Received: 5 December 2022 | Revised: 16 June 2023 | Accepted: 26 June 2023

DOI: 10.1111/pai.13992

ORIGINAL ARTICLE

WILEY

Potential sex differences in human milk fatty acids and their association with atopic dermatitis: Results of the Ulm SPATZ health study

Madeleine Ordnung[1] | Marko Mank[2] | Bernd Stahl[2,3] | Deborah Kurz[4] | Tamas Marosvölgyi[5,6] | Tamas Decsi[5] | Dietrich Rothenbacher[4,7] | Jon Genuneit[1,7] | Linda P. Siziba[1]

[1]Pediatric Epidemiology, Department of Pediatrics, Medical Faculty, Leipzig University, Leipzig, Germany

[2]Danone Nutricia Research, Utrecht, The Netherlands

[3]Department of Chemical Biology & Drug Discovery, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands

[4]Institute of Epidemiology and Medical Biometry, Ulm University, Ulm, Germany

[5]Department of Paediatrics, Medical School, University of Pécs, Pécs, Hungary

[6]Institute of Bioanalysis, Medical School, University of Pécs, Pécs, Hungary

[7]German Center for Child and Youth Health (DZKJ), Germany

Correspondence
Madeleine Ordnung, Pediatric Epidemiology, Department of Pediatrics, Medical Faculty, Leipzig University, Liebigstr. 20a, Haus 6, 04103 Leipzig, Germany.
Email: madeleine.ordnung@medizin.uni-leipzig.de

Funding information
Danone Nutricia Research; Medizinische Fakultät, Universität Leipzig; Medizinische Fakultät, Universität Ulm

Editor: Rachel Louise Peters

Abstract

Background: Polyunsaturated fatty acids (PUFAs) in human milk are essential in immune system maturation and might play a role in the development of allergic conditions, such as atopic dermatitis (AD) in infants. Immune system responses are modulated by sex, but data on the sex-specific associations with PUFAs are limited. We therefore explored sex-specific differences in human milk PUFAs and their association with AD up to 2 years.

Methods: PUFAs were measured in human milk samples from the Ulm SPATZ Health Study at 6 weeks ($n = 512$) and 6 months ($n = 367$). Associations with AD up to 2 years were evaluated using crude and multivariable logistic regression. Interactions between infant sex and PUFAs were explored by including the product term.

Results: No significant associations were observed with 6-week data. At 6 months, the median relative proportion of docosahexaenoic acid (DHA) was significantly higher in milk for female than male infants ($p = .001$). Female infants whose milk was lower in quintile proportions of alpha-linolenic acid (ALA) at 6 months had lower odds of AD compared to males [first vs. fifth quintile OR (95% confidence interval): 0.13 (0.02, 0.66), $p = .02$]. This interaction was not significant when correcting for multiple testing (α threshold: $p = .004$). No other statistically significant associations were observed.

Conclusion: Individual quintile PUFA proportions in human milk were not associated with AD, overall and in a sex-specific manner. More comprehensive and statistically powered longitudinal studies are needed to determine whether potential sex differences in human milk, if any, could be of clinical relevance for infants including the investigation of mediating factors.

KEYWORDS
atopic dermatitis, human milk, infant sex, polyunsaturated fatty acids

1 | INTRODUCTION

Globally, 15%–20% of children are affected by atopic dermatitis (AD) and the prevalence thereof varies across countries and age groups.[1] In addition, infants with AD usually have an increased risk for developing other allergic diseases as they grow older.[2] For allergy prevention, it has been recommended to breastfeed infants exclusively during the first 4–6 months of life.[3] In this regard, the health benefits of breastfeeding in general are well documented.[4] Yet, the evidence about the potential protective role of breastfeeding in allergy development, particularly in AD, remains inconclusive.[5]

Human milk comprises several components which are beneficial for the infant's health.[4] For instance, fatty acids in human milk are involved in the maturation of the growing infant's immune system.[6] Polyunsaturated fatty acids (PUFAs), in particular, are a branch of fatty acids which serve as precursors of eicosanoids. These are considered key elements in inflammatory processes and, as such, have been linked to atopic conditions.[7] In light of this, observational studies showed that elevated concentrations of n-3 PUFAs in human milk might protect infants from developing AD,[8,9] suggesting anti-inflammatory properties of these fatty acids.[10] N-6 PUFAs, on the contrary, are suggested to be pro-inflammatory, hence, increase the risk of AD.[11]

In addition, females and males are reported to differ in immunological responses which result in differential susceptibility to inflammatory and autoimmune diseases.[12] In infants, it has been shown that both sexes seem to synthesize anti-inflammatory n-3 PUFAs differently leading to sex differences in circulating PUFAs in blood plasma.[13] In fact, male infants are more likely to develop AD compared with female infants.[14] Furthermore, recent findings demonstrate that sex hormones modulate PUFA synthesis.[15]

Moreover, accumulating evidence suggests that human milk composition might differ between both sexes,[16] thus raising the question whether this could impact clinical outcomes differently between female and male infants. We are only aware of one recent study[17] that explored sex-specific associations of PUFAs and allergic conditions in 1-year-old infants. Findings from this study suggest that lower proportions of some n-3 PUFAs (e.g., docosahexaenoic acid, DHA, C22:6n-3) in human milk were associated with a lower prevalence of food sensitization in female infants. However, there were no statistically significant sex-specific associations with AD.

It is therefore still unclear whether PUFAs differ in human milk for male and female infants especially with respect to their associations with clinical outcomes, such as AD. Furthermore, the composition of human milk changes across lactation,[18] but the aforementioned study[17] only investigated samples collected at one time point.

Of note, fatty acids are highly correlated, thus when one fatty acid increases, another decreases and vice versa. This compositional aspect of fatty acids is typically neglected during statically analyses and could be one reason for inconsistent findings.[19]

The aim of the current study, therefore, was to investigate sex-specific associations between PUFAs in human milk samples collected at two time points during lactation, that is, 6 weeks and 6 months and infant AD up to 2 years, using data from a large birth cohort conducted in South Germany. To further strengthen the study, a sensitivity analysis using CLR-transformed data to account for the compositionality of fatty acids was done.

Key Message

It still remains unclear whether immunological responses to polyunsaturated fatty acids (PUFAs) in human milk are sex-specific. To moderate potential implications in child health, further research is needed to examine possible relationships to clinical outcomes and their mediators. This, in turn, could contribute to the development of adaptable nutritional strategies.

