To the Editor,

Allergies originate early in life, and food sensitization is often the first manifestation of allergic disease. Breastfeeding has been inconsistently associated with allergic conditions. These inconsistencies could reflect differences in human milk composition, which varies across different settings and populations. However, it remains poorly understood which of the bioactive components in human milk contribute to the developmental programming of allergic disease.

Human milk oligosaccharides (HMOs) are the third most abundant component of human milk, yet they are absent from most infant formulas. HMO composition is influenced by genetic fucosyltransferase-2 secretor status and also by lactation stage, gestational age, maternal health, ethnicity, geographic location, and breastfeeding exclusivity. Among their many functions, HMOs act as selective substrates to guide development of the infant gut microbiota. We have previously reported that gut microbiota richness in early infancy is associated with subsequent food sensitization, suggesting that HMOs and other determinants of early gut colonization could influence the development of allergic disease. This hypothesis is also supported by experimental research in rodents and a small clinical study where low concentrations of the HMO lacto-N-fucopentaose III (LNFPIII) were associated with higher incidence of cow’s milk allergy. However, the potential impact of other individual HMOs on food sensitization is not known, and the impact of overall HMO composition has not been studied, yet this may be important because breastfed infants are naturally exposed to complex combinations of HMOs in human milk.

In this study, among 421 mother–infant dyads from the Canadian Healthy Infant Longitudinal Development (CHILD) cohort, we examined the associations of 19 individual HMOs and overall HMO profiles with food sensitization at 1 year of age using Projection on Latent Structures-Discriminant Analysis (PLS-DA). Detailed methods are provided in Supplementary Materials.

Overall, 59/421 infants (14.0%) were sensitized to 1 or more food allergens at 1 year of age (Table S1). We did not observe any significant associations for the 19 individual HMOs or total HMOs and food sensitization (Figure 1A); however, overall HMO profiles differed significantly in milk consumed by sensitized vs nonsensitized infants (P < .001; robust to leave-one-out cross-validation) (Figure 1B). The discrimination performance was “fair,” with an area under the curve (AUC) of 0.73, 95% Confidence Interval (CI) 0.66-0.79 (robust to permutation testing with 100 replicates; P = .02) (Figure 1C). Similar results were observed in a sensitivity analysis excluding 22 infants with food allergy symptoms prior to milk sample collection (AUC 0.75, 95% CI: 0.69-0.81) (Figure S1).

Restricting our analysis to the top 10 most important HMOs contributing to the PLS-DA score resulted in similar discrimination (AUC 0.71; 95% CI: 0.64-0.78), indicating that these 10 HMOs are sufficient to explain the association of HMO profile and food sensitization. The rankings, PLS-DA scaled importance scores, and direction of association for these 10 HMOs are shown in Figure 1D. HMO profiles associated with lower risk of food sensitization were characterized by relatively higher concentrations of fucodisialyllacto-N-hexaose (FDNLNHI), lacto-N-fucopentaose II (LNFPII), lacto-N-neotetraose (LNnT), lacto-N-fucopentaose I (LNFP1), sialyl-lacto-N-tetraose c (LSTc) and fucosyllacto-N-hexaose (FLNH), and relatively lower concentrations of lacto-N-hexaose (LNH), lacto-N-tetrose (LNT), 2’-fucosyllactose (2’FL), and disialyllacto-N-hexaose (DSLNH).

Finally, to account for potential confounders and adjust for known allergy risk factors, we evaluated the PLS-DA score in multivariable logistic regression models (Table 1). Compared to infants consuming milk with a discriminant score in the highest quintile, those in the lowest quintile had a 90% lower risk of food sensitization (Odds Ratio [OR] 0.10 [95% CI: 0.03, 0.34]).

To our knowledge, only 1 previous study has explored the association of HMOs with food sensitization in children, where infants receiving milk with low LNFPIII concentrations were more likely to develop cow’s milk allergy. In contrast, we did not observe associations of any individual HMOs with food sensitization, and LNFPIII was not among the most discriminatory HMOs in our analysis. This may reflect differences in study populations (high-risk infants vs our general population cohort), timing of milk collection (1 month vs 3-4 months), or outcomes assessed (confirmed milk allergy vs sensitization to various food allergens).

