Lactational exposure to polychlorinated biphenyls is higher in overweight /obese women and associated with altered infant growth trajectory: A pilot study

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ABSTRACT

Background: Infant exposure to environmental chemicals, such as polychlorinated biphenyls (PCBs), may contribute to developmental programming of long-term metabolic disease risk. PCBs persist given their lipophilicity and long half-lives, allowing them to bio-accumulate in adipose tissue. These compounds can then be excreted into maternal milk resulting in infant exposure.

Objective: To determine the level of PCBs in milk from mothers with pre-pregnancy overweight and obese (OW/OB) versus normal weight status (NW) and evaluate the association of milk PCB levels with infant growth over the first 6 months of life.

Methods: A pilot study of a subset of milk samples from mothers with NW (pre-pregnancy body mass index (BMI) < 25 kg/m^2, n = 11) and OW/OB (pre-pregnancy BMI ≥ 25 kg/m^2, n = 8) were examined approximately 2-weeks postpartum. PCB congeners 138 + 163, 132 + 153, 180, and the sum were measured using gas chromatography/mass spectrometry and adjusted for milk fat content. Infant growth was monitored from birth to 6 months for weight-for-age (WFA), length-for-age (LFA), weight-for-length (WFL), head circumference-for-age (HCA), and associations with milk PCB content determined using linear mixed modeling.

Results: Total milk fat content did not differ by maternal weight status ($p = 0.88$). Milk from mothers with OW/OB had significantly higher PCB sum ($p = 0.02$) and PCB 138 + 163 ($p = 0.03$). PCB 132 + 153 (β −0.0008, $p = 0.0218$), PCB 180 (β −0.0010, $p = 0.0279$), and PCB sum (β −0.0006, $p = 0.0138$) were negatively associated with HCA Z-score growth to 6 months. PCB 180 was negatively associated with infant WFA (β −0.0015, $p = 0.0058$) and WFL Z-score (β −0.0016, $p = 0.0263$) to 6 months. There were no associations of PCB sum content with WFL, LFA, WFL Z-score over the first 6 months of life.

Conclusions: Maternal overweight and obesity are associated with higher levels of total PCB congeners (132, 138, 153, 163, 180) in human milk. PCB congeners have negative associations with infant head circumference and weight trajectory over the first 6 months of life.

1. Introduction

Infant exposure to adverse prenatal and postnatal environments has been shown to program long-term disease risk (Yessoufou and Moutairou, 2011). Early life exposure to environmental endocrine-disrupting chemicals (EDCs), including polychlorinated biphenyls (PCBs), bisphenol A, and pesticides, has been linked to increased childhood and adult metabolic disease risk (Mead, 2008; Vafeiadi et al., 2017). Regulation of EDCs now exists in the United States for some substances, including the ban placed on PCBs in the 1970s due to their carcinogenic properties as well as multi-organ impacts on the endocrine, reproductive, nervous, and immune system (U.S. EPA, 2006). Historically, 209 PCB congeners were produced and introduced into the environment through their use as plasticizers, heat transfer fluids, hydraulics, lubricants, solvents, and in carbonless copy paper (Keeler et al., 1993). Of those 209 congeners, 197 are non-dioxin-like PCBs while 12 are dioxin-like PCBs (Todaka et al., 2010). Highly detected non-dioxin-like PCBs include PCB 138, 153, and 180, which differ in their pathways of action compared to dioxin-like PCBs (Center for Disease Control (CDC), 2019). Despite being banned, PCBs persist today due to their lipophilicity and long half-lives, which allow them to bio-accumulate in the food chain. This persistent exposure is of particular interest in the state of Michigan due to contamination of the Great Lakes and inland areas (Mackay and Fraser, 2000; Venier et al., 2014).

PCB exposure poses increased risks to vulnerable populations, including pregnant and lactating women as well as the developing fetus and infant due to concerns for negative impacts on maternal metabolic health, fetal development, postnatal childhood growth, and neurodevelopment (Vafeiadi et al., 2017; Ando et al., 1985; Boersma and Lanting, 2000; Longnecker et al., 2001; Meeker, 2012). In addition to prenatal transfer across the placenta, infant PCB exposure after birth may occur through mother's milk. Throughout a mother's lifetime, PCBs are stored in adipose tissue and can then be excreted in human milk resulting in intergenerational transfer to offspring during the period of lactation (Todaka et al., 2010; Ando et al., 1985; Barr et al., 2005). The most predominant PCBs reported in human milk are PCB 138, 153, and 180 because of their use in commercial PCB mixtures (Brajenovic et al., 2018). Per kilogram body weight, human milk-fed infants have been shown to receive 50-times the daily PCB intake of adults (Patandin et al., 1999). Mothers with increased adiposity can accumulate higher levels of lipophilic chemicals, including PCBs, with subsequent enhanced excretion in human milk (Mead, 2008). This study will assess lactational PCB exposure in mothers with pre-pregnancy overweight or obesity and the impact on infant growth.

