International Journal of Obesity (2018) **42,** 36–43

www.nature.com/ijo

ORIGINAL ARTICLE

Adiponectin, leptin and insulin in breast milk: associations with maternal characteristics and infant body composition in the first year of life

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BACKGROUND/OBJECTIVES: Breastfeeding may protect against excessive weight gain during infancy. However, the breast milk components responsible for this effect are unknown. We examined the variation of three breast milk hormones (adiponectin, leptin and insulin) according to maternal characteristics and determined their association with infant body composition.
SUBJECTS/METHODS: We studied a representative subset of 430 breastfed infants in the CHILD birth cohort. Breast milk was collected at 4 months postpartum and hormone concentrations were measured using the MesoScale Discovery System. Weight-for-length (WFL) and body mass index (BMI) *z*-scores were calculated according to the World Health Organization reference standard from infant anthropometrics measured at 4 months and 1 year. Maternal BMI and demographics were self-reported.
RESULTS: Breast milk hormone concentrations varied widely between mothers. The geometric mean (range) was 19.4 (3.7–74.4) ng ml^{-1} for adiponectin; 361 (31–3968) pg ml^{-1} for leptin; and 589 (53–5557) pg ml^{-1} for insulin. Maternal BMI was positively correlated with breast milk insulin ($r = +0.40$, $P < 0.0001$) and leptin ($r = +0.71$, $P < 0.0001$), but not adiponectin ($r = -0.02$, $P = 0.68$). Breast milk hormone concentrations were also associated with maternal ethnicity, parity and breastfeeding exclusivity at sample collection. Independent of these factors and maternal diabetes, smoking and breastfeeding duration, higher breast milk leptin was associated with lower infant WFL *z*-score at 4 months (β −0.67, 95% confidence interval (CI): −1.17, −0.17 for highest vs lowest quintile) and 1 year (β −0.58, 95% CI: −1.02, −0.14). Insulin showed a U-shaped association, with intermediate concentrations predicting the lowest infant WFL *z*-score at 4 months (β −0.51, 95% CI: −0.87, −0.15 for third vs lowest quintile) and 1 year (β −0.35, 95% CI: −0.66, −0.04). Similar results were seen with infant BMI. Breast milk adiponectin was not significantly associated with infant body composition.
CONCLUSIONS: Breast milk hormone concentrations were associated with several fixed and modifiable maternal characteristics. Higher concentrations of leptin and intermediate concentrations of insulin were associated with lower infant WFL in the first year of life.

International Journal of Obesity (2018) **42,** 36–43; doi:10.1038/ijo.2017.189

INTRODUCTION

The current epidemic of childhood obesity is one of the largest public health challenges facing healthcare providers and policy-makers worldwide.[1–3] Global estimates from 2014 indicate that over 42 million preschool-aged children worldwide are overweight or obese,[4] and this figure is expected to reach 70 million by 2025.[3] Accumulating evidence suggests that growth trajectories and metabolic health are 'programmed' in early life,[5,6] and this theory is supported by recent data showing that high body mass index (BMI) in infancy predicts obesity later in childhood.[7]

It is well established that breastfeeding promotes healthy growth trajectories during infancy and may protect against childhood obesity. Recent systematic reviews synthesizing results from many studies report that breastfeeding affords a 10–22% reduction in obesity risk.[8–10] However, despite this large body of evidence, the underlying biological mechanisms remain unclear, and some breastfed infants still become overweight or obese. Although many factors influence infant feeding practices and the development of obesity, the inconsistent associations between breastfeeding and infant obesity risk could also be related to variations in human milk composition.[11] In addition to macro and micronutrients, human milk contains many bioactive components with the potential to influence infant growth and development,[11,12] including cytokines, microbiota, oligosaccharides and metabolic hormones such as adiponectin, leptin and insulin.[13,14]

Adiponectin and leptin are adipose-derived hormones that regulate satiety and whole-body metabolism.[15] Circulating

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Received 20 April 2017; revised 16 July 2017; accepted 1 August 2017; accepted article preview online 14 August 2017; advance online publication, 19 September 2017

adiponectin promotes tissue insulin sensitivity, stimulates glucose uptake and decreases energy expenditure,[16,17] whereas leptin regulates fat storage by suppressing appetite and increasing energy expenditure.[18] Insulin is a peptide hormone secreted by the pancreas to regulate glucose metabolism.[14] The physiological effects of these hormones in blood have been studied for decades, but their presence in breast milk is a relatively new discovery, and their impact on infant metabolism, appetite regulation and weight gain is not well understood. Receptors for all three hormones are expressed in the highly permeable infant gastrointestinal tract,[19–21] suggesting they may act locally to elicit endocrine effects or be absorbed into circulation and mediate growth effects at other sites.[14]

Some evidence suggests that breast milk adiponectin, leptin and insulin may contribute to the protective effect of breastfeeding against childhood obesity,[13,14] although most previous studies have been limited in size and none have simultaneously evaluated all three hormones. Moreover, aside from maternal BMI,[22] studies have not systematically investigated the maternal factors that might influence breast milk hormone concentrations. We analyzed breast milk samples and clinical data from 430 mother–infant dyads enrolled in the Canadian Healthy Infant Longitudinal Development (CHILD) Study to determine the association of breast milk adiponectin, leptin and insulin with infant body composition, and further explored the association of maternal characteristics with variations in breast milk hormone concentrations.

