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Maternal breast milk long-chain n-3 fatty acids are associated with increased risk of atopy in breastfed infants

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Summary

Background Australia has one of the highest prevalence rates internationally of allergic conditions, such as asthma and eczema. Atopy is one hallmark for the development of allergic disease and predisposes to allergic inflammation in the target organs. ω-3 (n-3) fatty acids (FAs) are thought to act as precursors to the formation of less active inflammatory mediators, with the potential to reduce inflammation.

Objective To investigate whether increased n-3 FA levels in maternal breast milk are associated with a lower risk of developing atopy in infancy.

Methods Subjects were part of the prospective Melbourne atopy cohort study, which involved 620 children born into families where at least one first-degree relative had an atopic disease. Some 224 women (mean age 31.4 ± 4.2 (SD) years, with 73.2% ($n = 164$) having self-reported atopy) provided either a colostrum ($n = 194$) or 3-month expressed breast milk (EBM) sample ($n = 118$). Maternal colostrum and 3-month EBM samples were analysed for FA content by gas chromatography. Skin prick tests (SPTs) to six common allergens were performed on infants at 6, 12 and 24 months of age and on mothers who agreed at study entry.

Results For infants sensitized to foods at 6 months ($n = 29$), the total n-3 FA level in the colostrum was significantly higher ($P = 0.004$) as were levels of individual long-chain n-3 FAs, docosoapentaenoic acid (DPA, C22:5, $P = 0.001$) and docosahexaenoic acid (DHA, C22:6, $P = 0.002$) than in non-sensitized infants. Infants with aero-allergen sensitization at 24 months ($n = 30$) had higher levels of the n-3 FA, DPA ($P = 0.002$) and DHA ($P = 0.007$), and similarly higher total n-3 FA ($P = 0.009$) in maternal colostrum than those infants who were not sensitized.

Conclusion Higher n-3 FA levels in the colostrum do not appear to confer protection against, but may be a risk factor for, the eventual development of atopy in high-risk breastfed infants.

Keywords atopy, breast milk, epidemiology, infants, polyunsaturated fatty acids
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Introduction

Allergic conditions such as asthma, rhinoconjunctivitis and eczema are common disorders worldwide, with Australia having one of the highest prevalences internationally [1, 2]. The prevalence of recent wheeze in the past 12 months has been found to be nearly 30% in Australian school aged children, with prevalences of current eczema and allergic rhinitis up to 11% and 20%, respectively, in this age group [3].

Atopy is the predisposition to produce IgE in response to common environmental allergen exposure. It is one hallmark for the development of the above allergic diseases and predisposes to allergic inflammation in the target organs. ω-3 (n-3) fatty acids (FAs, derived primarily from oily fish) act as precursors to the formation of less active inflammatory mediators than derivatives of the ω-6 (n-6) FAs, with the potential to reduce inflammation [4]. Previous cross-sectional studies in Australia have shown that regular fish consumption [5] and particularly consumption of oily fish [6] were associated with a reduced risk of children having asthma. However, intervention trials involving the dietary manipulation and/or supplementation of n-3 FAs have not had any favourable overall effect on asthma outcomes [7].

Since early life influences are critical in immunological development, particularly allergic responses to environmental allergens, it is possible that breast milk may confer a protective effect on the development of allergic diseases due to the n-3 FA content of the milk [8]. It is known that many factors, including parity, duration of lactation, diurnal rhythm, gestation, infections and maternal diet are associated with changes in the total lipid content of human milk [9]. Furthermore, there is evidence that the n-3 FA levels in breast

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milk can vary, depending on maternal diet. In a randomized placebo-controlled trial, supplementation with cod liver oil (three different dose levels) increased breast milk FA levels in a dose-dependent manner [10]. Therefore, this study sought to investigate the hypothesis that increased n-3 FA levels in breast milk were associated with a lower risk of developing atopy in breastfed infants.

Materials and methods

Design and subject recruitment

Subjects were part of the Melbourne atopy cohort study (MACS), which is a large prospective cohort study of the development of atopy [11]. This study involved 620 children born into families where at least one first-degree relative had atopic disease. The mothers of the children were prospectively recruited from women attending the antenatal clinic at the Mercy Maternity Hospital, Melbourne, Australia, from 1990 to 1994, if the mother answered 'yes' to the question – 'Do you, the father of your unborn child, or any other of your children have or have had eczema, asthma, hayfever, or severe food allergy?'.

