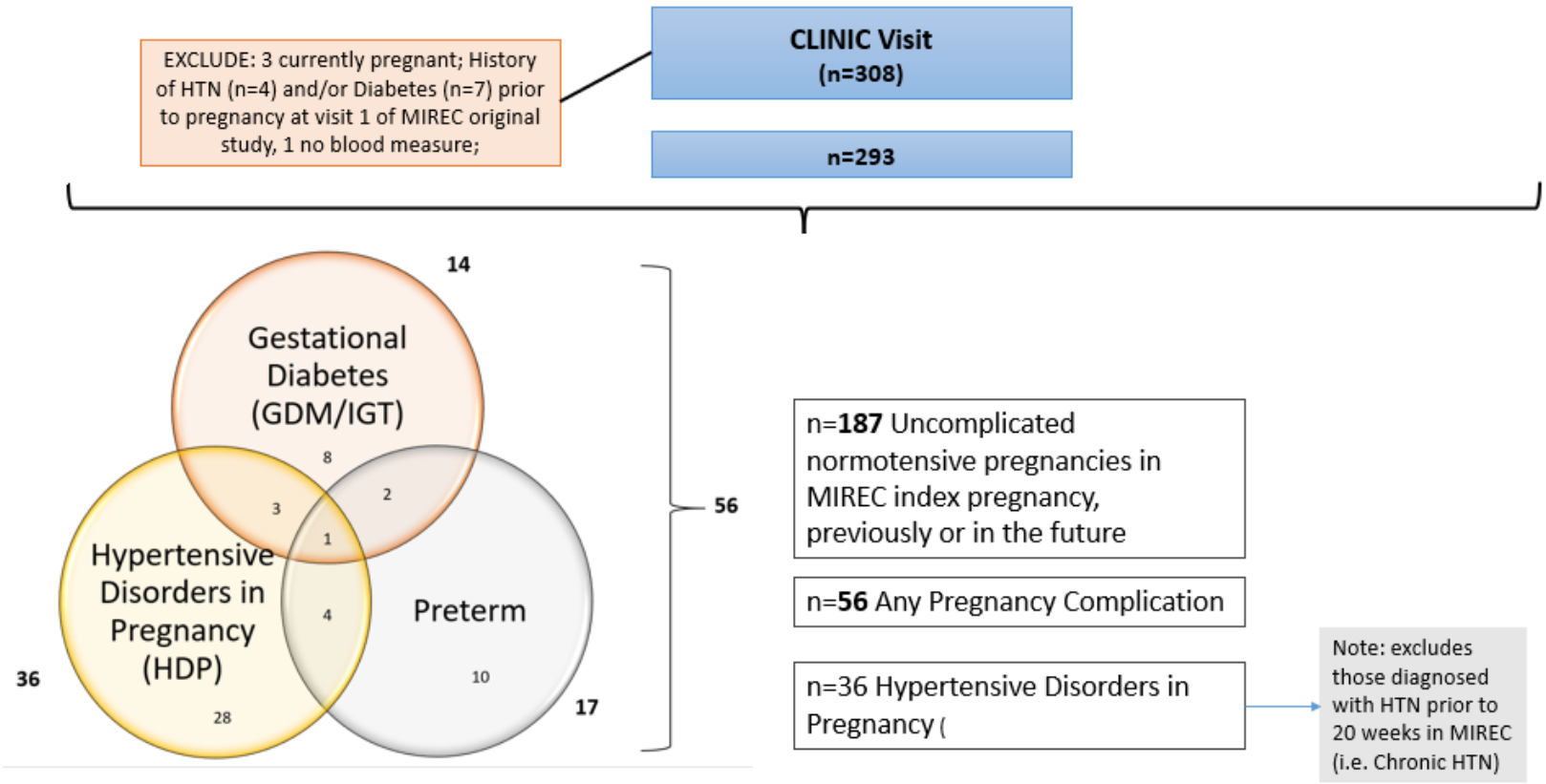
		Hypertensive Disorders in Pregnancy (HDP) only			Pregnancy Complications (HDP, preterm, IGT or GDM)		
OUTCOME	**MODEL**	**β Estimate**	**LCI**	**UCI**	**β Estimate**	**LCI**	**UCI**
Body Fat Percentage (%)	Crude	6.99	3.74	10.24	5.39	2.71	8.07
	Adjusted	2.48	-0.43	5.38	2.55	0.28	4.82
Systolic BP (mmHg)	Crude	16.01	11.35	20.66	11.83	7.88	15.77
	Adjusted	11.92	7.14	16.70	8.96	5.13	12.80
Diastolic BP (mmHg)	Crude	9.77	6.27	13.28	7.93	4.99	10.88
	Adjusted	6.03	2.42	9.63	5.47	2.57	8.36
Weight retention (kg)	Crude	0.35	-2.84	3.54	1.22	-1.41	3.85
	Adjusted	0.15	-3.33	3.64	1.11	-1.68	3.90
	Adjusted2[a]	0.15	-3.33	3.64	1.11	-1.68	3.90
Glucose (mg/dL)	Crude	6.76	2.73	10.79	8.52	4.72	12.32
	Adjusted	4.06	-0.14	8.27	5.63	1.84	9.43
	Adjusted2 [a]	1.84	-1.73	5.42	3.40	0.38	6.41
Log Transformed Outcomes[b]		**%change**	**%LCU**	**%UCI**	**%change**	**%LCU**	**%UCI**
Total Cholesterol	Crude	8.1	1.2	15.3	5.7	0.0	11.7
	Adjusted	5.3	-1.8	12.9	3.9	-1.8	10.0
LDL Cholesterol	Crude	12.4	1.4	24.5	9.7	0.7	19.5
	Adjusted	5.1	-5.7	17.1	5.0	-3.6	14.5
	Adjusted2 [a]	5.7	-4.9	17.5	5.5	-3.0	14.8
HDL Cholesterol	Crude	-8.1	-15.4	-0.2	-7.0	-13.2	-0.4
	Adjusted	0.5	-7.7	9.5	-0.1	-6.6	6.9
Triglycerides	Crude	27.9	7.9	51.6	17.9	1.9	36.4
	Adjusted	9.1	-8.8	30.6	2.9	-10.9	19.0
	Adjusted 2 [a]	6.2	-10.7	26.2	1.4	-11.8	16.6
C-Reactive Protein	Crude	102.6	32.1	210.8	82.4	26.3	163.3
	Adjusted	36.9	-11.4	111.5	28.2	-10.1	82.9

	Adjusted2 [a]	32.0	-14.6	104.0	26.2	-11.6	80.0
Leptin	Crude	78.5	28.2	148.6	63.5	23.6	116.3
	Adjusted	18.3	-15.0	64.8	20.5	-7.6	57.1
Adiponectin	Crude	-5.2	-14.1	4.6	-7.2	-14.6	0.8
	Adjusted	-2.6	-12.7	8.6	-4.5	-12.6	4.3

MICE imputation for missing covariate information (m=50 times)
adjusted for: maternal age, pre-pregnancy BMI, parity, smoking status, marital status, education level, income, time spent walking in pregnancy, fasting status

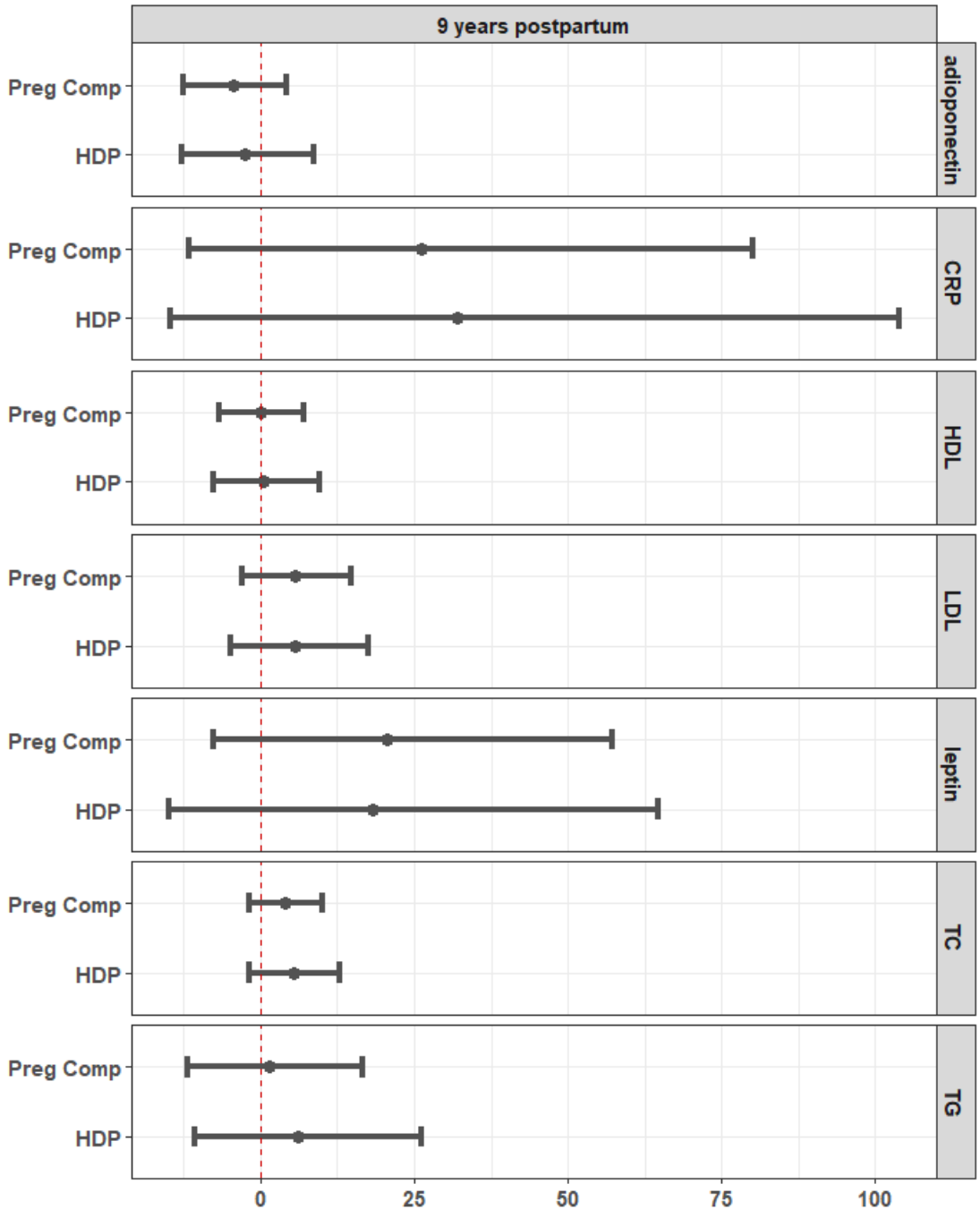
a. removal of outliers (3*IQR; upper or lower)
   Outlier details:
   weight retention lost > -23 kg, (n=1)
   glucose >136 mg/dL (n=2)
   LDL >216 mg/dL (n=1)
   TG >259 mg/dL(n=2)
   CRP >11 mg/L (n=5)
b. Log-transformed because of skewed distribution. Estimates expressed as percent change in those with HDP or Pregnancy complications verses uncomplicated pregnancies. $(e^{\beta} - 1) \times 100$

Figure 1: Study Flow Diagram MIREC-ENDO



50 participants were excluded from the main analysis because they had self-reported pregnancy complication prior to (n=27) or after (n=14) the MIREC index pregnancy but not in the MIREC Index pregnancy and therefore not considered in the "Uncomplicated pregnancies"; or they were missing pregnancy complication information in the MIREC Index pregnancy (n=9) (See Supplemental Figure S3).
1 person who had GDM also developed chronic HTN and is included in the "Any Pregnancy Complication" group but excluded from the Hypertensive Disorders in Pregnancy (HDP) group.
Note: 187 (uncomplicated)+56 (pregnancy complications)+50 (described above)=293

Figure 2: Percent change in cardiometabolic measure 9 years postpartum for participants who experienced a hypertensive disorder in pregnancy (HDP) or a pregnancy complication (Preg Comp) in the MIREC index pregnancy verses those that had uncomplicated pregnancies.



Estimates expressed as percent change in those with HDP or Pregnancy complications versus uncomplicated pregnancies ($e^{\beta} - 1$) x 100; CRP=high sensitivity C-reactive protein; LDL=low density lipoprotein; HDL=high density lipoprotein; TC=total cholesterol; TG=triglycerides

The Association Between Pregnancy Complications with Long-Term Maternal Cardiometabolic Health in the MIREC Cohort Study.

Supplemental Information

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8 - The Ottawa Hospital Research Institute;
9 – Dalhousie University
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Table SI: Criteria for Gestational Diabetes Mellitus (GDM) and Impaired Glucose Tolerance (IGT).

75g OGTT	100g OGTT
↓ fasting glucose ≥5.3 mmol/L, ↓ 1-hour glucose ≥10.6 mmol/L ↓ 2-hour glucose ≥ 8.9 mmol/L	↓ fasting glucose ≥5.8 mmol/L, ↓ 1-hour glucose ≥10.6 mmol/L ↓ 2-hour glucose ≥ 9.2 mmol/L ↓ 3-hour glucose ≥8.0 mmol/L
If 2 or more of these values are met or exceeded, diagnosis of GDM is made.	
If 1 of these values is met or exceeded, then a diagnosis of IGT is made.	

Berger, H., Crane, J. & Farine, D. Screening for Gestational Diabetes Mellitus. *J. Obstet. Gynaecol. Canada* **25**, 96 (2003).

Canadian Diabetes Association. Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2008. *Can J. Diabetes* **32**, S1–S201 (2008).

Table S2: Characteristics of those with missing key variables

	mean		geometric mean	
	not missing	missing	not missing	missing
Outcome				
Systolic BP (mmHg)	114.4	115.2	113.3	114.4
Diastolic BP (mmHg)	77.9	79.4	77.5	79.0
Body Fat Percentage	34.0	35.3	32.8	34.1
Fasting glucose	90.9	88.9	90.0	88.2
Total Cholesterol	178.9	161.0	175.9	159.2
LDL-Cholesterol	99.6	80.9	95.6	76.7
HDL-Cholesterol	61.3	61.3	59.7	59.7
Triglycerides	90.6	94.1	80.6	82.3
C-reactive Protein	2.4	2.6	1.2	1.0
Leptin	18.4	19.1	12.9	14.6
Adiponectin	13.0	12.3	12.6	11.6

Missing category (n=34 participants) =missing one of key covariates used in analysis (income, fasting status, pre-pregnancy BMI, extra weight (excess weight gain). The highest percent missing was for breastfeeding history. This variable was only used in the descriptive analysis but not in the modeling. Pre-pregnancy BMI and excess weight gain had slightly higher than 5% missing. We assumed the data was Missing at Random (MAR).

