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LETTER TO THE EDITOR

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Sex-specific association of human milk hormones and asthma in the CHILD cohort

To the Editor.

Asthma is one of the most common chronic childhood diseases, affecting 15%-20% of children worldwide.[1] The Developmental Origins of Health and Diseases hypothesis suggests that early childhood offers a critical opportunity to alter the development of chronic disease.[2] During this period, breastfeeding is increasingly recognized as serving a dual role of both nutrition and immune protection.[3] In the CHILD Cohort Study, we have observed a dose-dependent protective association between breastfeeding and possible or probable asthma at 3 years of age,[4] suggesting a potential role for immunomodulatory components of human milk. In addition to transferring maternal antibodies and cytokines, human milk contains adipokines and metabolic hormones including adiponectin, leptin, and insulin. While these hormones are most often studied in relation to growth, appetite, and satiety, they also have immunomodulatory properties;[5] yet, to our knowledge, they have never been studied in relation to asthma.

It is well established that asthma affects more boys than girls in early childhood,[1] but this sex difference is not fully understood. An emerging body of evidence suggests that human milk composition may differ when produced for sons vs. daughters.[6] However, this has not been widely studied, and even less is known about the potentially sex-specific effects of human milk components on clinical outcomes such as asthma.[5] Here, using data from the Canadian CHILD Cohort Study, we performed a sex-stratified analysis of human milk adiponectin, leptin, and insulin concentrations and their association with possible or probable asthma development by 3 years of age.

1 | METHODS

We studied a representative subset of 428 mother-infant dyads enrolled in the CHILD Cohort Study, a Canadian general population national birth cohort.[7] This study was approved by the Human Research Ethics Boards at McMaster University, University of Manitoba, University of Alberta, the Hospital for Sick Children, and University of British Columbia. As previously reported, breastmilk (one sample per mother) was collected at 3-4 months post-partum and adiponectin, leptin, and insulin were quantified in duplicate using the Mesoscale Discovery system (Gaithersburg, MD, USA).[8] Maternal age, ethnicity, education (completion of post-secondary degree), prenatal smoking, diabetes (type 1, type 2, or gestational),

TABLE 1 Human milk hormone concentrations in the CHILD cohort, stratified by sex of child

Human milk hormone	Boys N = 218	Girls N = 210	Sex difference *P*-value
Adiponectin (ng/mL)			
Geometric Mean	19.7	19.2	.55
Median (IQR)	21.6 (15.1-25.9)	19.5 (13.8-25.9)	
Range	6.4-54.0	3.7-74.4	
Leptin (pg/mL)			
Geometric Mean	337.6	386.8	.14
Median (IQR)	303.8 (178.8-626.1)	405.1 (214.5-733.4)	
Range	31.7-3165.0	30.6-3968.0	
Insulin (pg/mL)			
Geometric Mean	582.9	595.3	.77
Median (IQR)	577.8 (344.2-1063.0)	603.7 (338.3-943.0)	
Range	52.6-4831.0	80.1-5557	

IQR, interquartile range.

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parity (number of previous live births), and physician-diagnosed asthma were self-reported during pregnancy. Infant-feeding practices were reported at 3, 6, 12, 18, and 24 months. This information was compared with the infant's exact age on the date of breastmilk collection in order to classify feeding status as exclusive breastfeeding (breastmilk only, with no formula supplementation, other fluids or solid foods since birth) or partial breastfeeding.[8] Maternal and child BMI were measured as reported previously.[8] Subscapular skinfolds were measured using calipers by research staff, and asthma was diagnosed by trained healthcare professionals (physicians, nurses, or clinical research associates at each site) based on a physical examination and detailed history of respiratory symptoms.[4] For this analysis, asthma was classified as "possible or probable asthma" or "no asthma."

To normalize and facilitate comparison of effect estimates for different hormones, concentrations were log-transformed and converted to *z*-scores. Stepwise logistic regression models were used to assess associations between milk hormones and child asthma. Each hormone was assessed separately as follows: (a) crude unadjusted association, (b) adjusted model accounting for study site, maternal asthma, and key factors associated with milk hormone concentrations based on previous research [8] (maternal BMI, breastfeeding exclusivity, and infant age at the time of milk sample collection); (c&d) additional adjustment for child BMI z-score and subscapular skinfold thickness *z*-score at 3 years as potential mediators; and finally, (e) all three hormones were assessed in a single model adjusted for covariates and child BMI. Interaction terms were used to evaluate sex differences. Results are presented as crude and adjusted odds ratios (OR, aOR) with 95% confidence intervals (CI). ORs reflect the odds of asthma per standard deviation increase in the log-transformed hormone concentration. Analyses were performed using RStudio (version 0.99.896, R Foundation for Statistical Computing, Vienna).