2 | METHODS

2.1 | Study population

The current study used data from the Ulm SPATZ Health Study (SPATZ), an ongoing birth cohort study that enrolled 970 mothers and their 1006 newborn infants. They were recruited from the general population between April 2012 and May 2013, after delivery during their hospital stay at the University Medical Center Ulm.[20] All participants gave written informed consent in accordance with the Declaration of Helsinki. The study protocol was approved by the Ethics board of the University of Ulm (No. 311/11).

2.2 | Data collection and measurements

2.2.1 | Sample characteristics and atopic dermatitis (AD)

All socio-demographic, health- and birth-related data were assessed using self-administered questionnaires in the year prior to, as well as during pregnancy. AD was assessed yearly and was based on separate parent or doctor reports of doctor-diagnosed AD. In the parent questionnaire, they were asked: "Has a doctor diagnosed your child with one of the following diseases: neurodermatitis, endogenous eczema, atopic dermatitis, in the past 12 months?" The caring physician was asked in a separate self-administered questionnaire: "Has one of the following diseases: neurodermatitis, endogenous eczema, atopic dermatitis, been found/diagnosed by a physician until now?" Although the AD diagnosis was not entirely made by the caring physician themselves, both reports were based on a previous doctor's diagnosis.[21] The outcome AD at 2 years was then based on a positive report of either parent's or doctor's (i.e., caring physician) questionnaire at 1 year or 2 years.

2.2.2 | Human milk samples

The human milk samples and information about breastfeeding practices were collected at approximately 6 weeks (Median [min–max]; (6 weeks [4–10]) and 6 months (26 weeks [23–29])) after delivery. Using a sterile collection jar, mothers manually expressed or pumped human milk after breakfast and before lunch, between 9 AM and 12 AM, at least 1 h after the last breastfeed. Mothers were instructed to store the human milk samples in a refrigerator until they were collected by the study nurses and delivered to the study center. At the study center, the samples were stored at −80°C and then analyzed by high-resolution capillary gas–liquid chromatography applying the method of Bligh and Dyer[22] and Beermann et al.[23]

A total of 45 fatty acids were measured in the lab, but only selected polyunsaturated fatty acids (PUFAs) were used in the current study in order to replicate the previous report.[17] A full report on all measured fatty acids in SPATZ can be found elsewhere.[24] The PUFAs used for the current analysis included n-3 PUFAs: alpha-linolenic acid (ALA, 18:3n-3), eicosatetraenoic acid (ETA, 20:4n-3), eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (22:5n-3), DHA as well as n-6 PUFAs: linoleic acid (LA, 18:2n-6), gamma-linolenic acid (18:3n-6), dihomo-gamma-linolenic acid (DGLA, 20:3n-6), and arachidonic acid (ARA, 20:4n-6).

Among the 970 mothers in SPATZ, human milk samples were available from 786 (81%) and 581 (60%) lactating women at 6 weeks and 6 months, respectively. However, for the current analysis, we included singleton mother–infant pairs for whom human milk fatty acid samples at either 6 weeks or 6 months and complete data on AD up to 2 years of age were available. Consequently, the datasets analyzed in the current study comprised a total of $n = 512$ (53%) and $n = 367$ (38%) for 6 weeks and 6 months, respectively (Figure 1).

2.3 | Statistical analyses

Sex differences of sociodemographic, health- and birth-related variables as well as relative PUFA proportions were evaluated using Kruskal–Wallis tests. In case of significant differences, subsequent rank-based regression[25] analyses were performed in order to rule out potential confounding with infant age and exclusive breastfeeding at the time of sample collection as well as maternal history of allergies.

For the main analysis, similar methods from the previous report[17] were used in order to mimic what was presented in the paper. As such, relative PUFA proportions were first transformed into standardized z-scores and then into categorical variables with five levels using quintiles of the respective fatty acid z-score. All PUFAs were quantified within one metric to increase the comparability between the investigated PUFAs and to ensure concise interpretation of the results.[17] The associations between each PUFA and AD at 2 years were assessed using crude and multivariable logistic regression models. For the multivariable models, established risk factors such as infant sex, age, and exclusive breastfeeding at the time of sample collection as well as maternal history of allergies (hay fever, AD, and/or asthma) were entered as covariates. Interactions between sex and PUFAs were explored by including the product term in the multivariable models. Odds ratio (OR) estimates and their 95% confidence interval (CI) were calculated using the fifth level of the fatty acid categorical variable, that is, the one with the highest concentration, as a reference category. Male infants were used as the reference category in the models investigating sex-specific interactions. In order to correct for multiple comparisons, Bonferroni correction was applied resulting in an alpha threshold of $\alpha = 0.004$ (0.05/12). Given that compositional data are subject to the constant-sum constraint,[26] a sensitivity analysis was performed using the centered log ratio (CLR) transformed PUFA values in all aforementioned models. All statistical analyses were done using R (version 3.5.1; R Foundation for Statistical Computing).

3 | RESULTS

Among the 512 eligible infants with fatty acid data at 6 weeks and AD data up to 2 years, 138 (27%) were diagnosed with AD (Table 1).

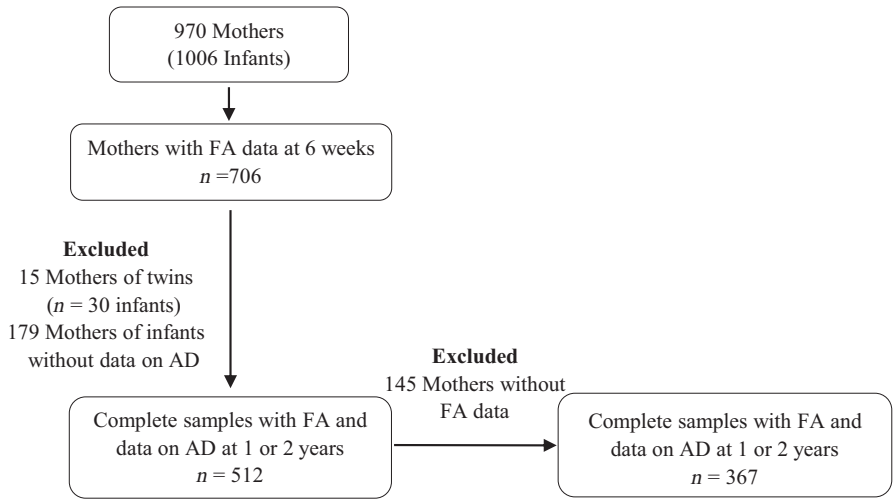

FIGURE 1 Flow chart of study population and final study sample size. AD, atopic dermatitis; FA, fatty acid.