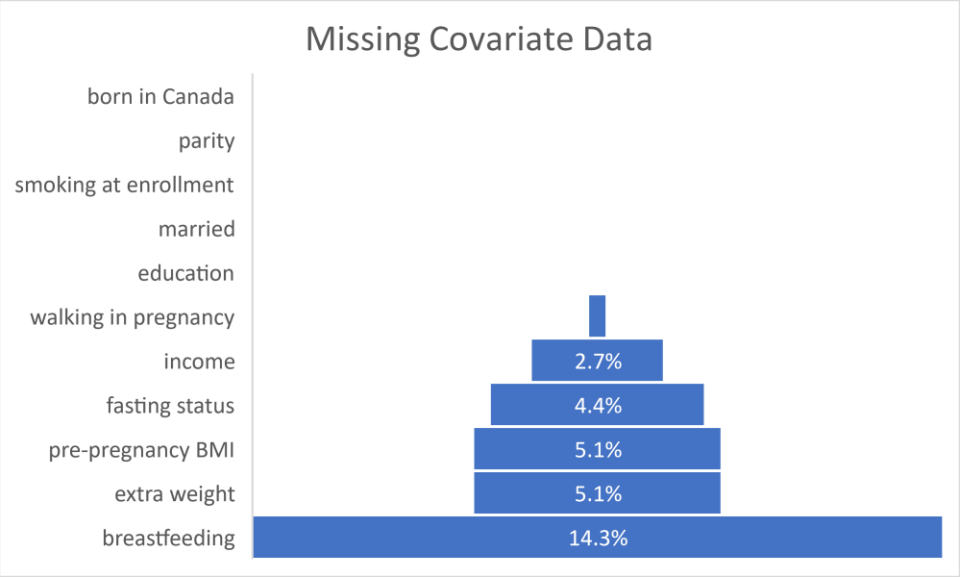


Table S3: Further Description of participants

Characteristic		Hypertensive Disorders in Pregnancy (HDP)		Any Pregnancy Complication		Uncomplicated Pregnancies	
		n=36		n=56		n=187	
		n	%	n	%	n	%
Assisted Reproductive Technology (ART)	no	35	97	53	95	173	93
(MIREC INDEX Pregnancy)	yes	1	3	3	5	14	7
Future pregnancy complication: after MIREC INDEX	no	27	84	43	84	175*	100
(GestHTN, PE or GD, Preterm singleton)	yes	5	16	8	16	0	0
Past Pregnancy complications (prior to MIREC INDEX)	no	24	75	36	71	175*	100
(GestHTN, PE or GD, Preterm singleton)	yes	8	25	15	29	0	0
Family Hx of HTN in pregnancy	no	30	83	47	84	169	90
(collected at baseline - MIREC)	yes	6	17	9	16	18	10
High Blood Pressure	no	33	94	51	93	184	98
(collected at follow-up - ENDO)	yes	2	6	4	7	3	2
If yes, do you take medication	yes	1	3	3	5	1	<1
	No	1	3	1	2	2	<1
Diabetes at follow-up	no	32	91	51	93	187	100
(collected in ENDO 9 years postpartum)	yes	3	9	4	7	0	0
	Type I	0	0	0	0	0	
	Type II	3	9	4	7	0	
If yes, do you take medication for your Diabetes							
	Take Hypoglycemic agents	2		3	6		
	Take Insulin	0		0	0		

*12 participants who did not have any pregnancy complications in the MIREC Index Pregnancy did not have information on past or future pregnancy complications.

Table S4: Sensitivity Analysis: Multivariable Linear Regression: average change in outcome in those with **Recurrent Pregnancy Complications** (n=19) vs those who had uncomplicated pregnancies (n=187)

Outcome	Model	β Estimate	LCI	UCI
Body fat %	Crude	6.43	2.36	10.49
	Adjusted	**3.62**	**0.21**	**7.04**
Systolic BP	Crude	12.66	6.64	18.68
	Adjusted	**11.18**	**5.32**	**17.03**
Diastolic BP	Crude	10.51	5.96	15.06
	Adjusted	**8.67**	**4.22**	**13.11**
Weight Retention	Crude	3.43	0.04	6.82
	Adjusted	2.76	-0.80	6.33
	Adjusted2*	**2.76**	**-0.80**	**6.33**
Glucose	Crude	12.09	6.72	17.45
	Adjusted	9.71	4.34	15.09
	Adjusted2*	**4.95**	**0.26**	**9.64**
		%change	%LCI	%UCI
Total Cholesterol	Crude	3.22	-5.29	12.50
	Adjusted	3.01	-5.66	12.48
LDL Cholesterol	Crude	8.40	-5.07	23.78
	Adjusted	5.76	-7.50	20.93
	Adjusted2*	5.53	-7.44	20.31
HDL Cholesterol	Crude	-11.21	-19.88	-1.60
	Adjusted	-4.76	-14.01	5.49
Triglycerides	Crude	20.56	-2.62	49.26
	Adjusted	8.55	-12.16	34.15
	Adjusted2*	9.66	-11.12	35.30
C-Reactive Protein	Crude	101.66	15.27	252.78
	Adjusted	45.77	-15.04	150.11
	Adjusted2*	41.00	-16.65	138.54
Leptin	Crude	99.82	30.57	205.78
	Adjusted	**53.53**	**2.72**	**129.49**
Adiponectin	Crude	-15.65	-25.42	-4.60
	Adjusted	**-12.04**	**-22.68**	**0.07**

See Figure S2 for more details on Recurrent Pregnancy Complications.

MICE imputation for missing covariate information (m=50 times)
adjusted for: maternal age, prepregnancy BMI, parity, smoking status, marital status, education level, income, time spent walking in pregnancy
*outliers removed 3*IQR

Table S5: Sensitivity Analysis: Multivariable Models for the association between Hypertensive Disorders in Pregnancy (HDP), and Pregnancy Complications (HDP, preterm or Gestational Diabetes/Impaired Glucose Tolerance) with additional covariate adjustment (**excess weight gain**).

Outcome	Model	HDP only			Pregnancy Complications (HDP, preterm, IGT or GDM)		
		β Estimate	LCI	UCI	β Estimate	LCI	UCI
Body fat percentage (%)	Adjusted	2.41	-0.52	5.33	2.49	0.21	4.76
Systolic BP (mmHg)	Adjusted	12.07	7.26	16.87	9.07	5.22	12.92
Diastolic BP (mmHg)	Adjusted	6.18	2.56	9.81	5.58	2.68	8.48
weight retention (kg)	Adjusted2*	-0.35	-3.76	3.06	0.80	-1.90	3.50
Glucose (mg/dL)	Adjusted2*	1.82	-1.77	5.41	3.37	0.35	6.40
Log transformed outcomes	Model	%change	%LCU	%UCI	%change	%LCU	%UCI
Total Cholesterol	Adjusted	5.19	-1.95	12.86	3.96	-1.77	10.02
LDL Cholesterol	Adjusted2*	5.80	-4.83	17.63	5.66	-2.88	14.96
HDL Cholesterol	Adjusted	0.49	-7.78	9.50	-0.02	-6.59	7.02
Triglycerides	Adjusted2*	5.51	-11.23	25.42	1.43	-11.79	16.63
C-Reactive Protein	Adjusted2*	31.05	-15.30	102.75	24.36	-11.69	75.12
Leptin	Adjusted	16.59	-16.20	62.22	19.32	-8.41	55.45
Adiponectin	Adjusted	-2.68	-12.79	8.59	-4.43	-12.51	4.39

MICE imputation for missing covariate information (m=50 times)
adjusted for: maternal age, prepregnancy BMI, parity, smoking status, marital status, education level, income, time spent walking in pregnancy, excess weight gain in pregnancy.
*adjusted for covariates and outliers removed 3*IQR

Log-transformed because of skewed distribution. Estimates expressed as percent change in those with HDP or Pregnancy complications verses uncomplicated pregnancies. $(e^{\beta} - 1) \times 100$

Excess weight gain defined according to Institute of Medicine Guidelines. National Research Council. *Guidelines on Weight Gain and Pregnancy*. *Guidelines on Weight Gain and Pregnancy* (2013). doi:10.17226/18291

Table S6: Sensitivity Analysis: Multivariable Models for the association between Hypertensive Disorders in Pregnancy (HDP), and Pregnancy Complications (HDP, preterm or Gestational Diabetes/Impaired Glucose Tolerance) with the **removal of those on hypertensive medication at follow-up**

Outcome	Model	HDP only			Pregnancy Complications (HDP, preterm, IGT or GDM)		
		β Estimate	LCI	UCI	β Estimate	LCI	UCI
Body fat percentage (%)	Adjusted	2.48	-0.44	5.39	2.43	0.10	4.76
Systolic BP (mmHg)	Adjusted	11.92	7.13	16.72	8.09	4.22	11.96
Diastolic BP (mmHg)	Adjusted	6.03	2.41	9.64	4.78	1.86	7.70
weight retention (kg)	Adjusted2*	0.15	-3.34	3.65	1.20	-1.63	4.03
Glucose (mg/dL)	Adjusted2*	1.30	-2.14	4.74	2.82	-0.17	5.81
Log transformed outcomes	Model	%change	%LCU	%UCI	%change	%LCU	%UCI
Total Cholesterol	Adjusted	5.3	-1.9	13.0	3.3	-2.4	9.4
LDL Cholesterol	Adjusted2*	6.0	-4.8	18.0	4.8	-3.8	14.1
HDL Cholesterol	Adjusted	0.3	-8.0	9.4	-0.3	-7.0	6.9
Triglycerides	Adjusted2*	5.2	-11.7	25.4	-0.6	-13.8	14.6
C-Reactive Protein	Adjusted2*	34.7	-11.7	105.3	27.3	-10.0	80.2
Leptin	Adjusted	15.4	-17.3	61.1	15.5	-12.0	51.5
Adiponectin	Adjusted	-1.9	-12.1	9.5	-3.7	-12.0	5.4

MICE imputation for missing covariate information (m=50 times)
adjusted for: maternal age, prepregnancy BMI, parity, smoking status, marital status, education level, income, time spent walking in pregnancy, fasting status
*adjusted for covariates and outliers removed 3*IQR

Log-transformed because of skewed distribution. Estimates expressed as percent change in those with HDP or Pregnancy complications verses uncomplicated pregnancies. $(e^{\beta} - 1) \times 100$