MATERIALS AND METHODS

Study design and population

We accessed data and breast milk samples from 430 mother–infant dyads enrolled in the general cohort of the national CHILD birth cohort.[23] Women with singleton pregnancies were enrolled between 2009 and 2012 across four sites in Canada (Vancouver, Edmonton, Winnipeg, and Toronto). Those who delivered a healthy infant >35 weeks gestation were eligible to remain in the study. We analyzed a representative subset of 430 infants who were breastfed for at least 3 to 4 months (the time of the CHILD Study home assessment and breast milk sample collection). Mother–infant dyads with available breast milk samples and 1 year clinical assessment data were randomly selected with equal weighting from the four study sites. Our sample size was chosen to provide at least 90% power at 5% significance to detect a 20% difference in breast milk leptin and adiponectin concentrations between normal weight and overweight infants, based on published milk composition data[24,25] and anticipating a prevalence of 20% overweight in our cohort. This study was approved by the Human Research Ethics Boards at McMaster University, University of Manitoba, University of Alberta, University of Toronto and University of British Columbia. Mothers provided written informed consent at enrollment, and at each subsequent follow-up assessment.

Milk collection and hormone analysis

Mothers were provided a sterile collection jar and instructed to hand-express, combine and refrigerate fore milk and hind milk (pre and post feed) from multiple feeds during the 24 h prior to the CHILD study home assessment at 3 to 4 months postpartum. The use of a pump was acceptable if mothers were unable to hand-express.[26] Samples were then collected and aliquoted by study staff, and stored at − 80 °C until analysis. The concentration of adiponectin, leptin and insulin were quantified in duplicate from 100 µl of skimmed breast milk using kits precoated with the antibody to each hormone purchased from Mesoscale Discovery (MSD; Gaithersburg, MD, USA; catalog numbers K151BXC, K151BYC, K151BZC), following the manufacturer's instructions as previously described.[27] Results were measured on the MesoScale Discovery Sector Imager 2400 plate reader and the Discovery Workbench 3.0 software was used to generate standard curves and calculate analyte concentrations in each sample (expressed as ng protein per ml for adiponectin; pg protein per ml for leptin; pg protein per ml for insulin). The lower limit of detection was 0.064 ng ml^{-1} for adiponectin, 137 pg ml^{-1} for leptin and 69 pg ml^{-1} for insulin. Acceptable sample values fell within the linear range of the standard curve and had a critical value $<10\%$.

Infant anthropometrics

Following a standardized protocol, infant weight (in kg) and length (in cm) were measured by study staff during the CHILD Study home visit at 3 to 4 months postpartum (mean 17.1 ± 5 weeks), and again during a clinical assessment at 1 year. These measurements were used to calculate weight-for-length (WFL, kg/m) and body mass index (BMI, kg m^{-2}). Exact age (in days) was calculated based on the date of birth and date of measurement. Sex-specific WFL and BMI-for-age *z*-scores were calculated according to the World Health Organization Child Growth standard.[28] Currently, the American Academy for Pediatrics and the Canadian Pediatric Society recommend WFL as the standard clinical measure for infant body composition, whereas the World Health Organization and European authorities recommend BMI for all ages.[29,30] Large cohort studies have recently demonstrated that infant BMI is an equivalent[31] or superior[32] predictor of childhood obesity, compared to infant WFL. Therefore, we evaluated both BMI and WFL in our cohort.

Covariates

Maternal age, ethnicity, education (completion of postsecondary degree), prenatal smoking, diabetes (type 1, type 2, or gestational) and parity (number of previous live births) were self-reported during pregnancy using standardized questionnaires. Maternal BMI was determined from measured height and self-reported pre-pregnancy weight ($n=256$), or estimated from measured weight 1 year after delivery if mothers could not recall their pre-pregnancy weight ($n=174$). Validation against pre-pregnancy weight from prenatal health records in a subset of mothers ($n=224$) showed that pre-pregnancy weight was slightly underestimated by maternal recall (mean difference, − 1 kg; 95% confidence interval (CI): − 1.5 to − 0.4) and slightly overestimated by measured weight at 1 year after birth (mean difference, +1.3 kg; 95% CI: 0.5–2.2).[33] Maternal overweight and obesity were defined as a body mass index >25 kg m^{-2} and >30 kg m^{-2} respectively. Gestational weight gain was self-reported and excessive gestational weight gain was defined according to the Institute of Medicine guidelines.[34] The nurse at delivery recorded infant sex, gestational age, birth weight and mode of delivery. Infant feeding practices were reported by mothers at 3, 6 and 12 months postpartum using standardized questionnaires that captured age at weaning and first introduction to formula, non-human milks, juices and solid foods. This information was compared with the infant's exact age on the date of breast milk collection to classify feeding status as: exclusive breastfeeding (breast milk only, with no formula supplementation, other fluids or solid foods since birth), partial breastfeeding without formula (breast milk supplemented with solid foods, but no formula) or partial breastfeeding with formula (breast milk supplemented with formula, with or without solid foods). Vitamins and water were not considered in our definition of mother-reported exclusive breastfeeding.