2. Methods

2.1. Overview

Eligible breastfeeding mothers were recruited at an academic delivery hospital in Michigan and completed written informed consent for their participation and assent for their infant's participation as part of the Infant Metabolism and Gestational Endocrinopathies (IMAGE) mother-infant cohort. Mother-infant dyads were recruited during hospital admission for delivery and completed the IRB-approved protocol. A subset of mother-infant dyads was included in this pilot study analysis of PCB content in milk samples based on availability of 2-week milk samples with adequate volume required for PCB analysis. Maternal milk samples were collected approximately 2-weeks postpartum following a detailed home milk collection protocol. Electronic medical records were reviewed for maternal demographics, weight status, and health characteristics. Infant growth parameters were determined using World Health Organization (WHO) Z-scores based on pediatrician well child visit documentation of infant growth. Documented nutritional intake was reviewed from the medical record. Participants received reimbursement for their participation in this study. This study protocol was approved by the University of Michigan Institutional Review Board (HUM00107801).

2.2. Participants

Nineteen mother-infant dyads were included in this pilot sub-study with 11 normal weight (NW) and 8 overweight/obese (OW/OB) mothers providing milk samples at near 2-weeks post-partum. Using maternal pre-pregnancy weight status as defined by medical record documentation of pre-pregnancy or early first trimester weight and height to calculate body mass index (BMI), maternal OW/OB was defined as BMI ≥ 25.0 kg/m^2, and NW was defined as BMI < 25.0 kg/m. Mothers with endocrinopathies including gestational diabetes, type 1 and type 2 diabetes, and polycystic ovary syndrome were not included. Eligibility criteria included maternal age 18 years or older, with a singleton gestation, delivery of a healthy infant at 35 weeks or greater, and planning to breastfeed her infant.

2.3. Human milk collection

Milk collection protocol was based on collection methods published by Fields & Demerath with modifications as previously described for the IMAGE Study (Ellsworth et al., 2020; Fields and Demerath, 2012). Briefly, mothers were provided written and verbal instructions for non-fasting morning milk collection to occur in their home on the morning of their infant's well child pediatrician appointment. Samples were briefly stored in a designated clinic freezer (-20 ° C) and then transported on ice for final storage at -80 °C.

2.4. Human milk Macronutrient analysis

Whole milk was measured for macronutrient composition of carbohydrate, protein, fat, and total energy using a mid-IR spectroscopy macronutrient milk analyzer (MIRIS Human Milk Analyzer (HMA)™, Uppsala, Sweden). Milk samples were thawed on ice at the time of analysis. For analyses, 5 mL of milk was warmed to 40 °C using a bead bath and processed with ultrasonic homogenization per the MIRIS HMA™ manufacturer's protocol. Standards for internal calibration were analyzed prior to sample analysis with verification of targeted values from fat, crude protein, true protein, and carbohydrate. Duplicate analyses were performed for each sample and the average values used for data analysis.

2.5. Human breast milk PCB analysis

Milk samples were analyzed through the M−LEEaD Exposure Assessment Core at the University of Michigan. Samples were frozen until prepared for extraction. At extraction, two subsamples were taken. First, 2 mL were spiked with surrogate standards (PCB 65 and 166), 1 mL of hydrochloric acid, and extracted with methanol/isopropanol (1:1) followed by hexane/methyl t-butyl ether (1:1). The organic layer was transferred to a new tube with 1% potassium chloride and extracted with hexane/methyl t-butyl ether again (3 mL). The two organic fractions were combined and evaporated under a stream of ultrapure nitrogen. The dry content was weighed for lipid determination.

The extract was dissolved in 4 mL of n-hexane for column clean-up and then silica column purified by hexane/dichloromethane (DCM) (9 mL 1:1 v/v). The analytes were eluted with hexane/DCM (8 mL 1:1 v/v). Solvent was then evaporated under the flow of nitrogen to

a volume of 0.5 mL, then 0.5 mL of n-nonane was added, and evaporated to 250 μL; 15 μL of internal standard (PCB136 and 204) was added before gas chromatography/mass spectrometry (GC/MS) analyses.