TABLE 1 Characteristics of mother–infant pairs at 6 weeks and 6 months post-partum.

	6 weeks' samples (*n* = 512)	6 months' samples (*n* = 367)
Maternal characteristics		
Age, y, mean (SD)	33.4 (4.4)	33.5 (4.2)
Education, *n* (%)		
No degree	25 (4.9)	10 (2.8)
Secondary school degree	126 (24.9)	78 (21.5)
High school degree	356 (70.2)	274 (75.7)
Parity, *n* (%)	233 (45.6)	168 (45.8)
BMI		
Prepregnancy, mean (SD)	24.3 (4.6)	24 (4.3)
Postpartum, mean (SD)	24.9 (4.3)	24 (4.4)
Smoking		
Prepregnancy smoking, *n* (%)	90 (17.6)	48 (13.2)
During pregnancy, *n* (%)	15 (3.0)	6 (1.7)
Alcohol		
Prepregnancy consumption, *n* (%)	367 (72.0)	264 (72.3)
During pregnancy, *n* (%)	28 (5.5)	19 (5.2)
Asthma, *n* (%)	44 (8.6)	31 (8.5)
Atopic dermatitis, *n* (%)	71 (13.9)	54 (14.8)
Hay fever, *n* (%)	127 (24.9)	98 (26.8)
Breastfeeding		
Age at human milk collection, wk, mean (SD)	5.8 (0.6)	25.8 (0.8)
EBF at sample collection, *n* (%)	395 (77.1)	107 (29.2)
Duration of total breastfeeding, wk, mean (SD)	42.5 (21.1)	49.2 (19.1)
Duration EBF, wk, mean (SD)	16.7 (10.5)	19.1 (9.9)
Feedings per day n, mean (SD)	2 (0.2)	1.7 (0.5)
<=5	114 (31.4)	7 (0.19)
5	249 (68.6)	357 (98.1)
Infant		
Sex, girls, *n* (%)	249 (48.6)	176 (48.0)
Birthweight, g, mean (SD)	3374 (468)	3364 (468)
Gestational age, wk, mean (SD)	39.13 (1.5)	39.16 (1.5)
Atopic dermatitis at 2 y, *n* (%)	138 (27.0)	94 (25.6)

Note: Frequencies may not add up to total n due to missing's for some variables. Data presented as *n* (%) for categorical and mean and standard deviation (SD) for continuous variables.

Abbreviations: BMI, Body mass index; EBF, Exclusive breastfeeding; wk, week; y, years.

More than two-thirds (*n* = 395; 77.1%) of all infants were exclusively breastfed at the time of sample collection. At 6 months post-partum, we used data from 376 infants from whom 94 (25.5%) had AD up to 2 years and almost one-third (*n* = 107; 29.2%) were exclusively breastfed. All of the included infants at both time points were born after the gestational age of 32 weeks. Apart from infant birth weight, there were no sex differences in other sociodemographic, health- and birth-related variables for 6 weeks and 6 months.

3.1 | Sex-specific comparisons of relative PUFA proportions

There were no statistically significant sex differences in the relative PUFA proportions in human milk samples collected at 6 weeks (Table 2). Following Bonferroni correction (α threshold = 0.004), only DHA proportions in 6-month samples were higher in the milk for female infants than in the milk for male infants (Table 3, Figure 2). This difference was confirmed by a subsequent rank-based regression analysis using infant sex to predict relative DHA proportions and remained statistically significant after controlling for potential confounders, such as maternal history allergy, infant age, and exclusive breastfeeding at the time of sample collection. Similar differences were observed when analyzing the CLR-transformed fatty acids in a sensitivity analysis (Tables S3 and S4). Note, the quintile ranges of relative PUFA proportions for both samples are given in Tables S1 and S2.

3.2 | Associations between quintile PUFA proportions and AD

Among the 6-week samples, crude and multivariable logistic regression analysis showed no statistically significant (α threshold = 0.004) associations between fatty acid quintiles and AD up to 2 years of age (Figures S1 and S2). However, at conventional level of statistical significance ($p < .05$) in the sensitivity analyses using CLR-transformed fatty acids, there were some associations between PUFAs and AD. For instance, the multivariable adjusted model showed that infants whose milk was in the lower quintile of n-3 PUFAs had lower odds of AD (second vs. fifth quintile: OR (95% CI) 0.49 (0.24, 0.96), $p = .04$). The sensitivity analysis also showed sex differences at conventional level of statistical significance ($p < .05$) for DGLA. That is, female infants whose human milk was in the lower quintile had lower odds of AD (second vs. fifth quintile: 0.26 (0.07, 0.93), $p = .04$; Figure S4) compared to their male counterparts.

Likewise, using 6-month samples, no statistically significant (α threshold = 0.004) crude or multivariable adjusted associations between PUFAs and AD up to 2 years were found after Bonferroni correction (Figures S1 and S2). But, at conventional level of significance ($p < .05$), the multivariable adjusted model showed that female infants whose milk was in the lower quintile of ALA at 6 months had lower odds of AD compared with males (first vs. fifth quintile: 0.13 (0.02, 0.66), $p = .02$) (Figure 3; Table S5). The sensitivity analysis using CLR-transformed fatty acids showed a similar association for ALA (first vs. fifth quintile: 0.17 (0.03, 0.85), $p = .04$; Figure S3) and another sex-specific association for ARA/DHA ratio (second vs. fifth quintile: 6.62 (1.41, 32.47), $p = .02$; Figure S5), both statistically significant at conventional level of significance ($p < .05$).

TABLE 2 Relative proportions (%) of polyunsaturated fatty acids in human milk samples for all children and stratified by infant sex at 6 weeks postpartum.