Statistical analysis

Because all breast milk hormone concentrations were positively skewed, they were normalized by log transformation. Spearman's correlation was used to determine the association between pre-pregnancy maternal BMI and breast milk hormone concentrations. Univariate analyses (*t*-test and ANOVA) were conducted to determine the association of maternal and infant characteristics with breast milk hormone concentrations, followed by multivariable linear regression to identify independent predictors for each hormone. Predictors that were significantly associated with any hormone were subsequently included as covariates in regression models investigating the independent association between each breast milk hormone (classified in quintiles) and infant WFL and BMI *z*-score. Results are presented as crude and adjusted beta estimates (β, $a\beta$) with 95% CI. Analyses were performed using RStudio (Version 0.99.896, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

In our study population, the mean maternal age was 32.9 ± 4.2 years and 35% of mothers were overweight or obese (Table 1). The majority were Caucasian (73%) and had a postsecondary degree (83%). The median duration of exclusive breastfeeding was 4.5 months and 56% of infants were still breastfeeding at 12 months. Half of mothers (50%) reported exclusively breastfeeding (meaning no exposure to formula or solid foods) from birth

Table 1. Maternal and infant characteristics among 430 dyads from the CHILD cohort

Characteristic	n *(%) or mean ± s.d.*
Maternal age	
18–29 years	109 (25.3)
30–34 years	195 (45.3)
35–44 years	126 (29.3)
Maternal ethnicity	
Caucasian	315 (73.3)
Asian	80 (18.6)
First Nations	17 (4.0)
Other	18 (4.2)
Maternal postsecondary degree	
No	72 (16.7)
Yes	355 (82.6)
Missing	3 (0.7)
Maternal pre-pregnancy BMI	
Normal weight	281 (65.3)
Overweight	88 (20.5)
Obese	61 (14.2)
Excessive gestational weight gain[a]	
No	144 (33.5)
Yes	123 (28.6)
Missing	163 (37.9)
Parity	
Primiparous	236 (54.9)
Multiparous	194 (45.1)
Maternal diabetes[b]	
No	403 (93.7)
Yes	27 (6.3)
Maternal smoking	
No	411 (95.6)
Yes	19 (4.4)
Mode of delivery	
Vaginal	324 (75.3)
Cesarean	100 (23.3)
Missing	6 (1.4)
Infant sex	
Boy	220 (51.2)
Girl	210 (48.8)
Infant gestational age	
34–36 weeks	16 (3.7)
37–38 weeks	101 (23.5)
39–40 weeks	252 (58.6)
⩾41 weeks	56 (13.0)
Missing	5 (1.2)
Infant birth weight	
< 3000 g	64 (14.9)
3000–3499 g	167 (38.8)
3500–3999 g	129 (30.0)
⩾4000 g	59 (13.7)
Missing	11 (2.6)
Breastfeeding status at milk sample collection	
Exclusive	217 (50.5)
Partial without formula	50 (11.6)
Partial with formula	149 (34.7)
Missing	14 (3.3)
Lactation stage at milk sample collection (weeks postpartum)	17.1 ± 5.0
Infant anthropometrics	
WFL *z*-score at 4 months	− 0.32 ± 1.16
BMI *z*-score at 4 months	− 0.28 ± 1.09
WFL *z*-score at 1 year	0.26 ± 1.03
BMI *z*-score at 1 year	0.19 ± 1.04

Abbreviations: BMI, body mass index; WFL, weight-for-length. [a]According to the Institute of Medicine guidelines. [b]Type 1, Type 2 and gestational diabetes.

until the time of breast milk sample collection at 3 to 4 months postpartum, whereas the remainder reported supplementing with formula (27%), solid foods (12%) or both formula and solid foods (8%). Breast milk hormone concentrations are summarized in Table 2 and Supplementary Figure S1. The geometric mean concentrations were: 19.4 (3.7–74.4) ng ml^{-1} for adiponectin, 361 (range: 31–3968) pg ml^{-1} for leptin; and 589 (53–5557) pg ml^{-1} for insulin. The mean infant WFL was − 0.32 (95% CI: − 0.42, − 0.22) at 3 to 4 months and 0.26 (95% CI: 0.17, 0.36) at 1 year (Table 1). The mean infant BMI *z*-score was − 0.28 (95% CI: − 0.39, − 0.18) at 3 to 4 months and 0.19 (95% CI: 0.09, 0.29) at 1 year (Table 1).

Maternal characteristics and breast milk hormones

Pre-pregnancy maternal BMI was strongly correlated with breast milk leptin (Spearman $r = +0.71$, $P = < 0.0001$) and insulin (Spearman $r = +0.40$, $P = < 0.0001$) (Figure 1). Although no linear correlation was observed for breast milk adiponectin (Spearman $r = -0.02$, $P = 0.68$), concentrations were lower in breast milk from obese vs normal weight mothers (17.1 vs 19.4 ng ml^{-1}; $P = 0.02$) (Supplementary Table S1).