Of the total number of women enrolled in the MACS, some 224 women provided either a colostrum ($n = 194$) or 3-month expressed breast milk (EBM) sample ($n = 118$). Eighty-eight women provided both colostrum and 3-month EBM samples. Their mean age was 31.4 ± 4.2 (SD) years, with 73.2% ($n = 164$) having self-reported atopy with either eczema, asthma or hayfever. Of these women, 41.5% ($n = 68$) reported one atopic disease, 36.6% ($n = 60$) had two atopic diseases, while the remaining 22% ($n = 36$) had all three atopic conditions. The majority of the women were born in Australia/New Zealand (84.8%), with the remaining women born in England/North America (8.9%), Europe (2.2%), Asia (1.8%) or elsewhere (2.2%). Ninety-three percent of the women were married, with a mean of 13.5 ± 1.8 years of formal education. Only 4% of the women currently smoked, while 17% reported smoking more than 6 months previously.

Of the infants born to these mothers, 55.5% ($n = 124$) were male and 44.5% ($n = 100$) were female.

This information was obtained from an interviewer-administered ante-natal baseline questionnaire. The demographic characteristics of the mothers described above were not significantly different from the total MACS population. All the infants were exclusively breastfed for up to 4 weeks.

On study entry, 84% of the women who provided a colostrum sample ($n = 162$ of 194) underwent skin prick testing (SPT), while 82% of women who provided 3-month EBM sample ($n = 96$ of 118) underwent SPT. These women formed the study population for the assessment of maternal breast milk FA composition by maternal SPT-defined atopic status. Of the women who provided a colostrum sample for which SPT data were available, 107 of the 162 women (66%) were atopic as defined by SPT. Of the women who provided 3-month EBM sample, who also underwent SPT testing at baseline, 60 of the 96 women (63%) were atopic.

The study was approved by the Ethics Committee of the Mercy Hospital for Women. All participants provided written informed consent.

Outcome measures

Skin prick tests SPTs to six common allergens were performed on infants at 6, 12 and 24 months of age by a specially trained allergy nurse according to a standard technique [12]. The following allergen extracts were used: cow's milk, egg, peanut, *Dermatophagoides pteronyssinus* (house dust mite), *Lolium perenne* (rye grass pollen) and cat extracts (Bayer Corporation Sydney, Australia). The mean diameter of the weal, after subtraction of the saline control solution, was compared with that induced by 1% histamine acid phosphate. A positive skin test was defined as an SPT > 2 to either food or aero-allergens according to the following grading system [13]:

> 4 reaction = > twice the area of the histamine standard;
4 reaction = from the area of the histamine standard up to twice the area of that standard;
3 reaction = from one half of the area of the histamine standard up to the area of the histamine standard;
2 reaction = from one quarter to one half of the area of histamine standards;
1 reaction = up to one quarter of the area of histamine standard, and greater than control solutions. In all cases, these exceeded the control weal by 2 mm or more.

For the current study, maternal atopy was defined as SPT > 2 to the above allergen panel.

FA profile of maternal breast milk Maternal colostrum (collected 2–4 days after delivery) and 3-month EBM samples were frozen initially at $-20\,^{\circ}C$, then transferred to a $-70\,^{\circ}C$ freezer for subsequent storage. Colostrum samples were hand expressed usually in the early morning coinciding with the first infant feed of the day. When insufficient volume was collected, sampling was conducted during additional feeds. Three-month EBM samples were similarly collected using a breast pump, but exact times were not recorded. Samples (2 mL) were aliquoted from the original stored specimens prior to transportation on dry ice to Flinders Medical Centre, Adelaide for subsequent analysis of FA profile in the year 2000. The samples were thawed in warm water to 38 °C and total lipids extracted from breast milk with chloroform : -methanol and methylated in 1% H_2SO_4 in methanol for 2 h at 70 °C [14]. When cooled, the resulting methyl esters were then extracted into *n*-heptane and transferred to vials containing anhydrous Na_2SO_4 as the dehydrating agent. FA methyl esters were separated and quantified using a Hewlett-Packard 6890 gas chromatograph equipped with a 50 m capillary column (0.33 mm internal diameter) coated with BPX70 (0.25 μm film thickness SGE Pty Ltd, Victoria, Australia). The injector temperature was set at 250 °C and the detector (flame ionization) temperature at 300 °C. The initial oven temperature was 140 °C and was programmed to rise to 220 °C at 5 °C/min. Helium was used as the carrier gas at a velocity of 35 cm/s. FA methyl esters C8–C24 were identified based on the retention time to authentic lipid standards obtained form Nuchek Prep Inc. (Elysian, MN, USA). FA results were expressed as weight percentage (wt%).

