



## Original Contribution

# Maternal Concentrations of Perfluoroalkyl Substances and Fetal Markers of Metabolic Function and Birth Weight

## The Maternal-Infant Research on Environmental Chemicals (MIREC) Study

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Perfluoroalkyl substances (PFAS) are ubiquitous, persistent chemicals that have been widely used in the production of common household and consumer goods for their nonflammable, lipophobic, and hydrophobic properties. Inverse associations between maternal or umbilical cord blood concentrations of perfluorooctanoic acid and perfluorooctanesulfonate and birth weight have been identified. This literature has primarily examined each PFAS individually without consideration of the potential influence of correlated exposures. Further, the association between PFAS exposures and indicators of metabolic function (i.e., leptin and adiponectin) has received limited attention. We examined associations between first-trimester maternal plasma PFAS concentrations and birth weight and cord blood concentrations of leptin and adiponectin using data on 1,705 mother-infant pairs from the Maternal Infant Research on Environmental Chemicals (MIREC) Study, a trans-Canada birth cohort study that recruited women between 2008 and 2011. Bayesian hierarchical models were used to quantify associations and calculate credible intervals. Maternal perfluorooctanoic acid concentrations were inversely associated with birth weight z score, though the null value was included in all credible intervals ( $\log_{10} \beta = -0.10$ , 95% credible interval:  $-0.34, 0.13$ ). All associations between maternal PFAS concentrations and cord blood adipocytokine concentrations were of small magnitude and centered around the null value. Follow-up in a cohort of children is required to determine how the observed associations manifest in childhood.

adiponectin; birth weight; environmental exposure; leptin; maternal exposure; perfluoroalkyl substances; pregnancy

Abbreviations: CrI, credible interval; GWG, gestational weight gain; MIREC, Maternal-Infant Research on Environmental Chemicals; PFAS, perfluoroalkyl substance(s); PFHxS, perfluorohexanesulfonate; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonate; PPAR- $\alpha$ , peroxisome proliferator-activated receptor alpha.

The fetal time period is a critical window of development. In utero exposures to environmental chemicals may induce fetal metabolic changes and produce lasting changes in phenotypic expression (1). Perfluoroalkyl substances (PFAS), including perfluorooctanoic acid (PFOA), perfluorooctanesulfonate (PFOS), and perfluorohexanesulfonate (PFHxS), have been widely used in the production of common household and consumer goods for

their nonflammable, lipophobic, and hydrophobic properties (2). Though production of PFOA and PFOS has been declining in recent decades, these substances are persistent in natural and human environments (3). PFOA, PFOS, and PFHxS are widely detected in Canadian women (4). Moreover, certain PFAS have been shown to cross the placenta, thereby creating the potential for direct fetal exposure (5).

Authors of recent reviews of the human and nonhuman literature concluded that PFOA is “known to be toxic” based on sufficient evidence of decreased fetal growth (6, 7). In a meta-analysis of 18 human studies, Johnson et al. (7) concluded that a 1-ng/mL increase in maternal PFOA exposure was associated with an 18.9-g decrease in birth weight. In a systematic review, Bach et al. (8) reported that 6 out of 8 studies found an inverse association between maternal PFOS and birth weight, with 3 of those studies showing statistically significant results. The epidemiologic studies included in both of these reviews had primarily investigated the independent associations between PFAS and birth weight without consideration of the potential effects of correlated exposures, namely other types of PFAS. Correlation among PFAS may be due to the presence of a common source of exposure or the same precursor compound (9). Alternatives to single chemical models that allow inclusion of correlated exposures may heighten the validity of findings of exposure-related associations with health outcomes.

Reliance on birth weight as an outcome measure precludes determination of whether PFAS exposure is associated with skeletal growth, organ growth, or adiposity. To our knowledge, there has been no indexed epidemiologic investigation of the associations among PFAS and umbilical cord blood markers of metabolic function, such as leptin and adiponectin. Leptin and adiponectin are adipocytokines that are detectable in cord blood and have been associated with adverse metabolic profiles (10, 11). Leptin is involved in appetite satiety and body weight regulation (11), whereas adiponectin is involved in insulin resistance (10). Low-dose gestational PFOA exposure has been associated with elevated leptin concentrations in an animal model (12) and with obesity at age 20 years in a Danish cohort (13).