Multivariable analyses revealed that, in addition to maternal BMI, several other maternal characteristics were independently associated with breast milk hormone concentrations (Table 3). Compared to Caucasian mothers, Asian mothers had lower concentrations of breast milk adiponectin ($P = 0.01$) and higher concentrations of breast milk insulin ($P < 0.0001$), while there was no association between ethnicity and breast milk leptin. Breast milk leptin concentrations were lower in older and multiparous women (both $P < 0.05$, independent of each other), and tended to be higher in those who smoked during pregnancy ($P = 0.08$). In contrast, maternal age, parity and smoking were not associated with breast milk adiponectin or insulin concentrations. Stage of lactation ranged from 8 to 40 weeks and was uniquely associated with breast milk adiponectin, with lower concentrations in milk collected later in lactation ($P = 0.005$). Breast milk insulin concentrations were higher in mothers with diabetes ($P = 0.02$, Supplementary Table S1), but this difference was not significant in adjusted models ($P = 0.16$, Table 3).

Independent of lactation stage and the above maternal characteristics, maternal-reported breastfeeding exclusivity at the time of milk sample collection was significantly associated with milk hormone concentrations (Table 3). Compared to mothers who were exclusively breastfeeding, those who were supplementing with formula had higher concentrations of breast milk leptin ($P = 0.006$) and insulin ($P = 0.05$), while there was no apparent effect from supplementation with solid foods ($P = 1.00$ and 0.77, respectively). By contrast, breast milk adiponectin was significantly higher in mothers who were supplementing with solid foods only ($P = 0.002$), while there was no significant association with formula supplementation ($P = 0.09$). Altogether, breastfeeding exclusivity and maternal characteristics explained 45.2% of the observed variation in breast milk leptin concentrations, 20.8% of the

Table 2. Breast milk hormone concentrations in the CHILD cohort ($N = 430$)

	Breast milk hormones		
	Adiponectin (ng ml^{-1})	*Leptin (pg ml^{-1})*	*Insulin (pg ml^{-1})*
Geometric mean	19.4	361	589
Median (IQR)	19.5 (14.5–25.9)	349 (186–689)	586 (340–1013)
Range	3.7–74.4	31–3968	53–5557
Median (range) by quintile			
Quintile 1	10.7 (3.7–13.4)	109 (31–161)	231 (53–293)
Quintile 2	15.5 (13.5–17.4)	220 (162–273)	408 (294–498)
Quintile 3	19.5 (17.5–21.6)	349 (274–426)	585 (499–705)
Quintile 4	24.8 (21.7–29.0)	593 (427–805)	878 (706–1127)
Quintile 5	35.2 (29.1–74.4)	1291 (805–3968)	1532 (1128–5557)

Milk hormone distributions are shown in Supplementary Figure S1.

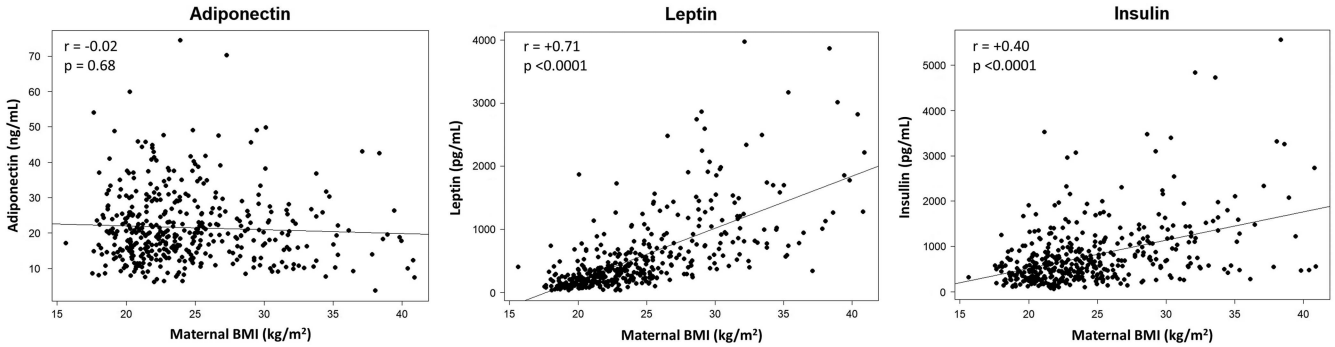

Figure 1. Correlation of breast milk hormones and pre-pregnancy BMI among 430 mothers in the CHILD cohort. BMI, body mass index; CHILD, Canadian Healthy Infant Longitudinal Development.

variation in breast milk insulin and 7% of the variation in breast milk adiponectin (Table 3).

Method of delivery, infant sex, birth weight and gestational age (Supplementary Table S1) were not independently associated with breast milk hormone concentrations (not shown). Among a subset of mothers with available gestational weight gain data ($N = 267$), gestational weight gain was not independently associated with milk hormone concentrations after adjusting for pre-pregnancy BMI (not shown).

