ORIGINAL ARTICLE

# Sex-specific associations of human milk long-chain polyunsaturated fatty acids and infant allergic conditions

Kozeta Miliku[1,2,3] | Jacqueline Richelle[1] | Allan B. Becker[1,2] | Elinor Simons[1,2] | Theo J. Moraes[4] | Turvey E. Stuart[5] | Piush J. Mandhane[6] | Malcolm R. Sears[3] | Padmaja Subbarao[4] | Catherine J. Field[7] | Meghan B. Azad[1,2]

[1]Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, MB, Canada

[2]Manitoba Interdisciplinary Lactation Centre (MILC), Children's Hospital Research Institute of Manitoba, Winnipeg, MB, Canada

[3]Department of Medicine, McMaster University, Hamilton, ON, Canada

[4]Department of Pediatrics, University of Toronto, Toronto, ON, Canada

[5]Department of Pediatrics, University of British Columbia, Vancouver, BC, Canada

[6]Department of Pediatrics, University of Alberta, Edmonton, AB, Canada

[7]Department of Agricultural Food, and Nutritional Science, University of Alberta, Edmonton, AB, Canada

**Correspondence**
Meghan B. Azad, Children's Hospital Research Institute of Manitoba, 504C-715 McDermot Avenue, Winnipeg, MB R3E 3P4, Canada.
Email: meghan.azad@umanitoba.ca

## Abstract

**Background:** Polyunsaturated fatty acids (PUFAs) may influence immune development. We examined the association of PUFAs in human milk with food sensitization and atopic dermatitis among breastfed infants.

**Methods:** In a selected subgroup of 1109 mother-infant dyads from the CHILD Cohort Study, human milk was analyzed by gas-liquid chromatography to quantify PUFAs including arachidonic acid (ARA) and docosahexaenoic acid (DHA). At 1 year of age, food sensitization was determined by skin-prick testing for egg, peanut, cow's milk, and soybean, and atopic dermatitis was diagnosed by pediatricians. Logistic regression analyses controlled for breastfeeding exclusivity, family history of atopy, and other potential confounders.

**Results:** Overall, 184 infants (17%) were sensitized to one or more food allergens and 160 (14%) had atopic dermatitis. Sex-specific associations were observed between these conditions and milk PUFAs. Girls receiving human milk with lower proportions of DHA had lower odds of food sensitization (aOR 0.35; 95% CI 0.12, 0.99 for lowest vs highest quintile), and a clear dose-dependent association was observed for the ARA/DHA ratio (aOR 2.98; 95% CI 1.10, 8.06 for lowest vs highest quintile). These associations were not seen in boys. Similar sex-specific tendencies were observed for atopic dermatitis.

**Conclusions:** Human milk PUFA proportions and their ratios are associated with infant atopic conditions in a sex-specific manner. In female infants, a higher ratio of ARA/DHA may reduce the risk of food sensitization and atopic dermatitis. Further research is needed to determine the underlying mechanisms and clinical relevance of this sex-specific association.

**Key Message**

To out knowledge, this is the first study exploring the associations between human milk polyunsaturated fatty acids (PUFAs) and infant allergic conditions in boys and girls separately. The results show that the ratios of n-6 to n-3 human milk PUFAs (in particular, ARA to DHA) are associated with infant atopic outcomes in a dose-dependent manner, and are evident only in girls. These findings could have implications for maternal and infant nutrition guidelines, and allergy prevention strategies.

## 1 | INTRODUCTION

Food allergy affects up to 7% of children in high-income countries.[1,2] The prevalence of atopic dermatitis is even higher at 10%-20%, reflecting a twofold to threefold increase over the past 30 years.[3] These conditions often arise during infancy, highlighting the importance of understanding their early origins.[4-6]

Breastfeeding is an important early-life exposure, providing optimal infant nutrition[7] along with immunologic benefits and possible protection against allergic conditions.[8-10] Polyunsaturated fatty acids (PUFA) are among the breastmilk components that may contribute to immune system development.[11] Human milk PUFAs vary greatly among mothers.[12,13] Essential long-chain PUFAs, linoleic acid (LA, 18:2n-6), and alpha-linolenic acid (ALA, 18:3n-3) are obtained from the diet (primarily found in vegetable oils), as are the n-3 PUFAs (eg, docosahexaenoic acid (DHA, 22:6n-3), primarily found in fish). In contrast, n-6 PUFAs (eg, arachidonic acid (ARA, 20:4n-6)) are mostly derived from maternal body stores, endogenous synthesis in the liver and mammary gland, and uptake from maternal plasma. These PUFAs are substrates in the biosynthetic pathway of eicosanoids, which are signaling molecules responsible for modulating the inflammatory response.[10,14] N-3 PUFAs in cell membranes may reduce allergic inflammation by decreasing inflammatory responses,[15] while n-6 PUFAs may enhance inflammation by opposing the actions of n-3 PUFAs, suggesting that the ratio between n-6 and n-3 PUFAs is important. International authorities recommend supplementation of both arachidonic acid (ARA) and docosahexaenoic acid (DHA) in formulas for infants who cannot be breastfed[16-18] to support growth[19] and immune development.[20]

A recent systematic review reported some inverse associations between human milk n-3 PUFAs and the risk of atopic dermatitis and sensitization in breastfed children,[21] although many studies found no association[22,23] and one reported that n-3 PUFAs were *positively* associated with sensitization.[24] Similarly, conflicting results were reported for n-6 PUFAs.[21] Most studies have focused on DHA and ARA separately, with only a few investigating PUFA ratios, showing inconsistent results.[22,24,25]

It is well known that allergic conditions develop differently in boys and girls,[26] with a higher tendency in boys during early childhood.[27] Emerging evidence suggests that milk composition may be different for male and female infants[28]; however, it is unknown whether the potential associations between human milk PUFAs and infant atopic conditions are sex-specific.

