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Associations between fatty acids in colostrum and breast milk and risk of allergic disease

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Summary

Background Exposure to n-3 polyunsaturated fatty acids (PUFA) in early life is hypothesized to offer protection against atopic disease. However, there is controversy in this area, and we have previously observed that high levels of n-3 fatty acid (FA) in colostrum are associated with increased risk of allergic sensitization.

Objective The aim of the study was to assess the relationship between FA profile in breast milk and risk of childhood atopic disease.

Methods A high-risk birth cohort was recruited, and a total of 224 mothers provided a sample of colostrum ($n = 194$) and/or 3-month expressed breast milk ($n = 118$). FA concentrations were determined by gas chromatography. Presence of eczema, asthma and rhinitis were prospectively documented up to 7 years of age.

Results High levels of n-3 22:5 FA (docosapentaenoic acid, DPA) in colostrum were associated with increased risk of infantile atopic eczema [odds ratio (OR) = 1.66 per 1 standard deviation increase, 95% confidence interval (CI) = 1.11–2.48], while total n-3 concentration in breast milk was associated with increased risk of non-atopic eczema (OR = 1.60, 95% CI = 1.03–2.50). Higher levels of total n-6 FA in colostrum were associated with increased risk of childhood rhinitis (OR = 1.59, 95% CI = 1.12–2.25). There was no evidence of associations between FA profile and risk of asthma.

Conclusion In this cohort of high-risk children, a number of modest associations were observed between FA concentrations in colostrum and breast milk and allergic disease outcomes. Further research in this area with larger sample sizes is needed.

Keywords allergy prevention, asthma, eczema, polyunsaturated fatty acids, rhinitis

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Introduction

An inverse association between fish consumption and childhood symptoms of asthma has been observed using cross-sectional studies [1, 2]. Recently, frequent maternal consumption of fish during pregnancy has been associated with reduced risk of infantile eczema, asthma and rhinitis [3–5]. Such observations have provided support to the hypothesis that dietary n-3 polyunsaturated fatty acids (PUFA), which are highly concentrated in oily fish, may reduce the production of pro-inflammatory eicosanoid mediators, including prostaglandins and leuco-

trienes, and thus reduce the risk of atopic disease [6]. Early infancy is believed to be the key time for the programming of the immune system [7]. A number of studies have examined fatty acid (FA) profile in breast milk as a risk factor for subsequent atopic disease [8–16]. These studies have shown mixed results, but have generally found that n-3 PUFAs in breast milk possibly protect against atopic diseases.

We have previously reported data from the Melbourne Atopy Cohort Study (MACS), which showed that n-3 FAs in colostrum were unexpectedly associated with increased risk of allergic sensitization at 6 and 24 months [17].

This apparently conflicted with the hypothesized association and with the body of literature in this area. We now present clinical outcomes from the same cohort (eczema up to 2 years of age, and asthma and allergic rhinitis at 6 and 7 years of age) to extend this work and further explore the associations between early life FA exposure and risk of atopic disease.

Methods

Participants

The MACS recruited 620 infants before birth, born between 1990 and 1994 in Melbourne, Australia. The recruitment process has been described in more detail elsewhere [18]. The Mercy Maternity Hospital's Human Research Ethics Committee approved this research. Infants were eligible to be enrolled in MACS if the mother reported that a first-degree family member of the infant had a history of asthma, hayfever, eczema or severe food allergy. An allergy-trained nurse conducted a telephone survey every 4 weeks until the infant was 64 weeks of age, and then at 78 weeks, 2 years and annually until 7 years of age. Skin prick tests (SPTs) were performed at 6, 12 and 24 months with six allergens (milk, egg, peanut, dust mite, rye grass and cat dander) using a standardized technique [19]. SPTs were not performed routinely from 3 to 7 years of age.

A subsample of mothers in the MACS provided hand-expressed colostrum (2–4 days after delivery) and expressed breast milk (EBM) samples at 3 months using a breast pump. FA extraction and analysis was performed by gas chromatography according to the method of Bligh and Dyer [20], and detailed in our previous paper [17].