Statistical analysis

Data were entered, verified and analyses conducted using the SPSS^{TM} computer software package (version 10, Chicago, IL, USA). Data were expressed as mean and SD, unless otherwise indicated. As the levels of individual FAs were normally distributed, Student's *t*-tests were used to compare the FA profiles between atopic and non-atopic mothers in both colostrum and 3-month EBM samples. Comparison of FA profiles in food and aero-allergen sensitized infants vs. non-sensitized infants at 6, 12 and 24 months of age were also compared by two-tailed unpaired Student's *t*-tests. Differences were confirmed by generalized linear models adjusting for maternal atopic status. Owing to multiple comparisons being made, a *P*-value <0.01 was considered statistically significant.

Power and sample size

There were over 200 breast milk specimens available for analysis. Based on the previous findings of Yu et al. [15] (mean n-3 FA level of atopic mothers = 0.64% with mean n-3 FA level of non-atopic mothers = 0.53%, SD = 0.014%), our study had a greater than 90% power to detect a true difference in n-3 FA levels between these two groups.

Results

Breast milk FA profile by SPT-defined maternal atopic status

The levels of the major saturated FA, palmitic acid (C16:0) and major monounsaturated FA, oleic acid (C18:1n-9) did not vary in colostrum or 3-month EBM samples in atopic vs. non-atopic mothers (Table 1). Similarly, the levels of the essential FAs linoleic acid (LA) and α-linolenic acid (LNA) did not vary according to maternal atopic status, nor did the levels of individual long-chain or total n-3 and n-6 FAs. This was subsequently reflected in a similar n-6 : n-3 FA ratio in atopic vs. non-atopic mothers for both colostrum and 3-month EBM samples. Thus, breast milk FA profile was found to be independent of maternal atopic status.

Table 1. Fatty acid composition of colostrum and 3-month EBM according to maternal atopic status*

	Colostrum ($n=162$)		3-month EBM ($n=96$)	
Type of fatty acids	Non-atopic ($n=55$)	Atopic ($n=107$)	Non-atopic ($n=36$)	Atopic ($n=60$)
Saturated				
12:0	2.23 ± 0.94	2.32 ± 0.86	2.81 ± 0.92	2.96 ± 1.30
14:0	4.49 ± 1.37	4.77 ± 1.37	4.68 ± 1.50	4.70 ± 1.66
16:0	23.1 ± 2.26	22.9 ± 1.96	21.1 ± 3.10	21.2 ± 2.27
18:0	8.25 ± 1.09	8.13 ± 1.14	8.61 ± 1.69	8.52 ± 1.60
Monounsaturated				
18:1	34.8 ± 3.38	34.4 ± 2.84	34.7 ± 3.32	33.9 ± 2.50
n-6 polyunsaturated				
18:2 LA	11.8 ± 3.64	12.2 ± 3.30	15.2 ± 5.23	15.8 ± 5.15
18:3 GLA	0.13 ± 0.04	0.12 ± 0.04	0.16 ± 0.03	0.16 ± 0.05
20:2	0.64 ± 0.25	0.66 ± 0.19	0.28 ± 0.07	0.29 ± 0.09
20:3 DHGLA	0.60 ± 0.18	0.57 ± 0.16	0.39 ± 0.09	0.39 ± 0.09
20:4 AA	0.63 ± 0.16	0.60 ± 0.13	0.38 ± 0.11	0.40 ± 0.09
22:2	0.13 ± 0.05	0.14 ± 0.04	0.04 ± 0.01	0.04 ± 0.02
22:4	0.26 ± 0.14	0.25 ± 0.09	0.08 ± 0.03	0.09 ± 0.02
22:5	0.09 ± 0.04	0.09 ± 0.03	0.03 ± 0.02	0.03 ± 0.01
n-3 polyunsaturated				
18:3 LNA	0.69 ± 0.26	0.70 ± 0.20	0.84 ± 0.31	0.84 ± 0.28
20:3	0.10 ± 0.03	0.10 ± 0.03	0.04 ± 0.02	0.04 ± 0.02
20:5 EPA	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.04	0.06 ± 0.04
22:5 DPA	0.25 ± 0.08	0.24 ± 0.06	0.19 ± 0.06	0.19 ± 0.05
22:6 DHA	0.45 ± 0.15	0.46 ± 0.18	0.26 ± 0.23	0.23 ± 0.14
Total				
SFA	39.6 ± 4.5	39.7 ± 4.1	38.7 ± 5.5	38.9 ± 5.1
MUFA	41.7 ± 3.8	41.3 ± 3.4	40.3 ± 3.7	39.5 ± 3.0
PUFA	18.7 ± 4.3	19.1 ± 3.7	21.0 ± 6.3	21.6 ± 5.9
n-6	14.2 ± 3.9	14.7 ± 3.4	16.5 ± 5.4	17.2 ± 5.3
n-3	1.87 ± 0.35	1.86 ± 0.35	1.68 ± 0.45	1.63 ± 0.40
n-6 : n-3	7.95 ± 3.4	8.18 ± 2.7	10.1 ± 3.1	11.3 ± 4.9