An Agilent 5973 gas chromatograph/mass spectrometer (Agilent Industries, Palo Alto, CA, USA) was used for analyte separation and quantification. The analyzer was equipped with a DB5MS column (30 m; inner diameter 0.25 mm; film thickness 0.25 μm) obtained from J&W Scientific (Folsom, CA, USA). Helium was used as the carrier gas. The MS detector was operated in negative chemical ionization selective ion monitoring mode using ultra-pure methane as the reagent gas.

The limit of detection (LOD) for this method was defined as the higher of LODs calculated by two methods: (i) by direct relation to method blanks prepared in parallel with the unknown samples, as 3 times the standard deviation of the method blanks, and (ii) according to the instrumental detection limit defined as the lowest point in the calibration curve (0.5 pg/μL or 5 pg/μL) verified to give a signal with a signal-to-noise equal to or greater than 3.0. For the target congeners in this study, the LOD was determined as 0.01 ng/g. In parallel with the above analyses, blank (corn oil) samples were analyzed. Linearity, drift check (repeat analysis of a standard), and spike recovery were performed.

Analysis of samples by GC/MS were used to measure PCB congeners (138 + 162, 153 + 132, and 180). The sum of these congeners was used to calculate a total PCB milk content. PCB concentrations were adjusted for fat content based on content of fat in the sample. Lipid percentage was determined gravimetrically, by first evaporating the milk extract until dry, and then adjusting the PCB concentration to the lipid base by dividing the wet weight concentration by the lipid percent and multiplying by 100.

2.6. Infant growth assessment

Through review of the infant's medical record, growth measurements were extracted from hospital delivery and well-child pediatrician appointments at academic affiliated clinics. Sex- and age-specific Z-scores for weight-for-age (WFA), length-for-age (LFA), head circumference-for-age (HCA), and weight-for-length (WFL) according to the WHO growth standards for infants 0–2 years were used (Onis, 2006). Growth data was reviewed from medical encounters at birth, 2 weeks, 2 months, and 6 months of age. After this point, complementary foods were anticipated to be included in the infant diet with consequential influences on further infant growth trajectories.

2.7. Statistical analysis

Participant characteristics for the 19 mother-infant dyads were defined using descriptive statistics. The relationship between maternal weight status and milk macronutrient composition or PCBs congener content were examined using Welch's unequal variances *t*-test. Maternal demographics and markers of socioeconomic status association with PCB milk content was evaluated using Welch's unequal variances *t*-test. Longitudinal trajectories of WFA, LFA, WFL, and HCA Z-scores at 2 weeks, 2 months, and 6 months were analyzed using linear mixed models. Models were fit for each anthropometric outcome and each PCB congener predictor, including random intercepts at the subject level and fixed effects for time (in months), PCB, birth anthropometric Z-score, and the interaction of PCB and time. Adjusted models including maternal pre-pregnancy BMI for exploratory analysis were then fit for each anthropometric outcome and each PCB congener predictor, including random intercepts at the subject level and fixed effects for time (in months), PCB, maternal BMI, birth anthropometric Z-score, and the interaction of PCB and time (to represent the effect of PCB on the slope of the Z-score trajectory). As a visual aid, loess regression curves were generated by plotting infant WFA, LFA, WFL, and HCA Z-score from birth to 6 months of age grouped by milk PCB congener sum above or below the median. All available infant growth measure-

ments through 6 months of age were included irrespective of type of milk or formula intake.

Analysis was carried out using R version 4.0.0 and RStudio (Boston, MA). The package *nlme* (v 3.1–147) was used for modeling, *simr* (v 1.0.5) for post-hoc power analysis, and *ggplot2* (v 3.3.0) for graphics. Statistical significance was set at p < 0.05. Statistical tests were not adjusted for multiple comparisons.

3. Theory

Increased exposure of human milk-fed infants to persistent organic pollutants, including PCBs, has been linked to childhood metabolic disease risk, impaired growth, and neurocognitive developmental impairments (Mead, 2008; Boersma and Lanting, 2000; Patandin et al., 1999a, 1999b; La Merrill and Birnbaum, 2011). Research on the link between PCB exposure and childhood growth has yielded variable results as summarized in reviews by La Merrill, Tang-Peronard, and Jorissen. (La Merrill and Birnbaum, 2011; Jorissen, 2007; Tang-Péronard et al., 2011). Studies on PCB exposure have largely centered around placental or fetal exposure. However, given their presence in human milk and the potential for increased exposure during states of maternal adiposity, the aim of this study is to evaluate the impact of lactational PCB exposure in mothers with pre-pregnancy overweight or obesity on infant growth rates.