Breast milk hormones and infant body composition

The association of milk hormones with infant WFL *z*-score was evaluated at the time of sample collection (mean 3.9 ± 1.2 months) (Figure 2; Table 4) and again at 1 year of age (mean 12.5 ± 1.9 months) (Figure 2; Supplementary Table S2). Infant BMI *z*-scores were also evaluated (Supplementary Tables S3 and S4).

Leptin. In crude analyses, there was no clear association between breast milk leptin concentration and infant BMI *z*-score. However, adjusting for maternal pre-pregnancy BMI revealed a strong inverse linear association, where higher concentrations of breast milk leptin were associated with lower infant WFL *z*-scores at 4 months (β -0.65, 95% CI: − 1.13, − 0.16, $P = 0.0009$ for highest vs lowest quintile) (Table 4). These associations persisted after further adjusting for maternal ethnicity, parity, diabetes, smoking, lactation stage and breastfeeding status at sample collection ($a\beta$ − 0.67, 95% CI: − 1.17, − 0.17, $P = 0.009$) (Figure 2; Table 4), and were similar for infant WFL *z*-score at 1 year ($a\beta$ − 0.58, 95% CI: − 1.02, − 0.14, $P = 0.0009$ for highest vs lowest quintile) (Figure 2; Supplementary Table S2). Similar associations were seen with infant BMI *z*-score ($a\beta$ − 0.67 and − 0.61 at 4 months and 1 year, respectively, both $P < 0.01$) (Supplementary Tables S3 and S4).

Insulin. At 4 months, a U-shaped association was observed whereby intermediate concentrations of breast milk insulin were associated with lower infant WFL *z*-score, independent of maternal pre-pregnancy BMI and other covariates ($a\beta$ − 0.51, 95% CI: − 0.87, − 0.15, $P = 0.006$ for middle vs lowest quintile) (Figure 2; Table 4). A similar but attenuated pattern of association was seen for infant WFL *z*-scores at 1 year ($a\beta$ − 0.35, 95% CI: − 0.66, − 0.04, $P = 0.03$ for middle vs lowest quintile) (Figure 2; Supplementary Table S2). Similar results were found for BMI *z*-scores ($a\beta$ − 0.46 and − 0.37 at 4 months and 1 year, respectively, for middle vs lowest quintile) (Supplementary Tables S3 and S4).

Adiponectin. At 4 months, breast milk adiponectin concentrations appeared to be inversely associated with infant WFL *z*-score (crude β − 0.34, 95% CI: − 0.70, 0.02, $P = 0.07$ for highest vs lowest quintile) (Table 4). However, this association was not statistically significant in adjusted models ($a\beta$ − 0.30, 95% CI: − 0.67, 0.08, $P = 0.12$) (Figure 2; Table 4), and there was no apparent association with infant WFL *z*-score by 1 year of age ($a\beta$ − 0.14, 95% CI: − 0.46, 0.18, $P = 0.38$) (Figure 2; Supplementary Table S2). Similar results were observed for infant BMI *z*-scores (Supplementary Tables S3 and S4).

DISCUSSION

In this analysis of mother–infant dyads representative of the CHILD birth cohort, we found that breast milk hormone concentrations were significantly associated with infant body

Table 3. Multivariable analysis of associations between maternal characteristics and breast milk hormone concentrations in the CHILD cohort

Maternal characteristics	*Association with breast milk hormones*[a]					
	Adiponectin		*Leptin*		*Insulin*	
	Beta (95% CI)	P	*Beta (95% CI)*	P	*Beta (95% CI)*	P
Age						
18–29 years	Ref		Ref		Ref	
30–34 years	−0.01 (−0.12, 0.10)	0.87	−0.07 (−0.24, 0.11)	0.46	0.01 (−0.16, 0.19)	0.88
35–44 years	0.01 (−0.11, 0.14)	0.83	**−0.21 (−0.40, −0.02)**	**0.03**	−0.04 (−0.23, 0.16)	0.72
Ethnicity						
Caucasian	Ref		Ref		Ref	
Asian	**−0.14 (−0.25, −0.03)**	**0.01**	0.04 (-0.14, 0.21)	0.68	**0.42 (0.24, 0.60)**	**<0.0001**
First Nations	−0.11 (−0.34, 0.13)	0.36	0.22 (−0.15, 0.60)	0.24	0.17 (−0.20, 0.54)	0.37
Other	−0.05 (−0.27, 0.18)	0.68	0.19 (−0.17, 0.56)	0.30	**0.37 (0.004, 0.74)**	**0.05**
Pre-pregnancy BMI						
Normal weight	Ref		Ref		Ref	
Overweight	0.06 (−0.06, 0.16)	0.34	**0.91 (0.73, 1.08)**	**<0.0001**	**0.37 (0.20, 0.55)**	**<0.0001**
Obese	**−0.15 (−0.27, −0.02)**	**0.03**	**1.51 (1.30, 1.71)**	**<0.0001**	**0.79 (0.59, 0.99)**	**<0.0001**
Parity						
Primiparous	Ref		Ref		Ref	
Multiparous	−0.06 (−0.16, 0.02)	0.13	**−0.15 (−0.29, −0.01)**	**0.04**	0.06 (−0.09, 0.20)	0.44
Diabetes[b]						
No	Ref		Ref		Ref	
Yes	−0.01 (−0.19, 0.18)	0.94	0.09 (−0.20, 0.38)	0.52	0.21 (−0.08, 0.50)	0.16
Smoking						
No	Ref		Ref		Ref	
Yes	0.10 (−0.10, 0.31)	0.32	0.30 (−0.04, 0.63)	0.08	0.17 (−0.16, 0.50)	0.30
Breastfeeding status at milk sample collection						
Exclusive	Ref		Ref		Ref	
Partial without formula	**0.28 (0.11, 0.45)**	**0.002**	0.00 (−0.27, 0.27)	1.00	−0.04 (−0.31, 0.23)	0.77
Partial with formula	0.08 (−0.01, 0.18)	0.09	0.21 (0.06, 0.36)	**0.006**	0.15 (−0.002, 0.30)	0.05
Lactation stage						
Per additional month	**−0.07 (−0.11, −0.02)**	**0.005**	−0.03 (−0.10, 0.05)	0.47	0.00 (−0.02, 0.01)	0.78
Total variation explained	**7.0%**	**0.006**	**45.2%**	**<0.0001**	**20.8%**	**<0.0001**