Figure S1: MIREC ENDO Study Participation

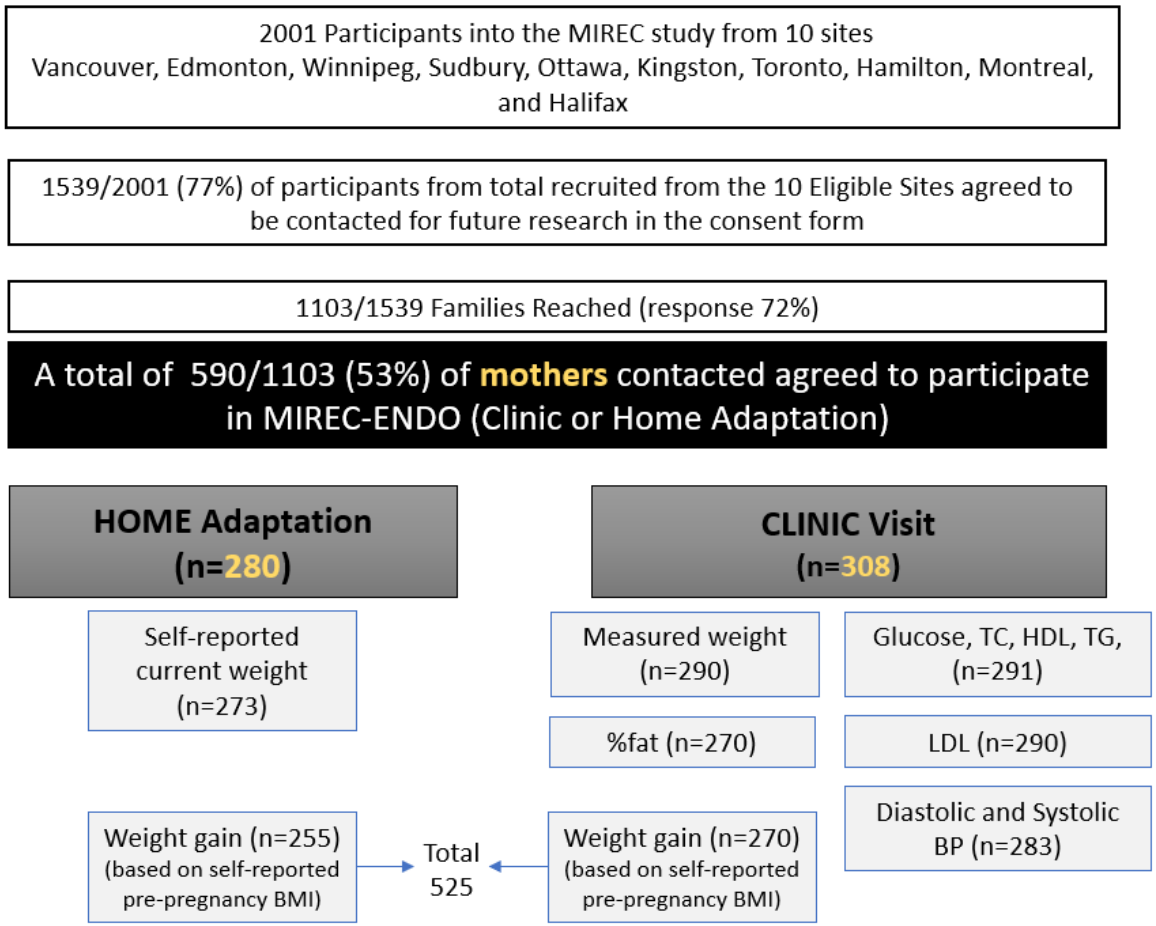
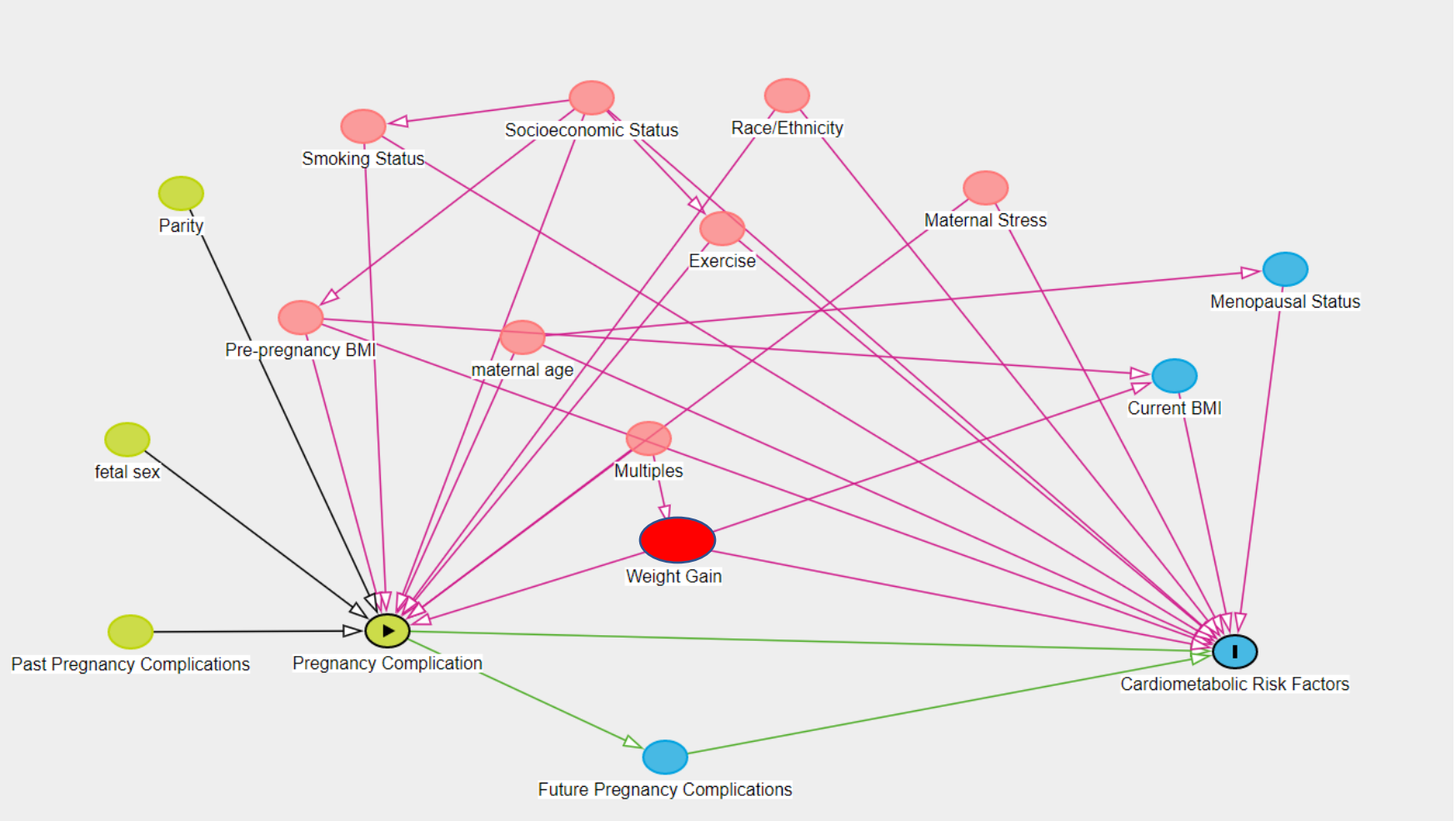
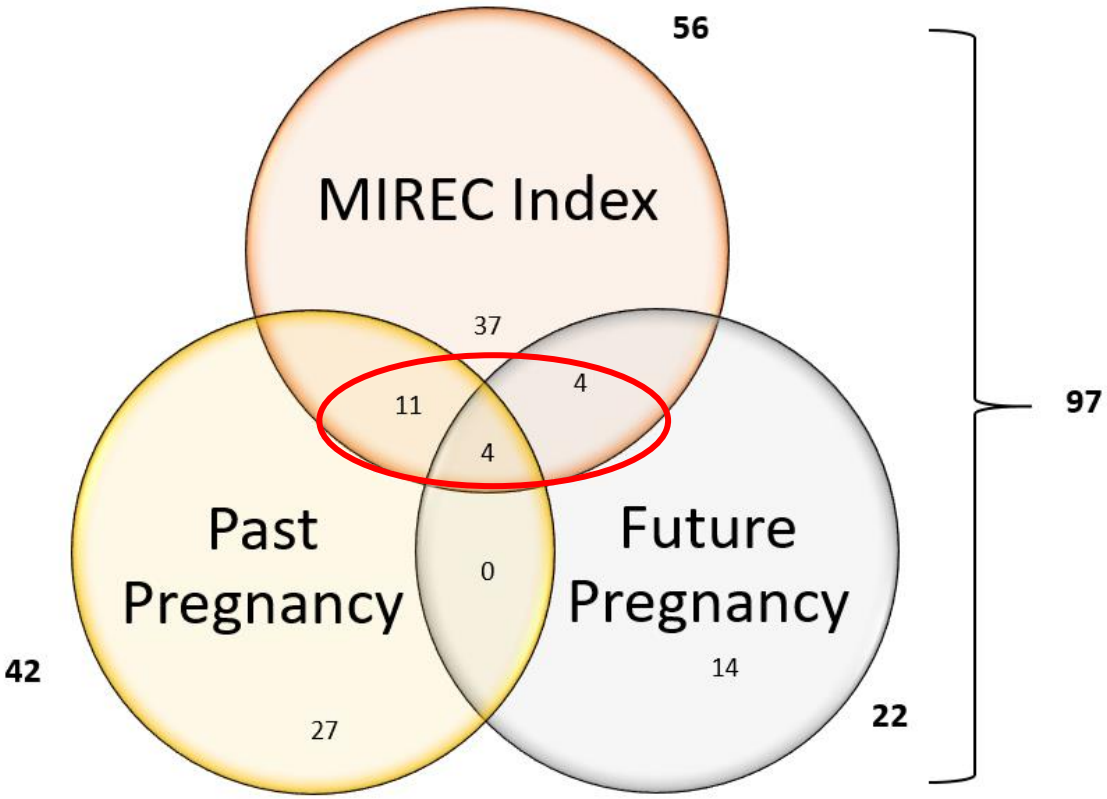


Figure S2: Directed Acyclic Graph (DAG) for Pregnancy Complications and Cardiometabolic Risk Factors



Note: Weight Gain may be along the causal pathway and was therefore adjusted for in a sensitivity analysis.

Figure S3: Timing of Pregnancy Complications in the MIREC Cohort (Preterm Birth, HDP, IGT or GDM)

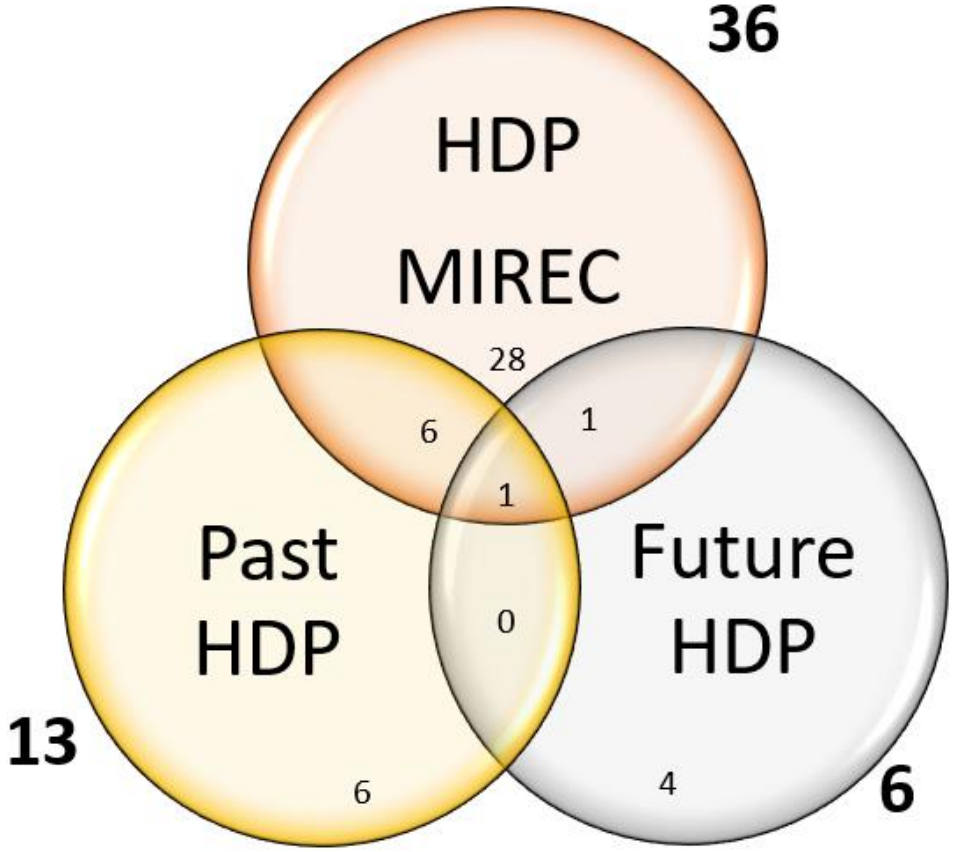


Because women gave information on their obstetrical history in the MIREC ENDO follow-up visit approximately 9 years postpartum we obtained information on all of their pregnancies. A total of 97 (37+14+27+11+4+4=97) participants had a pregnancy complication with their MIREC Index child, previous to the MIREC Index child (past pregnancy) or after the MIREC index child (future pregnancy). There were 56 women with a pregnancy complication (Preterm, GDM or IGT, HDP) in the MIREC Index Pregnancy, 42 women had a complication (Preterm, GDM, HDP) in a pregnancy prior to the MIREC Index pregnancy, and 22 women had a future pregnancy complication (Preterm, GDM, HDP). 11 women had a complication in a past pregnancy and the MIREC Index pregnancy, 4 had a pregnancy complication in MIREC and then again the future, and another 4 women had a pregnancy complication in a past pregnancy, the MIREC Index pregnancy and a future pregnancy. 19 participants had a recurrent pregnancy complication (red circle above).

50 women (27 past only complication + 14 future only compilation) were excluded from the main analysis because they had self-reported past or future pregnancy complications or missing information about pregnancy complications in MIREC (n=9)

19 participants had recurring pregnancy complications. Note: Past and Future Pregnancy Complications were self-report whereas the information on the MIREC index Pregnancy was compiled at study visits and through chart reviews.

Figure S4: Timing of Hypertensive Disorders in Pregnancy (HDP)



CHAPTER 5

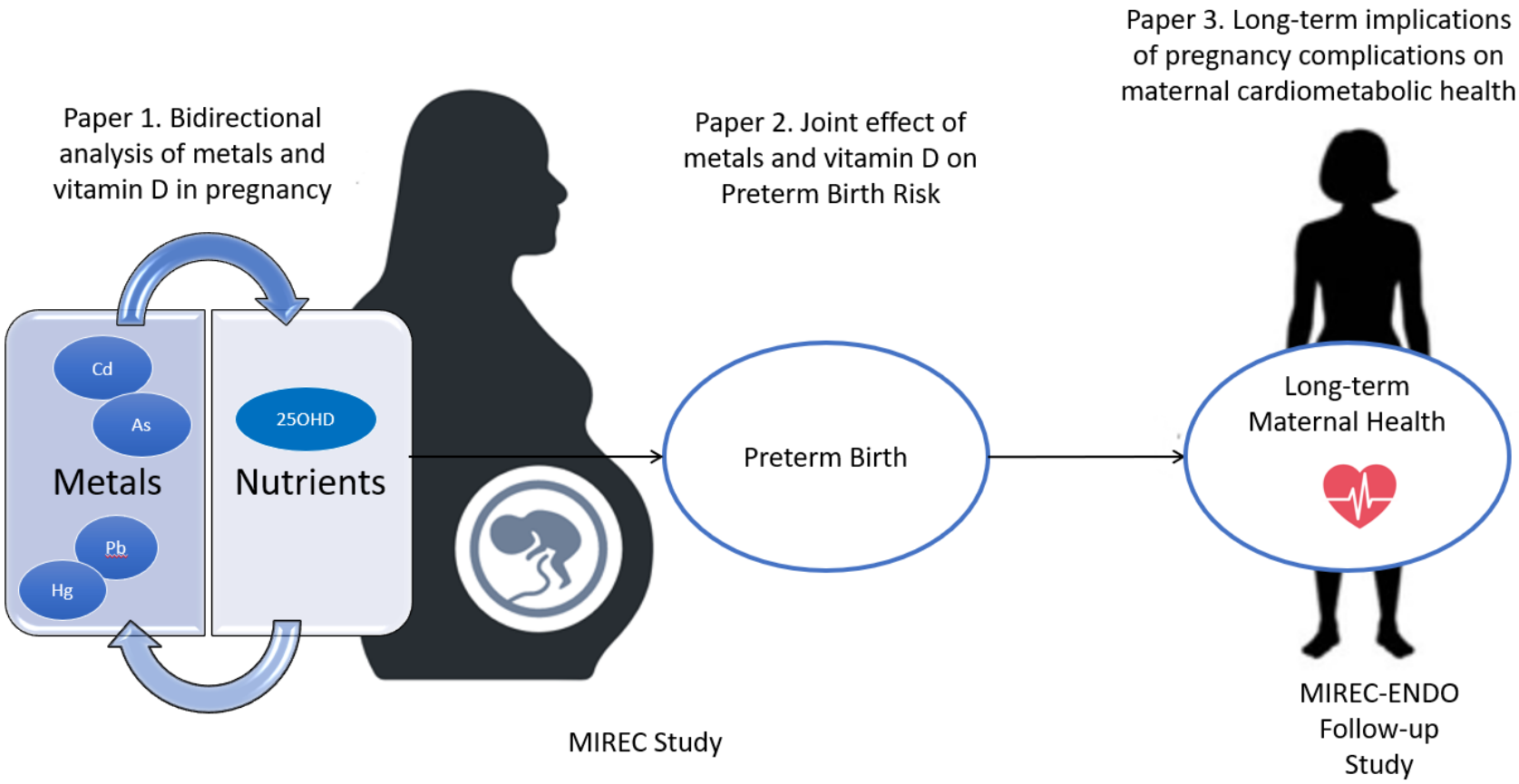
INTEGRATED DISCUSSION

1.0 Introduction

The Government of Canada's Chemicals Management Plan is "aimed at reducing the risks posed by chemical substances found in food and food products, consumer products, cosmetics, drugs, drinking water, and industrial releases to Canadians and the environment" [1]. This is done through risk assessment and management. It is clear that environmental chemicals are ubiquitous in our environment and among Canadians[2], and while many of these substances are used to improve the quality of our lives, many are also associated with disease risk [3,4]. Research has shown that some nutrients may modify the impact of chemical exposures by blocking their absorption or facilitating their excretion from the body. On the other hand, chemicals in the environment may disrupt the absorption and metabolism of nutrients. Nutrition is a practical means for intervention to help mitigate the effect of exposures given the pervasive nature of environmental chemicals [9, 10].