To address these knowledge gaps, we examined the associations of breastmilk PUFAs (and their ratios) with food sensitization and atopic dermatitis in the first year of life in the longitudinal CHILD Cohort Study.

## 2 | METHODS

The CHILD Cohort Study is a general population birth cohort that recruited 3455 pregnant women between 2008 and 2012 across four Canadian sites (Vancouver, Edmonton, Manitoba, and Toronto) to study the development of allergic diseases.[29] We studied a subsample of mother-infant dyads that breastfed for at least 3 months, had milk fatty acids analyzed (N = 1200), and had complete data on the outcomes of food sensitization and atopic dermatitis at 1 year (N = 1109) (Figure S1). This subsample consisted of a representative sample of 417 dyads and an additional 692 dyads enriched for maternal and infant allergy outcomes, but with similar general characteristics with the representative sample (Table S1).[13] Written informed parental consent was obtained at enrollment, and the study was approved by the Human Research Ethics Boards at McMaster University and the Universities of Manitoba, Alberta and British Columbia, and the Hospital for Sick Children.

### 2.1 | Infant atopic sensitization

Allergic sensitization to food allergens at 1 year of age was determined by standardized skin-prick testing to egg white, peanut, cow's milk, and soybean (ALK-Abello Pharmaceuticals Inc) using Duotip-Test® II devices (Lincoln Diagnostics Inc).[30,31] Allergic sensitization was defined as a positive skin-prick test with a wheal measuring ≥2 mm in diameter compared with the negative control (glycerin). The diagnosis of atopic dermatitis at 1 year of age was made by CHILD study pediatricians [32] and defined as a recurrent or persistent pruritic skin rash and at least 3 of the following: history of itching in

the skin creases or cheeks, history of hay fever or asthma, or first-degree relative with a history of an atopic condition; dry skin within the past year; and visible eczema on examination.

## 2.2 | Human milk collection and fatty acid analysis

Analysis of breastmilk fatty acids in the CHILD Cohort Study was described previously.[13] Briefly, breastmilk samples were collected at 3-4 months postpartum (median 15.1 weeks, 95% range 11.3, 28.1). In a sterile collection jar, mothers collected a mixture of foremilk and hindmilk from multiple feeds over a 24-hour period and kept the container refrigerated. Samples were stored at −80°C[33] and then analyzed by high-resolution capillary gas-liquid chromatography.[34] For this study, human milk PUFAs including long-chain PUFAs (poly-unsaturated fatty acids with chain lengths greater than 18 carbons) were analyzed, including n-3 PUFAs: alpha-linolenic acid (18:3n-3), eicosatetraenoic acid (20:4n-3), eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (22:5n-3), docosahexaenoic acid (DHA, 22:6n-3); and n-6 PUFAs: linoleic acid (18:2n-6), gamma-linolenic acid (18:3n-6), conjugated linoleic acid (CLA; 18:2c–9, t–11), dihomo-gamma-linolenic acid (DGLA, 20:3n-6), and arachidonic acid (ARA, 20:4n-6). Each PUFA level was expressed as relative percentage of total fatty acids (including SFAs, MUFAs, and PUFAs) as described earlier.[13] We also calculated total n-6 and n-3 PUFAs, their ratios, and the ARA/DHA ratio.

## 2.3 | Statistical analyses

To facilitate comparability and interpretation of results for PUFAs present across a wide range, we converted measurements into a common scale using standard deviation (SD) scores and further categorized these SD scores into quintiles. We used $t$ tests to compare PUFA proportions between boys and girls, and the chi-square linear-by-linear association test for trend to compare the proportion of infants with each allergic outcome across PUFA quintiles. In addition, we used crude (unadjusted) and multivariable-adjusted logistic regression analyses to quantify these associations, expressed as odds ratios (ORs) with 95% confidence intervals (CIs) using the highest quintile as the reference group. The multivariable models were adjusted for study site, infant sex, age at milk collection, exclusivity of breastfeeding at the time of milk sample collection, and potential maternal confounders (maternal ethnicity, post-secondary education, food allergy, atopic dermatitis, and fish oil supplementation). These covariates were selected based on previous reports in the literature or because of a resultant change in the adjusted odds ratio (aOR) by >10%.[21] In a sensitivity analysis, PUFA proportions (including SFAs, MUFAs, and PUFAs) were transformed using the centered log-ratio method (CLR) to control for constant-sum constraint (compositional nature of milk fatty acids),[35] and all of the above analyses were repeated using the CLR-transformed PUFA values. To assess whether associations differed by child sex or maternal allergies, we

evaluated the statistical interaction by including the product term with PUFA variables in the models. Interactions with $P < .10$ were further explored in stratified analyses. Analyses were performed using the Statistical Package for the Social Sciences version 21.0 for Windows (SPSS IBM).

## 3 | RESULTS

Among the 1109 infants in this study, 184 (17%) were sensitized to food and 160 (14%) had atopic dermatitis at 1 year of age (Table 1). About half (52%) of infants were exclusively breastfed at the time of milk sample collection. The mean ± SD weight percentages of total n-3 and n-6 PUFAs were 2.42% ± 0.76% and 14.76% ± 3.13%, respectively (Table 2), including the individual PUFAs ARA (0.38% ± 0.09%) and DHA (0.19% ± 0.14%). The mean ratio of total n-6/n-3 PUFA was 6.49 ± 1.74, and the mean ratio of ARA/DHA was 2.65 ± 1.45. There were no sex differences in the proportions or ratios of PUFAs (Table 2). The ranges of PUFA quintiles are given in Table 3.

