

1

2 MS DEBORAH CHAN (Orcid ID : 0000-0002-4717-0870)

3 DR MALCOLM R SEARS (Orcid ID : 0000-0002-7113-4917)

4 DR PADMAJA SUBBARAO (Orcid ID : 0000-0003-0394-1933)

5

6

7 Article type : Letter to the Editor

8

9

10 Corresponding author mail id :- Meghan.Azad@umantitoba.ca

11

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

13

14 **Impact Statement**

15 Breastfeeding may help prevent asthma, but current evidence is mixed and the mechanism is
16 unknown. Human milk contains many bioactive compounds, including leptin and insulin. These
17 endocrine hormones have immunomodulatory properties but they have not been studied in relation
18 to asthma. In the CHILD Cohort Study, we found that breastfed girls consuming milk with high
19 concentrations of leptin and insulin were more likely to develop asthma by 3 years of age. This
20 association was not mediated by body composition and was not seen in boys. Our results raise new
21 questions about the potential sex-specific role of human milk hormones in the development or
22 prevention of asthma.

23

24

25 To the Editor:

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/PAI.13219](#)

This article is protected by copyright. All rights reserved

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

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

44 **Methods**

45 We studied a representative subset of 428 mother-infant dyads enrolled in the CHILd Cohort Study,
46 a Canadian general population national birth cohort (7). This study was approved by the Human
47 Research Ethics Boards at McMaster University, University of Manitoba, University of Alberta, the
48 Hospital for Sick Children, and University of British Columbia. As previously reported, breast milk
49 (one sample per mother) was collected at 3-4 months postpartum and adiponectin, leptin, and insulin
50 were quantified in duplicate using the Mesoscale Discovery system (Gaithersburg, MD, USA) (8).
51 Maternal age, ethnicity, education (completion of postsecondary degree), prenatal smoking, diabetes
52 (type 1, type 2, or gestational), parity (number of previous live births) and physician-diagnosed
53 asthma were self-reported during pregnancy. Infant feeding practices were reported at 3, 6, 12, 18
54 and 24 months. This information was compared with the infant's exact age on the date of breastmilk
55 collection in order to classify feeding status as exclusive breastfeeding (breastmilk only, with no

56 formula supplementation, other fluids or solid foods since birth) or partial breastfeeding (8). Maternal
57 and child BMI were measured as reported previously (8). Subscapular skinfolds were measured
58 using calipers by research staff and asthma was diagnosed by trained healthcare professionals
59 (physicians, nurses or clinical research associates at each site) based on a physical examination and
60 detailed history of respiratory symptoms (4). For this analysis, asthma was classified as “possible or
61 probable asthma” or “no asthma”.

62
63 To normalize and facilitate comparison of effect estimates for different hormones, concentrations
64 were log-transformed and converted to z-scores. Stepwise logistic regression models were used to
65 assess associations between milk hormones and child asthma. Each hormone was assessed
66 separately as follows: 1) crude unadjusted association, 2) adjusted model accounting for study site,
67 maternal asthma, and key factors associated with milk hormone concentrations based on previous
68 research (8) (maternal BMI, breastfeeding exclusivity and infant age at the time of milk sample
69 collection); 3&4) additional adjustment for child BMI z-score and subscapular skinfold thickness z-
70 score at 3 years as potential mediators; and finally, 5) all three hormones were assessed in a single
71 model adjusted for covariates and child BMI. Interaction terms were used to evaluate sex
72 differences. Results are presented as crude and adjusted odds ratios (OR, aOR) with 95%
73 confidence intervals (CI). ORs reflect the odds of asthma per standard deviation increase in the log-
74 transformed hormone concentration. Analyses were performed using RStudio (Version 0.99.896, R
75 Foundation for Statistical Computing, Vienna).

76 Results

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

81
82 Milk hormone concentrations did not differ in milk produced for sons vs. daughters (**Table 1, Figure**
83 **1a**); however, sex-specific associations were observed between milk hormone concentrations and
84 child asthma (**Table 2, Figure 1b**). In girls only, higher concentrations of human milk insulin were
85 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=0.007). Sex-specific associations were also observed for milk leptin, where the association in boys trended towards a protective effect (aOR 2.58 95%CI 1.20, 6.01 for girls; aOR 0.65 95%CI 0.30, 1.19 for boys; p for interaction=0.03). 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)).