4. Results

Participant characteristics for the 19 enrolled mother-baby dyads are shown in Table 1. The mean infant age of evaluation at a well-

Table 1
Participant Demographics.

	NW n = 11	OW/OB n = 8	*p*-value
Maternal Age (years)	30.2 (3.9)	29.9 (3.4)	0.86
Maternal Pre-pregnancy BMI (kg/m²)	20.8 (2.2)	29.7 (4.5)	<0.001*
Maternal Race/Ethnicity (%)			0.30
Caucasian	8 (73)	7 (88)	
African American	0 (0)	1 (12)	
Hispanic/Lanito	1 (9)	0 (0)	
Asian/Pacific Islander	2 (18)	0 (0)	
Annual Total Income Level (%)			0.04*
>60,000	11 (100)	3 (37.5)	
<60,000	0 (0)	2 (25)	
Unknown	0 (0)	3 (37.5)	
Maternal Education (%)			0.02*
High School/Associates/Bachelors	3 (27)	7 (88)	
Master's/Professional	7 (64)	0 (0)	
Unknown	1 (9)	1 (12)	
County of Residence (%)			0.46
Ingham	0 (0)	1 (12)	
Livingston	1 (9)	2 (25)	
Oakland	1 (9)	0 (0)	
Washtenaw	8 (73)	5 (63)	
Wayne	1 (9)	0 (0)	
Obstetric History			
Pregnancies	1.9 (1.4)	2.3 (0.7)	0.09
Deliveries	1.5 (0.8)	2.0 (0.8)	0.33
Mode of Delivery (%)			0.31
Vaginal	6 (55)	7 (88)	
C-section	5 (45)	1 (12)	
Infant Sex (%)			1.0
Female	6 (55)	4 (50)	
Male	5 (45)	4 (50)	
Infant Birth Weight (kg)	3.4 (0.3)	3.7 (0.4)	0.19
Infant Age at 2 week evaluation (days)	16.2 (3.1)	15.6 (2.5)	0.67

Data presented as mean (standard deviation) or number (percentage) as appropriate. Abbreviations: normal weight (NW), overweight and obese (OW/OB). * represents statistical significance with *p* < 0.05.

child pediatrics visit and time of maternal milk collection was 16.2 days in NW and 15.6 days in OW/OB. At the time of human milk collection, 3 infants of OW/OB mothers received human milk with formula supplementation of which the amount of formula supplementation was not quantified.

Analysis of macronutrient composition of human milk revealed no significant difference in fat content based upon maternal pre-pregnancy weight status (NW: 3.77 ± 1.28 g/dL, OW/OB: 3.69 ± 1.04 g/dL, $p = 0.88$). All macronutrients were conserved between maternal groups with no difference in true protein (NW: 1.19 ± 0.15 g/dL, OW/OB: 1.32 ± 0.29 g/dL $p = 0.27$), carbohydrate (NW: 6.92 ± 0.39 g/dL, OW/OB: 6.84 ± 0.32 g/dL, $p = 0.62$), or energy content (NW: 69.05 ± 11.60 kcal/dL, OW/OB: 68.63 ± 10.08 kcal/dL, $p = 0.93$) between mothers with NW and OW/OB.

PCB congeners 138 + 162, 132 + 153, 180, and the sum PCB congeners (132, 138, 153, 163, 180) were selected for assessment due to reports of their presence in human milk with sum environmental exposures ranging from 41.6 to 365.1 ng/mL. Mothers with OW/OB had significantly higher levels of the sum PCB congeners (NW: 215.9 ± 101.0 ng/mL, OW/OB: 355.8 ± 122.8 ng/mL, $p = 0.02$), which was driven by significantly higher PCB 138 + 163 (NW: 54.96 ± 32.4 ng/mL, OW/OB: 101.2 ± 44.8 ng/mL, $p = 0.03$) (Fig. 1). There were also trends toward higher PCB 132 + 153 (NW: 80.9 ± 50.4 ng/mL, OW/OB: 136.7 ± 81.9 ng/mL, $p = 0.12$) and PCB 180 (NW: 80.0 ± 63.3 ng/mL, OW/OB: 117.9 ± 50.6 ng/mL, $p = 0.17$) in mothers with OW/OB. Levels of milk PCB were not associated with infant birth weight, birth weight Z-score, maternal place of residency by zip code, or maternal income. There was no significant interaction effect of OW/OB with number of pregnancies on PCB content.