## 3.1 | Human milk N-3 PUFAs and atopic conditions in infancy

Sex-specific associations were observed between human milk PUFAs and infant food sensitization. In univariate analyses and unadjusted regression models, we observed that girls receiving milk with lower proportions of DHA had a lower prevalence of food sensitization (lowest vs highest quintile: 8% vs 24%, OR (95% confidence interval (CI)) 0.28 (0.12, 0.65); $P$ for linear trend across quintiles < .001) (Figure 1 and Figure S2) ($P$ for sex interaction = .04). These differences persisted in the multivariable models adjusted for maternal food allergies and other risk factors aOR (95% CI) 0.35 (0.12, 0.99) (Figure 2). Similar associations were observed for total n-3 PUFA, EPA, and DPA, but not for ALA and eicosatetraenoic acid. For example, girls who received human milk with n-3 PUFAs in the lowest quintile had 66% lower odds of food sensitization compared with those in the highest quintiles (0.34 [0.12, 0.95]) (Figure 2). No associations were observed among boys (1.02 [0.47, 2.23]) (Figures 1 and 2 and Figure S2). Similar associations were observed using CLR-transformed fatty acid data (Figure S3). Similar though non-significant tendencies were observed for atopic dermatitis (eg, lowest vs highest quintile of milk n-3 PUFAs in girls: 0.51 [0.20, 1.28]) (Figure S4). Girls consuming milk with ALA proportions in the middle quintile (Q3 vs Q5) had lower odds of atopic dermatitis. Opposite tendencies were observed in boys (Figure S4).

## 3.2 | Human milk N-6 PUFAs and atopic conditions in infancy

In girls only, a trend was observed among human milk ARA proportions and food sensitizations (lowest vs highest quintile: 12%

**TABLE 1** Descriptive characteristics of mother-infant dyads in this subset of the CHILD Cohort (N = 1109)

| Maternal characteristics | |
|---|---|
| Age, y | 33.0 ± 4.3 |
| Primiparous, n (%) | 633 (57.1) |
| Completed post-secondary education, n (%) | 888 (80.1) |
| Ethnicity, n (%) | |
| Asian | 190 (17.1) |
| Caucasian | 803 (72.4) |
| First Nations | 41 (3.7) |
| Other | 73 (6.6) |
| Study site, n (%) | |
| Edmonton | 237 (21.4) |
| Toronto | 282 (25.4) |
| Vancouver | 296 (26.7) |
| Winnipeg | 294 (26.5) |
| BMI, kg/m$^2$ | 23.1 (18.3, 37.8) |
| Diet quality, HEI-2010 score | 73.9 ± 7.9 |
| Prenatal fish oil supplement use, n (%) | 256 (23.1) |
| Prenatal multivitamin intake, n (%) | 777 (70.1) |
| Prenatal smoking, n (%) | 53 (4.8) |
| Maternal food allergy, n (%) | 306 (27.6) |
| Maternal atopy, n (%) | 807 (72.8) |
| Breastmilk characteristics | |
| Age at breastmilk collection, weeks | 15.1 (11.3, 28.1) |
| Exclusive breastfeeding at sample collection, n (%) | 580 (52.3) |
| Season at breastmilk sample collection, n (%) | |
| Winter: Dec-Feb | 266 (24.0) |
| Spring: Mar-May | 304 (27.4) |
| Summer: Jun-Aug | 267 (24.1) |
| Fall: Sept-Nov | 270 (24.3) |
| Duration of exclusive breastfeeding, months | 4.5 (0.0, 6.0) |
| Duration of breastfeeding, months | 12.0 (3.8, 24.0) |
| Infant characteristics | |
| Sex, male, n (%) | 606 (54.6) |
| Gestational age, wk | 39.1 ± 1.4 |
| Birthweight, g | 3450 ± 497 |
| Atopic dermatitis at 1 y, n (%) | 160 (14.4) |
| Allergic sensitization to food at 1 y, n (%)[a] | |
| Any | 184 (16.6) |
| Egg | 113 (10.2) |
| Peanut | 83 (7.5) |
| Milk | 34 (3.1) |
| Soy | 14 (1.3) |
| Both atopic dermatitis and food sensitization, n (%)[a] | 67 (6.0) |

*Note:* Values reflect percentages of non-missing data for categorical variables, means ± SD for continuous variables with a normal distribution, or medians (95% range) for continuous variables with a skewed distribution.

[a]Positive SPT indicated by ≥2 mm wheal. Maternal atopy and food allergy are self-reported.

vs 20%, OR 0.56; 95% CI (0.25, 1.24); *P* for trend = .03, *P* for sex interaction = .01) (Figure 1 and Figure S5). No clear associations were observed for total n-6 PUFAs, LA, or GLA (Figure 3); however, girls consuming milk with CLA in the lowest vs highest quintile had 65% lower odds of food sensitization (0.35 [0.14, 0.84]) (Figure 3). This association was not observed in boys. Results were generally similar using CLR-transformed fatty acid data, with one exception: A new association was observed for total n-6 PUFAs in girls (lowest vs highest quintile: 0.39 [0.17, 0.92]) (Figure S6). Human milk n-6 PUFAs were not associated with infant atopic dermatitis in either sex (Figure S7).