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 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 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 3-6 months (12). Further research is needed to understand why boys and girls might respond differently to milk hormones, and to determine if these sex-specific responses contribute to the well-known sex differences in asthma development.

117

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

126 *Deborah Chan¹, Allan B. Becker¹, Theo J. Moraes², Piushkumar J. Mandhane³, Malcolm R. Sears⁴,
127 Stuart E. Turvey⁵, Padmaja Subbarao², Catherine J. Field⁶ and Meghan B. Azad^{1*}*

128

129 *¹Department of Pediatrics and Child Health, University of Manitoba and Children's Hospital Research
130 Institute of Manitoba, Winnipeg, Manitoba; ²Department of Pediatrics, Hospital for Sick Children,
131 University of Toronto, Toronto, Ontario; ³Department of Pediatrics, University of Alberta, Edmonton,
132 Alberta; ⁴Department of Medicine, McMaster University, Hamilton, Ontario; ⁵Department of
133 Pediatrics, Child and Family Research Institute and BC Children's Hospital, University of British
134 Columbia, Vancouver, British Columbia; ⁶Department of Agricultural Food, and Nutritional Science,
135 University of Alberta, Edmonton, Alberta, Canada.*

136

137

138 **Competing Interests**

139 The authors declare that they have no competing interests

140

141 **Funding/Support**

142 This research was funded by the Manitoba Medical Service Foundation and the Children's Hospital
143 Research Institute of Manitoba. The Canadian Institutes of Health Research and the Allergy, Genes
144 and Environment Networks of Centres of Excellence of Canada provided core support for the CHILD
145 Study. This research was supported, in part, by the Canada Research Chairs Program. MBA holds
146 the Tier 2 Canada Research Chair in the Developmental Origins of Chronic Disease. These agencies
147 did not have a role in the design of the study and collection, analysis, and interpretation of data and
148 in writing the manuscript.

149

150 **Acknowledgements**

151 We are grateful to all the families who took part in this study, and the whole CHILD team, which
152 includes interviewers, nurses, computer and laboratory technicians, clerical workers, research
153 scientists, volunteers, managers, and receptionists.

154 **References**

- 155 1. Ferrante G. & La Grutta S. The burden of pediatric asthma. *Front. Pediatr* 2018; 6:186. doi:
156 10.3389/fped.2018.00186
- 157 2. Gluckman PD & Hanson MA. Developmental Origins of Disease Paradigm: A Mechanistic and
158 Evolutionary Perspective. *Pediatric Research* 2004; 56: 311-317”
- 159 3. Geddes D & Perrella S. Breastfeeding and human lactation. *Nutrients* 2019; 11: 802.
160 doi:10.3390/nu11040802
- 161 4. Klopp A, Vehling L, Becker AB, Subbarao P, Mandhane PJ, Turvey SE, Lefebvre DL, Sears MR,
162 the CHILD Study Investigators & Azad MB, Modes of infant feeding and the risk of childhood
163 asthma: A prospective birth cohort study. *J Peds* 2017; 190.
164 <https://doi.org/10.1016/j.jpeds.2017.07.012>
- 165 5. Field CJ. The immunological components of human milk and their effect on immune
166 development in infants. *J. Nutr.* 2005; 135: 1-4.
- 167 6. Galante L, Milan AM, Reynolds CM, Cameron-Smith D, Vickers MH, & Pundir S. Sex-specific
168 human milk composition: The role of infant sex in determining early life nutrition. *Nutrients* 2018;
169 10: 1194. doi:10.3390/nu10091194
- 170 7. Subbarao P, Anand SS, Becker AB, Befus AD, Brauer M, Brook JR et al. The Canadian Healthy
171 Infant Longitudinal Development (CHILD) Study: examining developmental origins of allergy and
172 asthma. *Thorax* 2015; 70: 998–1000.
- 173 8. Chan D, Goruk S, Becker AB, Subbarao P, Mandhane PJ, Turvey SE, Lefebvre D, Sears MR,
174 the CHILD Study Investigators, Field CJ, & Azad MB. Adiponectin, leptin and insulin in breast
175 milk: associations with maternal characteristics and infant body composition in the first year of
176 life. *Int J Obesity* 2017; doi:10.1038/ijo.2017.189
- 177 9. Papoutsakis C, Priftis KN, Drakouli M, Prifti S, Konstantaki E, Chondronikola M, Antonogeorgos
178 G, & Matziou V. Childhood overweight/obesity and asthma: Is there a link? A systematic review