Associations between fat-adjusted PCB content in human milk at 2 weeks and infant growth trajectory by WHO Z-score for WFA, LFA, WFL, and HCA from 2 weeks to 6 months, adjusted for birth Z-score, are shown in Table 2 including unadjusted models and the models adjusted for maternal pre-pregnancy BMI for exploratory analysis. Significant negative associations of the change in infant HCA Z-score with PCB 132 + 153 (β −0.0008, $p = 0.0218$), PCB 180 (β −0.0010, $p = 0.0279$), and PCB sum (β −0.0006, $p = 0.0138$) were evident in the unadjusted models. PCB 180 was negatively associated with the change in infant WFA Z-score (β −0.0015, $p = 0.0058$) and WFL (β −0.0016, $p = 0.0263$) in the unadjusted models. There were no associations of PCB 132 + 153, PCB 138 + 163, or PCB sum with change in WFL, LFA, or WFL Z-score over the first 6 months of life.
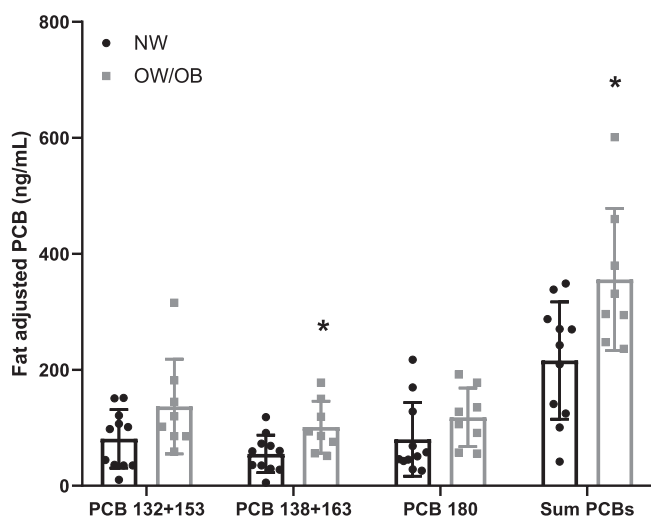
Maternal BMI was not a significant predictor in any of the adjusted models, and did not substantially alter the estimated effect or significance of PCB on infant growth. In Fig. 2, unadjusted loess curves for infant WHO Z-score growth trends are shown with higher milk PCB above the median for sum PCBs related to lower infant HCA Z-score over the first 6 months of life.

Due to the small sample size, post-hoc power analysis was carried out using simulated mixed models. The simulations assumed fixed effects of covariates and variance components to be equal to those estimated for the model predicting HCA Z-score with sum PCB congeners. Using the observed parameter estimates and sample size (time:PCB β −0.0005, n = 19), the model is estimated to have 87.5% power (95% CI: 85.29, 89.49) to detect the interaction of PCB and time. If the true interaction term β = -0.0004, the sample size of 19 achieves 69.8% power (95% CI: 66.85, 72.63), and if β = -0.0003, 45.2% power (95% CI: 42.08, 48.34). To detect these smaller interaction effects with greater than 80% power, a sample size of 25 to 40 would be required. Therefore, future larger scale studies to definitively determine the effects of lactational PCB exposure on infant growth should measure breast milk PCB in a sample of 40 mothers.

5. Discussion

Our pilot study results suggest that the concentrations of human milk PCB congener 138 + 163 and sum of PCB congeners (132, 138, 153, 163, 180) are higher in milk from mothers with elevated pre-pregnancy BMI. Importantly, higher milk PCB levels were negatively associated with early infant growth trajectory during the window of lactation, prior to the introduction of complementary foods. This study, albeit involving a small sample size, brings to attention the importance of studying PCB exposures during the first weeks of life through milk from mothers with increased pre-pregnancy weight in the setting of rising levels of obesity that complicate pregnancy and lactation.

Since PCBs accumulate in adipose tissue, the rising incidence of excess maternal adiposity poses a risk for increased infant exposure to PCBs through human milk (Müllerová and Kopecký, 2007). Human milk composition has been shown to be altered in the setting of maternal obesity with past studies focusing on hormone and nutrient levels (Fields et al., 2017; Ellsworth et al., 2018). However, studies examining the impact of maternal obesity on human milk environmental toxin levels are limited. The elevated PCB concentrations in OW/OB mothers milk during a period of transition from colostrum to mature milk, as captured in our study, may reflect increased adipose stores of PCBs. Importantly, maternal weight status does not appear to alter overall milk fat content.