## 3.3 | Human milk PUFA ratios and atopic conditions in infancy

Sex-specific and dose-dependent associations were observed between ARA/DHA ratio quintiles and food sensitization (*P* for trend < .001 in girls only; *P* for sex interaction = .07) (Figure 1 and Figure S8). Girls consuming milk with an ARA/DHA ratio in the lowest vs highest quintile had a threefold higher odds of food sensitization (2.98 [1.10, 8.06]) (Figure 4). Total n-6/n-3 PUFA ratios were also associated with food sensitization in girls (Figure 4). No associations were observed in boys. Similar sex-specific associations were observed for atopic dermatitis (Figure S9). There was no evidence of interaction between any PUFAs and maternal allergies.

## 4 | DISCUSSION

Our research provides new evidence that human milk PUFAs may influence the development or prevention of atopic conditions in breastfed infants. Unlike prior studies on this topic, we considered PUFA ratios and evaluated sex differences, revealing two important nuances. First, the associations were only evident in girls. Second, when considered separately, it appeared that lower proportions of both n-3 and n-6 LC-PUFAs (in particular, DHA and ARA) were associated with lower odds of food sensitization and atopic dermatitis; however, when considered as a ratio, it became clear that the proportion of these PUFA *in relation to each other* was associated with infant atopic outcomes in a dose-dependent manner. Specifically, we found that girls receiving human milk with a higher ratio of ARA/DHA at 3 months had lower odds of developing food sensitization and atopic dermatitis by 1 year of age. While further research is needed to replicate our findings and explore the potential role of endogenous fatty acid conversion pathways, our results suggest that a higher ARA-to-DHA ratio may be beneficial for infants who cannot be breastfed.

The role of n-3 and n-6 PUFAs in the inflammatory response is well recognized.[10,14] N-3 PUFAs may reduce allergic inflammation by decreasing inflammatory responses,[15] while n-6 PUFAs may enhance inflammation by opposing the actions of n-3 PUFAs; yet, inconsistent results are reported on the associations between

**TABLE 2**   Human milk PUFAs at 3 mo postpartum in the CHILD Cohort Study, stratified by child sex (N = 1109)

| LC-PUFA | | All children | Milk for girls | Milk for boys |
|---|---|---|---|---|
| **Total n-3 PUFA (%)** | | 2.42 ± 0.76 | 2.42 ± 0.73 | 2.41 ± 0.80 |
| α-linolenic acid (ALA) | 18:3n-3 | 1.92 ± 0.66 | 1.92 ± 0.63 | 1.93 ± 0.68 |
| Eicosatetraenoic acid | 20:4n-3 | 0.08 ± 0.03 | 0.08 ± 0.03 | 0.08 ± 0.03 |
| Eicosapentaenoic acid (EPA) | 20:5n-3 | 0.08 ± 0.07 | 0.08 ± 0.07 | 0.08 ± 0.07 |
| Docosapentaenoic acid (DPA) | 22:5n-3 | 0.13 ± 0.06 | 0.13 ± 0.06 | 0.13 ± 0.05 |
| Docosahexaenoic acid (DHA) | 22:6n-3 | 0.19 ± 0.14 | 0.19 ± 0.14 | 0.19 ± 0.14 |
| **Total n-6 PUFA (%)** | | 14.76 ± 3.13 | 14.66 ± 3.17 | 14.84 ± 3.11 |
| Linoleic acid (LA) | 18:2n-6 | 13.60 ± 3.05 | 13.51 ± 3.08 | 13.68 ± 3.03 |
| Conjugated linoleic acid (CLA) | 18:2c–9, t–11 | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.01 |
| γ-Linolenic acid (GLA) | 18:3n-6 | 0.10 ± 0.06 | 0.10 ± 0.06 | 0.10 ± 0.06 |
| Dihomo-γ-linolenic acid (DGLA) | 20:3n-6 | 0.35 ± 0.11 | 0.34 ± 0.11 | 0.35 ± 0.11 |
| Arachidonic acid (ARA) | 20:4n-6 | 0.38 ± 0.09 | 0.38 ± 0.09 | 0.38 ± 0.10 |
| Ratio of PUFAs | | | | |
| **Total n-6/ Total n-3 PUFA** | | 6.49 ± 1.74 | 6.39 ± 1.67 | 6.56 ± 1.79 |
| ARA/DHA | | 2.65 ± 1.45 | 2.63 ± 1.55 | 2.67 ± 1.36 |

*Note:* Values are mean ± SD. Individual PUFAs are reported as percentages of total fatty acids (g/100 g of fatty acids). No sex differences were observed when compared by the *t* test or the non-parametric Mann-Whitney test.

Abbreviation: PUFA, polyunsaturated fatty acid.