Recently, a randomized trial reported that infants receiving formula supplemented with 2’FL had more similar immune responses to breastfed controls, compared to infants receiving formula without...
In addition, a rodent study showed that 2FL and 6SL can reduce symptoms of food allergy. In contrast, we did not find an association of 2FL or 6SL or any other individual HMO with infant food sensitization. Instead, in our study, overall HMO composition was associated with food sensitization, reflecting the complexity of human milk and its evolution to supply the breastfed infant with many different HMOs.

While the causality of these associations remains to be determined, there are several plausible mechanisms by which HMO profiles could influence food sensitization. For example, HMOs modulate immune development through their prebiotic effects on gut bacteria, and by influencing lymphocyte maturation. Further research is needed to determine whether the “beneficial” HMO profile we have identified can optimally stimulate these developmental processes.

Figure 1. A-B. Association of individual and total HMOs (A) and overall HMO profile (B) at 3-4 months with food sensitization at 1 year in the CHILD cohort (N = 421). C. Receiver operating characteristic (ROC) curve. D. Scaled importance scores, ranking and direction of association for the top 10 HMOs contributing to the overall PLS-DA score. Boxes indicate interquartile range; white dots indicate median values; whiskers indicate range. Mann-Whitney U test (P < .001). Abbreviations: PLS-DA, Projection on Latent Structures-Discriminant Analysis, AUC, area under the curve. 2FL, 2'-fucosyllactose; 3FL, 3'-fucosyllactose; LNnT, lacto-N-neotetraose; 3SL, 3'-sialyllactose; DFLac, difucosyllactose; 6SL, 6'-sialyllactose; LNT, lacto-N-tetrose; LNFP I, lacto-N-fucopentaose-I; LNFP II, lacto-N-fucopentaose-II; LNFP III, lacto-N-fucopentaose-III; LSTb, sialyl-lacto-N-tetraose b; LSTc, sialyl-lacto-N-tetraose c; DFLNT, difucosyllacto-N-tetrose; LNH, lacto-N-hexaose; DSLNT, disialyllacto-N-tetrose; FLNH, fucosyllacto-N-hexaose; DFLNH, difucosyllacto-N-hexaose; FDSLNH, fucodisialyllacto-N-hexaose; DSLNH, disialyllacto-N-hexaose.
processes, and to identify the maternal and environmental factors that promote a “beneficial” HMO profile.

To our knowledge, this is the largest study to examine the association of HMOs and allergy development in infants, and the first to evaluate overall HMO profiles. Key strengths include the prospective design within a large population-based cohort, and standardized skin testing to assess food sensitization. Our methods allowed absolute quantification of HMOs, and we applied a novel multivariate approach to account for the natural occurrence of HMOs in complex combinations within human milk. The main limitation of our study is the lack of external validation; however, our PLS-DA results were robust to cross-validation. Finally, we acknowledge that food sensitization during infancy does not always persist into later childhood; however, it is an important clinical outcome and a strong predictor of future atopic disease.1

In conclusion, our results demonstrate that HMO composition is associated with the development of food sensitization in the first year of life, and emphasize that overall profiles should be considered when examining the health effects of HMOs or considering their utility for therapeutic interventions. Further research is warranted to confirm our findings in other populations, explore the underlying biological mechanisms, and establish the long-term consequences of HMO composition on confirmed allergic disease later in childhood.

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**CONFLICT OF INTEREST**

None.

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**ORCID**

K. Miliku http://orcid.org/0000-0002-9614-7191
M. B. Azad http://orcid.org/0000-0002-5942-4444

1Manitoba Developmental Origins of Chronic Diseases in Children Network (DEVOTION), Children’s Hospital Research Institute of Manitoba, Winnipeg, MB, Canada
2Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, MB, Canada
3The Generation R Study Group, Departments of Pediatrics and Epidemiology, Erasmus University Medical Center Rotterdam, Rotterdam, The Netherlands
4Department of Pediatrics and Larsson-Rosenquist Foundation Mother-Milk-Infant Center of Research Excellence, University of California San Diego, La Jolla, CA, USA
5George & Fay Yee Centre for Healthcare Innovation, University of...
To the Editor,

We report an open-label study of the effects of bilastine at 20, 40 and 80 mg daily for 2 weeks on the signs and symptoms of chronic spontaneous urticaria (CSU) in patients who had not responded sufficiently to licensed doses of other H1-antihistamines. The study was designed to mimic the real-life situation in which the antihistamine dose is increased gradually up to fourfold the licensed dose, depending on the effectiveness of the previous dose. Its limitations include the relatively small number of patients and uncontrolled design.

Updosing of bilastine is effective in moderate to severe chronic spontaneous urticaria: A real-life study

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