Outcome measures

The primary outcomes were presence of eczema in the first 2 years of life, and current asthma and rhinitis at 6 or 7 years of age. Eczema in the first 2 years of life was defined as parental report of either a doctor diagnosis of eczema or any rash that was treated with topical steroid preparation (excluding rash that only affected the scalp or nappy region) [21]. A positive SPT was defined as a weal of at least 3 mm (mean) diameter. Infantile *atopic eczema* was defined as eczema within the first year of life with sensitization detected at 6, 12 or 24 months, while *non-atopic eczema* was defined as eczema without sensitization at these times [22]. Current childhood asthma was defined as parental report during the telephone interview of one or more events of wheezing illness attributed to asthma by the family physician in the previous 12 months. Current childhood rhinitis was defined as one or more episodes of nasal discharge and/or congestion, in the absence of an upper respiratory tract infection, which

either the family physician or parent attributed to hayfever and required treatment with an anti-histamine and/or nasal steroid in the previous 12 months [22].

Statistical analysis

The risk of eczema in the first 2 years of life and current childhood asthma and rhinitis (at age 6 and 7 years) were analysed separately using logistic regression models. The primary predictors were total n-3 and n-6 FA concentrations, and the n-6:n-3 ratio, in colostrum and 3-month EBM samples assessed as continuous measurements. We elected to report the associations for colostrum and 3-month EBM separately because not all participants provided both samples, and although the concentrations of each of the FAs were correlated between the time points, these correlations were generally modest (the strongest Pearson's *r* being 0.581 for total n-6). The possibility of non-linear associations between FA concentrations and risk of allergic disease was assessed by examining 44 fractional polynomial models to determine if they fit the observed data better than a linear model (using the 'fracpoly' command in Stata). There was no evidence of non-linearity in any of these associations.

The associations between individual FAs and allergic disease outcomes were reported as secondary outcomes. The range of values for the concentration of n-6 FA in breast milk was much greater than that of n-3 FA. To make the interpretation of the odds ratios (ORs) comparable between n-3 and n-6 FAs, the individual values were converted into Z scores based on the observed distribution. The reported ORs represent the increase in risk of the outcome per 1 standard deviation increase in the concentration of that particular FA. Associations with infantile atopic and non-atopic eczema were assessed using multinomial logistic regression, with no-eczema as the reference group. Logistic regression models for asthma and rhinitis were estimated by the generalized estimating equations approach, in order to allow for repeated measures on the same subject at the two time points (6 and 7 years) [23]. Potential interactions between FA concentrations and sex on risk of atopic disease were assessed using Wald tests.

The following variables were included in the final model if they caused important confounding [$\geq 15\%$ change in primary OR or predicted the outcome ($P < 0.05$ for the effect in the multivariable model)]: presence of allergic diseases in the mother, presence of allergic diseases in the father, infant gender, presence of older siblings, parental smoking (within past 6 months at recruitment), maternal and paternal age and years of education, use of gas heating and cooking in the home and pet exposure in the first year of life. Stata (release 9.2, Stata Corporation, College Station, TX, USA, 2005) was used for all statistical analysis.

Results

Study population

Colostrum samples were provided by 194 women, while 118 women provided an EBM sample (224 in total, with 88 women providing both). There was no evidence of differences between infants whose mothers provided breast milk and those who did not in terms of infant gender, parental and sibling history of allergic disease, parental age and level of education, parental smoking status and family pet ownership. The concentrations of total n-6 and n-3 FA were consistent with previously published data [24] (Table 1). When compared with colostrum, 3-month EBM had an increased proportion of n-6 FAs and a reduced proportion of n-3 FAs, when assessed for the 88 women who provided both samples.