Abbreviations: BF, breastfeeding; CI, confidence interval. [a]Log-transformed. [b]Type 1, Type 2, gestational. Significant associations ($P < 0.05$) shown in bold. No significant associations were found for mode of delivery, infant sex, gestational age or birthweight (Supplementary Table S1). Associations determined by multivariable linear regression, adjusted for all maternal characteristics in table. $N = 413$ mothers with complete data.

composition during the first year of life. Our results show that higher concentrations of breast milk leptin and intermediate concentrations of breast milk insulin were associated with lower infant WFL and BMI *z*-scores at 4 months of age, whereas breast milk adiponectin concentrations were not significantly associated with infant body composition. These patterns of association persisted to 1 year of age, and were independent of pre-pregnancy maternal BMI, total breastfeeding duration and other known obesity risk factors. In addition, we identified several maternal characteristics associated with breast milk hormone concentrations, including BMI, ethnicity, age, parity, lactation stage and breastfeeding exclusivity.

Milk hormones and infant body composition

Previous studies provide mixed evidence for the association of breast milk leptin with infant growth and body composition.[14] Several small studies ($N < 30$) suggest that breast milk leptin levels are inversely correlated with infant weight or BMI,[25,35–37] although others have found no association with body composition during infancy[38–40] or early childhood.[15,41] Our current results from 430 infants in the CHILD cohort show an inverse dose-dependent relationship between breast milk leptin at 4 months postpartum and infant WFL and BMI *z*-scores at 4 months and 1 year. This strong association was both statistically and clinically significant. Our fully adjusted model predicts that infants consuming breast milk in the lowest leptin quintile have a 0.67-unit higher WFL *z*-score ($P = 0.009$) compared with infants in the highest quintile—a difference that exceeds the estimated impact of maternal BMI ($\beta = 0.04$, $P = 0.02$) and maternal smoking ($\beta = 0.41$, not significant), two established obesity risk factors evaluated in the same model.

Fewer studies have examined adiponectin in breast milk. In cohorts from Germany,[38] USA[42] and Mexico,[42] higher milk adiponectin was associated with lower infant weight[38] or WFL *z*-score[42] at 4 to 6 months of age; however, in the same children, higher milk adiponectin was subsequently associated with higher weight gain and adiposity in the second year of life.[38,24] A large German cohort[15] did not measure body composition during infancy, but found that higher levels of breast milk adiponectin at 6 weeks postpartum were associated with higher odds of overweight at 2 years of age. In our Canadian study, limited to the first year of life, we observed a trend toward lower infant WFL and BMI *z*-scores at 4 months among infants consuming breast milk with higher adiponectin concentrations; however, this