This thesis aimed to understand the impact of toxic metals on maternal health and the potential modifying role of vitamin D (Figure 1). Using data from the Maternal-Infant Research on Environmental Chemicals (MIREC) study, first, we investigated the direction of the relationship between the toxic metals cadmium (Cd) and lead (Pb) with 25-hydroxyvitamin D (25OHD) during pregnancy. Then, we investigated the association between metals and preterm birth and the potential modification of that association by vitamin D status, given what we learned in the first paper. Finally, we considered the longer-term impact of pregnancy complications, such as preterm birth, on maternal cardiometabolic health approximately 9 years after pregnancy (based on the MIREC index birth) using data from the most recent follow-up study of this cohort.

Figure 1: Dissertation Outline

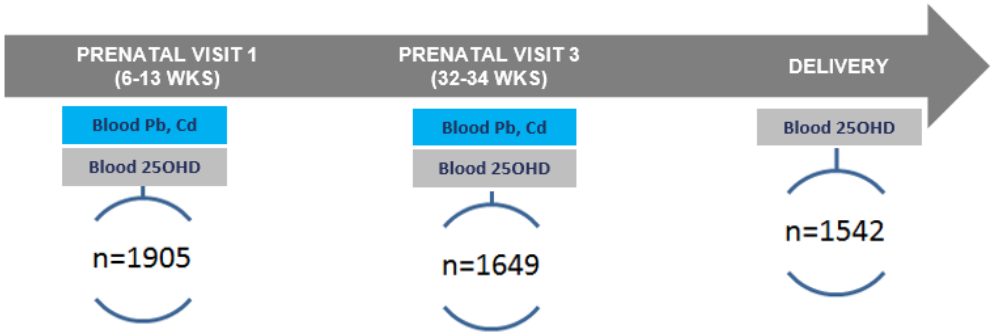


As=arsenic, Cd=cadmium, Pb=lead, Hg=mercury, 25OHD=25-hydroxyvitamin D

1.1 Summary of Paper #1: Blood Metals and Vitamin D Status in a Pregnancy Cohort: A Bidirectional Biomarker Analysis

Both toxic metals and low vitamin D status have shown associations with adverse pregnancy outcomes, such as preterm birth and preeclampsia [5–10]. Observational studies indicate that long-term, high level exposure to metals such as cadmium (Cd) and lead (Pb) affect vitamin D status in pregnancy [11–13]. Other observational studies bring into question the directionality of vitamin D and toxic metals, particularly at low-level exposures typical of current environmental exposures among the general population in North America. A number of studies suggest that higher vitamin D status or intake are associated with lower blood metals in pregnant women and children [14–16]. Therefore, the objectives of this study were to investigate the potential relationships between 25OHD, a biomarker of vitamin D status, and the blood metals cadmium

Figure 2: Biomarker Data by Visit



(Cd) and lead (Pb) measured in the 1st and 3rd trimester of pregnancy. We used longitudinal data from the MIREC study and investigated different statistical techniques (multivariable linear regression, latent growth curve analysis, and cross lagged panel models) to estimate the associations cross-sectionally (with 25OHD as the outcome), longitudinally (with 25OHD as the

outcome) and bidirectionally. The traditional multivariable linear regression analyses showed a weak inverse association between metals and 25OHD suggesting that higher Cd was associated with lower vitamin D concentrations cross-sectionally and longitudinally while the latent growth model displayed higher Pb to be associated with lower vitamin D at the intercept (baseline) but not with the vitamin D trajectory in pregnancy. In contrast, when we considered the potential for a bidirectional relationship using the crossed lagged panel model, the reverse held true. Higher 1st trimester 25OHD was associated with lower 3rd trimester Cd (-9%, 95% CI: -15%, -3%) and Pb (-3%, 95% CI: -7, 0.1%) . Higher metals in the 1st trimester were no longer associated with lower 25OHD. This method focuses on the causal direction by controlling for contemporaneous effects (cross-sectional), and the persistence of a biomarker over time, in order to rule out reverse causation. Thus, our conclusion was that 25OHD may modify the concentration of metals during pregnancy. We have not observed the use of cross lagged panel models in any other biomonitoring study of environmental chemicals; these models are used in psychological and sociological research.

1.2 Summary of Paper #2: Association between toxic metals, vitamin D and Preterm Birth in the Maternal-Infant Research on Environmental Chemicals Study (Paediatric and Perinatal Epidemiology, accepted for publication)

In this paper, we investigated the association between toxic metals and preterm birth and the potential modifying effect of vitamin D, given that our first paper suggested 25OHD may be associated with lower concentrations of Pb and Cd. We wanted to consider time in our analysis given the important role that time plays in pregnancy and preterm birth and in order to use all of

our data (time varying exposures and covariates). We also wanted to consider the mechanism of action by looking specifically at spontaneous preterm birth, a sub-category of preterm birth that could potentially be caused by inflammation or oxidative stress related to metals. Using discrete time survival analysis, we found that a 1 µg/dL increase in blood Pb concentration during pregnancy was associated with an increased risk of preterm birth (RR: 1.48, 95% CI: 1.00, 2.20); the relative risk was higher for spontaneous preterm birth (RR: 1.71, 95% CI: 1.13, 2.60). These associations were found to be stronger in those with low vitamin D concentrations (25OHD <50 nmol/L). A 1 µg/L increase in blood arsenic was also associated with a higher risk of preterm birth (RR=1.10, 95% CI: 1.02, 1.19) and spontaneous preterm birth (RR=1.11, 95% CI: 1.03, 1.20) but these associations were unaffected by vitamin D status. Few studies have explored the association between toxic metals at the low concentrations found in most Canadians and preterm birth; no other studies have examined the modifying effect of vitamin D.

1.3 Summary of Paper #3: The Association between Pregnancy Complications and Long-Term Maternal Cardiometabolic Health in the MIREC Cohort Study.

Reflecting the broader implications of exposures associated with pregnancy complications, this 3rd paper in the dissertation explored the potential long-term impact of pregnancy complications, such as preterm birth, on maternal health. Using data from MIREC's most recent follow-up study, MIREC-Endocrine and Metabolic function (MIREC-ENDO) we investigated the association of having a pregnancy complication in the MIREC index pregnancy with maternal cardiometabolic health approximately 9 years postpartum. We created a composite variable (pregnancy complications) that contained a combination of pregnancy complications (gestational

diabetes or impaired glucose tolerance, preterm birth, or hypertensive disorders in pregnancy) as well as hypertensive disorders in pregnancy (HDP) only. Unfortunately, we had too few preterm births to look at them as a separate category. Although a number of studies indicate that having a pregnancy complication increases the risk of cardiovascular disease and Type II diabetes later in life, few studies have the depth of directly measured cardiometabolic endpoints, collected at the approximately the same point in time (median 9 years, range 8-13 y) as the MIREC study. We observed that participants with HDP during pregnancy had significantly higher systolic (mean difference of 11.9 mm Hg, 95% CI 7.1, 16.7) and diastolic (6.0 mm Hg, 95% CI: 2.4, 9.6) blood pressure, and 32% (-14%, 104%) and 18% (-15%, 65%) higher high sensitivity-C-reactive protein (hsCRP) and leptin respectively, compared to participants who had uncomplicated pregnancies. We also observed higher percent body fat in those who had a pregnancy complication (percent change: 2.6%, 95% CI: 0.3%, 4.8%) or a recurrent pregnancy complication (percent change: 3.6%, 95% CI: 0.2%, 7.0%), compared to those who had uncomplicated pregnancies after controlling for a number of covariates including pre-pregnancy BMI. This paper gives some insight into the association of pregnancy complications with cardiometabolic health endpoints, just 9 years post index pregnancy in a population with only 4% of participants reporting that they were post-menopausal, and few reporting a diagnosis of hypertension (n=7) or Type II diabetes (n=4).

2.0 Main Points of Integration

2.1 Pregnancy as a Sensitive Window

Our research examined the association between environmental chemicals and preterm birth and then examined the long-term impact of pregnancy complications on maternal health. During

pregnancy, there are necessary physiological adaptations to accommodate the fetus [17] and an accumulation of evidence suggests that environmental chemicals may interrupt these adaptations causing pregnancy complications that have implications for both the mother and child [18]. For example, one area of physiological change during pregnancy involves matrix malloproteinases (MMPs), which act broadly to regulate the inflammatory process [19] and are involved in embryo implantation, placentation, and cervical dilation [20,21]. Studies have shown associations between metals and MMPs during pregnancy,[22,23] including the MIREC study [23]. Since inflammation and infection are considered risk factors for preterm birth [24], it is possible that this is part of the mechanism explaining the observed relationship between metals and preterm birth.

In turn, preterm birth is not only the leading cause of death among children under 5 years of age globally [25] but may also impact the health of the child for rest of their lives. The impact of the *in utero* environment on the future health of the child has been realized over the past 30 years with the developmental origins of health and disease (DoHaD) hypothesis [26,27], and this has become a key priority for research in some funding agencies [28]. The premise of DoHaD is that the fetus adapts to the maternal conditions during development shaping the structure and function of their organs and in turn affecting health and disease throughout the lifespan. Preterm birth is an independent risk factor for neurodevelopmental disorders [29] and is associated with an increased risk of childhood asthma [30], and cardiovascular diseases in young adulthood [31].

The impact of pregnancy on the long-term health of the mother was more recently recognized [32]. Studies have found that women who experience a pregnancy complication such as a hypertensive

disorder in pregnancy, preterm birth or gestational diabetes have at least double the risk of cardiovascular disease and Type II diabetes later in life [33–35]. The first maternal health clinic with a structured postpartum screening program for women who experienced a hypertensive disorder in pregnancy was established in Kingston Ontario in 2011[36]. In 2013, the task force for the American College of Obstetricians and Gynecologist recommended that women with a history of preeclampsia with a preterm birth or recurrent preeclampsia be assessed yearly for blood pressure, lipids, fasting blood glucose and BMI based on low quality evidence [37]. Further, an article by Rich-Edwards in 2009 [38] argued that many aspects of women's reproductive health, beyond pregnancy complications, provide important information about subclinical disease trajectories as the factors that contribute to cardiovascular disease (CVD) and cancers (inflammation, angiogenesis, thrombosis, insulin resistance and endocrine profiles) are also linked to abnormal menstrual patterns, female infertility and pregnancy complications. For example, age at menarche is linked with breast cancer risk [39], and women with irregular or long periods are more likely to develop CVD compared to women with regular periods. Our research found associations between pregnancy complications and markers of cardiometabolic health, just nine years postpartum. These markers were associated with increased body fat percentage, blood pressure, and fasting plasma glucose. Some of these associations were even stronger in those with recurrent pregnancy complications. Further research is required to better understand shared mechanisms behind a women's reproductive health and future risk of chronic disease. For example, are the factors such as underlying metabolic dysfunction that put women at increased risk of pregnancy complications, exacerbated by environmental chemical exposure, poor nutritional status and lifestyle factors, and setting these women on a trajectory of poorer long-term cardiometabolic health?

2.2 Nutrition as Potential Modifier

Environmental chemicals are everywhere and difficult to avoid given there is rarely just one source of any particular chemical. For example, exposure to toxic metals such as lead and arsenic can occur from several sources that are connected to eating habits, occupation, hobbies, the age of residential buildings, and geographical region. In addition, Canadians are simultaneously exposed to many chemicals at low concentrations [40]. There is a growing recognition of the need to identify preventive measures to address multiple environmental exposures in order to mitigate their potential impact, as highlighted in a workshop report on chemical mixtures [41]. In a series of papers by Hennig [42–44] a number of researchers have collectively stressed the need to further explore the nutritional paradigm in environmental toxicology. Specifically, over the past few decades there has been a growing body of research suggesting that nutrients may modify the toxicity of environmental chemicals [45,46]. In addition to our first paper showing that increasing 25OHD concentrations in maternal blood during pregnancy may be associated with reduced concentrations of the metals Pb and Cd, other studies in the MIREC cohort have found that folate may modify the association between phthalate exposures in pregnancy and autistic traits in offspring [47]. Arbuckle et al. also showed that calcium and vitamin D intake, as measured by a food frequency questionnaire, was inversely associated with maternal and cord blood metals [48].

Our papers build on the limited studies examining vitamin D as a potential modifier of metal exposures. Our 1st and 2nd papers raise important questions about the association between toxic metals and vitamin D and how low vitamin D status may increase the effect of metals on preterm birth risk. Studies have also demonstrated associations between toxic metals and a number of

other pregnancy outcomes in the MIREC cohort including preeclampsia, gestational diabetes and small for gestational age [49–52]. The implications of these collective findings may extend beyond birth for both the mother and child. This may be particularly important for low-income and marginalized communities where there may be a heavier burden of air pollution, toxic chemicals and inadequate nutrition [53–55].

2.3 Application of statistical techniques used in other substantive areas

The MIREC dataset has detailed and repeated exposure measures which allowed us to use a variety of statistical techniques in order to investigate relationships. For our first paper, we wanted to consider all of the repeated exposure data available in order to investigate bidirectional relationships using the cross lagged panel model. The main objective of this method is to examine the causal direction between variables [56]. It allows for simultaneous regressions and uniquely, makes it possible to specify reciprocal associations by allowing for a variable to be a response variable in one equation and a predictor variable in another [57,58]. Studies utilizing the cross lagged panel model method have so far been mainly from the fields of psychology, education and kinesiology/sports medicine. Some recent examples are studies that have used this method to examine reciprocal relationships between:

- Physical activity and gross motor skills [59]
- Posttraumatic stress symptoms and cannabis and alcohol use [60]
- Appetite behavior and BMI in childhood [61]
- Depressive symptoms and breastfeeding [62]
- Developmental trajectories and video game engagement [63]

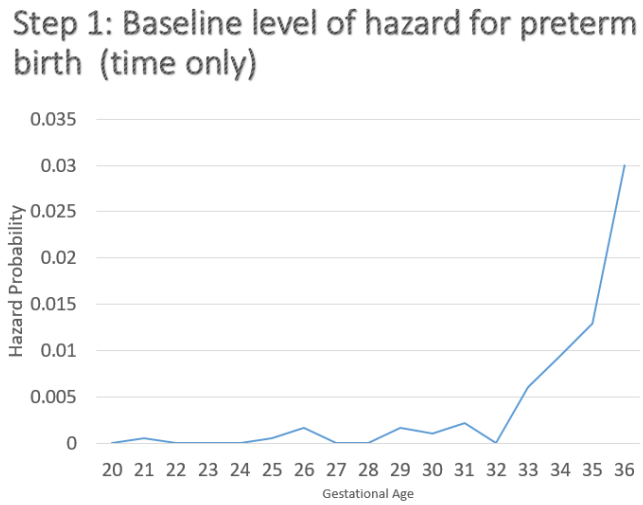
As far as we are aware, there are no previous applications of this method in biomonitoring research or environmental epidemiology.