	6 weeks postpartum (median [Q1, Q3]) ($N = 512$)			
PUFA	**All children**	**Male infants ($n = 263$)**	**Female infants ($n = 249$)**	***p***
n-3 PUFA				
Total n-3 PUFA	1.48 [1.23, 1.74]	1.46 [1.21, 1.72]	1.48 [1.24, 1.78]	.240
α-linolenic acid (ALA, 18:3n-3)	0.84 [0.67, 1.09]	0.84 [0.67, 1.06]	0.85 [0.68, 1.11]	.409
Eicosatetraenoic acid (ETA, 20:4n3)	0.09 [0.07, 0.11]	0.09 [0.07, 0.11]	0.09 [0.07, 0.10]	.256
Eicosapentaenoic acid (EPA, 20:5n3)	0.06 [0.05, 0.08]	0.06 [0.05, 0.08]	0.06 [0.05, 0.09]	.365
Docosapentaenoic acid (DPA, 22:5n3)	0.14 [0.13, 0.17]	0.14 [0.12, 0.17]	0.14 [0.13, 0.17]	.432
Docosahexaenoic acid (DHA, 22:6n3)	0.25 [0.19, 0.32]	0.24 [0.19, 0.31]	0.25 [0.19, 0.33]	.236
n-6 PUFA				
Total n-6 PUFA	11.03 [9.66, 12.82]	11.07 [9.70, 12.80]	11.02 [9.62, 12.82]	.868
Linoleic acid (LA, 18:2n6)	9.70 [8.35, 11.38]	9.70 [8.36, 11.39]	9.68 [8.34, 11.22]	.915
γ-Linolenic acid (GLA, 18:3n6)	0.12 [0.09, 0.15]	0.12 [0.10, 0.14]	0.12 [0.09, 0.15]	.336
Dihomo-γ-linolenic acid (DGLA, 20:3n6)	0.35 [0.30, 0.41]	0.35 [0.30, 0.41]	0.35 [0.30, 0.41]	.818
Arachidonic acid (ARA, 20:4n6)	0.43 [0.37, 0.48]	0.42 [0.38, 0.48]	0.43 [0.37, 0.48]	.713
PUFA ratios				
n-6/n-3 PUFA	7.37 [6.15, 8.84]	7.36 [6.19, 9.01]	7.38 [6.14, 8.73]	.386
ARA/DHA	1.76 [1.32, 2.26]	1.77 [1.36, 2.30]	1.74 [1.26, 2.23]	.288

Note: Displayed are median values and their first (Q1) and third quartile (Q3). The given *p*-values are based on Kruskal–Wallis tests.
Abbreviation: PUFA, Polyunsaturated fatty acids.

Of note, when correcting for multiple testing (α threshold = 0.004), all associations were not statistically significant. Furthermore, the fifth quintile representing the highest PUFA concentration was used as the reference category in all analyzed models.

4 | DISCUSSION

The current study investigated sex-specific associations of PUFAs in human milk samples collected at 6 weeks and 6 months of lactation with AD in infants up to 2 years of age. Although fatty acids in human milk did not differ at 6 weeks, relative proportions of DHA measured at 6 months were higher in human milk for female infants compared with male infants. This sex difference was statistically significant (α threshold = 0.004). Although there were some overall and sex-specific associations between PUFAs and AD up to 2 years at conventional level of significance ($p < .05$), none of them were statistically significant following Bonferroni correction (α threshold = 0.004).

4.1 | Sex differences in relative DHA proportions at 6 months

Our finding of statistically significant higher DHA in the milk for female infants compared with that for male infants at 6 months is in contrast to other studies[18,26,27] that did not show any sex differences. We are aware of one other study[28] in which human milk for male infants contained significantly higher relative proportions of LA than for female infants, while all other fatty acids did not differ. Of note, approximately 30% of LA found in human milk is obtained directly from the diet and the rest is synthesized from maternal endogenous stores.[27] Thus, the authors from the aforementioned study[28] attributed their finding to maternal metabolic regulations specific to LA rather than being related to dietary habits.

However, elevated levels of the sex hormone testosterone in mothers carrying a male fetus[29] could influence dietary preferences prenatally[30] which could have subsequent biological consequences on milk production. On the one hand, given that DHA in human milk is particularly sensitive to dietary intake, it is plausible that the sex difference in DHA proportions could have been driven by maternal diet. Although we did not assess maternal dietary intake, we previously showed a potentially higher PUFA intake of lactating mothers in SPATZ over the years.[31] Using maternal age and higher education status as proxies of socioeconomic status, lactating women in SPATZ presumably had a higher socioeconomic status.[31] This combined with an overall higher PUFA intake over the years could indicate that they have consumed or taken fish oil/*n*-3 fatty acid supplements.[31]

On the other hand, we also cannot rule out that the small sample size in our study could have led to a potential chance finding. It is therefore plausible that if the study were larger, this chance effect would be less likely to occur.

Furthermore, some of these previous studies[17,32,33] which showed no sex-specific differences in PUFAs only included data

TABLE 3 Relative proportions (%) of polyunsaturated fatty acids in human milk samples for all children and stratified by infant sex at 6 months postpartum.

	6 months postpartum (median [Q1, Q3]) (*N* = 367)			
PUFA	**All children**	**Male infants (*n* = 191)**	**Female infants (*n* = 176)**	***p***
n-3 PUFA				
Total n-3 PUFA	1.43 [1.17, 1.79]	1.39 [1.17, 1.77]	1.47 [1.17, 1.85]	.309
α-linolenic acid (ALA,18:3n-3)	0.88 [0.70, 1.15]	0.87 [0.70, 1.15]	0.88 [0.70, 1.16]	.852
Eicosatetraenoic acid (ETA, 20:4n3)	0.06 [0.05, 0.07]	0.06 [0.05, 0.07]	0.06 [0.05, 0.07]	.487
Eicosapentaenoic acid (EPA, 20:5n3)	0.06 [0.04, 0.08]	0.06 [0.04, 0.07]	0.06 [0.05, 0.08]	.030
Docosapentaenoic acid (DPA, 22:5n3)	0.15 [0.12, 0.17]	0.14 [0.12, 0.16]	0.15 [0.13, 0.17]	.127
Docosahexaenoic acid (DHA, 22:6n3)	0.19 [0.16, 0.27]	0.18 [0.15, 0.24]	0.21 [0.17, 0.30]	.001*
n-6 PUFA				
Total n-6 PUFA	10.56 [9.56, 12.42]	7.54 [6.42, 9.01]	7.23 [6.04, 9.03]	.796
Linoleic acid (LA, 18:2n6)	9.50 [8.57, 11.25]	9.43 [8.51, 11.36]	9.67 [8.57, 11.13]	.802
γ-Linolenic acid (GLA, 18:3n6)	0.10 [0.07, 0.12]	0.10 [0.07, 0.12]	0.09 [0.08, 0.12]	.515
Dihomo-γ-linolenic acid (DGLA, 20:3n6)	0.25 [0.22, 0.29]	0.25 [0.22, 0.29]	0.24 [0.22, 0.29]	.622
Arachidonic acid (ARA, 20:4n6)	0.38 [0.33, 0.44]	0.38 [0.33, 0.43]	0.38 [0.32, 0.45]	.490
PUFA ratios				
n-6/n-3 PUFA	7.36 [6.31, 9.02]	7.54 [6.42, 9.01]	7.23 [6.04, 9.03]	.387
ARA/DHA	1.95 [1.42, 2.42]	2.05 [1.57, 2.45]	1.79 [1.23, 2.40]	.012