2 | RESULTS

In this subset of the CHILD cohort, mothers were primarily primiparous (55%) and Caucasian (73%) and had a post-secondary degree (83%). Nearly 20% of mothers (85/428) reported having physician-diagnosed asthma, while 13% (29/218) of boys and 10% (21/210) of girls were diagnosed with possible or probable asthma at 3 years.

Milk hormone concentrations did not differ in milk produced for sons vs. daughters (Table 1, Figure 1A); however, sex-specific associations were observed between milk hormone concentrations and child asthma (Table 2, Figure 1B). In girls only, higher concentrations of human milk insulin were associated with higher odds of possible or probable asthma at 3 years (crude OR 2.00 95%CI 1.26, 3.27 per standard deviation increase). This association persisted after adjusting for maternal BMI, maternal asthma, lactation stage, breastfeeding exclusivity, and study site (aOR 2.95 95%CI 1.60, 5.96). It was also independent of child BMI z-score (aOR 2.95 95%CI 1.59, 5.98) and subscapular skinfold thickness z-score (aOR 2.87 95%CI 1.51, 5.97) at 3 years, indicating that child body weight and composition are not mediators of this association. These patterns of association were not seen in boys (aOR 0.91 95%CI 0.57, 1.46 in the fully adjusted model) (*P* for sex interaction = .007). Sex-specific associations were also observed for milk leptin, where the association in boys trended toward a protective effect (aOR 2.58

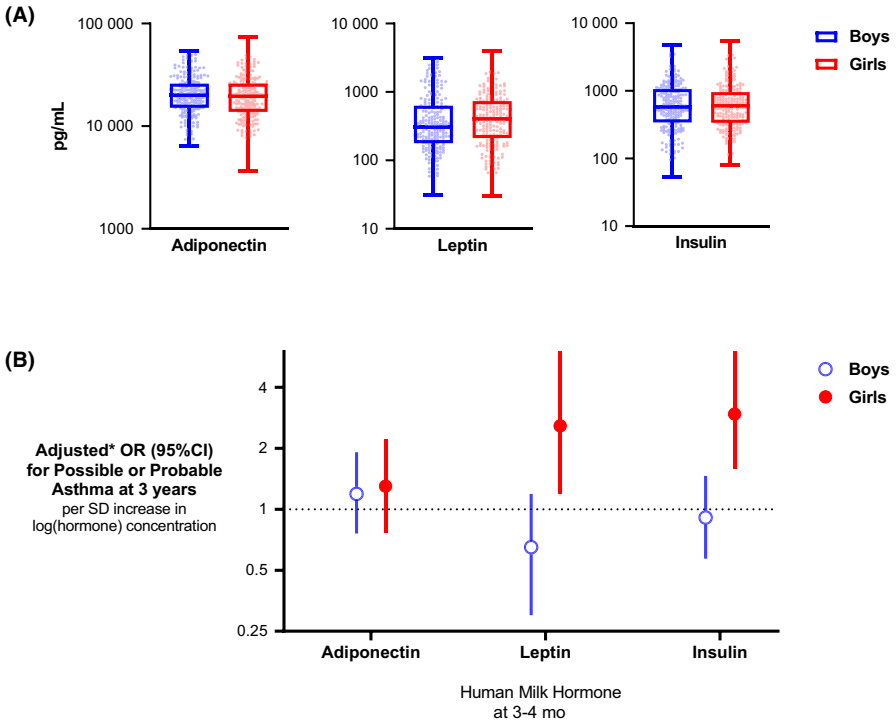

FIGURE 1 Human milk adiponectin, leptin, and insulin at 3-4 mo and asthma at 3 y among boys and girls in the CHILD cohort (N = 428). A) Human milk hormone concentrations at 3-4 mo, stratified by infant sex. Boxes indicate interquartile range; lines indicate medians; and whiskers indicate range. B) Odds ratio (OR) and 95% confidence interval (CI) for possible or probable asthma at 3 y, per standard deviation increase in log(hormone) concentration. *Adjusted for study site, maternal BMI, maternal self-report of physician-diagnosed asthma, breastfeeding exclusivity, and lactation stage (ie, exact infant age) at the time of milk sample collection, and child BMI z-score at 3 y. *P*-values for sex interaction: .75 (adiponectin), .03 (leptin), and .007 (insulin) [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Sex-specific associations of human milk hormones at 3-4 mo with possible or probable asthma at 3 y in the CHILD cohort