This method reinforced two key messages: 1) the importance of ruling out contemporaneous effects; and 2) the importance of considering the persistence of a risk factor over time. These are highly relevant concepts to biomonitoring research generally, especially with persistent chemicals and those that are encountered in daily life. An important current limitation of this field is that much of the available evidence of health effects of environmental chemicals is cross-sectional, reflecting the nature of population-based biomonitoring programs such as the Canadian Health Measures Survey (CHMS) and the National Health and Nutrition Examination Survey (NHANES).

In our second paper, we used discrete time survival analysis to examine the joint association between metals and vitamin D in association with preterm birth. Traditionally, preterm birth has been analyzed as a dichotomous variable with logistic regression, with the cut-off being at 37 completed weeks of gestation. Term gestation extends from 37-42 weeks while preterm gestation has a much wider window, extending from 22-36 weeks. An important advantage of survival analysis is the ability to control for time varying exposures and covariates [64]. In the MIREC study, we were able to access and account for time varying measurements for metals (1st and 3rd trimester) and vitamin D (1st and 3rd trimester) as well as several time varying covariates (e.g., weight gain in pregnancy, season of blood collection, smoking). Survival analysis is usually not used for continuous exposures when there are only a few repeated exposure measurements; for continuous exposures, the method has been primarily used in air pollution

studies that have daily or weekly measurements [65]. However, we wanted to take into account all the data available to us. We also did not want to ignore time in our analysis or assume that the risk was constant over time, without first checking this. In the Figure 3, it is apparent that time plays an important role in the risk of preterm birth. If the risk of the event were independent of time, the hazard probability would be flat. Discrete time survival analysis allowed us to consider each week at risk separately and correctly capture the covariate data with the ultimate goal of improving the precision of the estimated associations [66].

Figure 3. Considering just time and the risk of preterm birth



3.0 Strengths and Limitations of the Dissertation

A major strength of this thesis is the comprehensive dataset that allowed us to do the extensive analysis in paper 1 that compared methods for examining cross-sectional, longitudinal, and bi-directional associations; permitted the examination of vitamin D/metal interactions with the risk of preterm birth in paper 2, which is a contribution that, if replicated in future studies may have

important implications for preventive intervention; and facilitated paper 3 which allowed us to empirically investigate the broader and long-term implications of our research on maternal health and include cardiometabolic markers that are often not measured in other studies in this area.

Although the MIREC cohort study has a number of notable strengths including its prospective nature and depth of exposure measures, its overarching limitation is its lack of ethnic and socioeconomic diversity. Therefore, the generalizability of our findings in all three papers is limited to predominately white, highly educated, married women. However, while the homogeneity of our sample is an important limitation with respect to external validity, it is possible that this increased internal validity, by limiting residual confounding in our studies. Another overarching limitation is a relatively low sample size. Our statistical power was limited in the 2nd and 3rd papers where we investigated preterm birth and maternal cardiometabolic health.

To address some of the sample size limitations, in our future work beyond this dissertation, we plan to replicate the analysis used in our 2nd paper using the Avon Longitudinal Study of Parents and Children (https://www.bristol.ac.uk/alspac/), to study the joint effect of vitamin D and lead on preterm birth in a different cohort. This cohort has a larger sample size (n>4000), and more variability in blood lead and vitamin D concentrations [67].

4.0 Implications for Field Environmental and Perinatal Epidemiology

When I reflect on what I have learned throughout this dissertation there are 3 key take-home messages. They are:

- Relationships between biomarkers can be bidirectional.

- There are opportunities for perinatal epidemiologists to further consider the implications of time and integrate this into our analyses.
- We need to keep following our participants to better elucidate the long-term impact of exposures and complications that occur in pregnancy.

4.1 Bidirectional Relationships

In our first paper we wanted to understand the relationships between metals and vitamin D and used the cross lagged panel model. This method could be used to further explore biomarkers of exposure and effect in environmental epidemiology. For example, in a previous paper our team at Health Canada investigated the association between blood concentrations of perfluoroalkyl substances (PFAS) and plasma lipids and liver enzymes in the general population [66] and pregnant women [67]. We found that certain PFAS were associated with worse lipid profiles and elevated liver enzymes. However, there could be reverse causation because those with worse lipid profiles or elevated liver enzymes may be less able to clear PFAS from the body. The challenge, however, is having repeated measures of both biomarkers over similar time periods in order to be able to explore directionality. Ideally, the bidirectional nature of relationships that we plan to investigate should be considered in the planning stages of a study.

4.2 What about considering time when looking at pregnancy outcomes?

Considering time in future studies investigating pregnancy outcomes, using statistical methods that better account for longitudinal data with repeated exposure measures compared with traditional logistic regression, is an important priority. When a person is pregnant, time is intimately linked to the risk of pregnancy complications [70]. For example the timing of preeclampsia is linked to its severity [71] and neonatal morbidities are shown to differ even within the term period [72]. Discrete time survival analysis allows us to look at the week-specific risk of

an outcome and is amenable to time dependent (gestational age) or time varying (e.g. smoking status may change throughout pregnancy) variables in the analysis. It also allows us to correctly capture the covariate information. For example, if we were interested in gestational weight gain as an exposure or covariate, we could accurately represent it across pregnancy provided data were available. Gestational weight gain is often non-uniform and influenced by pregnancy length, with a substantial portion of weight gain occurring in the term period of a pregnancy (37-42 weeks) [66]. When time-varying covariates are available survival analysis allows us to utilize all the data available and ultimately improve the precision of our estimates.

4.3 Maintain follow-up of our Participants

Our research, along with that of others [33,35], suggests that pregnancy complications have long lasting effects. Pregnancy cohorts should consider consistent and long-term follow-up of mothers as well as children in their studies. In the MIREC cohort study we conducted two follow-up studies, the MIREC-Infant Development (MIREC-ID) study and MIREC-Child Development (MIREC-CD Plus) study, focused on offspring but not maternal health. Only recently in our MIREC-ENDO study did we consider the mother's health as well as the child's. The U.S. National Institute of Health has recognized this need for longer-term data in a recent call for funding aimed at examining the long-term effect of environmental chemical exposures during pregnancy on maternal health [73]. One recent study found that early pregnancy PFAS were associated with poorer anthropometry, blood pressure, and cardiometabolic health at 3 years postpartum in women from the United States [74]. Given the paucity of data in this area, additional future research investigating the association between toxic metals and vitamin D insufficiency on cardiometabolic health in this MIREC cohort is warranted.

4.4 Public Health Implications and Considerations

In this thesis, we found that higher blood 25-hydroxyvitamin D levels were associated with lower blood cadmium and lead levels in pregnancy and that the association between lead and preterm birth was stronger in those with insufficient vitamin D status (<50 nmol/L). If further studies corroborate these relationships, there could be wide-ranging implications given that a number of reviews have identified toxic metals as potential reproductive toxicants, showing associations not only with preterm birth but also miscarriage and preeclampsia [75–77]. These pregnancy complications could affect the health of both the child and mother, as discussed in detail above. Preterm birth is the leading cause of death in children under 5 years of age, and the World Health Organization (WHO) estimated that over 13 million babies were born preterm in 2020 [78]. Toxic metals in pregnancy may also have long-term effects on the child's neurodevelopment [79]. Thus, the potential modification of the impact of toxic metals, such as lead, by vitamin D is important to consider. About 75% of Canadian females between the age of 19-50 are considered vitamin D sufficient (≥50 nmol/L) according to the 2007-2009 CHMS Survey[80]; however, in northern Canada only 27% (95% CI: 11%, 42%) of Inuit participants had vitamin D levels above 50 nmol/L[81]. According to our study findings, these populations with lower vitamin D levels may be at higher risk of exposure/absorption of Pb and Cd and the harmful effects of metals such as Pb. In addition, northern populations may be more highly exposed to toxic metals due to the higher prevalence of smoking [82] and some traditional foods [55]. On the contrary, several traditional foods are excellent sources of vitamin D [81]. We acknowledge the potential for unmeasured confounding in our studies, as vitamin D may be a marker of overall mineral status [83] or an indicator of physical activity, as seen in studies of adolescents and post-menopausal women [84,85]. However, a recent study did not see an association between

physical activity and 25OHD in postpartum lactating women [86]. Additional research is needed to further elucidate the biological mechanisms explaining the potential relationship between 25OHD and toxic metals during pregnancy.

5.0 Overall Conclusions

In summary, this dissertation used a variety of rather unique statistical methods to examine the potential interplay between nutrients and environmental chemicals. I've learned of the importance of thinking about causal direction and uniquely used a statistical method that allowed us to investigate the bidirectional nature of this associations, with the results suggesting that higher vitamin D earlier in pregnancy may be associated with lower levels of some toxic metals in the blood later in pregnancy. We then investigated the potential modifying effect of 25OHD on the association between metals and preterm birth and learned about the potential for nutrition to modify the harmful effects of chemical exposures. We found blood Pb to be associated with preterm birth and this association was strongest in women with lower vitamin D status. Finally, we gained insight into the importance of long-term follow-up in pregnancy cohort studies as we investigated the long-term impact of pregnancy complications on maternal health, 9 years post-partum. We found evidence to suggest that having a pregnancy complication worsens cardiometabolic health. Elevations in body fat percentage, blood pressure and markers of inflammation and insulin resistance were observed among women who experienced a pregnancy complication, especially hypertensive disorders in pregnancy. Going forward our results suggest environmental epidemiology should consider the potential role of nutrition in the modification of exposures or their effects. Further, chemicals that are associated with pregnancy complications, such as preterm birth, could have lasting effects on the child and the mother. Pregnancy cohorts should consider follow-up of both the mother and child in the long-term.

REFERENCES

1. CMP. Chemicals Management Plan. vol. 2016 (2016).

2. Health Canada. *Fifth Report on Human Biomonitoring if Environmental Chemicals in Canada: Results of the Canadian Health Measures Survey Cycle 5 (2016-2017)*. (2019).

3. Egusquiza, R. J. & Blumberg, B. Environmental obesogens and their impact on susceptibility to obesity: New mechanisms and chemicals. *Endocrinol. (United States)* **161**, 1–14 (2020).

4. Gore, A. C. *et al.* EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. *Endocrine Reviews* at https://doi.org/10.1210/er.2015-1010 (2015).

5. Qin, L. L., Lu, F. G., Yang, S. H., Xu, H. L. & Luo, B. A. Does maternal Vitamin D deficiency increase the risk of preterm birth: A meta-analysis of observational studies. *Nutrients* **8**, (2016).

6. Schröder-Heurich, B., Springer, C. J. P. & von Versen-Höynck, F. Vitamin D effects on the immune system from periconception through pregnancy. *Nutrients* **12**, 21–30 (2020).

7. Taylor, C. M., Golding, J. & Emond, A. M. Adverse effects of maternal lead levels on birth outcomes in the ALSPAC study: A prospective birth cohort study. *BJOG An Int. J. Obstet. Gynaecol.* **122**, 322–328 (2015).

8. Baghurst, P. A. *et al.* Lead in the placenta, membranes, and umbilical cord in relation to pregnancy outcome in a lead-smelter community. *Environ. Health Perspect.* **90**, 315–320 (1991).

9. Jelliffe-Pawlowski, L. L., Miles, S. Q., Courtney, J. G., Materna, B. & Charlton, V. Effect of magnitude and timing of maternal pregnancy blood lead (Pb) levels on birth outcomes. **26**, 154–162 (2006).

10. Vigeh, M. *et al.* Blood lead at currently acceptable levels may cause preterm labour. *Occup. Environ. Med.* **68**, 231–234 (2011).

11. Kido, T. *et al.* alpha1-Microglobulin determination in urine for the early detection of renal tubular dysfunctions caused by exposure to cadmium. **24**, 195–201 (1985).

12. Nogawa, K. *et al.* Mechanism for bone disease found in inhabitants environmentally exposed to cadmium: decreased serum 1α, 25-dihydroxyvitamin D level. *Int. Arch. Occup. Environ. Health* (1987) doi:10.1007/BF00377675.

13. Nogawa, K. *et al.* Serum vitamin D metabolites in cadmium-exposed persons with renal damage. *Int. Arch. Occup. Environ. Health* **62**, 189–193 (1990).

14. Chang, L. *et al.* Association of 25-hydroxyvitamin D with Hb and lead in children: A Chinese population-based study. *Public Health Nutr.* **17**, 827–832 (2014).

15. Rosen, J. F. *et al.* Reduction in 1,25-Dihydroxyvitamin D in Children with Increased Lead Absorption. *N. Engl. J. Med.* (1980) doi:10.1056/NEJM198005153022006.

16. Arbuckle, T. E. *et al.* Maternal and fetal exposure to cadmium, lead, manganese and mercury: The MIREC study. *Chemosphere* **163**, 270–282 (2016).

17. Chang, J. & Streitman, D. Physiologic Adaptations to Pregnancy. *Neurol. Clin.* **30**, 781–789 (2012).

18. Barrett, E. S., Groth, S. W., Preston, E. V., Kinkade, C. & James-Todd, T. Endocrine-Disrupting Chemical Exposures in Pregnancy: a Sensitive Window for Later-Life Cardiometabolic Health in Women. *Curr. Epidemiol. Reports* **8**, 130–142 (2021).

19. Manicone, A. M. & McGuire, J. K. Matrix Metalloproteinases as Modulators of Inflammation. **19**, 34–41 (2008).

20. Tu, F. F. *et al.* Prenatal Plasma Matrix Metalloproteinase-9 Levels to Predict Spontaneous Preterm Birth. *Obstet. Gynecol.* **92**, 446–449 (1999).

21. Lee, S. M., Park, K. H., Jung, E. Y., Cho, S. & Ryu, A. Prediction of spontaneous preterm birth in women with cervical insuf fi ciency : Comprehensive analysis of multiple proteins in amniotic fl uid. **42**, 776–783 (2016).