Data are mean ± SD, wt%.
*No *P*-value <0.01, maternal atopy defined as SPT >2. EBM, expressed breast milk; LA, linoleic acid; GLA, γ-linolenic acid; DHGLA, dihomo γ-linolenic acid; AA, arachidonic acid; LNA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SD, standard deviation.

Maternal FA profile and infant atopy

Further analyses were undertaken to investigate whether differences in maternal breast milk FA profiles were associated with the subsequent development of sensitization to food and inhalant allergens in infants at 6, 12 and 24 months of age. At 6 months of age, 16% ($n = 29$, from the available sample population of $n = 177$) of the infants whose mothers provided a colostrum sample showed a positive skin reaction to at least one of the three food allergens tested (cow's milk, egg, peanut). For these food-sensitized infants, the total n-3 FA level in colostrum was significantly *higher* ($P = 0.004$) as were levels of individual long-chain n-3 FAs; docosoapentaenoic acid (DPA, C22:5, $P = 0.001$) and docosahexaenoic acid (DHA, C22:6, $P = 0.002$) than in non-sensitized infants (Table 2). FA composition of total lipids in colostrum was not significantly different between the groups defined by aero-allergen sensitization, although the number of sensitized infants was small at this age ($n = 9$, 5% of the sample population) (Table 2).

No significant differences in FA composition of maternal colostrum were seen at 12 months for either food ($n = 36$ of 139 infants) or aero-allergen-sensitized infants ($n = 20$ of 155 infants) (data not shown, but available from authors if requested). SPT data were unavailable for 19 infants.

At 24 months, food-sensitized infants (11%, $n = 16$ of available sample population $n = 146$) were more likely to have *higher* levels of DPA in their maternal colostrum, as compared with non-sensitized infants ($P = 0.001$) (Table 3). Furthermore, levels of the n-6 FAs, C22:4n-6 and C22:5n-6 were also higher in food-sensitized infants ($P = 0.002$ and 0.008, respectively) (Table 3). The number of infants who were aero-allergen sensitized increased by 24 months to 21% ($n = 30$) of the available population. Infants with aero-allergen sensitization had *higher* levels of the n-3 FA, DPA ($P = 0.002$) and DHA ($P = 0.007$), and similarly higher total n-3 FA ($P = 0.009$) in their maternal colostrum than those infants who were not sensitized to the allergens tested.

When comparisons were made for the 3-month EBM samples, the only significant finding was an increased level of C22:5n-6 in aero-allergen-sensitized vs. non-sensitized infants at 24 months ($0.206 \pm 0.06\%$ vs. $0.183 \pm 0.04\%$, $P = 0.007$). No other significant differences in FA composition of maternal 3-month EBM were found between food- or aero-allergen-sensitized infants at 6, 12 or 24 months of age (data not shown, but available from authors if requested).

The infants were fully breastfed for a median (interquartile range) of 36 (12–52) weeks. There was no relationship between the duration of breastfeeding and skin test results at 6, 12 or 24 months. Infants were introduced to a median (IQR) of three (1–4) solid foods by the age of 24 months. Solid foods were introduced before 4 months in 19.7% of infants. There were no significant relationships between either the number of solids introduced in the first 24 months or the early introduction of solids and skin test results at any age tested.