The primary objective of the present study was to determine the association between prenatal exposure to 3 PFAS (PFOA, PFOS, and PFHxS) and infant birth weight and umbilical cord blood concentrations of leptin and adiponectin. We employed a Bayesian hierarchical model to account for the potential effects of correlated exposures. A secondary objective was to examine the influence of gestational weight gain on the PFAS–birth weight association.

## METHODS

### Study population

Data and biospecimens were obtained from the Maternal-Infant Research on Environmental Chemicals (MIREC) Study, a trans-Canada cohort study of 2,001 pregnant women. Study participants were recruited from 10 Canadian cities between 2008 and 2011. Briefly, women were eligible for inclusion if the fetus was at <14 weeks' gestation at the time of recruitment and they were  $\geq 18$  years of age, able to communicate in French or English, and planning on delivering at a local hospital. Women with known fetal or chromosomal anomalies in the current pregnancy and women with a history of medical complications (including renal disease, epilepsy, hepatitis, heart disease, pulmonary disease, cancer, hematological disorders, threatened spontaneous abortion, and illicit

drug use) were excluded from the study (14). The population in the present investigation included mothers who had a singleton, term live birth and no missing chemical or outcome data ( $n = 1,705$ ). The leptin and adiponectin analyses were further restricted to participants with a cord blood sample suitable for analysis. The analytical sample was restricted to term births because the association of interest was the effect of exposure on birth weight rather than on preterm birth (15). Moreover, leptin and adiponectin concentrations are reportedly lower in preterm infants (16).

### Exposure and outcome assessment

Chemical analysis of plasma samples was carried out at the Laboratoire de Toxicologie, Institut National de Santé Publique du Québec (Quebec City, Quebec, Canada), which is accredited by the Standards Council of Canada. Three PFAS (PFOA, PFOS, and PFHxS) were measured in first-trimester plasma using ultra-high-pressure liquid chromatography (ACQUITY UPLC System; Waters Corporation, Milford, Massachusetts) coupled with tandem mass spectrometry, operated in the multiple reaction monitoring mode with an electrospray ion source in negative mode.

Birth weight was recorded in each study participant's chart and extracted for inclusion in the MIREC database. Leptin and adiponectin were measured in plasma of 1,363 umbilical cord blood samples (68.7% ( $n = 1,983$ ) of the cohort). Analysis was conducted at Mt. Sinai Laboratory (Toronto, Ontario, Canada) using immunoassay kits from Meso Scale Discovery (Rockville, Maryland). Analyses of all samples with coefficients of variation greater than 15% were repeated. The inter- and intraassay coefficients of variation were 11.8% and 9.3%, respectively, for leptin and 8% and 9%, respectively, for adiponectin. All samples were within the range of detection.

### Statistical analysis

Descriptive statistics for maternal demographic factors and pregnancy and infant characteristics were calculated. Due to right-skewed distributions, data on PFAS, leptin, and adiponectin concentrations were all log-transformed prior to inclusion in multivariate models. Pearson's correlation coefficient was used to examine correlations among the log-transformed chemical values. The small percentage of samples with exposure concentrations below the limit of detection were substituted as (limit of detection)/2. Because of the low percentage (<5%) of samples below the limit of detection, this substitution method is not thought to introduce sufficient bias to substantively affect findings (17). Sex-specific birth weight  $z$  scores were calculated to account for sex differences in birth weight using sex-specific birth weight means and standard deviations from the analytical sample.

Bayesian hierarchical linear regression models were employed to compute the parameter estimates and 95% credible intervals for the associations between the log-transformed continuous exposure variables and continuous measures of birth weight  $z$  score,  $\log_{10}$  leptin, and  $\log_{10}$  adiponectin. We created a separate model for each outcome

(leptin, adiponectin, and birth weight), where each model included the 3 PFAS. This type of model facilitates inclusion of correlated exposures and is not subject to the challenges of convergence and unstable estimates faced by maximum likelihood regression models (18). This approach offers the advantage of lowering the possibility of type 1 error by shrinking estimates away from the maximum likelihood estimate and towards the prior mean (18). To aid in interpretation of results, we also conducted analyses using frequentist models where all 3 PFAS were included in the model. We assessed collinearity among the PFAS by evaluating the variance inflation factor, where a variance inflation factor less than 10 was indicative of no collinearity. We used frequentist analyses to replicate the Bayesian models with birth weight  $z$  score, leptin, and adiponectin as the outcomes. In addition, we conducted a sex-stratified analysis of the relationship between PFAS concentrations and birth weight to facilitate comparison with previous literature.