**TABLE 3**   Human milk PUFAs at 3 mo postpartum in the CHILD Cohort Study (N = 1109): median and quintile ranges

| LC-PUFA | Median 95% CI | Q1 | Q2 | Q3 | Q4 | Q5 |
|---|---|---|---|---|---|---|
| n-3 PUFA (%) | 2.32 (1.14, 4.12) | 0.90-1.80 | 1.80-2.18 | 2.18-2.51 | 2.51-2.96 | 2.96-6.62 |
| ALA | 1.86 (0.84, 3.40) | 0.44-1.40 | 1.40-1.71 | 1.71-2.00 | 2.00-2.38 | 2.38-5.82 |
| Eicosatetraenoic acid | 0.08 (0.03, 0.17) | 0.01-0.06 | 0.06-0.07 | 0.07-0.08 | 0.08-0.10 | 0.10-0.27 |
| EPA | 0.06 (0.03, 0.30) | 0.01-0.04 | 0.04-0.05 | 0.05-0.07 | 0.07-0.11 | 0.11-0.54 |
| DPA | 0.12 (0.06, 0.28) | 0.04-0.09 | 0.09-0.11 | 0.11-0.13 | 0.13-0.16 | 0.16-0.48 |
| DHA | 0.15 (0.06, 0.59) | 0.02-0.09 | 0.09-0.13 | 0.13-0.18 | 0.18-0.26 | 0.26-1.09 |
| n-6 PUFA (%) | 14.48 (9.44, 21.90) | 7.13-12.00 | 12.00-13.70 | 13.70-15.10 | 15.10-16.92 | 16.92-27.35 |
| LA | 13.33 (8.52, 20.64) | 6.17-11.00 | 11.00-12.54 | 12.54-14.00 | 14.00-15.74 | 15.74-26.20 |
| CLA | 0.02 (0.00, 0.04) | 0.00-0.01 | 0.01-0.02 | 0.02-0.02 | 0.02-0.03 | 0.03-0.18 |
| GLA | 0.09 (0.03, 0.26) | 0.17-0.06 | 0.06-0.08 | 0.08-0.10 | 0.10-0.14 | 0.14-0.40 |