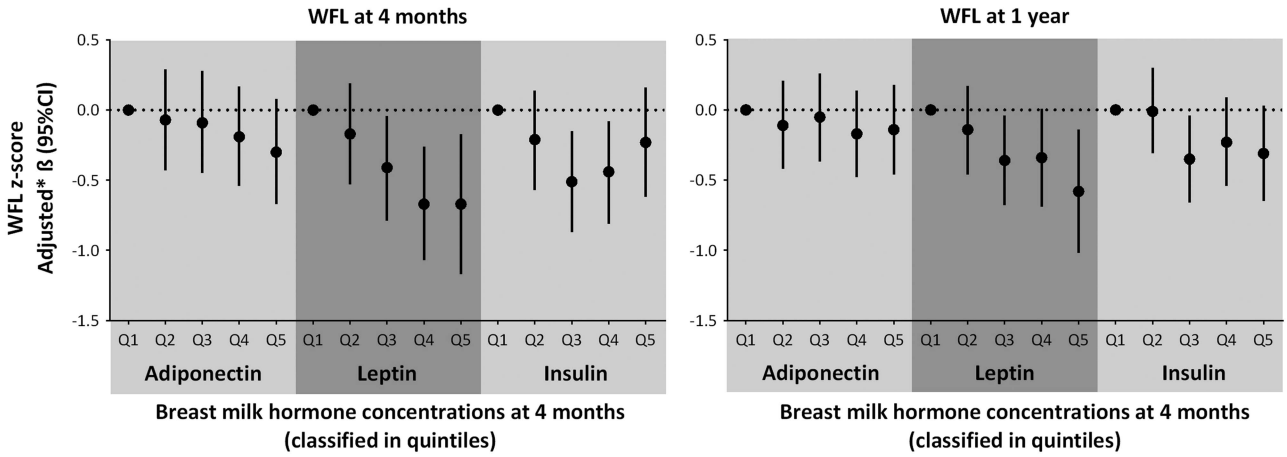

Figure 2. Associations between breast milk hormones and infant WFL *z*-score at 4 months and 1 year in the CHILD cohort. Breast milk adiponectin, leptin and insulin were measured at 4 months and classified in quintiles with Q1 (lowest quintile) as the reference group in multivariable linear regression models for infant WFL *z*-score at 4 months ($N = 388$) and 1 year ($N = 406$). *Models were adjusted for maternal pre-pregnancy BMI, ethnicity, parity, diabetes, smoking, lactation stage, breastfeeding status at sampling and (for 1-year BMI models) duration of any breastfeeding by 1 year. Results correspond to fully adjusted models in Table 4 and Supplementary Table S2. WFL, weight-for-length; CHILD, Canadian Healthy Infant Longitudinal Development; CI, confidence interval.

Table 4. Associations of breast milk hormone concentrations with infant WFL *z*-score at 4 months in the CHILD cohort

Breast milk hormone quintiles	*Association with infant WFL z-score at 4 months*					
	Crude		*Adjusted for maternal BMI*		*Adjusted for maternal BMI and covariates*[a]	
	Beta (95% CI)	P	*Beta (95% CI)*	P	*Beta (95% CI)*	P
Adiponectin						
1 (lowest)	0.00 (Reference)		0.00 (Reference)		0.00 (Reference)	
2	−0.08 (−0.44, 0.27)	0.64	−0.07 (−0.43, 0.29)	0.69	−0.07 (−0.43, 0.29)	0.71
3	−0.14 (−0.50, 0.22)	0.43	−0.14 (−0.50, 0.22)	0.46	−0.09 (−0.45, 0.28)	0.65
4	−0.22 (−0.58, 0.13)	0.22	−0.22 (−0.57, 0.14)	0.23	−0.19 (−0.54, 0.17)	0.30
5 (highest)	−0.34 (−0.70, 0.02)	0.07	−0.33 (−0.69, 0.04)	0.08	−0.30 (−0.67, 0.08)	0.12
Leptin						
1	0.00 (Reference)		0.00 (Reference)		0.00 (Reference)	
2	−0.09 (−0.44, 0.26)	0.62	−0.15 (−0.51, 0.20)	0.40	−0.17 (−0.53, 0.19)	0.35
3	−0.28 (−0.64, 0.09)	0.13	**−0.39 (−0.76, −0.01)**	**0.04**	**−0.41 (−0.79, −0.04)**	**0.03**
4	**−0.38 (−0.73, −0.02)**	**0.04**	**−0.61 (−1.01, −0.20)**	**0.003**	**−0.67 (−1.07, −0.26)**	**0.001**
5	−0.26 (−0.62, 0.11)	0.17	**−0.65 (−1.13, −0.16)**	**0.009**	**−0.67 (−1.17, −0.17)**	**0.009**
Insulin						
1	0.00 (Reference)		0.00 (Reference)		0.00 (Reference)	
2	−0.22 (−0.58, 0.13)	0.22	−0.23 (−0.58, 0.13)	0.21	−0.21 (−0.57, 0.14)	0.24
3	**−0.50 (−0.85, −0.15)**	**0.006**	**−0.52 (−0.89, −0.17)**	**0.004**	**−0.51 (−0.87, −0.15)**	**0.006**
4	**−0.38 (−0.73, −0.03)**	**0.04**	**−0.40 (−0.76, −0.04)**	**0.03**	**−0.44 (−0.81, −0.08)**	**0.02**
5	−0.12 (−0.47, 0.23)	0.49	−0.18 (−0.56, 0.20)	0.34	−0.23 (−0.62, 0.16)	0.25

Abbreviations: BMI, body mass index; CI, confidence interval. [a]Covariates include: pre-pregnancy maternal BMI, ethnicity, parity, diabetes, smoking, breastfeeding exclusivity at sampling and lactation stage. Associations determined by linear regression. Significant associations ($P < 0.05$) shown in bold. $N = 388$ dyads with complete covariate and infant WFL data at 4 months.

association was not statistically significant in adjusted models. Associations later in childhood will be the subject of future research in our CHILD cohort, where follow-up at 3 and 5 years is in progress.