Our findings are consistent with the Faroe Islands cohort study that demonstrated that sum of PCB congeners (138, 153, 180) in milk was associated with increased maternal BMI (Tang-Péronard et al., 2014). Interestingly, another study documented increased levels of placental PCBs in women with obesity (Jeong et al., 2018). However, conflicting data exist with other cohorts showing negative associations of maternal serum PCB 153 and mature human milk PCB with maternal pre-pregnancy BMI when levels are lipid-adjusted (Lauritzen et al., 2016; Rawn et al., 2017). Maternal serum and milk levels of PCBs have the potential to vary over time, which may account for these conflicting results. These discrepant results point to the need for further research focusing on specific stages of lactation. Additional maternal factors that may impact milk composition and excretion of PCBs include maternal historical risk for exposure to PCBs, maternal dietary intake, daily milk volume produced, and infant sex differences; these factors were not evaluated in our study (Komprda et al., 2019). The assessment of infant chemical exposure could further benefit from modeling previously described for polybrominated diphenyl ethers to estimate exposure risk (Marchitti et al., 2013).

Fig. 1. Fat adjusted polychlorinated biphenyl (PCB) content. *$p < 0.05$ by Welch two-sample *t*-test. (PCB 138 + 163$p = 0.03$; Sum PCBs $p = 0.02$).

Table 2

Interaction between human milk PCB content with infant growth trajectory across 2 weeks to 6 months.

Variables		WFAZ	LFAZ	WFLZ	HCAZ
	N	β (*p*-value) Difference in infant anthropometric change in Z-score			
PCB 132 + 153					
Unadjusted	19	−0.0003 (0.5000)	−0.0002 (0.6773)	−0.0004 (0.5032)	**−0.0008 (0.0218)***
Adjusted for maternal BMI	19	−0.0003 (0.5022)	−0.0002 (0.6777)	−0.0004 (0.5051)	**−0.0008 (0.0209)***
PCB 138 + 163					
Unadjusted	19	0.0006 (0.4393)	0.0004 (0.5065)	0.0006 (0.5173)	−0.0003 (0.5625)
Adjusted for maternal BMI	19	0.0006 (0.4386)	0.0004 (0.5042)	0.0006 (0.5121)	−0.0003 (0.5722)
PCB 180					
Unadjusted	19	**−0.0015 (0.0058)***	−0.0007 (0.1245)	**−0.0016 (0.0263)***	**−0.0010 (0.0279)***
Adjusted for maternal BMI	19	**−0.0015 (0.0058)***	−0.0007 (0.1233)	**−0.0016 (0.0259)***	**−0.0010 (0.0243)***
Sum PCBs					
Unadjusted	19	−0.0003 (0.1961)	−0.0001 (0.5002)	−0.0004 (0.2594)	**−0.0005 (0.0138)***
Adjusted for maternal BMI	19	−0.0003 (0.1983)	−0.0001 (0.4985)	−0.0004 (0.2649)	**−0.0005 (0.0130)***