Prior to inclusion in the hierarchical models, the linearity of each exposure-outcome association was assessed using restricted cubic spline models. The Bayesian analysis was conducted with 3 chains and 10,000 iterations, with the first 500 iterations being discarded as a burn-in period. Prior distributions for the exposure parameters were modeled as normal distributions (0,  $\Phi$ ), where  $\Phi$  was modeled as a half-normal distribution (underlying normal mean = 0, variance = 100). This prior distribution was chosen to represent an uninformative prior distribution (19). A half-normal prior for variance has been recommended for hierarchical linear regression models as a robust means of modeling the variance of the prior for the parameter estimates (19). Model convergence was assessed through visual assessment of trace plots and by means of convergence diagnostic tests. The Web Appendix (available at <http://aje.oxfordjournals.org/>) provides further details on the Bayesian hierarchical model.

Covariates were chosen for inclusion in multivariate models by identifying predictors of exposures (20) and predictors of outcome variables (21, 22). Birth weight models were adjusted for maternal age (20, 22), prepregnancy body mass index (weight (kg)/height (m)<sup>2</sup>) (20), parity (22), income (20), and maternal smoking (22). The adipocytokine models adjusted for maternal age, prepregnancy body mass index, infant sex, and parity (21). We also analyzed the role of birth weight  $z$  score in adipocytokine parameter estimates by conducting an additional analysis with birth weight  $z$  score included as a covariate. This was done to facilitate analysis of exposure-related changes in adipocytokine concentrations for a given birth weight. All analyses were carried out as complete-case analyses.

While some research has found that gestational weight gain (GWG) may confound the association between maternal exposure to persistent organic pollutants and birth weight (23), other investigators have noted that their findings differed according to GWG stratum (24, 25). In light of this literature, we conducted 2 additional analyses with the results 1) adjusted for GWG as a continuous variable and 2) stratified by GWG category, defined according to the US Institute of Medicine (26). GWG was calculated on

the basis of rates of weekly weight gain during the second and third trimesters.

Descriptive statistics were calculated in SAS, version 9.2 (SAS Institute, Inc., Cary, North Carolina). Bayesian modeling was performed using R, version 3.0.3 (R Foundation for Statistical Computing, Vienna, Austria) and OpenBUGS, version 3.2.1 (OpenBUGS Project Management Group). This study received ethical approval from Health Canada, Sainte Justine's Hospital (Montreal, Quebec, Canada), and the IWK Health Centre (Halifax, Nova Scotia, Canada), and all participants provided informed consent.

## RESULTS

Of the 2,001 women recruited into the MIREC Study, 18 withdrew and asked that all of their data and biospecimens be destroyed. Of the remaining 1,983 subjects, 1,705 women had a singleton, term live birth with no missing data on birth weight or exposure. There were 1,363 umbilical cord blood samples available for analysis in the overall cohort. Upon accounting for exclusions (multiple gestation, cord blood unsuitable for analysis, missing chemical data), there were 1,247 cord blood samples available for analysis.

Demographic characteristics of the study population and outcome statistics are provided in Table 1. Cord blood leptin concentrations were higher among female infants (median, 15.9 ng/mL) than among male infants (median, 8.8 ng/mL). Adiponectin concentrations did not differ by infant sex. Birth weight ranged from 1,765 g to 5,620 g, with a median value of 3,486 g. The majority of study participants were over 30 years of age, had a household income greater than Can\$50,000, were nonsmokers, and had a normal body mass index. Table 2 gives descriptive statistics for the 3 PFAS. Over 95% of all maternal plasma samples had detectable concentrations of PFOA, PFOS, and PFHxS. The Pearson correlation coefficients for correlations between log-transformed PFAS ranged from 0.5 (between PFOA and PFHxS) to 0.6 (between PFOA and PFOS) (all  $P$ 's < 0.05) (Web Table 1).