Note: Displayed are median values and their first (Q1) and third quartile (Q3). The given *p*-values are based on Kruskal–Wallis tests.

Abbreviation: PUFA, Polyunsaturated fatty acids.

*Indicates significant values after Bonferroni correction.

on human milk samples collected up to 4 months post-partum. However, we have previously shown that fatty acid composition changes during the first 12 months of lactation.[24] Likewise, while in this current study, we did not find sex differences in PUFA concentrations at 6 weeks, we show a decrease of DHA at 6 months which was more pronounced in the milk of male infants. Our results, therefore, further confirm that human milk collected on one time point in lactation may not depict an accurate picture of overall human milk composition. As such, these potential sex differences might depend on the time interval for sample collection. Moreover, the aforementioned research[17,32,33] has neglected the compositional aspect of fatty acids. As a result, there is a limited availability of compositional analyses in this regard.[16] Considering this, we additionally performed a sensitivity analysis using CLR-transformed fatty acid data which confirmed the observed sex differences in DHA. Thus, such an analytical approach could be more appropriate and suitable for compositional data compared to reporting relative proportions.

4.2 | Associations between PUFAs and AD

There were no statistically significant associations between human milk PUFAs and AD up to 2 years, neither were there sex-specific associations, following correction for multiple testing. Similarly, a recent study[17] showed no statistically significant associations between PUFA proportions and AD in 1-year-old infants. However, in comparison to our study, the authors[17] reported lower odds (non-statistically significant tendencies) of AD for female infants whose human milk contained proportions of n-3 PUFAs in the lowest quintile and proportions of ALA in the third quintile. Whereas the same proportions of ALA in the third quintile were associated with higher odds for males. The authors also did not adjust for multiple comparisons and did not report data on CLR-transformed fatty acid data with respect to AD. As such, the evidence for sex-specific associations with human milk fatty acids remains inconclusive.

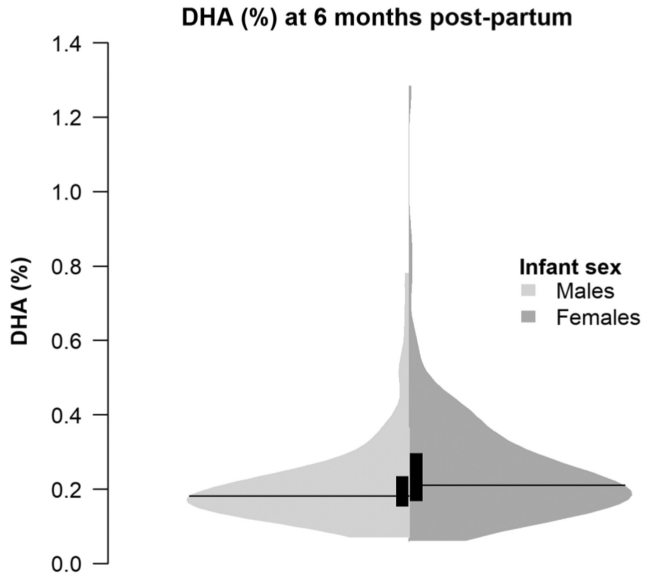

FIGURE 2 Distribution of the percentage proportions of docosahexaenoic acid (DHA) in the milk of male and female infants. The borders of each box display the first (Q1) respectively third quartile (Q3) with the horizontal line denoting the median.

4.3 | Biological plausibility of sex differences in PUFAs

In general, sex differences in atopic conditions are widely reported[34] with slightly higher prevalence and earlier onset in male than female infants.[35,36] While the evidence for sex-specific fatty acid composition in human milk in general and their relationship to AD is still largely elusive, sex dimorphic manifestations have been demonstrated for other body compartments. For instance, the plasma of 12-month-old female infants is suggested to contain higher concentrations of PUFAs, such as DHA compared with males.[37] Elevated levels of n-3 PUFAs, in particular DHA, in plasma or cord blood might protect from the development of wheezing, asthma, and AD in early childhood.[38,39] It has also been shown that both sexes differ in their ability to synthesize n-3 PUFAs potentially leading to differences in circulating PUFA concentrations.[13]

With respect to human milk, other bioactive molecules, such as adipokines (e.g., leptin, adiponectin), are shown to be sexually dimorphic.[40] For instance, testosterone, which increases during pregnancy in women carrying a male fetus,[29] inhibits the production of adiponectin.[41] Lower levels of adiponectin have been shown to change fatty acid concentrations in milk and to increase inflammatory cytokines.[42]

All this taken together, sex hormones could modulate the involvement of PUFAs in immune development leading to sex differences in the expression of atopic conditions in early childhood. However, the extent to which PUFAs provided through human milk might contribute to this remains unclear since many other factors besides hormones, such as maternal diet, genetics, and environment, could modify potential relationships.[43] Consequently, the absence of sex-specific associations of PUFAs with AD in infants up to 2 years in the current study could be also due to some of these factors which were not assessed.

4.4 | Limitations and strengths

A limitation of this study is the lack of dietary information, as this could have shed more light on the diet-sensitive fatty acids in human milk. As such, we cannot determine if the sex difference in DHA concentrations was driven by maternal diet or other factors that were not measured in this study. Of note, there could also be other determinants of DHA in human milk not measured in our study which are unequally distributed between female and male infants by chance, resulting in the difference we observed. In addition, the sample size of infants with AD up to 2 years might have been too small with respect to the given research question which, in combination with how fatty acids were quantified, may have led to large confidence intervals especially at 6 months post-partum. And given that AD was assessed retrospectively, we also cannot rule out recall bias.