Associations between fatty acid profiles and risk of eczema

Approximately 45% of the infants developed eczema in the first 2 years of life (Tables 2 and 3). There was no evidence that colostrum or 3-month EBM FA profiles were associated with overall risk of eczema within the first year of life (Tables 2 and 3). There was, however, very weak evidence for high levels of n-3 FA in colostrum to be associated with increased risk of *atopic* eczema (Table 2). This effect was driven by 22:5n-3 (docosapentaenoic acid, DPA), which was associated with an increased risk of atopic eczema both in colostrum and 3-month EBM (Tables 2 and 3). When compared with infants without eczema,

those infants with atopic eczema had a higher proportion of DPA in 3-month EBM [mean (SD) = 0.22 (0.05) compared with 0.18 (0.05)]. High levels of 22:5n-3 (DPA) in colostrum appeared to confer greater risk of atopic eczema in girls [OR = 3.00, 95% confidence interval (CI) = 1.57–5.72] than in boys (OR = 0.94, 95% CI = 0.51–1.72, *P* for interaction = 0.011). There was a similar but weaker gender difference for the association with 22:5n-3 in 3-month EBM

Table 1. Descriptive statistics for fatty acid content of colostrum (*n* = 194) and 3-month breast milk (118)

Fatty acid	Colostrum (<i>n</i> = 194)		3-month EBM (<i>n</i> = 118)	
	Mean (SD)	Range	Mean (SD)	Range
n-6 series				
18:2	12.05 (3.50)	5.74–30.20	15.40 (5.03)	6.04–29.06
18:3	0.12 (0.04)	0.00–0.25	0.16 (0.04)	0.08–0.30
20:3	0.58 (0.16)	0.27–1.26	0.38 (0.09)	0.22–0.62
20:4	0.61 (0.14)	0.28–1.06	0.40 (0.10)	0.13–0.70
22:4	0.25 (0.11)	0.08–0.79	0.08 (0.02)	0.03–0.16
22:5	0.09 (0.03)	0.03–0.20	0.03 (0.02)	0.00–0.10
Total n-6 series	14.49 (3.67)	7.96–32.72	16.77 (5.18)	7.05–30.89
n-3 series				
18:3	0.69 (0.22)	0.29–1.78	0.86 (0.31)	0.42–2.32
20:5	0.06 (0.02)	0.00–0.14	0.06 (0.04)	0.00–0.29
22:5	0.25 (0.07)	0.11–0.47	0.19 (0.05)	0.08–0.38
22:6	0.46 (0.17)	0.15–0.97	0.24 (0.17)	0.07–1.17
Total n-3 series	1.87 (0.35)	1.12–2.82	1.67 (0.41)	0.82–2.99
n-6/n-3 ratio	8.06 (2.88)	4.35–27.07	10.57 (4.07)	4.72–26.50

Data are weight percentage.

EBM, expressed breast milk; SD, standard deviation.

Table 2. Unadjusted associations between fatty acid concentrations in colostrum (*n* = 194) and risk of atopic and non-atopic eczema within the first 2 years of life

	Any eczema (<i>n</i> = 80/183)*		Atopic eczema (<i>n</i> = 27/172)†		Non-atopic eczema (<i>n</i> = 42/172)†	
	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>
n-6 series						
18:2	1.08 (0.81–1.44)	0.602	1.12 (0.76–1.67)	0.560	1.04 (0.74–1.48)	0.811
18:3	0.97 (0.72–1.29)	0.812	0.78 (0.50–1.22)	0.274	1.15 (0.81–1.63)	0.442
20:3	1.09 (0.82–1.45)	0.548	1.14 (0.77–1.70)	0.510	1.03 (0.73–1.47)	0.855
20:4	1.00 (0.75–1.34)	0.995	1.12 (0.73–1.71)	0.598	0.75 (0.52–1.10)	0.147
22:4	1.15 (0.86–1.53)	0.342	1.30 (0.89–1.90)	0.178	0.88 (0.60–1.31)	0.544
22:5	1.10 (0.82–1.48)	0.514	1.45 (0.97–2.17)	0.069	0.77 (0.51–1.16)	0.206
Total n-6 series	1.10 (0.82–1.47)	0.520	1.16 (0.78–1.71)	0.469	1.04 (0.73–1.47)	0.842
n-3 series						
18:3	1.01 (0.76–1.35)	0.935	1.10 (0.22–1.63–)	0.744	0.98 (0.69–1.40)	0.917
20:5	1.04 (0.77–1.41)	0.779	1.50 (0.99–2.27)	0.056	0.82 (0.55–1.23)	0.343
22:5	1.29 (0.96–1.73)	0.091	1.66 (1.11–2.48)	0.014	1.00 (0.69–1.46)	0.995
22:6	1.06 (0.79–1.43)	0.675	1.37 (0.92–2.04)	0.123	0.84 (0.58–1.23)	0.379
Total n-3 series	1.11 (0.82–1.48)	0.503	1.47 (0.96–2.24)	0.078	0.91 (0.63–1.31)	0.598
n-6/n-3 ratio	0.96 (0.72–1.29)	0.807	0.89 (0.57–1.40)	0.622	0.99 (0.70–1.40)	0.965