Because no nonlinear associations were detected using splines, the PFAS were entered into the model as continuous, log-transformed variables with linear terms (PFOA spline shown in Web Figure 1). Results from the Bayesian hierarchical model are presented in Table 3. All 95% credible intervals for all outcomes included the null value. A 1-unit increase in log<sub>10</sub> PFOA level was associated with a 0.10-unit decrease in birth weight  $z$  score (95% credible interval: -0.34, 0.13). Results were nearly identical when a frequentist model was used (Web Table 2). Results also did not vary when data were stratified by infant sex or were adjusted for GWG (data not shown).

PFOS concentrations were inversely associated with cord blood leptin levels ( $\beta = -0.09$ , 95% credible interval (CrI): -0.23, 0.04). The associations between both PFOA and PFHxS and cord blood leptin were positive, yet the magnitude became negligible after adjustment. The direction of association between all 3 PFAS and cord blood adiponectin concentrations was positive and of small magnitude, with

**Table 1.** Characteristics of Participants in the MIREC Study, 2008–2011

Characteristic	No. of Persons	%	Median	IQR	Range
Maternal age, years	1,705		33.0	29–37	18.0–48.0
Gestational age, weeks	1,705		39.2	38–40	37–42
Birth weight, g	1,705		3,486.0	3,201–3,800	1,765–5,620
Females			3,420.0	3,150–3,740	1,765–5,070
Males			3,560.0	3,247–3,865	2,155–5,620
Umbilical cord blood leptin level, ng/mL	1,247		11.4	5.3–24.2	0.1–243.2
Females			15.9	7.3–33.3	0.1–243.2
Males			8.8	4.2–18.1	0.1–241.8
Umbilical cord blood adiponectin level, $\mu\text{g/mL}^{\text{a}}$	1,246		16.6	10.7–23.6	0.2–239.7
Females			16.6	11.1–23.7	0.2–59.6
Males			16.7	10.5–23.1	0.7–239.7
Prepregnancy body mass index <sup>b</sup>					
Underweight (<18.5)	45	2.8			
Normal weight (18.5–24.9)	982	62.0			
Overweight (25–29.9)	342	21.6			
Obese ( $\geq 30$ )	215	13.6			
Missing data	121				
Gestational weight gain <sup>c</sup>					
Inadequate	265	17.7			
Adequate	386	25.8			
Excess	846	56.5			
Missing data	208				
Household income (Can\$)					
$\leq 30,000$	127	7.8			
30,001–50,000	159	9.8			
50,001–100,000	674	41.5			
>100,000	666	41.0			
Missing data	79				
Ethnicity					
Caucasian	1,457	85.5			
Other	248	14.6			
Missing data	0				
Maternal smoking					
Never smoked or quit prior to pregnancy	1,493	87.6			
Quit when knew pregnant	120	7.0			
Current smoker	91	5.3			
Missing data	1				
Parity					
0	734	43.1			
1	684	40.2			
$\geq 2$	285	16.7			
Missing data	2				
Infant sex					
Male	895	52.5			
Female	809	47.5			
Missing data	1				

Abbreviations: IQR, interquartile range; MIREC, Maternal Infant Research on Environmental Chemicals.

<sup>a</sup> One cord blood sample was not available for adiponectin analyses.

<sup>b</sup> Weight (kg)/height (m)<sup>2</sup>.

<sup>c</sup> US Institute of Medicine guidelines (26).

**Table 2.** First-Trimester Plasma Concentrations of Perfluoroalkyl Substances Among Women in the MIREC Study, 2008–2011

PFAS	LOD, ng/mL	% With Levels >LOD	PFAS Concentration, ng/mL		
			Median	IQR	Range
PFOA	0.1	99.8	1.7	1.2–2.4	LOD–16
PFOS	0.3	99.8	4.6	3.2–6.8	LOD–36
PFHxS	0.3	96.0	1.0	0.7–1.6	LOD–25

Abbreviations: IQR, interquartile range; LOD, limit of detection; MIREC, Maternal Infant Research on Environmental Chemicals; PFAS, perfluoroalkyl substance(s); PFHxS, perfluorohexanesulfonate; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonate.

95% credible intervals overlapping the null value (Table 3). Results were similar in a frequentist model (Web Table 2). There were no differences in associations in the leptin or adiponectin models when results were stratified by GWG or adjusted for birth weight  $z$  score (data not shown).

An inverse association was observed between PFOA exposure and birth weight in each GWG category, though the magnitude was strongest among women with adequate weight gain (adjusted  $\beta = -0.36$ , 95% CrI:  $-0.85$ ,  $0.11$ ) (Table 4). There were no notable differences in results when this analysis was performed in a frequentist model (data not shown).