| DGLA | 0.33 (0.16, 0.59) | 0.06-0.24 | 0.24-0.30 | 0.30-0.36 | 0.36-0.44 | 0.44-0.83 |
| ARA | 0.37 (0.22, 0.59) | 0.15-0.30 | 0.30-0.35 | 0.35-0.39 | 0.39-0.45 | 0.45-0.85 |
| Ratio of PUFAs | | | | | | |
| Total n-6/n-3 PUFA | 6.24 (3.91, 10.72) | 2.35-5.10 | 5.10-6.00 | 6.00-6.60 | 6.60-7.60 | 7.60-17.28 |
| ARA/DHA | 2.47 (0.66, 5.42) | 0.28-1.41 | 1.41-2.10 | 2.10-2.90 | 2.90-3.70 | 3.70-17.60 |

*Note:* Values are medians and 95% CI, and quintiles ranges of PUFAs are reported as percentages of total fatty acids, calculated by weight (g/100 g of fatty acids).

Abbreviations: ALA, α-linolenic acid; ARA, arachidonic acid; CLA, conjugated linoleic acid; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; LA, linoleic acid; PUFA, polyunsaturated fatty acid.
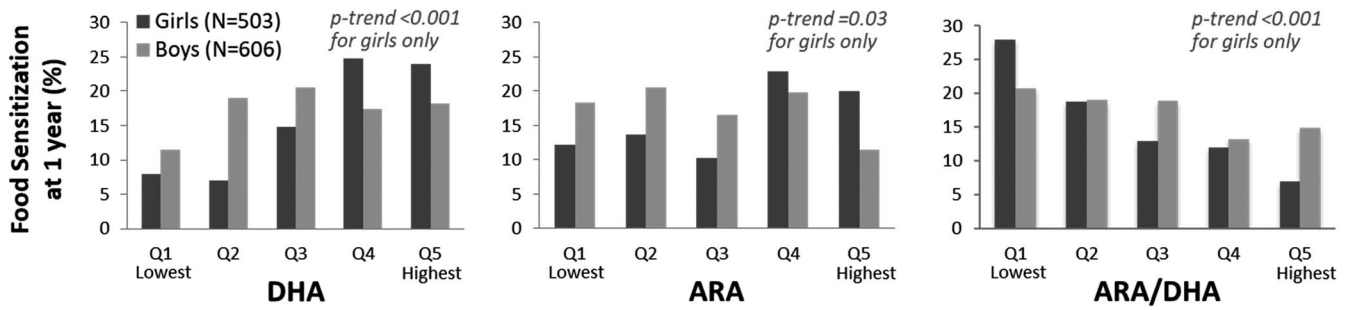
**FIGURE 1** Prevalence of food sensitization at 1 y according to human milk DHA, ARA, and ARA/DHA ratio quintiles (Q1 = lowest, Q5 = highest) in breastfed boys and girls in the CHILD Cohort Study. P-trend values are P-values from the chi-square linear-by-linear association test for trend
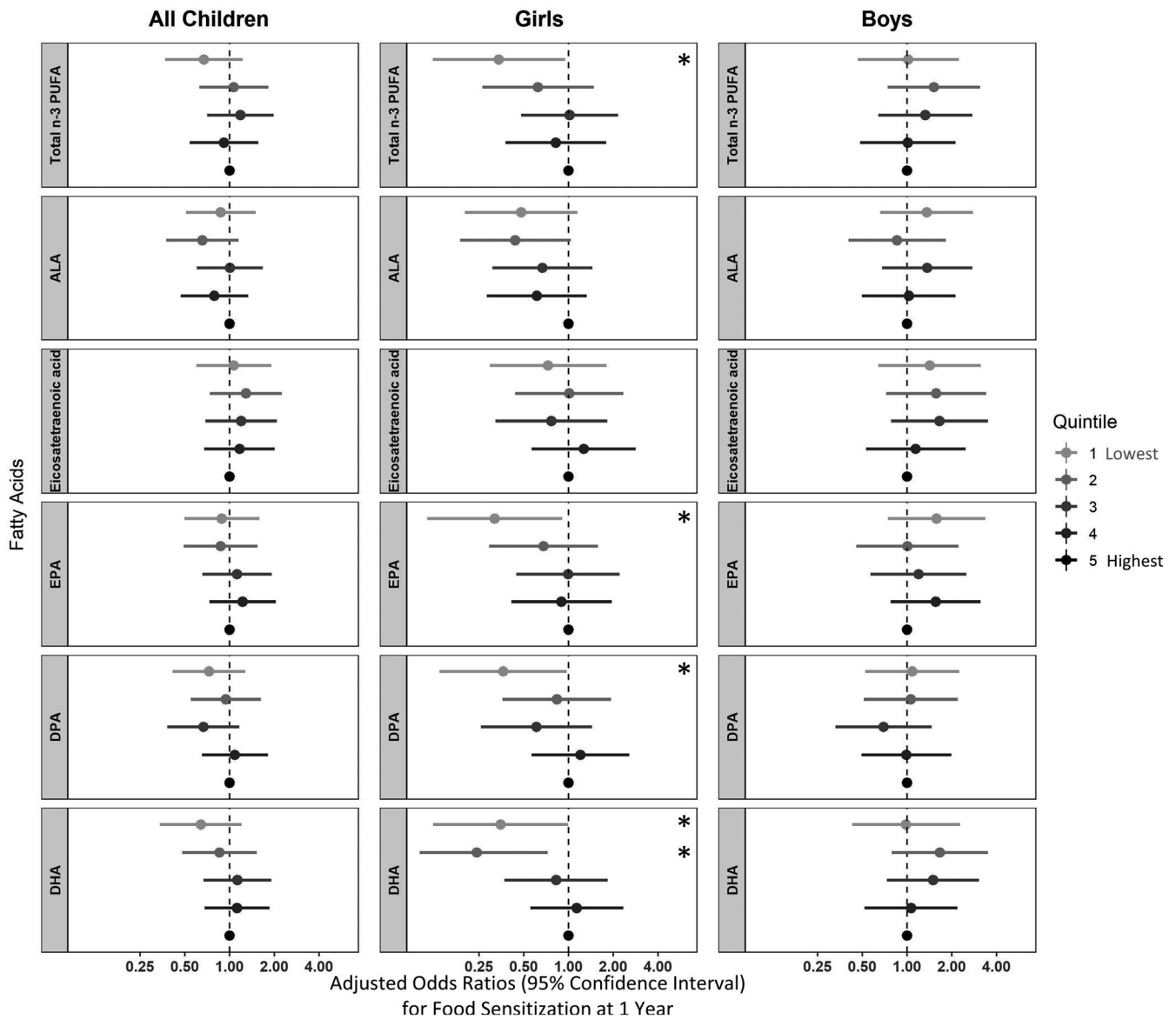