179 of recent epidemiologic evidence. J Acad Nutr Diet. 2013; 113: 77-105. doi:
180 10.1016/j.jand.2012.08.025

181 10. Mai X, Chen Y, & Krewski D. Does leptin play a role in obesity–asthma relationship? Pediatr
182 Allergy Immunol 2009; 20: 207–212. doi: 10.1111/j.1399-3038.2008.00812.x

183 11. Lemas DJ, Young BE, Li PRB, Tomczik AC, Soderborg TK, Hernandez TL et al. Alterations in
184 human milk leptin and insulin are associated with early changes in the infant intestinal
185 microbiome. Am J Clin Nutr 2016; 103: 1291–1300.

186 12. Lanciotti L, Cofini M, Leonardi A, Penta L, & Esposito S. Up-to-date review about minipuberty
187 and overview on hypothalamic-pituitary-gonadal axis activation in fetal and neonatal life. Front.
188 Endocrinol. 2018; 9:410. doi: 10.3389/fendo.2018.00410

189

Tables & Figures

Table 1. Human milk hormone concentrations in the CHILD cohort, stratified by infant sex.

Human milk hormone		Boys N=218	Girls N=210	Sex Difference p-value
Adiponectin (ng/mL)	Geometric Mean	19.7	19.2	0.55
	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	0.14
	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	0.77
	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.

Table 2. Sex-specific associations of human milk hormones at 3-4 months with possible or probable asthma at 3 years in the CHILD cohort.

Human milk hormone	Model	Association with Asthma at 3 years		Sex Difference p-value
		Boys (N=186) OR* (95% CI)	Girls (N=185) OR* (95% CI)	
Adiponectin	1) Crude	1.04 (0.69, 1.58)	1.10 (0.72, 1.71)	0.85
	2) Adjusted	1.23 (0.78, 1.98)	1.31 (0.78, 2.25)	0.81
	3) Model 2 + child BMI at 3 years	1.19 (0.76, 1.91)	1.30 (0.77, 2.22)	0.75
	4) Model 2 + child subscapular skinfolds at 3 years	1.10 (0.68, 1.81)	1.26 (0.72, 2.26)	0.77
	5) Model 3 with mutual adjustment for all hormones	1.20 (0.75, 1.97)	1.04 (0.60, 1.82)	0.71
Leptin	1) Crude	0.82 (0.55, 1.19)	1.59 (0.98, 2.68)	0.04
	2) Adjusted	0.68 (0.38, 1.23)	2.62 (1.21, 6.13)*	0.03
	3) Model 2 + child BMI at 3 years	0.65 (0.30, 1.19)	2.58 (1.20, 6.01)*	0.03
	4) Model 2 + child subscapular skinfolds at 3 years	0.77 (0.40, 1.47)	2.60 (1.18, 6.26)	0.14
	5) Model 3 with mutual adjustment for all hormones	0.62 (0.32, 1.20)	1.70 (0.70, 4.27)	0.04
Insulin	1) Crude	0.91 (0.62, 1.34)	2.00 (1.26, 3.27)**	0.01
	2) Adjusted	0.95 (0.60, 1.51)	2.95 (1.60, 5.96)**	0.009
	3) Model 2 + child BMI at 3 years	0.91 (0.57, 1.46)	2.95 (1.59, 5.98)**	0.007
	4) Model 2 + child subscapular skinfolds at 3 years	0.94 (0.57, 1.58)	2.87 (1.51, 5.97)	0.04

5) Model 3 with mutual adjustment for all hormones 1.04 (0.62, 1.74) **2.51 (1.31, 5.24)**** 0.007

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. ¹Adjusted for study site, maternal BMI, maternal self-report of physician-diagnosed asthma, breastfeeding exclusivity at sampling, and lactation stage. Significant associations ($p < 0.05$) shown in bold. * $p < 0.05$, ** $p < 0.01$. BMI, body mass index; OR, odds ratio. CI, confidence interval.