To our knowledge, only one small cross-sectional study has previously reported on breast milk insulin and infant body composition, finding a negative correlation with WFL *z*-score at 1 month[25] among 19 infants, with no association at 6 months.[43] In our much larger longitudinal study, we observed a U-shaped association between breast milk insulin and infant body composition with intermediate concentrations predicting the lowest WFL and BMI *z*-scores at 4 and 12 months. We speculate that intermediate concentrations of breast milk insulin may optimally support infant metabolism while the immature pancreas develops its capacity to produce insulin, whereas insufficient or excessive insulin in breast milk may impair this process. Further research is warranted to address this intriguing hypothesis.

Potential mechanisms

There are several possible mechanisms by which breast milk hormones could influence infant growth and development.

Receptors for leptin, insulin and adiponectin are expressed in the infant gut,[19–21] where these hormones may act locally or be absorbed across the immature gastrointestinal tract to exert systemic effects. Research in rodents has shown that leptin supplied orally during the suckling period is absorbed into circulation, resulting in lower body weight and adiposity later in life.[44] Also in rodents, oral insulin promotes maturation of the gut[45] and pancreas,[46] and has favorable effects on blood glucose and lipid profiles.[47] A recent human study suggests that breast milk leptin and insulin may also influence the infant gut microbiome, favouring beneficial microbial metabolic pathways that reduce intestinal inflammation.[48] These potential mechanisms can be investigated in the CHILD cohort, where biobanked blood samples are available[26] to study circulating hormones and metabolic profiles, and gut microbiota have already been analyzed.[49,50]

Maternal characteristics and breast milk hormones

Consistent with previous studies, reviewed recently by Andreas *et al.*,[22] we found that maternal BMI was highly correlated with breast milk leptin, whereas adiponectin was not strongly associated with maternal BMI. We also contribute new evidence showing that maternal BMI is strongly correlated with breast milk insulin, which has been relatively understudied compared to leptin and adiponectin.[22] These results are consistent with findings from Young *et al.*[51] and Ley *et al.*,[52] where milk insulin was consistently higher in overweight and obese women compared with their normal weight counterparts. In addition, we found that breast milk adiponectin and insulin were associated with maternal ethnicity, suggesting that genetic factors may influence the concentration of these hormones in breast milk. Previous studies have reported that breast milk adiponectin levels are associated with maternal smoking, length of gestation and parity,[15,52] but we did not observe these associations in our cohort, possibly due to differences in our study population where smoking rates are low and pre-term births were excluded.

We observed a trend towards higher breast milk insulin concentrations in mothers with diabetes, suggesting a possible area for future research. Indeed, Young *et al.*[51] have recently shown that maternal insulin sensitivity is a powerful predictor of milk insulin concentrations. This association may partially explain the ethnic differences in our study, as insulin sensitivity and diabetes risk vary by ethnicity. Adjustment for maternal diabetes did not fully explain the ethnic differences we observed; however, we were unable to account more specifically for insulin sensitivity as it was not measured in our cohort.

An interesting and novel finding in our study is that concentrations of all three breast milk hormones were lower among mothers who were exclusively breastfeeding at the time of sample collection, compared to those who were supplementing with formula (for insulin and leptin) or solid foods (for adiponectin). This association persisted after adjusting for lactation stage and other maternal factors. Although further research is needed to confirm and study this phenomenon, our results suggest that breast milk hormone concentrations may naturally adjust (or be diluted), depending on the quantity of breast milk being produced. Alternatively, these findings could reflect associations with postpartum weight loss, which is known to be greater with exclusive breastfeeding.[53] However, we did not document postpartum weight loss and are unable to directly test this hypothesis in our cohort.

Strengths and limitations

The major strengths of our study include the large unselected sample of healthy infants, longitudinal design with 12 months follow-up, and ability to evaluate and control for maternal BMI and other important covariates. Pooling of breast milk samples across a 24 h period allowed us to obtain a good estimate of infant exposure to breast milk hormones. In addition, infant anthropometrics were measured using a standardized protocol by trained staff, although we did not measure fat mass and lean mass separately as some smaller studies have done.[25,38,43] Our study was limited by the collection of a single breast milk sample from each mother, which precluded the analysis of changes in breast milk hormone concentrations over time. However, we documented and controlled for exact lactation stage, and the hormone concentrations measured in our cohort were comparable to previous studies.[22]

CONCLUSION

In the CHILD cohort, higher concentrations of breast milk leptin and intermediate concentrations of breast milk insulin were associated with lower infant WFL and BMI in the first year of life. These associations may partially explain the increased risk of obesity among formula-fed infants,[12] who are not exposed to breast milk hormones, as well as the inconsistent risk among breastfed infants, who are exposed to variable hormone concentrations depending on multiple fixed and modifiable maternal characteristics. Further research is needed to understand the origins and determinants of breast milk hormones, and to characterize their impact on infant growth and childhood obesity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We are grateful to all the families who took part in this study, and the whole CHILD team, which includes interviewers, nurses, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers and receptionists. We also thank Drs Atul Sharma and Arthur Owora for their statistical expertise. This research was funded by the Manitoba Medical Service Foundation and the Children's Hospital Research Institute of Manitoba and supported by the Canadian Institutes of Health Research and the Allergy, Genes and Environment Networks of Centres of Excellence of Canada.

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Supplementary Information accompanies this paper on International Journal of Obesity website (http://www.nature.com/ijo)