22. Dagouassat, M., Lanone, S. & Boczkowski, J. Interaction of matrix metalloproteinases with pulmonary pollutants. **39**, 1021–1032 (2012).

23. Au, F. *et al.* Blood metal levels and third trimester maternal plasma matrix metalloproteinases (MMPs). *Chemosphere* **159**, 506–515 (2016).

24. McElrath, T. F. *et al.* Pregnancy disorders that lead to delivery before the 28th week of gestation: An epidemiologic approach to classification. *Am. J. Epidemiol.* **168**, 980–989 (2008).

25. Blencowe, H. *et al.* Born Too Soon: The global epidemiology of 15 million preterm births. *Reprod. Health* (2013) doi:10.1186/1742-4755-10-S1-S2.

26. Swanson, J. M., Entriger, S., Buss, C. & Wadhwa, P. D. Developmental Origins of Health and Disease: Environmental Exposures. *Semin Reprod Med* **27**, 391–402 (2009).

27. Gluckman, P. D. & Hanson, M. *Developmental Origins of Health and Disease*. (Cambridge University Press, 2006). doi:10.1017/CBO9780511544699.

28. NIH. National Institute of Environmental Health Sciences: Developmental Origins of Health and Disease Program Description. (2022).

29. Sutton, P. S. & Darmstadt, G. L. Preterm birth and neurodevelopment: A review of outcomes and recommendations for early identification and cost-effective interventions. *Journal of Tropical Pediatrics* at https://doi.org/10.1093/tropej/fmt012 (2013).

30. Sonnenschein-Van Der Voort, A. M. M. *et al.* Preterm birth, infant weight gain, and childhood asthma risk: A meta-analysis of 147,000 European children. *J. Allergy Clin.*

Immunol. (2014) doi:10.1016/j.jaci.2013.12.1082.

31. Abitbol, C. L. & Rodriguez, M. M. The long-term renal and cardiovascular consequences of prematurity. *Nature Reviews Nephrology* at https://doi.org/10.1038/nrneph.2012.38 (2012).

32. Rich-Edwards, J. W., Fraser, A., Lawlor, D. A. & Catov, J. M. Pregnancy characteristics and women's future cardiovascular health: An underused opportunity to improve women's health? *Epidemiol. Rev.* **36**, (2014).

33. Grandi, S. M. *et al.* Cardiovascular Disease-Related Morbidity and Mortality in Women with a History of Pregnancy Complications: Systematic Review and Meta-Analysis. *Circulation* **139**, 1069–1079 (2019).

34. Bellamy, L., Casas, J. P., Hingorani, A. D. & Williams, D. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. *Lancet (London, England)* **373**, 1773–1779 (2009).

35. Bellamy, L., Casas, J., Hingorani, A. & Williams, D. Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. *BMJ* **335**, (2007).

36. Cusimano, M. C., Pudwell, J., Roddy, M., Cho, C. K. & Smith, G. N. The maternal health clinic: an initiative for cardiovascular risk identification in women with pregnancy-related complications. *Am. J. Obstet. Gynecol.* **210**, 438.e1-438.e9 (2014).

37. The American College of Obstetricans and Gynecologists. Hypertension in Pregnancy. *Obs. Gynecol* **122**, 1122–1131 (2013).

38. Rich-Edwards, J. Women's Reproductive Health as a Sentinel of chronic disease in women. *Women's Heal.* **5**, 101–105 (2009).

39. Cancer, C. G. on H. F. in B. Menarche, menopause, and breast cancer risk: individual participant meta-analysis, including 118 964 women with breast cancer from 117 epidemiological studies. *The Lancet.Oncology* **13**, 1141–1151 (2012).

40. Willey, J. B. *et al.* Exposure Load: Using biomonitoring data to quantify multi-chemical exposure burden in a population. *Int. J. Hyg. Environ. Health* **234**, 113704 (2021).

41. Taylor, K. W. *et al.* Statistical approaches for assessing health effects of environmental chemical mixtures in epidemiology: Lessons from an innovative workshop. *Environ. Health Perspect.* **124**, 227–229 (2016).

42. Hennig, B. *et al.* The role of nutrition in influencing mechanisms involved in environmentally mediated diseases. *Reviews on Environmental Health* at https://doi.org/10.1515/reveh-2017-0038 (2018).

43. Hennig, B. *et al.* Using nutrition for intervention and prevention against environmental chemical toxicity and associated diseases. *Environ. Health Perspect.* **115**, 493–495 (2007).

44. Hoffman, J. B. & Hennig, B. Protective influence of healthful nutrition on mechanisms of environmental pollutant toxicity and disease risks. *Ann. N. Y. Acad. Sci.* **1398**, 99–107 (2017).

45. Gamble, M. V. *et al.* Folic acid supplementation lowers blood arsenic. *Am. J. Clin. Nutr.* **86**, 1202–1209 (2007).

46. Ettinger, A. S. *et al.* Influence of maternal bone lead burden and calcium intake on levels of lead in breast milk over the course of lactation. *Am. J. Epidemiol.* **163**, 48–56 (2006).

47. Oulhote, Y. *et al.* Gestational exposures to phthalates and folic acid, and autistic traits in Canadian children. *Environ. Health Perspect.* **128**, 1–12 (2020).

48. Arbuckle, T. E. *et al.* Maternal and fetal exposure to cadmium, lead, manganese and mercury: The MIREC study. *Chemosphere* **163**, 270–282 (2016).

49. Ashley-Martin, J. *et al.* Association between maternal urinary speciated arsenic concentrations and gestational diabetes in a cohort of Canadian women. *Environ. Int.* **121**, 714–720 (2018).

50. Thomas, S. *et al.* Metals exposure and risk of small-for-gestational age birth in a Canadian birth cohort: The MIREC study. *Environ. Res.* **140**, 430–439 (2015).

51. Shapiro, G. D. *et al.* Exposure to phthalates, bisphenol A and metals in pregnancy and the association with impaired glucose tolerance and gestational diabetes mellitus: The MIREC study. *Environ. Int.* **83**, 63–71 (2015).

52. Borghese, M. M. *et al.* Individual, independent, and combined associations of toxic metals and manganese on hypertensive disorders of pregnancy: results from the MIREC Canadian pregnancy cohort. *Enviromental Heal. Perspect.*

53. Liu, J. *et al.* Disparities in Air Pollution Exposure in the United States by Race/Ethnicity and Income, 1990-2010. *Environ. Health Perspect.* **129**, 127005 (2021).

54. Eaves, L. A. *et al.* Generation of the Chemical and Social Stressors Integration Technique (CASS-IT) to Identify Areas of Holistic Public Health Concern: An Application to North Carolina. *Sci. Total Environ.* **862**, 160409 (2023).

55. Arctic Monitoring and Assessment Program (AMAP). *AMAP Assessment 2020: POPs adn Chemicals of Emerging Arctic Concern: Influence of Climate Change*. (2021).

56. Kearney, M. Cross-lagged panel analysis. *SAGE Encycl. Commun. Res. Methods* **355**, 165–174 (2017).

57. Allison, P. D., Williams, R. & Moral-Benito, E. Maximum Likelihood for Cross-lagged Panel Models with Fixed Effects. *Socius Sociol. Res. a Dyn. World* **3**, 237802311771057 (2017).

58. Amorim, L. D. A. F. *et al.* Structural equation modeling in epidemiology. *Cad. Saude Publica* **26**, 2251–2262 (2010).

59. Burns, R. D. *et al.* Bidirectional relationships of physical activity and gross motor skills before and after summer break: Application of a cross-lagged panel model. *J. Sport Heal. Sci.* **00**, 1–9 (2020).

60. Cheesman, A. & Read, R. Bidirectional relationship between posttraumatic stress symptoms, and cannabis and alcohol use: A random intercept cross-lagged panel model. *Alcohol. Clin. Exp. Res.* **44**, 80A (2020).

61. Costa, A., Severo, M., Vilela, S., Fildes, A. & Oliveira, A. Bidirectional relationships between appetitive behaviours and body mass index in childhood: a cross-lagged analysis in the Generation XXI birth cohort. *Eur. J. Nutr.* (2020) doi:10.1007/s00394-020-02238-9.

62. Haga, S., Lisoy, C., Drozd, F., Valla, L. & Slinning, K. A population-based study of the relationship between perinatal depressive symptoms and breastfeeding: a cross-lagged panel study. *Arch. Womens. Ment. Health* **21**, 235–242 (2018).

63. Krossbakken, E. *et al.* A cross-lagged study of developmental trajectories of video game engagement. *J. Behav. Addict.* **7**, 99 (2018).

64. O'Neill, M. S., Hertz-Picciotto, I., Pastore, L. M. & Weatherley, B. D. Have studies of urinary tract infection and preterm delivery used the most appropriate methods? *Paediatr. Perinat. Epidemiol.* **17**, 226–233 (2003).

65. Stieb, D. M. *et al.* Air pollution in the week prior to delivery and preterm birth in 24 Canadian cities: A time to event analysis. *Environ. Heal. A Glob. Access Sci. Source* **18**, 1–11 (2019).

66. Mitchell, E. ., Hinkle, S. N. & Schisterman, E. F. It's About Time: A Survival Approach to Gestational Weight Gain and Preterm Delivery. *Epidemiology* **27**, 182–187 (2016).

67. Taylor, C. M., Golding, J., Hibbeln, J. & Emond, A. M. Environmental Factors Predicting Blood Lead Levels in Pregnant Women in the UK: The ALSPAC Study. *PLoS One* **8**, (2013).

68. Fisher, M., Arbuckle, T. E., Wade, M. & Haines, D. Do perfluoroalkyl substances affect metabolic function and plasma lipids? Analysis of the 2007-2009, Canadian Health Measures survey (CHMS) Cycle 1. *Environ. Res.* **121**, 95–103 (2013).

69. Borghese, M. M., Liang, C. L., Owen, J. & Fisher, M. Individual and mixture effects of perfluoroalkyl substances on liver function biomarkers in the Canadian Health Measures Survey. **(under int**, (2023).

70. Joseph, K. S. & Kramer, M. S. The fetuses-at-risk approach: survival analysis from a fetal perspective. *Acta Obstet. Gynecol. Scand.* **97**, 454–465 (2018).

71. Raymond, D. & Peterson, E. A critical review of early-onset and late-onset preeclampsia. *Obstet. Gynecol.* **66**, 497–506 (2011).

72. Parikh, L. I. *et al.* Neonatal outcomes in early term birth. *Am. J. Obstet. Gynecol.* **211**, 265.e1-265.e11 (2014).

73. NIH. Research on Portfolio Online Reporting Tools (RePORT). *NIH REPORTER* https://report.nih.gov/ (2022).

74. Mitro, S. D. *et al.* Per- and Polyfluoroalkyl Substance Exposure, Gestational Weight Gain, and Postpartum Weight Changes in Project Viva. *Obesity* **28**, 1984–1992 (2020).

75. Dettwiler, M., Flynn, A. C. & Rigutto-Farebrother, J. Effects of Non-Essential "Toxic" Trace Elements on Pregnancy Outcomes: A Narrative Overview of Recent Literature Syntheses. *Int. J. Environ. Res. Public Health* **20**, (2023).

76. Singh, L., Anand, M., Singh, S. & Taneja, A. Environmental toxic metals in placenta and their effects on preterm delivery-current opinion. *Drug Chem. Toxicol.* **43**, 531–538 (2020).

77. Stone, J., Sutrave, P., Gascoigne, E., Givens, M. B. & Fry, R. C. Exposure to toxic metals and per- and polyfluroalkyl substances (PFAS) and the risk of preeclampsia and preterm birth in the United States: A review. **3**, (2021).

78. World Health Organization. Preterm Birth. *WHO Fact Sheets* https://www.who.int/news-room/fact-sheets/detail/preterm-birth (2023).

79. Alampi, J. D. *et al.* Association between Gestational Exposure to Toxicants and Autistic Behaviors Using Bayesian Quantile Regression. *Am. J. Epidemiol.* **190**, 1803–1813 (2021).

80. Health Canada. Plasma 25-hydroxyvitamin D (nmol/L), Canadian Health Measures Survey 2007 to 2009. **25**, 2009 (2009).

81. El Hayek Fares, J. & Weiler, H. A. Vitamin D status and intake of lactating Inuit women living in the Canadian Arctic. *Public Health Nutr.* **21**, 1988–1994 (2018).

82. Government of Canada. Original quantitative research – Tobacco, alcohol and marijuana use among Indigenous youth attending off-reserve schools in Canada: cross-sectional results from the Canadian Student Tobacco, Alcohol and Drugs Survey. *Heal. Promot. Chronic Dis. Prev. Canada Res. Policy Pract.* **39**, (2019).

83. Schwalfenberg, G. K. & Genuis, S. J. Vitamin D, essential minerals, and toxic elements: Exploring interactions between nutrients and toxicants in clinical medicine. *Sci. World J.* (2015) doi:10.1155/2015/318595.

84. Kluczynski, M. A. *et al.* Duration of Physical Activity and Serum 25-hydroxyvitamin D Status of Postmenopausal Women. *Ann Epidemiol* **21**, 440–449 (2011).

85. Black, L. J. *et al.* Vitamin D status and predictors of serum 25-hydroxyvitamin D concentrations in Western Australian Adolescents. *Br. J. Nutr.* **112**, 1154–1162 (2014).

86. Hall, J. T., Ebeling, M., Shary, J. R., Forestieri, N. & Wagner, C. L. The Relationship between Physical Activity and Vitamin D Status in Postpartum Lactating and Formula-Feeding Women. *J Steroid Biochem Mol Biol.* **177**, 261–265 (2018).