* Statistical significance threshold at $p < 0.05$. Estimations based on fixed effects in linear mixed models for the interaction between milk PCB content and time based on unadjusted model and adjusted model for maternal pre-pregnancy BMI. Abbreviations: Polychlorinated biphenyl (PCB) weight-for-age Z-score (WFAZ), length-for-age Z-score (LFAZ), weight-for-length Z-score (WFLZ), head circumference-for-age Z-score (HCA), and body mass index (BMI).
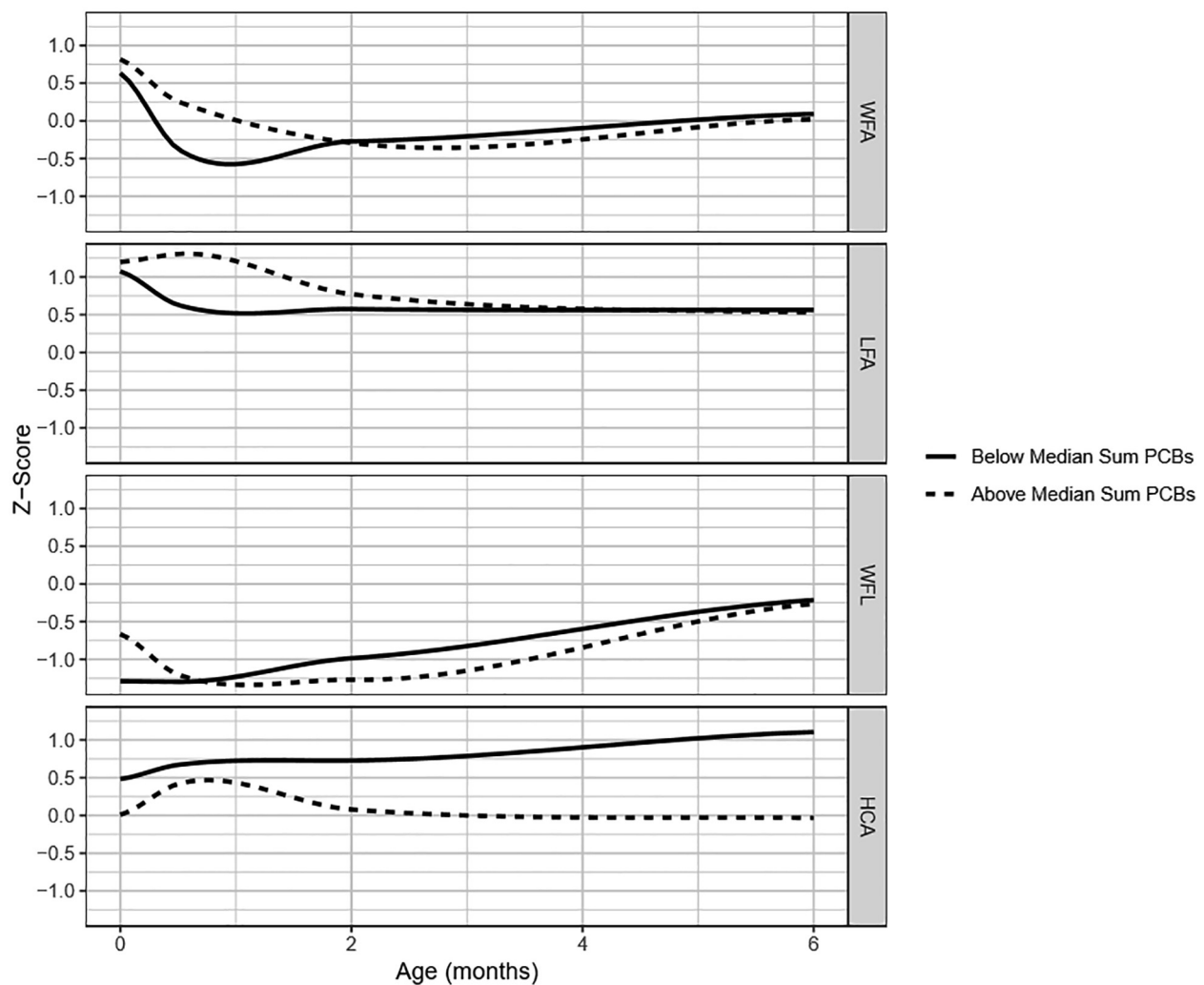

Fig. 2. Loess curves (unadjusted) of human milk polychlorinated biphenyl (PCB) content (ng/mL) with infant weight, length, weight/length, head circumference Z-score from birth to 6 months of age.

Postnatal exposure studies evaluating milk PCBs are limited, as attention has focused upon prenatal exposure and the impact on infant outcomes. Both the prenatal and postnatal timeframes are periods of ongoing organ development and maturation during which toxicant exposures have the potential to program long-term metabolic responses and growth (Ellsworth et al., 2018). Current literature evaluating associations between human milk PCB levels and infant growth reveals conflicting results. Our study further adds to these findings by

focusing on the lactation window of PCB exposure at approximately 2 weeks postpartum and showing that elevated levels of PCB 180 in milk were negatively associated with infant WFA and WFL Z-score, while multiple PCBs and overall sum PCB content were negatively associated with infant head circumference trajectory in the first 6 months of life.

Animal models of PCB 180 exposure in adult rats show transient reduced food intake and body weight, alterations in behavior, and thyroid dysregulation, which may point to mechanisms underlying our clinical findings during this early period of growth (Viluksela et al., 2014). Past human studies have demonstrated a continued negative association between milk PCBs and change in weight and length at 18 months to 2 years, when complimentary foods comprise the majority of dietary intake (Iszatt et al., 2015; Jackson et al., 2010; Grandjean et al., 2003). Another study, however, showed increased BMI at 7 years of age associated with high lactational PCB exposure in girls born to mothers who are overweight (Tang-Péronard et al., 2014). Interestingly, Tang-Perondad report that their results are consistent with previous literature noting that high PCB exposure in their study correlate with outcomes seen for low PCB exposure in other studies (Valvi et al., 2012; Verhulst et al., 2009). This may indicate a possible dose-dependent relationship between PCB exposure and infant growth. Finally, some studies show a lack of association between postnatal PCB exposure and infant growth through 3 to 42 months (Patandin et al., 1999; Criswell et al., 2017; Pan et al., 2010; Rogan et al., 1987). The differences amongst studies on the relationship between milk PCB concentration and infant growth outcomes are potentially attributed to the different PCB congeners evaluated, the absolute concentration detected, the stage in lactation, the overall volume of milk taken in by an infant impacting the dose received, modulation by formula supplementation, and the role of infant sex.