*Eleven children were lost to follow-up within the first 2 years of life.

†An additional 11 children were missing SPT data on one or more occasions within the first 2 years of life, and negative SPT at other occasions.

EBM, expressed breast milk; SPT, skin prick test; OR, odds ratio; CI, confidence interval.

Table 3. Unadjusted associations between fatty acid concentrations in 3-month EBM ($n = 118$) and risk of atopic and non-atopic eczema within the first 2 years of life

	Any eczema ($n = 51/112$)*		Atopic eczema ($n = 15/105$)†		Non-atopic eczema ($n = 29/105$)†	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
n-6 series						
18:2	1.23 (0.84–1.78)	0.284	1.06 (0.60–1.87)	0.846	1.30 (0.84–2.02)	0.232
18:3	0.99 (0.68–1.43)	0.941	1.06 (0.61–1.87)	0.827	1.08 (0.69–1.67)	0.744
20:3	1.04 (0.72–1.51)	0.844	1.12 (0.64–1.97)	0.684	1.04 (0.66–1.62)	0.871
20:4	1.27 (0.87–1.86)	0.221	1.12 (0.73–1.71)	0.598	0.75 (0.52–1.10)	0.147
22:4	1.15 (0.79–1.68)	0.464	1.39 (0.81–2.40)	0.229	1.07 (0.68–1.68)	0.774
22:5	1.18 (0.81–1.72)	0.381	1.07 (0.60–1.92)	0.814	1.43 (0.90–2.27)	0.128
Total n-6 series	1.23 (0.84–1.78)	0.284	1.07 (0.60–1.88)	0.827	1.31 (0.84–2.02)	0.231
n-3 series						
18:3	1.42 (0.94–2.13)	0.091	0.96 (0.48–1.91)	0.906	1.60 (1.02–2.50)	0.041
20:5	1.10 (0.76–1.59)	0.610	1.34 (0.84–2.14)	0.225	1.07 (0.69–1.68)	0.759
22:5	1.34 (0.91–1.96)	0.135	2.01 (1.17–3.45)	0.012	1.21 (0.75–1.93)	0.435
22:6	1.09 (0.75–1.57)	0.651	1.30 (0.81–2.09)	0.280	1.12 (0.72–1.73)	0.625
Total n-3 series	1.42 (0.96–2.09)	0.076	1.33 (0.76–2.34)	0.315	1.60 (1.03–2.50)	0.037
n-6/n-3 ratio	0.85 (0.58–1.24)	0.391	0.77 (0.41–1.42)	0.397	0.82 (0.51–1.29)	0.386

*Six children were lost to follow-up within the first 2 years of life.

†An additional seven children were missing SPT data on one or more occasions within the first 2 years of life, and negative SPT at other occasions. EBM, expressed breast milk; SPT, skin prick test; OR, odds ratio; CI, confidence interval.

(P for interaction = 0.158). Apart from gender, no other factors were associated with risk of infantile eczema.

Concentrations of FA in colostrum were not associated with the risk of non-atopic eczema (Table 2), but high levels of n-3 in EBM were associated with an increased risk of non-atopic eczema [mean (SD) in children with non-atopic eczema = 1.80 (0.43) compared with infants with no eczema = 1.59 (0.44)]. This effect was predominantly due to 18:3n-3 (α -linolenic acid, LNA) (Table 3). There was no evidence of an interaction with sex for this effect.