The strengths of this study include the sensitivity analysis using CLR-transformed fatty acid data and consequently correcting for multiple testing. Not accounting for the compositionality of fatty acids as in aforementioned studies could result in spurious correlations.[26] Especially with regard to infant outcomes such as allergic conditions, this approach could bear the risk for unconcise clinical inferences.[19] Moreover, we have fatty acid data from two time points of lactation, that is, 6 weeks and 6 months post-partum. With this, we accounted for temporal changes in fatty acid composition, and secondly, we attempted to narrow down the timing of exposure with regard to potential sex-specific associations with AD.

5 | CONCLUSIONS

In conclusion, there were no statistically significant associations between human milk fatty acids and AD up to 2 years. However, we do report potential sex differences in relative proportions of DHA in human milk samples collected at 6 months of lactation. Future studies are needed to determine whether these potential sex differences in human milk could be of clinical relevance for infants. Potential sex-specific nutritional and hormonal requirements of the growing infant, in particular, highlight the need for future multi-modal observational studies in order to optimize child development. More precisely, prospective studies should investigate fatty acids and hormones in human milk as well as maternal and infant blood including hormonal, genetic, and immunology components. As such it might be possible to disentangle when and how sex-specific modulations occur, to relate these to clinical infant outcomes and, as a result, to tailor sex-specific nutritional strategies. Moreover, future studies need to carefully consider the compositional nature of fatty acids and use appropriate statistical methods to account for this especially regarding infant health outcomes.[19]

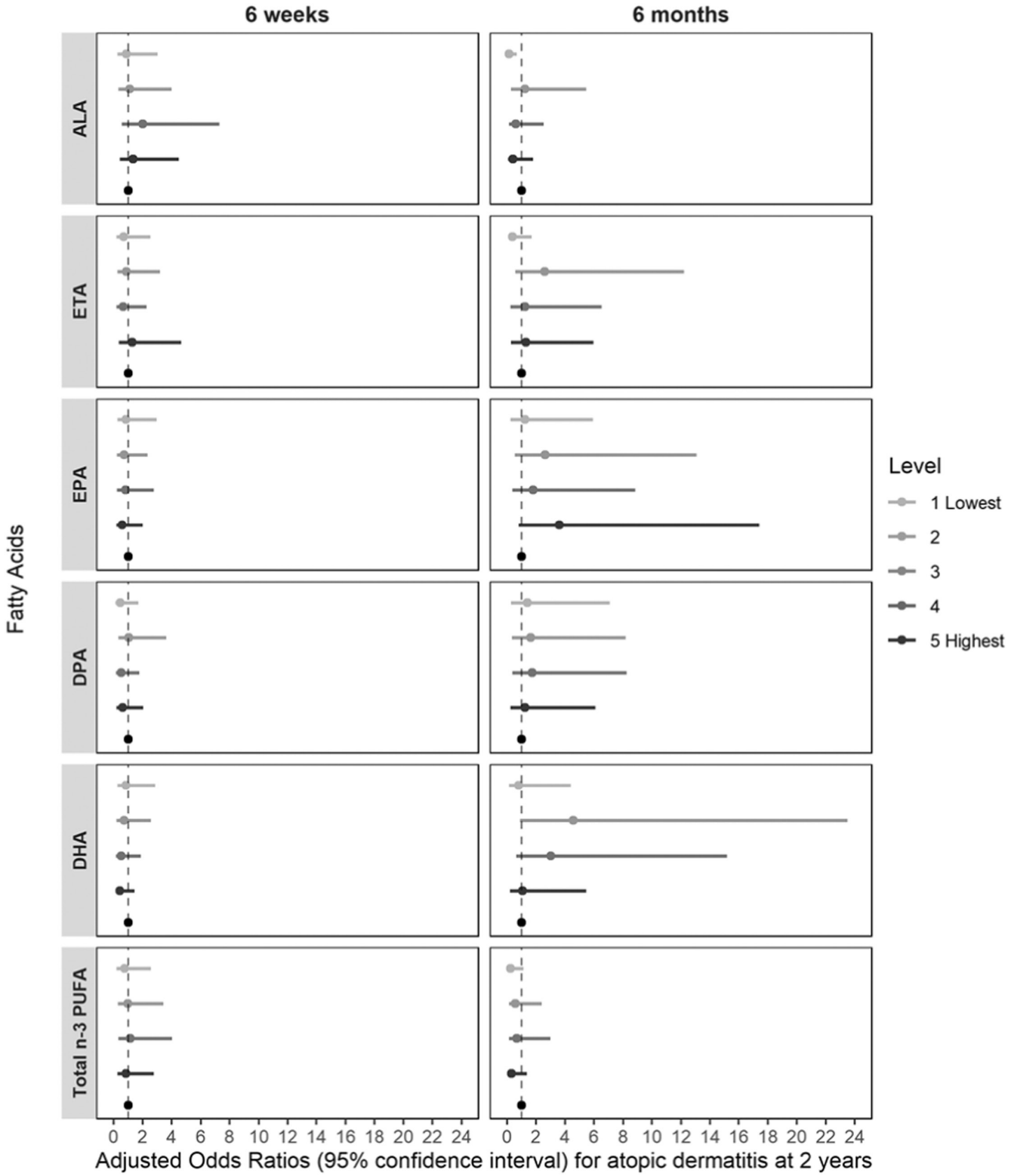

FIGURE 3 Adjusted odds ratios (95% confidence interval) of atopic dermatitis (AD) predicted by infant sex and n-3 polyunsaturated fatty acids (PUFAs) in human milk at 6 weeks and 6 months post-partum. The models were adjusted for age and exclusive breastfeeding at time of sample collection and maternal allergies (hay fever, atopic dermatitis, and/or asthma). ALA, α-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; ETA, eicosatetraenoic acid.

We display extensive information, in particular in the supplementary tables, so that future studies can determine appropriate sample sizes for hypothesis testing. Studying these early differences might pave the way to sex-specific nutritional strategies which might help to prevent the occurrence of early but also later allergic conditions.