Human milk hormone	Model	Association with asthma at 3 y		Sex difference
		Boys (N = 186)	Girls (N = 185)	
		OR* (95% CI)	OR* (95% CI)	*P*-value
Adiponectin	1) Crude	1.04 (0.69, 1.58)	1.10 (0.72, 1.71)	.85
	2) Adjusted	1.23 (0.78, 1.98)	1.31 (0.78, 2.25)	.81
	3) Model 2 + child BMI at 3 y	1.19 (0.76, 1.91)	1.30 (0.77, 2.22)	.75
	4) Model 2 + child subscapular skinfolds at 3 y	1.10 (0.68, 1.81)	1.26 (0.72, 2.26)	.77
	5) Model 3 with mutual adjustment for all hormones	1.20 (0.75, 1.97)	1.04 (0.60, 1.82)	.71
Leptin	1) Crude	0.82 (0.55, 1.19)	1.59 (0.98, 2.68)	**.04**
	2) Adjusted	0.68 (0.38, 1.23)	**2.62 (1.21, 6.13)***	**.03**
	3) Model 2 + child BMI at 3 y	0.65 (0.30, 1.19)	**2.58 (1.20, 6.01)***	**.03**
	4) Model 2 + child subscapular skinfolds at 3 y	0.77 (0.40, 1.47)	**2.60 (1.18, 6.26)**	.14
	5) Model 3 with mutual adjustment for all hormones	0.62 (0.32, 1.20)	1.70 (0.70, 4.27)	**.04**
Insulin	1) Crude	0.91 (0.62, 1.34)	**2.00 (1.26, 3.27)****	**.01**
	2) Adjusted	0.95 (0.60, 1.51)	**2.95 (1.60, 5.96)****	**.009**
	3) Model 2 + child BMI at 3 y	0.91 (0.57, 1.46)	**2.95 (1.59, 5.98)****	**.007**
	4) Model 2 + child subscapular skinfolds at 3 y	0.94 (0.57, 1.58)	**2.87 (1.51, 5.97)**	**.04**
	5) Model 3 with mutual adjustment for all hormones	1.04 (0.62, 1.74)	**2.51 (1.31, 5.24)****	**.007**

Associations determined by logistic regression. ORs reflect odds of asthma per standard deviation increase in log (milk hormone). N = 371 dyads with complete data for all covariates. Separate models were created for each hormone (Models 1-4), except for Model 5, which includes all hormones.

Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio.

[a]Adjusted for study site, maternal BMI, maternal self-report of physician-diagnosed asthma, breastfeeding exclusivity at sampling, and lactation stage. Significant associations ($P < .05$) shown in bold.

*$P < .05$

**$P < .01$.

95%CI 1.20, 6.01 for girls; aOR 0.65 95%CI 0.30, 1.19 for boys; *P* for interaction = .03). Milk adiponectin was not associated with asthma in either sex. When simultaneously adjusting for all three milk hormones, only insulin remained independently associated with asthma in girls (aOR 2.51, 95%CI 1.31, 5.24).

3 | DISCUSSION AND CONCLUSION

There are several possible mechanisms that could explain the association of human milk hormones ingested during infancy with asthma later in childhood. First, since milk hormones influence weight gain in breastfed infants [8] and high body weight is associated with asthma later in childhood,[9] it is possible that milk hormones affect asthma risk via their impact on body weight and/or composition. However, this hypothesis is not supported by our current results because the associations we observed were independent of infant weight gain velocity (not shown), subscapular skinfolds, and child BMI at 3 years (Table 2), suggesting a mechanism unrelated to growth or body composition. Alternatively, milk hormones could influence inflammation and immune development. It is known that circulating leptin can stimulate the production of pro-inflammatory cytokines, potentially mediating the asthma-obesity axis [10]; however, it is not known whether leptin in human milk can trigger similar responses. Finally, milk leptin and insulin may influence the development of the neonatal gut microbiome, which could impact intestinal inflammation and immune function,[11] with potential implications for asthma development.

The sex differences observed in our study could reflect an interaction between ingested milk hormones and circulating sex hormones, which are increased during the "mini-puberty" occurring between 3 and 6 months.[12] Further research is needed to understand why boys and girls might respond differently to milk hormones and to determine whether these sex-specific responses contribute to the well-known sex differences in asthma development.

In summary, we found that breastfed girls consuming milk with high concentrations of leptin and insulin were more likely to develop possible or probable asthma by 3 years of age. This association was not mediated by body weight and composition and was not seen in boys. We acknowledge that asthma diagnosis is uncertain at this age and will continue to study these associations as more definitive asthma diagnoses become available in the ongoing CHILD Cohort Study. Meanwhile, these findings suggest a potential role for human milk leptin and insulin in asthma development or prevention and highlight the importance of considering sex differences in breastfeeding and human milk research.

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CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

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