Associations between fatty acid profiles and risk of rhinitis

Higher levels of total n-6 FA in colostrum were associated with increased risk of rhinitis (Table 4) [mean (SD) in children with allergic rhinitis = 15.14 (4.89) compared with those without allergic rhinitis = 14.05 (3.12)]. This effect was primarily driven by 18:2n-6 (linoleic acid, LA) and 20:3n-6 (dihomo γ -linolenic acid, DHGLA) (Table 4). Male gender and maternal hayfever were also associated with increased risk of childhood rhinitis, while paternal smoking was associated with a reduced risk. Adjustment for these factors did not greatly alter the association between n-6 in colostrum and risk of rhinitis (OR = 1.46, 95% CI = 1.02–2.10, $P = 0.038$), nor was there evidence of an interaction between sex and FA profile. There was no evidence of any associations between total n-3 or n-6 FAs in EBM samples and risk of rhinitis; however, there was some evidence that high levels of 22:5n-6 were associated with increased risk (Table 5).

Table 4. Unadjusted associations between fatty acid concentrations in colostrum ($n = 194$) and risk of childhood asthma and rhinitis

	Asthma ($n = 51/168$)*		Rhinitis ($n = 37/168$)*	
	OR (95% CI)	P	OR (95% CI)	P
n-6 series				
18:2	0.95 (0.68–1.32)	0.752	1.53 (1.09–2.16)	0.014
18:3	1.10 (0.80–1.52)	0.545	1.23 (0.86–1.75)	0.252
20:3	0.88 (0.63–1.23)	0.450	1.44 (1.03–2.02)	0.033
20:4	0.87 (0.63–1.21)	0.413	1.10 (0.77–1.57)	0.607
22:4	0.90 (0.64–1.25)	0.522	1.22 (0.88–1.71)	0.237
22:5	0.87 (0.62–1.22)	0.415	1.24 (0.87–1.76)	0.231
Total n-6 series	0.93 (0.67–1.29)	0.662	1.59 (1.12–2.25)	0.009
n-3 series				
18:3	1.14 (0.85–1.55)	0.386	1.23 (0.89–1.71)	0.211
20:5	1.06 (0.76–1.46)	0.742	1.01 (0.70–1.47)	0.941
22:5	0.87 (0.63–1.21)	0.417	1.01 (0.70–1.45)	0.966
22:6	0.80 (0.57–1.12)	0.202	0.97 (0.67–1.40)	0.865
Total n-3 series	0.96 (0.69–1.32)	0.794	1.08 (0.75–1.55)	0.678
n-6/n-3 ratio	0.94 (0.68–1.31)	0.712	1.32 (0.96–1.81)	0.088

*26 children were lost to follow-up by age 6.

CI, confidence interval; OR, odds ratio.

Associations between fatty acid profiles and risk of asthma

There were no clear associations between FA profile in colostrum or EBM and risk of childhood asthma (Tables 4 and 5). Male sex, paternal education and use of gas heating in the home were associated with an increased risk of asthma. Adjusting for these factors did not alter the

Table 5. Unadjusted associations between fatty acid concentrations in 3-month expressed breast-milk ($n = 118$) and risk of childhood asthma and rhinitis

	Asthma ($n = 26/100$)*		Rhinitis ($n = 25/100$)*	
	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>
n-6 series				
18:2	1.08 (0.69–1.67)	0.740	1.20 (0.77–1.86)	0.425
18:3	0.91 (0.58–1.43)	0.692	1.10 (0.71–1.71)	0.656
20:3	0.91 (0.57–1.44)	0.679	1.24 (0.79–1.94)	0.341
20:4	1.06 (0.69–1.64)	0.783	1.26 (0.82–1.96)	0.296
22:4	1.10 (0.72–1.68)	0.657	1.39 (0.91–2.11)	0.125
22:5	1.08 (0.70–1.67)	0.733	1.69 (1.06–2.72)	0.029
Total n-6 series	1.07 (0.69–1.67)	0.751	1.21 (0.78–1.88)	0.398
n-3 series				
18:3	0.78 (0.45–1.34)	0.362	0.84 (0.49–1.44)	0.521
20:5	0.84 (0.50–1.41)	0.511	1.21 (0.80–1.82)	0.366
22:5	0.89 (0.56–1.43)	0.638	1.47 (0.95–2.29)	0.083
22:6	0.74 (0.41–1.36)	0.334	1.37 (0.90–2.08)	0.148
Total n-3 series	0.74 (0.45–1.20)	0.224	1.11 (0.71–1.74)	0.648
n-6/n-3 ratio	1.31 (0.88–1.96)	0.187	1.02 (0.66–1.57)	0.924