APPENDIX A: VITAMIN D BACKGROUNDER

List of Abbreviations

- $1,25(OH)_2D$: 1,25-dihydroxyvtitamin D (also known as calcitriol)
- 1-Ohase: 1α-hydroxylase
- 24-Ohase: 24-hydroxylase
- 25-Ohase: 25-hydroxylase
- 25OHD : 25-hydroxyvitamin D
- CYPS: P450 mixed function oxidases (CYPs): CYP2R1, CYP27A1, and CYP24B1.
- $D_{2:}$ ergocalciferol
- $D_{3:}$ cholecalciferol
- DBP: vitamin D binding protein
- DHC: 7- dehydrochlesterol
- FGF23: Fibroblast growth factor 23
- PTH: parathyroid hormone
- Retinoid X receptors (RXRα, RXRβ, or RXRγ)
- RPLs : remnant lipoproteins (RPLs)
- UVB : Ultraviolet B Rays
- VDR: vitamin D receptor
- VDREs: vitamin D responsive elements

Vitamin D Metabolism

There are two main types of vitamin D: D_2 (ergocalciferol) found naturally from ultraviolet B (UVB) irradiation of the ergosterol in plants and fungi and D_3 (cholecalciferol) from dietary sources (meat, fish, and eggs) and produced in the skin [1] (Figure 1). Few naturally occurring food sources are rich in vitamin D aside from oily fish. Other sources include egg yolks, meat, liver, supplements or fortified foods such as cow's milk and margarine, in Canada. Other foods permitted to be fortified with vitamin D include goat's milk, plant based beverages, and some calcium-fortified orange juices [2]. Both D_2 and D_3 can be commercially synthesized to use in supplements, and bioenrichment of some foods can be done using UVB irradiation of ergosterol from plants and fungi (D_2) and 7- dehydrochlesterol (DHC) from sheep's wool (D_3) [3].

Vitamin D is a unique nutrient for two key reasons: 1) it is obtained both exogenously from diet and produced endogenously in the skin; 2) it is technically not a vitamin, but rather a "prohormone" that needs to be metabolically activated and converted to its hormonal form 1,25-dihydroxyvtitamin D ($1,25(OH)_2D$) through the vitamin D endocrine system. Vitamin D_2 and D_3 obtained through the diet (food or supplements) are absorbed in the intestines and then delivered to various tissues through peripheral circulation via chylomirons[a], and subsequently transported to the liver through remnant lipoproteins (RPLs)[b] . Endogenous production of vitamin D occurs during exposure to UVB (spectrum 280-320) rays from sunlight. 7-DHC in the skin is converted to previtamin-D_3 , and then further converted to D_3 by enthalpy [4]. This process can be altered by a

[a] Chylomicrons: transport most dietary fatty acids and are the largest lipoproteins found in circulation [29]

[b] Remnant Lipoproteins (RPLs): the degradation product of partially catabolized chylomicrons. These particles are smaller and denser than chylomicrons and are linked to cardiovascular disease [30]; Julve *et al.*, 2016)

number of factors that influence UVB intensity (air pollution, season, time of day, latitude, atmospheric components), cutaneous exposure (clothing, sunscreen), and skin pigmentation. In addition aging is related to a decline in epidermal concentrations of 7-DHC [5].

D_3 is then transported to the liver via DBP. The liver is the main organ where vitamin D is converted to 25OHD and the kidney is the main source of conversion of 25OHD to $1,25(OH)_2D$. Vitamin D metabolism involves 3 main hydroxylations (25-hydroxylation, 1α-hydroxylation and 24-hydroxylation) and all are performed by the P450 mixed function oxidases (CYPs): CYP2R1, CYP27A1, and CYP24B1. Cytochrome P450-dependent hydroxylases are found in the mitochondria and smooth endopasmic reticulum (microsomes) and are important for hormone synthesis as well as the clearance of many compounds [6,7]. The major site of 25OHD production from D_2 and D_3 is in the liver. The liver contains a number of CYPs with 25-hydroxylase activity but CYP2R1 seems to be the major player in 25OHD synthesis. CYP2R1 gene knockout mice showed a greater than 50% reduction in 25OHD serum, suggesting it is a major, but not the sole player in 25-hydroxylation of vitamin D [8]. The production of 25OHD in the liver does not appear to be under tight regulation [7].

25OHD is transported from the liver to the kidneys via the vitamin D binding protein (DBP). In the kidneys 25OHD is further hydroxylated (1α-hydroxylation) to $1,25(OH)_2D$ (calcitriol) which is the biologically active form of vitamin D. CYP27B1 is the only recognized enzyme to have 1α-hydroxylation activity in the kidney and mutations within this gene cause $1,25(OH)_2D$ deficiency leading to rickets [9]. Although the kidney is the main site of 25OHD hydroxylation to $1,25(OH)_2D$,

there are several other extra-renal tissues, such as the placenta, bone, prostate, keratinocytes[c], and cells of the immune system (macrophages, T-lymphocyets, dendritic cells[d]) that are also able to convert 25OHD to $1,25(OH)_2D$ on a local tissue level [10,11]. Renal 1α-hydroxylase is tightly regulated through three main mechanisms [7,12–14]:

1. A direct negative feedback by $1,25(OH)_2D$ itself.
    - $1,25(OH)_2D$ itself directly inhibits CYP27B1 involved in renal 1α-hydroxylase;
    - $1,25(OH)_2D$ limits CYP27B1 activity by inhibiting PTH and increasing Fibroblast growth factor 23 (FGF23) production
    - Reducing $1,25(OH)_2D$ by inducing CYP24A1 expression in the kidney through a complex mechanism involving the vitamin D receptor (VDR) and the vitamin D inhibitory receptor;

2. Parathyroid hormone (PTH) as a signal of calcium status.
    - Low calcium and elevated PTH stimulates CYP27B1 and the renal production of $1,25(OH)_2D$;

3. Fibroblast growth factor 23 (FGF23) synthesized in the bone (osteoblasts, osteoclasts) as a peptide is essential to the maintenance of phosphate homeostasis by modulating intestinal phosphate absorption, renal phosphate reabsorption and/or bone metabolism [15].
    - $1,25(OH)_2D$ enhances FGF23 production and;
    - FGF23 inhibits CYP27B1 and reduces $1,25(OH)_2D$ production.

[c] Epidermal cell that produces keratin
[d] Antigen presenting cells

The Vitamin D binding protein (DBP) is the key transport protein for 25OHD and $1,25(OH)_2D$. It is similar to other ligand specific serum carriers of other steroid hormones such as corticosteroid-binding globulin, sex hormone-binding globulin (SHBG; estrogens and androgens) and thyroid hormone-binding globulin [16]. DBP is almost entirely produced in the liver and its synthesis is influenced by estrogen, glucocorticoids, and inflammatory cytokines. Noteworthy, its production is not influenced by vitamin D itself [17]. DBP binds approximately 85% of circulating vitamin D metabolites while albumin binds close to 15%. Around 0.4% of total $1,25(OH)_2D$ and 0.03% of 25OHD are 'free'. The 'bioavailable' vitamin D has been defined as the sum of 'free' vitamin D metabolite and that which is bound to albumin (15% in healthy non-pregnant individuals) (See Figure 2). The "free hormone" hypothesis proposes that only the non-bound portion ('free') of $1,25(OH)_2D$ can enter cells and exert a biological effect. Nevertheless, in the proximal tubules of the kidney megalin, a multi-ligand receptor, acts as a cell-surface receptor for DBP so 25OHD bound to DBP can be internalized and subsequently hydroxylated to $1,25(OH)_2D$ [16,18]. Although the action of megalin in the kidney is clear, its functional significance in a number of other vitamin D target tissues (placenta, mammary gland, parathyroid gland) is unknown [16].

$1,25(OH)_2D$ is transferred to target organs with the help of the DBP and reacts with the VDR to regulate calcium (Ca) and phosphate metabolism and PTH secretion, immunomodulation, neuroregulation, cell function (proliferation, differentiation, apoptosis) and angiogenesis. The VDR is the principal mediator for cellular effects of Vitamin D through its natural ligand with $1,25(OH)_2D$ [4,6].

The final step of vitamin D metabolism is the hydroxylation of vitamin D through the CYP24A1 enzyme, which has both 24- and 23-hydroxylase activity in humans (See Figure 1). The main function of CYP24A1 is to prevent the accumulation of 25OHD and $1,25(OH)_2D$ causing toxicity [7]. Both 25OHD and $1,25(OH)_2D$ are 24-hydroxyated but its preferred substrate is $1,25(OH)_2D$. The 5-step 24-hydroxyation pathway of $1,25(OH)_2D$ leads to $1,24,25(OH)_3D$ and the biologically inactive and water-soluble calcitroic acid which is subsequently excreted in urine [6]. $1,24,25(OH)_3D$ is biologically active, although about 10 times less biologically active than $1,25(OH)_2D$ [19], and has a high affinity for the VDR and may play a role in bone growth plate development [7]. 24-hydroxylation of 25OHD leads to $24,25(OH)_2D$ and subsequently to calcitroic acid, excreted in urine. The 23-hydroxylation of 25OHD and $1,25(OH)_2D$ leads to a form of lactone. Lactone may play a role as a VDR antagonist and as a potential "fail-safe" mechanism to more efficiently and rapidly downregulate the effect of vitamin D [19].

The regulation of CYP24A1 is essentially the reciprocal of CY27B1 in the kidney. CYP24A1 is induced by $1,25(OH)_2D$ and FGF23 while PTH lowers the induction of the CYP24A1 enzyme by $1,25(OH)_2D$ [7].

Table 1: Summary of Regulation of CYP enzymes

	CYP2R1 (liver 25-hydroxylase) 25OHD	CYP27B1 (kidney 1α-hydroxylase) $1,25(OH)_2D$ synthesis	CYP24A1 (catabolism of $1,25(OH)_2D$ and 25OHD so it can be excreted as calcitroic acid
$1,25(OH)_2D$		↓	↑
FGF23		↓	↑
PTH		↑	↓

↑ = induces enzymatic activity
↓ = inhibits enzymatic activity

D_2 vs D_3

D_2 and D_3 are similar in structure and they both undergo the same two-step hydroxylation process in their metabolism. However, D_3 is suggested to be superior in its efficacy in raising serum 25OHD due to several reasons:

1) D_3 is the preferred substrate for hepatic 25-hyrdoxylation in the liver with D_3 being converted to $25OHD_3$ five times as fast as D_2 to $25OHD_2$;
2) its higher binding affinity to DBP compared to D_2 and;
3) its slower rate of degradation - the formation of $1,24,25(OH)_3D_2$ deactivates the D_2 molecule however $1,24,25(OH)_3D_3$ requires an addition side-chain oxidation to be biologically deactivated [3,20].

Randomized controlled trials comparing the efficacy of D_2 to D_3 in raising serum 25OHD concentrations have been inconsistent. A systematic review of RCTs that directly compared the effects of vitamin D_2 and D_3 on serum 25OHD suggested that D_3 was more efficacious than D_2 is raising serum 25OHD [21]. However, these trials have suffered from being underpowered and heterogeneity in intervention dose and frequency [3].

Calcium, Phosphate and Vitamin D

Adequate dietary intake of calcium and phosphate are essential for bone mass and bone quality because they constitute the mineral component of bone. Intestinal absorption of both is regulated by vitamin D ($1,25(OH)_2D$), parathyroid hormone and FGF23 [22]. In states of adequate dietary intake of calcium and phosphate these ions are passively absorbed however, when the dietary intake is low (which is common for calcium) the parathyroid glands increase production of PTH which in turn induces: 1) increased intestinal lumen absorption of Ca (indirectly through the synthesis of $1,25(OH)_2D$ which depends on the availability of 25OHD, the substrate for the

CYP24A1 enzyme); 2) reabsorption of Ca in the kidneys (glomerular filtrate); and finally 3) Ca resorption from the bone to the blood stream if intestinal absorption is insufficient due to inadequate intake, or other disease factors, in order to maintain normal serum calcium levels. This may result in both bone loss and limited bone mineralization [22].

Table 2: Summary of Hormonal regulation of vitamin D metabolism

	FGF23	PTH	Bone resorption of Ca and P	$1,25(OH)_2D$ synthesis (CYP27B1)	Ca and P absorption in intestine (indirectly through $1,25(OH)_2D$)	Ca reabsorption in kidney	P excretion in kidney	Catabolism of $1,25(OH)_2D$ (CYP24A1) so it can be excreted as calcitroic acid
FGF23		↓	↓ (through inhibition of PTH)	↓	↓ (through inhibition of CYP27B1)	↓ (through inhibition of PTH)	↑	↑
PTH (parathyroid hormone)	↑		↑	↑	↑	↑	↑ (through FGF23)	↓
$1,25(OH)_2D$	↑	↓		↓				↑
Low Ca in blood		↑						
Increased Phosphorus in blood		↑						

Vitamin D in pregnancy

In pregnancy, DBP has been shown to increase in order to meet the increased calcium demands and enhance tolerance to paternal and fetal alloantigens [23]. A positive correlation has been seen between DBP and total calcitriol [$1,25(OH)_2D$] in non-pregnant and pregnant women [24]. In pregnancy, $1,25(OH)_2D$ increases by two fold in 1st trimester and a further 2 to 3 fold throughout

the course of the remainder of the pregnancy, and then rapidly declines at delivery. Usually the kidney is the major site of $1,25(OH)_2D$ synthesis, however PTH concentrations are reduced during pregnancy, suggesting that other tissues, cells or hormones may be involved in its production. The placenta also produces local $1,25(OH)_2D$ but does not contribute to circulating 25OHD levels. 25OHD crosses the placenta and $1,25(OH)_2D$ is produced in fetal kidneys. Cord blood 25OHD is about 50-80% of the concentration of maternal serum 25OHD [10].

Vitamin D Receptor (VDR)

The vitamin D receptor (VDR) is a member of the steroid receptor family and is an essential mediator of $1,25(OH)_2D$ [25]. $1,25(OH)_2D$ promotes the formation of a heterodimer[e] between VDR and one of three retinoid X receptors[f] (RXRα, RXRβ, or RXRγ) that function to regulate gene transcription through the binding of vitamin D responsive elements[g] (VDREs) within the promoter regions of the $1,25(OH)_2D$ regulated genes. The binding of the VDR-RXR complex to the VDRE promoter region attracts a complex of coactivator proteins connecting VDRE with RNA polymerase II[h] leading to gene

Table 3: Tissues that express the VDR for $1,25(OH)_2D$ in humans [13]

Adipose	Muscle (cardiac, embryonic, smooth)
Adrenal	Osteoblast
Bone	Ovary
Bone marrow	Pancreas β cell
Brain	Parathryroid
Breast	Paratid
Cancer cells	Pituitary
Cartilage	Placenta
Colon	Prostate
Epididymis	Retina
Hair follicle	Skin
Intestine	Stomach
Kidney	Testis
Liver (fetal)	Thymus
Lung	Thyroid
Lymphocytes (B & T)	Uterus

[e] **Heterodimer** – two non-identical molecules that form a bond (https://www.biologyonline.com/dictionary/dimer)

[f] **RXR** - specific members of the steroid receptor family

[g] **VDREs** – specific sequence of nucleotides in promoter region of VDR regulated genes.

[h] **RNA polymerase II**: multiprotein complex that catalyzes DNA-dependent synthesis of messenger *RNA* (mRNA) (https://www.cell.com/fulltext/S0092-8674(02)00646-3)

transcription and producing mRNA transcripts that leave the cell nucleus and head for coded protein in the cytoplasm [26,27].