In a frequentist linear regression model, PFOA was inversely associated with birth weight among boys and girls, though neither association was statistically significant (Table 5). Neither PFOS nor PFHxS was associated with birth weight among boys or girls in a statistically significant manner.

## DISCUSSION

In this population of Canadian women, we observed inverse associations between maternal PFOA concentrations and birth weight and between maternal PFOS and umbilical cord blood leptin concentrations, though the null value was included in all credible intervals. Other than an inverse association between PFOS and cord blood leptin concentrations, we observed no associations

between any of the PFAS and cord blood adipocytokine concentrations.

Our findings are consistent with previous studies and reviews that demonstrated an inverse association between PFOA exposure and birth weight, though lacking in statistical and likely medical significance (8, 27, 28), and are similar in direction and magnitude to those of the recent meta-analysis in which Johnson et al. reported an 18-g decrease in birth weight (95% CrI:  $-29.8$ ,  $-7.9$ ) per 1-ng/mL increase in PFOA (7). The range of PFOA concentrations within MIREC Study participants is also comparable to the ranges reported among studies included in the meta-analysis (7). In one of the few studies that examined log-transformed PFOA concentrations as a continuous variable, Apelberg et al. (29) reported that a 1-unit increase in log-transformed PFOA concentration was associated with a 69-g decrease in birth weight, though this association was not statistically significant and stratification by sex was not performed.

The epidemiologic literature on PFOA and birth weight is consistent. In a review of 21 animal studies, Koustas et al. (6) reported that mean pup birth weight decreased significantly with increasing levels of gestational PFOA exposure. PFOA-induced activation of the peroxisome proliferator-activated receptor alpha (PPAR- $\alpha$ ) pathway is one hypothesized mechanism underlying the inverse relationship between PFOA exposure and reduced birth weight (30, 31). The PPAR- $\alpha$  pathway is a known regulator of energy metabolism and lipid

**Table 3.** Bayesian Hierarchical Linear Regression Estimates ( $\beta$ ) of Log<sub>10</sub> PFAS Concentration (ng/mL) According to Umbilical Cord Blood Log<sub>10</sub> Leptin (ng/mL) and Adiponectin ( $\mu$ g/mL) Level and Birth Weight  $z$  Score, MIREC Study, 2008–2011

PFAS	Leptin (n = 1,176)				Adiponectin (n = 1,175)				Birth Weight $z$ Score (n = 1,509)			
	Crude		Adjusted <sup>a</sup>		Crude		Adjusted <sup>a</sup>		Crude		Adjusted <sup>b</sup>	
	$\beta$	95% CrI	$\beta$	95% CrI	$\beta$	95% CrI	$\beta$	95% CrI	$\beta$	95% CrI	$\beta$	95% CrI
PFOA	0.03	-0.11, 0.16	0.01	-0.15, 0.13	0.05	-0.04, 0.13	0.04	-0.05, 0.12	-0.16	-0.39, 0.06	-0.10	-0.34, 0.13
PFOS	-0.09	-0.23, 0.05	-0.09	-0.23, 0.04	-0.01	-0.10, 0.07	0.02	-0.11, 0.07	0.06	-0.17, 0.29	0.05	-0.18, 0.29
PFHxS	0.04	-0.06, 0.13	0.01	-0.08, 0.10	-0.02	-0.08, 0.04	0.02	-0.08, 0.04	0.06	-0.11, 0.22	0.04	-0.12, 0.20

Abbreviations: CrI, credible interval; MIREC, Maternal Infant Research on Environmental Chemicals; PFAS, perfluoroalkyl substance(s); PFHxS, perfluorohexanesulfonate; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonate.

<sup>a</sup> Adjusted for maternal age, prepregnancy body mass index, sex, and parity.

<sup>b</sup> Adjusted for maternal age, prepregnancy body mass index, parity, household income, and smoking.