*Eighteen children were lost to follow-up by age 6.

CI, confidence interval; OR, odds ratio.

association between FA and risk of asthma. There was no evidence of an interaction between sex and FA profile on risk of asthma.

Discussion

We have previously demonstrated that high levels of n-3 FA in colostrum were associated with atopy (positive SPT) at both 6 and 24 months of age [17]. Using further data from the same prospective birth cohort, we found that high levels of 22:5n-3 (DPA) in both colostrum and EBM were associated with an increased risk of infantile atopic eczema, and higher concentrations of n-6 FA in colostrum were associated with increased risk of childhood rhinitis. There were no associations observed between FA profile and risk of childhood asthma.

Population-based cross-sectional studies have found high rates of dietary consumption of n-3 PUFA, predominantly from oily fish consumption, to be associated with reduced asthma symptoms [1, 2]. The MACS cohort was recruited on family history of atopic disease, and so we have over sampled for genetic risk factors for the development of atopy and atopic disease. In this group, high levels of n-3 FA in colostrum were associated with both increased risk of atopy and atopic eczema, which is a clear risk factor for the development of childhood asthma [22, 25]. Possible immunological mechanisms for this effect have been described in our previous paper [17]. Despite this increased risk of atopy and atopic eczema, there was

no evidence that infants exposed to high levels of n-3 in colostrum were at increased risk of childhood asthma.

Only one previous study has reported associations between breast milk FAs and childhood asthma [9], and the results reported here are consistent with the findings of that study. We found a similar reduced risk of asthma associated with high levels of docosahexaenoic acid (DHA; 22:6n-3) and low n-6:n3 ratios [9], but these associations failed to reach statistical significance in the current study. This may have been due to lack of statistical power, as we observed an effect in the same direction and of similar magnitude as Wijga et al [9]. Also unlike the previous study, we have reported asthma symptoms at age 7 rather than at 4 years.

Previous reports on the association between FA concentrations in breast milk and risk of eczema have been mixed. As with some previous research [9–11], we did not see any clear associations between FA profile and eczema as a whole in early life. We were not able to replicate the reported finding of an association between high n-6/n-3 ratio and increased risk of non-atopic eczema [8]. In fact, we found that high levels of n-3 FA were associated with an increased risk of non-atopic eczema. We also observed that 22:5n-3 (DPA) in both colostrum and EBM was associated with increased risk of atopic eczema. Interestingly, this effect seemed stronger in girls than in boys, but this observation needs to be replicated before acceptance. This highlights the complex mechanisms underlying the pathogenesis of both atopic and non-atopic eczema, which are not yet clearly understood, and the need to consider gender-specific effects.

This is the first study to report the associations between FA profile in breast milk and risk of childhood rhinitis. Our observations suggest that high levels of n-6 in colostrum may be an important risk factor for the development of rhinitis. Colostrum is important as the very first source of neonatal dietary exposure and contains a wide range of immunologically active components. Nevertheless, the duration of colostrum feeding generally only lasts up to 3–4 days, so it is difficult to postulate that this brief exposure is a long-acting modifier of risk for atopic disease.