AUTHOR CONTRIBUTIONS

Madeleine Ordnung: Conceptualization; formal analysis; investigation; writing – review and editing; writing – original draft. **Marko Mank:** Methodology; project administration; validation; resources; writing – review and editing. **Bernd Stahl:** Methodology; project administration; resources; validation; writing – review and editing. **Deborah Kurz:** Writing – review and editing; investigation. **Tamas Marosvölgyi:** Methodology; resources; validation; writing – review and editing. **Tamas Decsi:** Methodology; resources; validation; writing – review and editing. **Dietrich Rothenbacher:** Conceptualization; data curation; funding acquisition; project administration; investigation; resources; writing – review and editing. **Jon Genuneit:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; project administration; resources; writing – original draft; writing – review and editing. **Linda P. Siziba:** Conceptualization; data curation; formal analysis; investigation; project administration; writing – original draft; writing – review and editing.

ACKNOWLEDGMENTS

We thank the midwives, nurses, and obstetricians of the Department of Gynaecology and Obstetrics, University Medical Centre Ulm and the caring pediatricians and mothers and their families for their study support and participation. The authors would also like to thank Mrs. Gerlinde Trischler for providing excellent technical assistance. Open Access funding enabled and organized by Projekt DEAL.

FUNDING INFORMATION

The Ulm SPATZ Health Study was funded through an unrestricted grant by the Medical Faculty of Ulm University. This research was co-funded by Medical Faculty, Leipzig University and by Danone Nutricia Research, Utrecht, The Netherlands.

CONFLICT OF INTEREST STATEMENT

JG is the project manager and LPS is a scientist on unrestricted grants from Danone Nutricia Research to Ulm University and Leipzig University for research on other aspects of human milk composition in the Ulm Birth Cohort Studies. MM and BS are employees of Danone Nutricia Research.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/pai.13992.

ORCID

Madeleine Ordnung https://orcid.org/0009-0008-6819-2973
Marko Mank https://orcid.org/0000-0001-7695-3906
Tamas Marosvölgyi https://orcid.org/0000-0002-4244-5513
Jon Genuneit https://orcid.org/0000-0001-5764-1528
Linda P. Siziba https://orcid.org/0000-0002-4773-252X

REFERENCES

1. Asher MI, Montefort S, Björkstén B, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC phases one and three repeat multicountry cross-sectional surveys. *Lancet*. 2006;368(9537):733-743. doi:10.1016/S0140-6736(06)69283-0
2. Tsuge M, Ikeda M, Matsumoto N, Yorifuji T, Tsukahara H. Current insights into atopic march. *Children*. 2021;8:11. doi:10.3390/children8111067
3. Muraro A, Halken S, Arshad SH, et al. EAACI food allergy and anaphylaxis guidelines. Primary prevention of food allergy. *Allergy: European J Allergy Clin Immunol*. 2014;69(5):590-601. doi:10.1111/all.12398
4. Mosca F, Giannì ML. Human milk: composition and health benefits. *Pediatria Medica e Chirurgica*. 2017;39(2):47-52. doi:10.4081/PMC.2017.155
5. Nuzzi G, Di Cicco ME, Peroni DG. Breastfeeding and allergic diseases: What's new? *Children*. 2021;8(5):330. doi:10.3390/children8050330
6. Bobiński R, Bobińska J. Fatty acids of human milk- a review. *Int J Vitam Nutr Res*. 2022;92:92. doi:10.1024/0300-9831/a000651
7. Calder PC, Kremmyda LS, Vlachava M, Noakes PS, Miles EA. Is there a role for fatty acids in early life programming of the immune system? *Proc Nutr Soc*. 2010;69(3):373-380. doi:10.1017/S0029665110001552
8. Thijs C, Müller A, Rist L, et al. Fatty acids in breast milk and development of atopic eczema and allergic sensitisation in infancy. *Allergy*. 2011;66(1):58-67. doi:10.1111/j.1398-9995.2010.02445.x
9. Soto-Ramírez N, Karmaus W, Zhang H, et al. Fatty acids in breast milk associated with asthma-like symptoms and atopy in infancy: a longitudinal study. *J Asthma*. 2012;49(9):926-934. doi:10.3109/02770903.2012.719251
10. Prescott SL, Calder PC. N-3 polyunsaturated fatty acids and allergic disease. *Curr Opin Clin Nutr Metab Care*. 2004;7(2):123-129. doi:10.1097/00075197-200403000-00004
11. Miles EA, Calder PC. Can early omega-3 fatty acid exposure reduce risk of childhood allergic disease? *Nutrients*. 2017;9(7):784. doi:10.3390/nu9070784
12. Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol*. 2016;16(10):626-638. doi:10.1038/nri.2016.90
13. Childs CE, Romeu-Nadal M, Burdge GC, Calder PC. Gender differences in the n-3 fatty acid content of tissues. *Proc Nutr Soc*. 2008;67(1):19-27. doi:10.1017/S0029665108005983
14. Möhrenschlager M, Schäfer T, Huss-Marp J, et al. The course of eczema in children aged 5-7 years and its relation to atopy: differences between boys and girls. *Br J Dermatol*. 2006;154(3):505-513. doi:10.1111/j.1365-2133.2005.07042.x
15. Bakewell L, Burdge GC, Calder PC. Polyunsaturated fatty acid concentrations in young men and women consuming their habitual diets. *Br J Nutr*. 2006;96(1):93-99. doi:10.1079/bjn20061801
16. Galante L, Milan AM, Reynolds CM, Cameron-Smith D, Vickers MH, Pundir S. Sex-specific human milk composition: the role of infant sex in determining early life nutrition. *Nutrients*. 2018;10(9):1194. doi:10.3390/nu10091194
17. Miliku K, Richelle J, Becker AB, et al. Sex-specific associations of human milk long-chain polyunsaturated fatty acids and infant allergic conditions. *Pediatr Allergy Immunol*. 2021;32(6):1173-1182. doi:10.1111/pai.13500
18. Thum C, Wall C, Day L, et al. Changes in human Milk fat globule composition throughout lactation: a review. *Front Nutr*. 2022;9:722. doi:10.3389/FNUT.2022.835856/BIBTEX
19. Logan CA, Brandt S, Wabitsch M, et al. New approach shows no association between maternal milk fatty acid composition and childhood wheeze or asthma. *Allergy*. 2017;72(9):1374-1383. doi:10.1111/all.13161