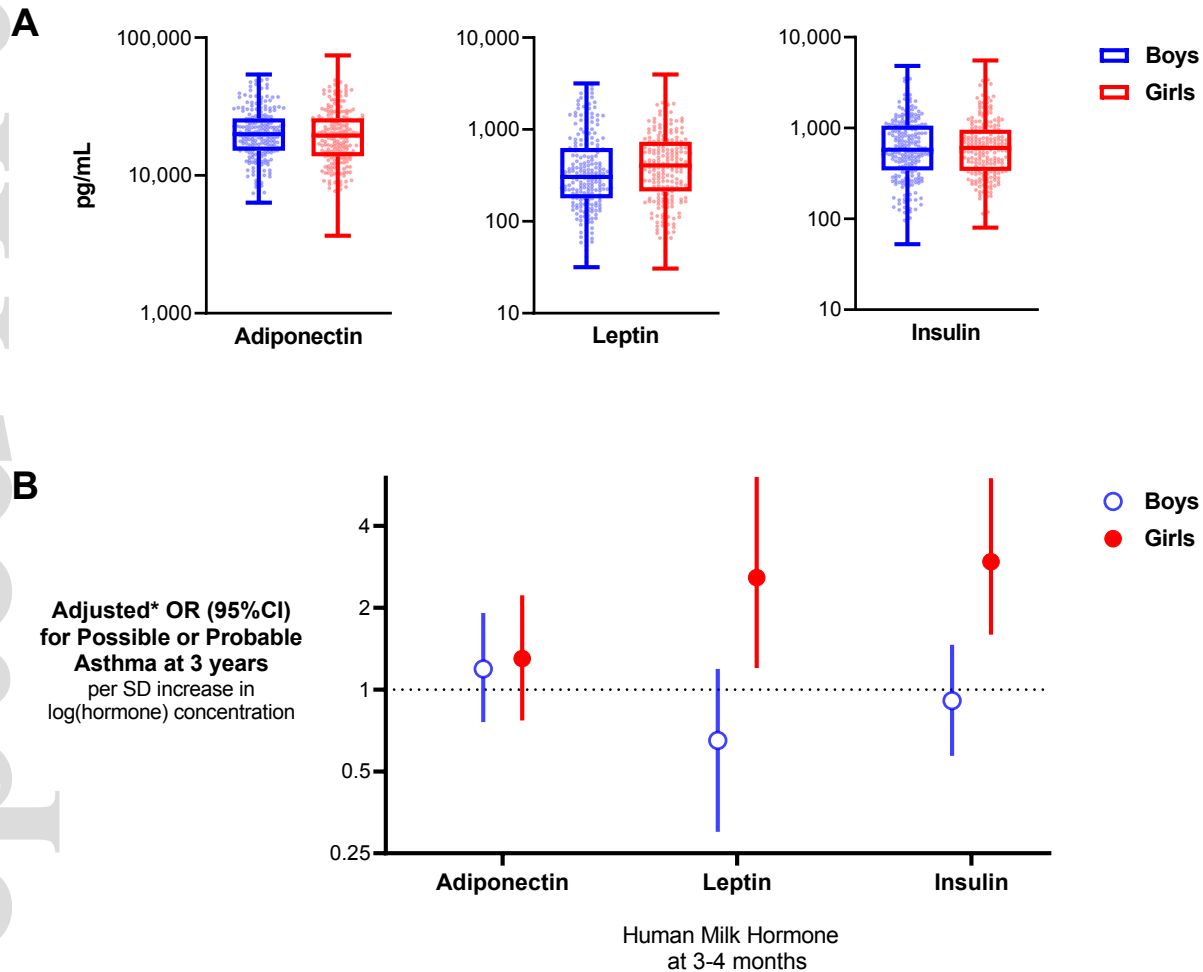


Figure 1. Human milk adiponectin, leptin and insulin at 3-4 months and asthma at 3 years among boys and girls in the CHILD cohort (N=428). A) Human milk hormone concentrations at 3-4 months, stratified by infant sex. Boxes indicate interquartile range; lines indicate medians; whiskers indicate range. B) Odds ratio (OR) and 95% Confidence Interval (CI) for possible or probable asthma at 3 years, 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 (i.e. exact infant age) at the time of milk sample collection, and child BMI z-score at 3 years. p-values for sex interaction: 0.75 (adiponectin), 0.03 (leptin), 0.007 (insulin).