Maternal supplementation with fish oil during pregnancy increases levels of n-3 FA in 3-day breast milk concentrations, particularly 20:5n-3 (eicosapentaenoic acid, EPA), 22:5n-3 (DPA) and 22:6n-3 (DHA) [26], and modifies neonatal immune response [27]. Moreover, frequent maternal intake of fish has recently been associated with reduced risk of childhood asthma, eczema and rhinitis [3–5]. Two of these studies [3, 4] have used prospective collection of maternal diet during pregnancy, thus avoiding possible recall bias. It is possible that FA exposure in the intrauterine environment critically influences prenatal immunological development, and subsequent expression of atopy and allergic disease. Colostrum FA levels may simply be a surrogate measure of fetal exposure to

maternal dietary FAs. Further work is required to test this hypothesis.

To date, only one study has modified the maternal intake of FAs during pregnancy in an attempt to alter the neonatal immune response [26, 27]. This study recruited 98 highly atopic mothers and randomized them to receive either daily fish oil (high in long chain n-3) or olive oil capsules. Fish oil supplementation resulted in reduced neonatal IL-10 production, and weak signals of reduced risk of a number of markers of atopic disease, including reduced risk of positive SPT for egg at 12 months. Interestingly, there was also a suggestion of increased risk of eczema in infants whose mothers were supplemented with fish oil. Unfortunately, this study was not powered to detect clinical effects of fish oil supplementation, and follow-up data are not available on asthma and rhinitis in childhood.

The Childhood Asthma Prevention Study (CAPS) found that increasing infant dietary consumption of n-3 FA did not alter the risk of atopy or allergic disease outcomes up to 5 years of age [28–30] nor did plasma FA levels [31]. Importantly, CAPS did not attempt to alter the maternal diet during pregnancy. It is possible that dietary exposure in infancy missed the window of opportunity, whereas maternal exposure during pregnancy may have had a greater impact on allergic disease outcomes.

Strengths of the current study include prospective assessment of FA profiles in both colostrum and 3-month EBM and assessment of asthma and rhinitis at ages 6 and 7 when the phenotypes are clearer than in earlier childhood. We have attempted to determine if there was non-linearity in the associations between FA composition of breast milk and risk of allergic disease outcomes, but we were unable to detect any such effects. Compared with previous literature, FA concentrations were assessed in a substantial number of mothers, but this study was underpowered to detect some potentially important associations, including interactions with sex, which have been implicated in the development of atopic diseases [32].

There are some limitations with this study. The results reported, like all previous studies in this area, are based on multiple hypothesis tests, and must recognize the increased risk of type-1 errors. There remains a real possibility that at least some of the observed associations in this study are spurious. Replication of these findings will be necessary.

Finally, the definitions used in this study do limit the conclusions that can be made. The definition of childhood asthma and allergic rhinitis are a limitation of this study. We do not yet have objective assessments of lung function, atopy and asthma phenotype (e.g. eosinophilic vs. non-eosinophilic) on this cohort in late childhood. It may be that the protective effect of n-3 FA exposure is limited to only one of the asthma phenotypes. It seems likely that any misclassification of asthma and allergic rhinitis may have been non-differential, so may have resulted in biasing the associations towards the null. The definitions of

infantile eczema used in this cohort have not been validated, but as we have previously noted, there are currently no validated definitions of eczema available for children under the age of 2 years [21]. These results are based on infants at high risk of allergic disease due to a family history, so they cannot be directly generalized to the whole population. Also, only a proportion of mothers in this cohort (224/620) provided a breast milk sample at either time point, allowing for possible selection bias. However, this seems unlikely, as we were unable to detect any differences between the infants who contributed to this analysis and those who were excluded.

Conclusion

Further work in large cohorts and clinical trials is required to further assess potential associations between FA profiles during the intra-uterine period and early life dietary exposures and risk of atopic disease. If replicated, the finding of an association between FA in colostrum and risk of rhinitis observed in these data could warrant interventions to modify this dietary constituent and reduce the burden of these conditions. Despite our earlier finding that n-3 FA in colostrum was associated with increased risk of atopy in infancy, at this time, there is no reason to question dietary guidelines that recommend pregnant women to consume one to two portions of sea fish per week, including oily fish [33], although care needs to be taken to avoid excessive mercury intake.

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