20. Logan C, Zittel T, Striebel S, et al. Changing societal and lifestyle factors and breastfeeding patterns over time. *Pediatrics*. 2016;137(5):e20154473. doi:10.1542/peds.2015-4473
21. Peng Z, Braig S, Kurz D, et al. Trajectory and determinants of agreement between parental and physicians' reports of childhood atopic dermatitis. *Pediatr Allergy Immunol*. 2022;33(9):e13855. doi:10.1111/PAI.13855
22. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol*. 1959;37(8):911-917. doi:10.1139/o59-099
23. Beermann C, Green A, Möbius M, Schmitt JJ, Boehm G. Lipid class separation by HPLC combined with GC FA analysis: comparison of seed lipid compositions from different *Brassica napus* L. varieties. *J Am Oil Chem Soc*. 2003;80(8):747-753. doi:10.1007/s11746-003-0767-0
24. Siziba LP, Lorenz L, Stahl B, et al. Changes in human milk fatty acid composition during lactation: the Ulm SPATZ health study. *Nutrients*. 2019;11(12):2842. doi:10.3390/nu11122842
25. Kloke JD, McKean JW. Rfit: rank-based estimation for linear models. *R Journal*. 2012;4(2):57-64. doi:10.32614/rj-2012-014
26. Aitchison J, Egozcue JJ. Compositional data analysis: where are we and where should we be heading? *Math Geol*. 2005;37(7):829-850. doi:10.1007/s11004-005-7383-7
27. Demmelmair H, Kuhn A, Dokoupil K, Hegele V, Sauerwald T, Koletzko B. Human lactation: oxidation and maternal transfer of dietary13C-labelled α-linolenic acid into human milk. *Isotopes Environ Health Stud*. 2016;52(3):270-280. doi:10.1080/10256016.2015.1071362
28. Thakkar SK, Giuffrida F, Cristina CH, et al. Dynamics of human milk nutrient composition of women from Singapore with a special focus on lipids. *Am J Hum Biol*. 2013;25(6):770-779. doi:10.1002/ajhb.22446
29. Meulenberg PMM, Hofman JA. Maternal testosterone and fetal sex. *J Steroid Biochem Mol Biol*. 1991;39(1):51-54. doi:10.1016/0960-0760(91)90012-T
30. Tamimi RM, Lagiou P, Mucci LA, Hsieh CC, Adami HO, Trichopoulos D. Average energy intake among pregnant women carrying a boy compared with a girl. *Br Med J*. 2003;326(7401):1245-1246. doi:10.1136/bmj.326.7401.1245
31. Siziba LP, Lorenz L, Brenner H, et al. Changes in human milk fatty acid composition and maternal lifestyle-related factors over a decade: a comparison between the two Ulm birth cohort studies. *Br J Nutr*. 2021;126(2):228-235. doi:10.1017/S0007114520004006
32. Giuffrida F, Fleith M, Goyer A, et al. Human milk fatty acid composition and its association with maternal blood and adipose tissue fatty acid content in a cohort of women from Europe. *Eur J Nutr*. 2022;61(4):2167-2182. doi:10.1007/s00394-021-02788-6
33. Zagierski M, Krukowska A, Kawska K, Sznurkowska K, Martysiak-Żurowska D, Szlagatys-Sidorkiewicz A. No evidence for sex-specificity in vitamins C, E, and fatty acid content of human Milk from healthy polish mothers. *J Pediatr Gastroenterol Nutr*. 2021;73(1):e20-e25. doi:10.1097/MPG.0000000000003136
34. Kanda N, Hoashi T, Saeki H. The roles of sex hormones in the course of atopic dermatitis. *Int J Mol Sci*. 2019;20(19):4660. doi:10.3390/ijms20194660
35. Mohn CH, Blix HS, Halvorsen JA, Nafstad P, Valberg M, Lagerløv P. Incidence trends of atopic dermatitis in infancy and early childhood in a Nationwide prescription registry study in Norway. *JAMA Netw Open*. 2018;1(7):e184145. doi:10.1001/jamanetworkopen.2018.4145
36. Dirven-Meijer PC, Glazenburg EJ, Mulder PGH, Oranje AP. Prevalence of atopic dermatitis in children younger than 4 years in a demarcated area in Central Netherlands: the west Veluwe study group. *Br J Dermatol*. 2008;158(4):846-847. doi:10.1111/j.1365-2133.2007.08407.x
37. Ellul S, Ponsonby AL, Carlin JB, et al. Sex differences in infant blood metabolite profile in association with weight and adiposity measures. *Pediatr Res*. 2020;88(3):473-483. doi:10.1038/s41390-020-0762-4
38. Newson RB, Shaheen SO, Henderson AJ, Emmett PM, Sherriff A, Calder PC. Umbilical cord and maternal blood red cell fatty acids and early childhood wheezing and eczema. *J Allergy Clin Immunol*. 2004;114(3):531-537. doi:10.1016/j.jaci.2004.05.010
39. Montes R, Chisaguano AM, Castellote AI, Morales E, Sunyer J, López-Sabater MC. Fatty-acid composition of maternal and umbilical cord plasma and early childhood atopic eczema in a Spanish cohort. *Eur J Clin Nutr*. 2013;67(6):658-663. doi:10.1038/ejcn.2013.68
40. Galante L, Lagström H, Vickers MH, et al. Sexually dimorphic associations between maternal factors and human milk hormonal concentrations. *Nutrients*. 2020;12(1):152. doi:10.3390/nu12010152
41. Horenburg S, Fischer-Posovszky P, Debatin KM, Wabitsch M. Influence of sex hormones on adiponectin expression in human adipocytes. *Horm Metab Res*. 2008;40(11):779-786. doi:10.1055/s-0028-1083780
42. Jin Z, Du Y, Schwaid AG, et al. Maternal adiponectin controls milk composition to prevent neonatal inflammation. *Endocrinology*. 2015;156(4):1504-1513. doi:10.1210/en.2014-1738
43. Miliku K, Duan QL, Moraes TJ, et al. Human milk fatty acid composition is associated with dietary, genetic, sociodemographic, and environmental factors in the CHILD cohort study. *Am J Clin Nutr*. 2019;110(6):1370-1383. doi:10.1093/ajcn/nqz229

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Ordnung M, Mank M, Stahl B, et al. Potential sex differences in human milk fatty acids and their association with atopic dermatitis: Results of the Ulm SPATZ health study. *Pediatr Allergy Immunol*. 2023;34:e13992. doi:10.1111/pai.13992