**Table 4.** Bayesian Hierarchical Linear Regression Estimates ( $\beta$ ) of Log<sub>10</sub> PFAS Concentration (ng/mL) According to Birth Weight z Score and US Institute of Medicine Category of Recommended Gestational Weight Gain,<sup>a</sup> MIREC Study, 2008–2011

PFAS	Birth Weight z Score											
	Inadequate Weight Gain (n = 246)				Adequate Weight Gain (n = 371)				Excess Weight Gain (n = 810)			
	Crude		Adjusted <sup>b</sup>		Crude		Adjusted <sup>b</sup>		Crude		Adjusted <sup>b</sup>	
	$\beta$	95% CrI	$\beta$	95% CrI	$\beta$	95% CrI	$\beta$	95% CrI	$\beta$	95% CrI	$\beta$	95% CrI
PFOA	-0.14	-0.58, 0.31	-0.08	-0.78, 0.63	-0.40	-0.80, -0.03	-0.36	-0.85, 0.11	-0.10	-0.40, 0.18	-0.08	-0.44, 0.27
PFOS	-0.13	-0.57, 0.31	-0.24	-0.95, 0.45	-0.07	-0.43, 0.28	-0.03	-0.49, 0.41	0.17	-0.12, 0.45	0.25	-0.11, 0.62
PFHxS	-0.14	-0.64, 0.32	-0.09	-0.58, 0.40	0.09	-0.19, 0.38	0.11	-0.22, 0.45	0.03	-0.18, 0.24	0.02	-0.22, 0.24

Abbreviations: CrI, credible interval; MIREC, Maternal Infant Research on Environmental Chemicals; PFAS, perfluoroalkyl substance(s); PFHxS, perfluorohexanesulfanoate; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonate.

<sup>a</sup> Defined according to the US Institute of Medicine (26).

<sup>b</sup> Adjusted for maternal age, prepregnancy body mass index, parity, household income, and smoking.

homeostasis. The greater magnitude of association between PFOA and birth weight could be partially explained by the fact that PFOA has been shown to be a stronger agonist of PPAR- $\alpha$  than PFOS (31).

The literature is scarce on the potential associations between exposure to PFAS and cord blood concentrations of leptin or adiponectin. Hines et al. (12) reported that low-dose prenatal PFOA exposure was associated with increased leptin concentrations in adult mice. Similarly, in a Danish cohort study, Halldorsson et al. (13) reported that prenatal PFOA concentrations were associated with increases in leptin levels and decreases in adiponectin levels among 20-year-old females. In a Japanese cohort study, Kishi et al. (32) reported an inverse association between maternal PFOS concentrations and lipid levels during pregnancy but not between PFOA concentrations and pregnancy lipid levels. The observed lack of association between PFOA and fetal markers of leptin and adiponectin suggests that any growth-related PFOA toxicity is not likely to operate through an adiposity-related pathway. The trend towards an inverse association between maternal PFOS concentrations and cord blood leptin but not between maternal PFOS concentrations and birth weight raises interesting questions regarding the mechanisms

underlying potential PFOS toxicity. Specifically, considering the observed associations between leptin, insulin resistance, and body mass index (11), it would be informative to determine whether the observed reduction in leptin levels translates into detectable anthropometric changes in childhood.

The present study benefited from a relatively large population recruited from diverse regions of the country and collection of data on numerous potential confounders. This study contributes to existing literature on this topic by the use of multiple statistical methods, the inclusion of leptin and adiponectin as outcomes, and adjustment and stratification for GWG. By restricting our analyses to term infants, we removed the potential influence of prematurity on the observed associations. In addition, our analytical approach offers strengths over traditional single-chemical statistical models. Using hierarchical models may have reduced the potential for type 1 error by shrinking parameter estimates towards the prior mean.

Potential limitations of this analysis need to be considered. First, it is not clear whether first-trimester PFAS measurements are representative of the target window of exposure. These chemicals have half-lives of several years (33), and a high degree of correlation has been observed between consecutive pregnancies (34). The single measurement of exposure, therefore, is unlikely to present a material threat to the internal validity of this study. Second, it is possible that our findings were subject to uncontrolled confounding due to maternal or fetal characteristics that are predictors of both the studied exposures and birth outcomes. For example, changes in maternal plasma volume may affect both PFAS metabolism and placental perfusion, a potential cause of reduced birth weight (27). As plasma volume increases throughout pregnancy (35), the protein-bound PFAS concentrations may be diluted. On the other hand, reduced plasma volume (as a result of hypertension (36)) may artificially elevate PFAS concentrations. Since PFAS concentrations were measured during the first trimester, prior to the time of maximal volume expansion (35), we anticipate this effect to have been minimal. Reduced glomerular filtration rate has also been hypothesized to confound the association between PFAS exposure and birth weight (37). We did not have the capacity to account for either of these physiological characteristics;