Our findings indicate that levels of PCB 132 + 153, PCB 180, and the sum of PCB congeners (132, 138, 153, 163, 180) in human milk are negatively associated with HCA Z-score over the first six months of life, which is concerning given the potential for adverse neurodevelopmental outcomes previously linked to PCB exposure (Darvill et al., 2000; Gladen et al., 2000). Fein showed that mothers who consumed PCB-contaminated lake fish had infants with 0.6 cm smaller head circumference at birth (Fein et al., 1984). At 11 years, these children were then 3-times more likely than children from mothers not exposed to PCBs to have lower intelligence quotient scores, reading comprehension, and attention span (Jacobson and Jacobson, 1996). Overall, however, studies examining the role of neonatal PCB exposure on neurodevelopmental outcomes are limited. Examining lactational PCB exposure, Walkowiak revealed significant negative associations between PCB levels in maternal milk and Bayley Scales of Infant Development scores after 30 months (Walkowiak et al., 2001). However, Patandin showed that lactational exposure to PCBs (118, 138, 153, 180) was not associated with 42-month cognitive abilities, and Rogan similarly showed that prenatal, but not postnatal, PCB exposure decreases psychomotor scales at 18 and 24 months (Patandin et al., 1999; Rogan and Gladen, 1991). The clinical implications of our findings of reduced HCA growth are unclear as the absolute difference in HCA was small. A larger sample size with associated developmental assessment would be needed to address this concern.

The small sample size is the main limitation of this study. Given this limitation, conclusions about the associations of PCB and infant growth are preliminary and more data are needed. Due to small sample size, this study is unable to adequately address the numerous potential confounders impacting milk PCB content and infant growth which are not limited to maternal parity, maternal age, maternal diet, maternal smoking, and infant sex. Despite its small sample size, this study provides novel insights into the impact of maternal weight status on milk PCB content during the period of transitional milk near 16 days post-partum. A strength of this study is that this is typically a time during which formula supplementation of newborns is limited. Therefore,

an exposure to infants through human milk would likely be higher at this time. This study also provides longitudinal growth outcomes on infants from a higher risk population living near the Great Lakes. A limitation of the study is that growth outcomes were not performed by trained research staff, but rather were extracted from the medical record, which may contribute to errors in measurements. Another limitation of our study is an inability to distinguish between prenatal and postnatal exposure to PCBs since maternal serum and infant cord blood were not collected. Prenatal PCB exposure studies indicate that PCB exposure can influence fetal development with demonstrated impacts on birth weight (Verhulst et al., 2009; Patandin et al., 1998); however, this was not seen in our study. While PCBs can be transferred across the placenta, human milk exposure has been shown to be a more significant contributor to infant PCB exposure (Jorissen, 2007). Further research is necessary to better differentiate exposure during prenatal and early postnatal life to identify potential target windows for intervention through larger cohort studies where potential confounders may also be addressed.

6. Conclusion

We have shown human milk levels of PCBs are higher in mothers with elevated pre-pregnancy weight status, and this increase may be related to the degree of adiposity in mothers. The higher concentration of PCBs in milk was negatively associated with infant head circumference, and PCB 180 was negatively associated with infant weight Z-score trajectory over the first 6 months of life. Together, these findings indicate that infant exposures to PCBs during lactation alters early infant growth outcomes. This early period of environmental toxin exposure through nutrition during a critical window of ongoing organ development may offer opportunities to study the mechanisms of PCB toxicity during this window and identify potential intervention strategies.

CRediT authorship contribution statement

Lindsay Ellswort: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Writing - original draft. **Harlan McCaffery:** Formal analysis, Data curation, Methodology, Writing - original draft. **Sergei Chernyak:** Methodology, Validation, Investigation, Resources, Writing - original draft. **Stephanie Lam:** Investigation, Writing - original draft. **Robert M. Sargis:** Conceptualization. **Vasantha Padmanabhan:** Conceptualization. **Brigid Gregg:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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