**FIGURE 2** Sex-stratified associations of total and individual human milk n-3 PUFAs with infant food sensitization (n/N = 184/1109) in the CHILD Cohort Study. Values are odds ratios (95% confidence interval) based on multivariable-adjusted logistic regression models. The models are adjusted for study site, age at milk collection, exclusivity of breastfeeding at the time of milk sample collection, maternal ethnicity, post-secondary education, food allergy, and fish oil supplementation. ALA, alpha-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid. (Quintile 1 = lowest, Quintile 5 = highest) *P-values < .05
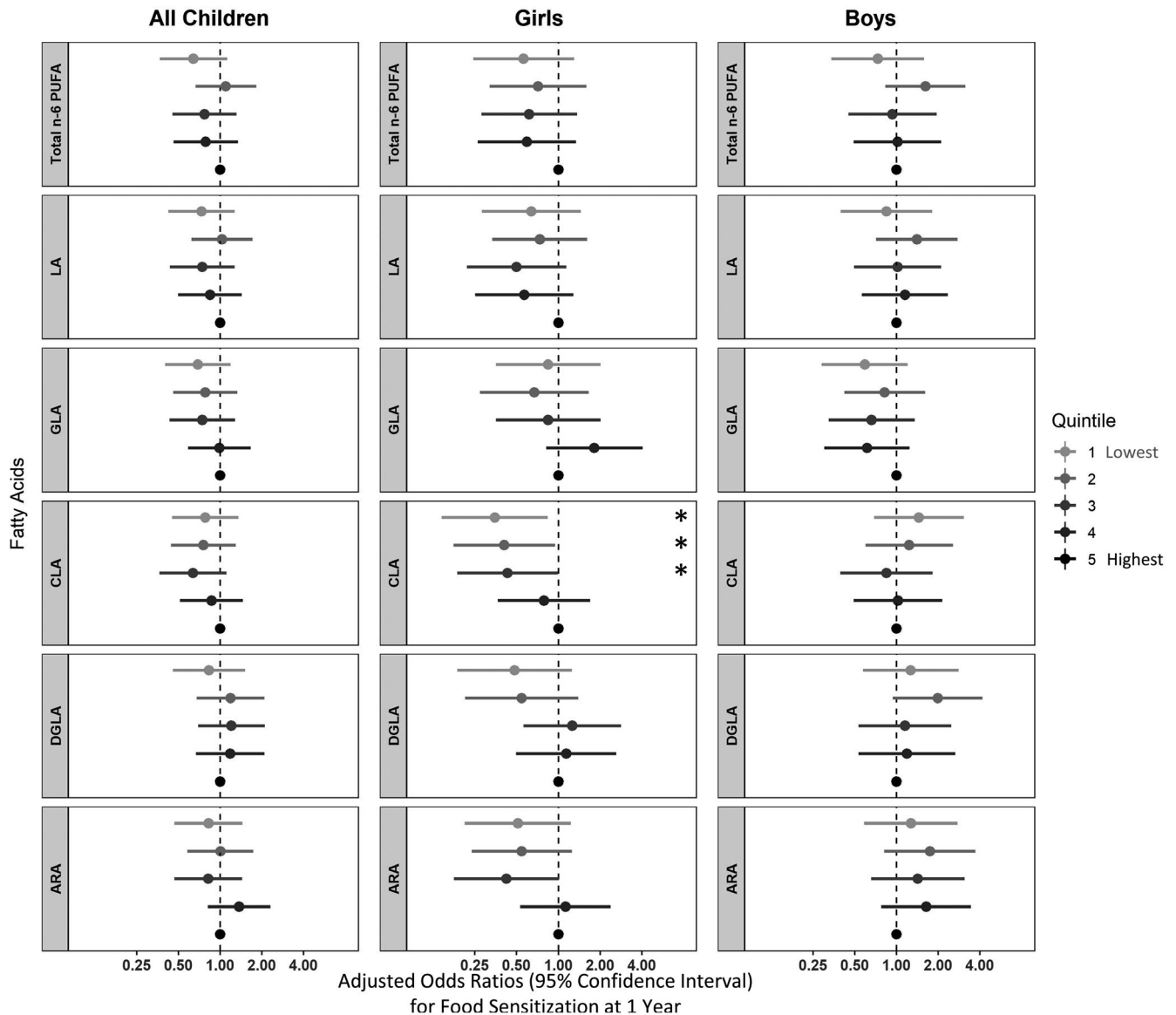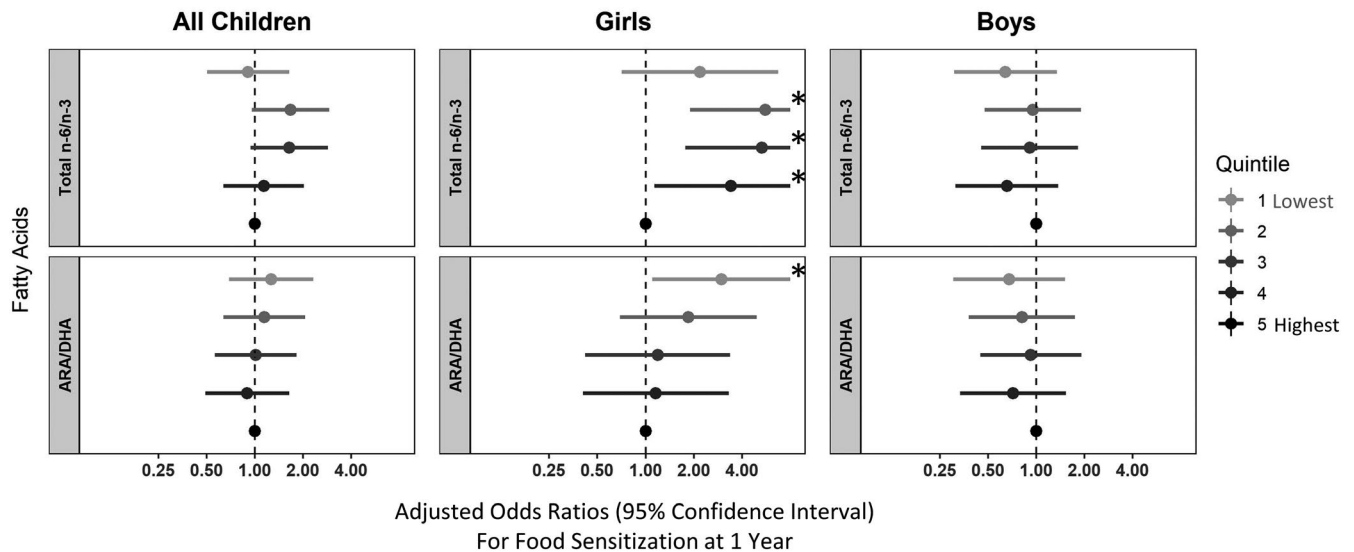
**FIGURE 3** Sex-stratified associations of total and individual human milk n-6 PUFAs with infant food sensitization (n/N = 184/1109) in the CHILD Cohort Study. Values are odds ratios (95% confidence interval) based on multivariable-adjusted logistic regression models. The models are adjusted for study site, age at milk collection, exclusivity of breastfeeding at the time of milk sample collection, maternal ethnicity, post-secondary education, food allergy, and fish oil supplementation. ARA, arachidonic acid; CLA, conjugated linoleic acid; DGLA, dihomo-gamma-linolenic acid; GLA, gamma-linolenic acid; LA, linoleic acid. (Quintile 1 = lowest, Quintile 5 = highest) *P-values < .05