**Table 5.** Linear Regression Estimates ( $\beta$ ) of the Association Between First-Trimester Log<sub>10</sub> PFAS Concentration (ng/mL) and Birth Weight (g), MIREC Study, 2008–2011

PFAS	Males (n = 797)		Females (n = 712)	
	$\beta^a$	95% CrI	$\beta^a$	95% CrI
PFOA	-35.51	-198.99, 127.97	-89.51	-263.40, 84.38
PFOS	-11.15	-174.26, 151.95	94.31	-76.30, 264.92
PFHxS	53.72	-53.71, 161.15	-24.72	-140.00, 90.55

Abbreviations: CrI, credible interval; MIREC, Maternal Infant Research on Environmental Chemicals; PFAS, perfluoroalkyl substance(s); PFHxS, perfluorohexanesulfanoate; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonate.

<sup>a</sup> Adjusted for age, body mass index, parity, income, smoking, and each PFAS.

however, both factors would be expected to have more of an effect on PFAS measures later in pregnancy. Third, although the Bayesian model allows the inclusion of correlated exposures, it does not allow assessment of potential synergy among chemicals. It is possible that exposure to numerous other chemicals (phthalates, metals, flame retardants) may affect the biological activity of PFAS.

There was a potential for selection bias in the GWG analysis (and in the leptin/adiponectin analyses) resulting from differences between the analytical sample and participants excluded for missing GWG data (or missing data on the adipokines). Among the eligible subset of participants, those with complete GWG data ( $n = 1,497$ ) had median first-trimester PFOA concentrations (1.7 ng/mL vs. 1.7 ng/mL) and birth weights (3,510 g vs. 3,537 g) comparable to those of participants without GWG data ( $n = 208$ ). In the analytical subset with complete GWG data, there were fewer women with a prepregnancy body mass index indicating obesity (13.4% vs. 16.1%) and fewer current smokers (4.8% vs. 9.2%) than among women with no GWG data. Among those missing data on leptin or adiponectin concentrations, the characteristics of the cohort members with and without missing cord blood samples were similar, except that women who were underweight or normal weight had a slightly higher proportion of missing cord blood samples. It is difficult to ascertain the potential influence of bias due to missing data, but given the small magnitude of the differences in characteristics between participants with missing data and those with nonmissing data, we do not expect our results to have been strongly influenced by selection bias. Last, determining the implications of these findings at birth necessitates follow-up in a cohort of children, because cord blood leptin measures alone may not be indicative of childhood body composition. Studies using data from the Project Viva cohort found that cord blood leptin was inversely associated with body composition measures at 6 months of age and at 3 years of age (38, 39). However, leptin levels at age 3 years were positively associated with adiposity at age 7 years (39). Planned follow-up studies in a subset of MIREC Study participants will examine associations with anthropometric measures and, possibly, leptin and adiponectin in childhood.

We conducted a sensitivity analysis to determine the differences in estimates obtained when using gestational-age-specific birth weight  $z$  scores as defined by Kramer et al. (40). We observed that parameter estimates did not change when we used the gestational-age-specific  $z$  scores, probably because of the exclusion of preterm births.

In this population of Canadian women, we observed no statistically significant associations between maternal PFAS concentrations and infant birth weight, leptin, or adiponectin concentrations. However, the relationship between PFOA and birth weight was consistently inverse in both the Bayesian and frequentist models and regardless of whether the outcome was birth weight  $z$  score or birth weight. Caution is warranted in generalizing these findings to other populations, because MIREC participants were on average older, more educated, had higher incomes, and were less likely to smoke than other women giving birth in Canada. Based on our findings, we support the previously

articulated argument that any inverse association between maternal PFOA exposure and birth weight may have biological relevance (41). However, further investigation is required to determine whether a potential association with birth weight persists beyond birth and becomes clinically detectable in childhood. In addition, further research is needed to determine whether the observed inverse association between PFOS and cord blood leptin concentrations persists and manifests in a clinically detectable manner in childhood. Elucidating these mechanisms requires continued efforts among toxicologists and epidemiologists to account for potential physiological confounders and the effects of chemical mixtures.

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