There are several tissues (See Table 1) that express the VDR-RXR complex for the steroid hormone 1,25$(OH)_2$D suggesting that vitamin D may play a role in many other processes beyond calcium homeostasis [13]. Close to 200 genes are regulated by the VDR [28].

References

1. Wagner, C. L. & Hollis, B. W. The implications of vitamin D status during pregnancy on mother and her developing child. *Front. Endocrinol. (Lausanne).* **9**, 1–11 (2018).

2. Health Canada. Vitamin D and Calcium: Updated Dietary Reference Intakes. https://www.canada.ca/en/health-canada/services/food-nutrition/healthy-eating/vitamins-minerals/vitamin-calcium-updated-dietary-reference-intakes-nutrition.html (2019).

3. Wilson, L. R., Tripkovic, L., Hart, K. H. & Lanham-new, S. A. Conference on ' New technology in nutrition research and practice ' Postgraduate Symposium Vitamin D de fi ciency as a public health issue : using vitamin D 2 or vitamin D 3 in future forti fi cation strategies Proceedings of the Nutrition Society. **76**, 392–399 (2017).

4. Holick, M. F. Vitamin D Status: Measurement, Interpretation, and Clinical Application. *Ann. Epidemiol.* **19**, 73–78 (2009).

5. MacLaughlin, J. & Holick, M. F. Aging decreases the capacity of human skin to produce vitamin D3. *J. Clin. Invest.* **76**, 1536–1538 (1985).

6. Mousavi, S. E., Amini, H., Heydarpour, P., Amini Chermahini, F. & Godderis, L. Air pollution, environmental chemicals, and smoking may trigger vitamin D deficiency: Evidence and potential mechanisms. *Environ. Int.* **122**, 67–90 (2019).

7. Bikle, D. D. Vitamin D Metabolism, Mechanisms of Action, and Clinical Applications. **21**, 319–329 (2014).

8. Zhu, J. G., Ochalek, J. T., Kaufmann, M., Jones, G. & Deluca, H. F. CYP2R1 is a major , but not exclusive , contributor to 25-hydroxyvitamin D production in vivo. **110**, 15650–15655 (2013).

9. Cho, J. H., Kang, E., Kim, G., Lee, B. H. & Choi, J. Long-term clinical outcome and the identification of homozygous CYP27B1 gene mutations in a patient with vitamin D hydroxylation-deficient rickets type 1A. 169–173 (2016).

10. Pilz, S. *et al.* The role of vitamin D in fertility and during pregnancy and lactation: A review of clinical data. *Int. J. Environ. Res. Public Health* **15**, (2018).

11. Kaseda, R., Hosojima, M., Sato, H. & Saito, A. Role of Megalin and Cubilin in the Metabolism of Vitamin D3. *Ther. Apher. Dial.* **15**, 14–17 (2011).

12. Henry, H. L. Regulation of vitamin D metabolism. *Best Pract. Res. Clin. Endocrinol. Metab.* **25**, 531–541 (2011).

13. Norman, A. W. From vitamin D to hormone D: Fundamentals of the vitamin D endocrine system essential for good health. *Am. J. Clin. Nutr.* **88**, (2008).

14. Fukumoto, S. Phosphate metabolism and vitamin D. *Bonekey Rep.* **3**, 1–5 (2014).

15. Saito, T. & Fukumoto, S. Fibroblast Growth Factor 23 (FGF23) and Disorders of Phosphate Metabolism. **2009**, (2009).

16. Chun, R. F. *et al.* Vitamin D and DBP: The free hormone hypothesis revisited. *J. Steroid*

Biochem. Mol. Biol. **144**, 132–137 (2014).

17. Bikle, D. D. & Schwartz, J. Vitamin D Binding Protein , Total and Free Vitamin D Levels in Different Physiological and Pathophysiological Conditions. **10**, 1–12 (2019).

18. Chapron, B. D. *et al.* Reevaluating the Role of Megalin in Renal Vitamin D Homeostasis Using a Human Cell-Derived Microphysiological System. **35**, (2018).

19. St-Arnaud, R. & Jones, G. *Vitamin D, Volume 1: Biochemistry, Physiology and Diagnostics, Fourth Edition; Chapter 6: CYP24A1: Structure, Function, and Physiological Role*. *Vitamin D: Fourth Edition* vol. 1 (Elsevier Inc., 2018).

20. Houghton, L. A. & Vieth, R. The case against ergocalciferol (vitamin D2) as a vitamin supplement. *Am. J. Clin. Nutr.* **84**, 694–697 (2006).

21. Tripkovic, L. *et al.* Comparison of vitamin D2 and vitamin D3 supplementation in raising serum 25-hydroxyvitamin D status: a systematic review and meta-analysis. *Am. J. Clin. Nutr.* **95**, 1357–64 (2012).

22. Christakos, S., Lieben, L., Masuyama, R. & Carmeliet, G. Vitamin D endocrine system and the intestine. *Bonekey Rep.* **3**, 1–7 (2014).

23. Karras, S. N., Koufakis, T., Fakhoury, H. & Kotsa, K. Deconvoluting the biological roles of Vitamin D-Binding protein during pregnancy: A both clinical and theoretical challenge. *Front. Endocrinol. (Lausanne).* **9**, (2018).

24. Bouillon, R., van Assche, F. A., van Baelen, H., Heyns, W. & De Moor, P. Influence of the vitamin D-binding protein on the serum concentration of 1,25-dihydroxyvitamin D3. Significance of the free 1,25-dihydroxyvitamin D3 concentration. *J. Clin. Invest.* **67**, 589–596 (1981).

25. Pike, J. W., Meyer, M. B., Lee, S. M., Onal, M. & Benkusky, N. A. *Genome-Wide Perspectives on Vitamin D Receptor-Mediated Control of Gene Expression in Target Cells*. *Vitamin D: Fourth Edition* vol. 1 (Elsevier Inc., 2018).

26. Campbell, F. C., Xu, H., El-Tanani, M., Crowe, P. & Bingham, V. The yin and yang of vitamin D receptor (VDR) signaling in neoplastic progression: operational networks and tissue-specific growth control. *Biochem. Pharmacol.* **79**, 1—9 (2010).

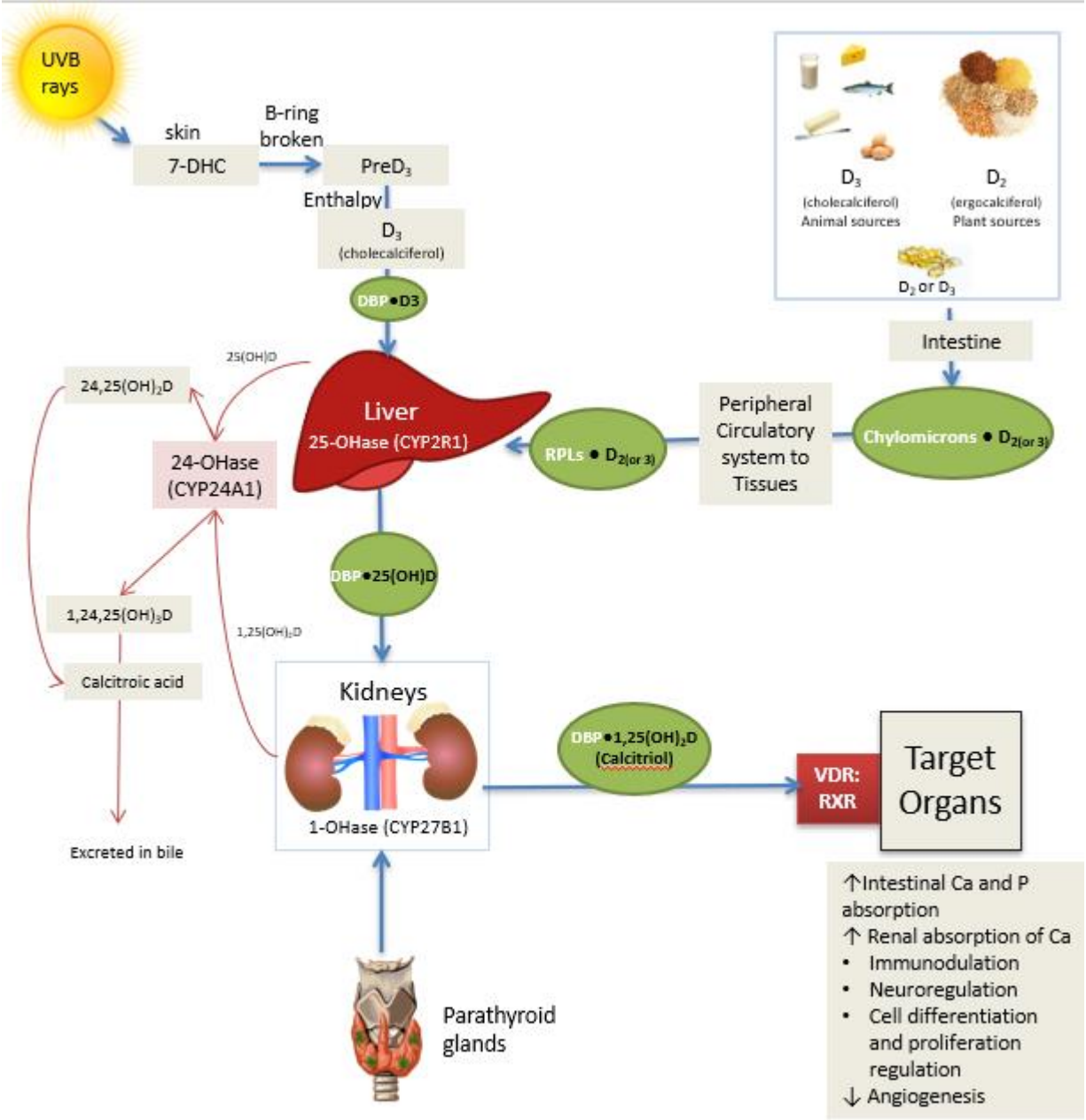
27. Raman, M., Milestone, A. N., Walters, J. R. f. & Ghosh, S. Vitamin D and gastrointestinal diseases: Inflammatory bowel disease and colorectal cancer. *Therap. Adv. Gastroenterol.* **4**, 49–62 (2011).

28. Santos, B. R., Mascarenhas, L. P. G., Satler, F., Boguszewski, M. C. S. & Spritzer, P. M. Vitamin D deficiency in girls from South Brazil: a cross-sectional study on prevalence and association with vitamin D receptor gene variants. *BMC Pediatr.* (2012) doi:10.1186/1471-2431-12-62.

29. Julve, J., Martín-Campos, J. M., Escolà-Gil, J. C. & Blanco-Vaca, F. Chylomicrons: Advances in biology, pathology, laboratory testing, and therapeutics. *Clin. Chim. Acta* **455**, 134–148 (2016).

30. Kim, J. Y. *et al.* High levels of remnant lipoprotein cholesterol is a risk factor for large artery atherosclerotic stroke. *J. Clin. Neurol.* **7**, 203–209 (2011).

Figure 1: Vitamin D Metabolism



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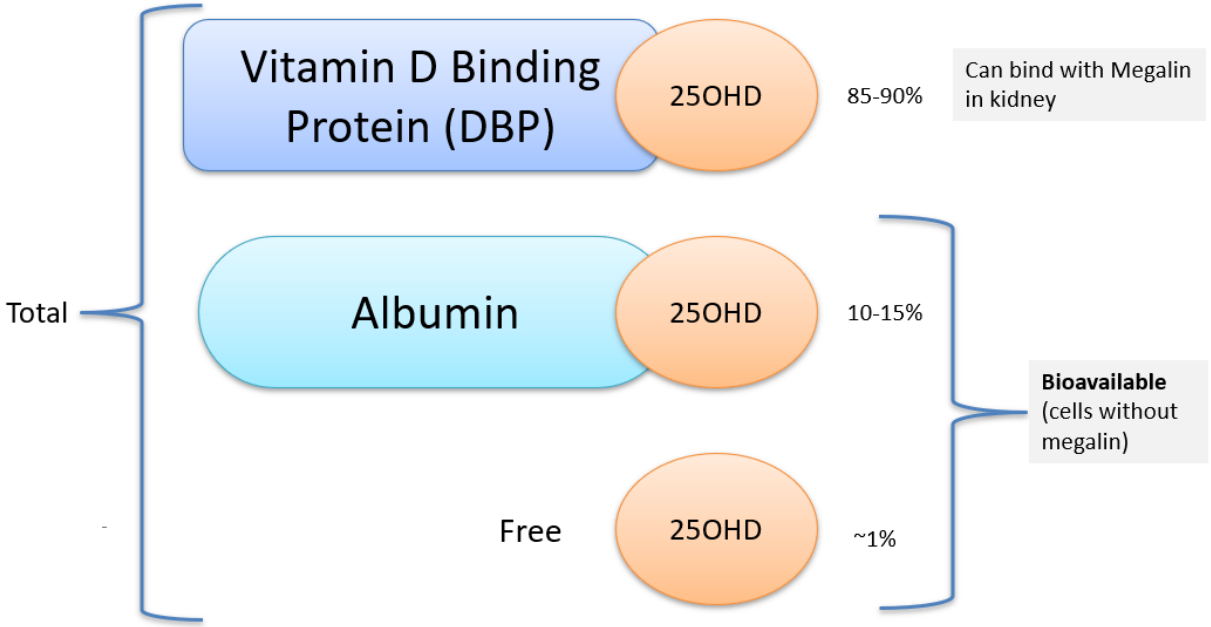
References:
Holick, M. F. Vitamin D Status: Measurement, Interpretation, and Clinical Application. Ann. Epidemiol. **19**, 73–78 (2009)
Mousavi, S. E., Amini, H., Heydarpour, P., Amini Chermahini, F. & Godderis, L. Air pollution, environmental chemicals, and smoking may trigger vitamin D deficiency: Evidence and potential mechanisms. Environ. Int. **122**, 67–90 (2019).

DBP: vitamin D binding protein; RPL: remnant lipoproteins
25-Ohase: 25-hydroxylase; 1-Ohase: 1α-hydroxylase; 24-Ohase: 24-hydroxylase
7-DHC: 7-dehydrochlesterol; VDR: vitamin D receptor; PTH: parathyroid Hormone; Ca: Calcium,; P: Phosphate

Figure 2: Vitamin D Status



This Figure was created by Mandy Fisher.

Reference: Chun & Neilson. Vitamin D (4th ed). Vol 1: biochemistry, Physiology, and Diagnostics. Ch 51. 2018. pages 925-937