PUFAs and atopic conditions in children.[21] Previous studies have not explored sex differences, and many have not assessed total n-3 PUFA and n-6 PUFA and ARA/DHA ratios—which are key features of these associations in our study. Our findings on n-3 LC-PUFAs (including EPA, DPA, DHA) and sensitization are consistent with the findings of the Australian MACS study, where higher n-3 LC-PUFAs including DPA and DHA proportions were seen in non-sensitized children at six and 24 months of age.[24] However, results from the Dutch PIAMA study showed no association between human milk n-3 PUFAs and sensitization at four years of age.[22] Inconsistent results are also reported for n-6 PUFAs (including CLA, DGLA, and ARA) and atopic conditions in children.[22,24,36-38] In our study, we observed that higher CLA proportions were

associated with higher odds of food sensitizations in girls, which contradicts previous findings from the Dutch KOALA birth cohort study, where CLA appeared to be protective—although sex-stratified analyses were not performed.[37]

There is a strong rationale for studying the ratio of total and individual n-3 and n-6 PUFA in the context of allergic disease because their biosynthesis pathways compete for the same enzymes, and their derivatives can have antagonistic pro- and anti-inflammatory effects. When we examined the proportions of individual and total PUFA in relation to each other, we found dose-dependent inverse association between n-6/n-3 PUFAs ratios and atopic conditions in 1-year-old girls. Altogether, these findings suggest that when studying allergic conditions, the ratios of individual or total n-6/n-3 PUFAs

**FIGURE 4** Sex-stratified associations of human milk PUFA ratios with infant food sensitization (n/N = 184/1109) in the CHILD Cohort Study. Values are odds ratios and 95% confidence intervals. Models are adjusted for study site, age at milk collection, breastfeeding exclusivity at the time of milk sample collection, maternal ethnicity, post-secondary education, food allergy, and fish oil supplementation. ARA/DHA, arachidonic/docosahexaenoic acid ratio. (Quintile 1 = lowest, Quintile 5 = highest) *P-values < .05

may be more clinically relevant than the individual or total PUFA proportions.

Another novel aspect of our study is that our results reveal sex differences, with associations observed in girls only. Sex differences in atopic disease are widely reported, and while the underlying pathogenesis is poorly understood, one hypothesis has attributed these differences to sex hormones.[39] For example, females show higher antibody responses against infections due to the enhanced immune responses promoted by female sex hormones compared with the immunosuppressive effects of male hormones.[40,41] During infancy, specifically in the first 3-6 months of life, a rise in sex hormone levels occurs.[42] Therefore, we speculate that the enhanced pro-inflammatory character of female sex hormones combined with an altered pro/anti-inflammatory balance of human milk n-6 and n-3 PUFAs could influence susceptibility for atopy in girls.

To our knowledge, this is one of the largest (N = 1109) prospective cohort studies of human milk PUFAs and infant atopic conditions. Other strengths of our study include the detailed assessments of atopic dermatitis and standardized skin testing to assess food sensitization, and the assessment of sex differences. The main limitation of our study is that we studied food sensitization (not clinical allergy) during infancy, which does not always persist into later childhood, although convincing evidence shows that food sensitization at 1 year predicts future atopic disease.[31] In addition, breastmilk samples were collected only once during lactation (preventing us from analyzing the longitudinal variation of PUFAs during lactation) and were stored for up to 5-8 years at −80°C before analysis. The storage time might have affected the total fat content but is unlikely to have altered the fatty acid composition. While we could reliably calculate the proportions of different human milk fatty acids, we could not quantify their absolute concentration because total milk fat content changes over the course of a feeding and diurnally, and we did not collect a full breast expression or standardize the timing of milk collection. We did not assess maternal PUFA status during pregnancy; therefore, we could not address the impact of in utero exposure to PUFA, which is likely correlated with PUFA levels in human milk. Although we have captured n-3 and n-6 PUFAs in the breastmilk samples, there are other less common PUFAs (eg, n-9 PUFAs) that may be of interest to study in relation to human health. Finally, while we adjusted for many potential confounders, residual confounding is still possible in this observational study.

## 5 | CONCLUSIONS

This study provides evidence that PUFAs and their ratios are associated with infant atopic conditions in a sex-specific manner. Our research suggests that a higher ratio of ARA/DHA in human milk may reduce the risk of food sensitization and atopic dermatitis in female infants. This suggests it is important to consider the amount of n-6 relative to n-3 PUFAs (ARA to DHA) consumed or supplemented during infancy. Further research is needed to validate these findings and determine the optimal ratio, explain the sex-specific associations, and investigate how the maternal-infant transfer and balance of PUFAs may influence immunity, inflammation, and allergy development.

## AUTHOR CONTRIBUTION

**Kozeta Miliku:** Conceptualization (equal); Data curation (lead); Formal analysis (lead); Funding acquisition (supporting); Methodology (equal); Project administration (equal); Supervision (equal); Visualization (lead); Writing-original draft (lead); Writing-review & editing (lead). **Jacqueline Richelle:** Conceptualization (supporting); Formal analysis (supporting); Writing-original draft (equal). **Allan B. Becker:** Conceptualization (supporting); Writing-review & editing (supporting). **Elinor Simons:** Methodology (supporting); Writing-review & editing (supporting). **Theo J. Moraes:** Conceptualization (supporting); Writing-review & editing (supporting). **Turvey E. Stuart:** Conceptualization (supporting); Writing-review & editing (supporting). **Piush J. Mandhane:** Conceptualization (supporting); Writing-review & editing (supporting). **Malcolm R. Sears:** Conceptualization (supporting); Funding acquisition (supporting); Methodology (supporting); Writing-review & editing (supporting). **Padmaja Subbarao:** Conceptualization (supporting); Funding acquisition (supporting); Methodology (supporting); Writing-review & editing (supporting). **Catherine J. Field:** Conceptualization (supporting); Data curation (equal); Methodology (equal); Writing-review & editing (supporting). **Meghan B. Azad:** Conceptualization (equal); Funding acquisition (lead); Methodology (supporting); Project administration (equal); Supervision (equal); Writing-original draft (equal); Writing-review & editing (equal).

## PEER REVIEW

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## ORCID

*Kozeta Miliku* https://orcid.org/0000-0002-9614-7191
*Padmaja Subbarao* https://orcid.org/0000-0003-0394-1933
*Meghan B. Azad* https://orcid.org/0000-0002-5942-4444

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.