Discussion

This cohort study found higher levels of the long-chain n-3 FAs; DPA and DHA and subsequently total n-3 FA levels, in maternal colostrum of infants who were food-allergen sensitized at 6 months and aero-allergen sensitized at 24 months of age. These results appear contrary to what might have been expected from the current knowledge of the role of essential FAs in the formation of inflammatory mediators, and in allergic inflammation. However, they raise intriguing issues about the possible role of dietary n-3 FA in early

Table 2. Long-chain fatty acid profile of colostrum according to infant atopic sensitization to both food and aero-allergens at 6 months*

	Food allergen sensitive (SPT > 2)			Aeroallergen sensitive (SPT > 2)		
Type of fatty acid	Atopic ($n = 29$)	Non-atopic ($n = 148$)	Adjusted *P*-value	Atopic ($n = 9$)	Non-atopic ($n = 168$)	Adjusted *P*-value
n-6 series polyunsaturated						
18:2 LA	11.3 ± 3.06	12.3 ± 3.67	0.24	13.0 ± 3.99	12.1 ± 3.57	0.72
18:3 GLA	0.11 ± 0.04	0.12 ± 0.04	0.08	0.15 ± 0.05	0.12 ± 0.04	0.04
20:3 DHGLA	0.56 ± 0.17	0.58 ± 0.17	0.66	0.63 ± 0.21	0.57 ± 0.16	0.16
20:4 AA	0.65 ± 0.14	0.60 ± 0.13	0.016	0.67 ± 0.14	0.60 ± 0.13	0.044
22:4	0.27 ± 0.10	0.24 ± 0.11	0.098	0.25 ± 0.09	0.25 ± 0.11	0.53
22:5	0.10 ± 0.03	0.08 ± 0.03	0.02	0.09 ± 0.02	0.09 ± 0.03	0.27
n-3 series polyunsaturated						
18:3 LNA	0.71 ± 0.22	0.69 ± 0.23	0.81	0.73 ± 0.31	0.70 ± 0.23	0.96
20:5 EPA	0.07 ± 0.02	0.05 ± 0.02	0.027	0.07 ± 0.02	0.06 ± 0.02	0.31
22:5 DPA	0.29 ± 0.07	0.24 ± 0.06	0.001*	0.27 ± 0.04	0.24 ± 0.07	0.37
22:6 DHA	0.55 ± 0.19	0.44 ± 0.16	0.002*	0.50 ± 0.14	0.45 ± 0.17	0.56
Total						
n-6	13.8 ± 3.2	14.7 ± 3.9	0.33	15.6 ± 4.0	14.5 ± 3.8	0.59
n-3	2.05 ± 0.34	1.82 ± 0.34	0.004*	1.96 ± 0.37	1.86 ± 0.35	0.70
n-6 : n-3	6.84 ± 1.8	8.37 ± 3.1	0.027	7.95 ± 1.5	8.13 ± 3.0	0.95

Data are mean ± SD, wt%.
*$P < 0.01$ was considered statistically significant, *P*-values have been adjusted for maternal atopic status. SPT data unavailable for $n = 17$ infants. SPT, skin prick test; LA, linoleic acid; GLA, γ-linolenic acid; DHGLA, dihomo γ-linolenic acid; AA, arachidonic acid; LNA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; SD, standard deviation.

Table 3. Long-chain fatty acid profile of colostrum according to infant atopic sensitization to both food and aero-allergens at 24 months*

	Food allergen sensitive (SPT > 2)			Aeroallergen sensitive (SPT > 2)		
Type of fatty acid	Atopic ($n = 16$)	Non-atopic ($n = 130$)	Adjusted *P*-value	Atopic ($n = 30$)	Non-atopic ($n = 116$)	Adjusted *P*-value
n-6 series polyunsaturated						
18:2 LA	13.1 ± 3.97	11.9 ± 3.55	0.36	12.8 ± 4.54	11.8 ± 3.31	0.35
18:3 GLA	0.11 ± 0.02	0.13 ± 0.04	0.23	0.12 ± 0.04	0.13 ± 0.04	0.32
20:3 DHGLA	0.62 ± 0.14	0.58 ± 0.18	0.14	0.59 ± 0.15	0.58 ± 0.18	0.65
20:4 AA	0.66 ± 0.19	0.60 ± 0.13	0.03	0.64 ± 0.16	0.60 ± 0.13	0.034
22:4	0.33 ± 0.14	0.24 ± 0.10	0.002	0.29 ± 0.11	0.24 ± 0.11	0.032
22:5	0.11 ± 0.05	0.09 ± 0.03	0.008	0.10 ± 0.03	0.08 ± 0.03	0.026
n-3 series polyunsaturated						
18:3 LNA	0.72 ± 0.23	0.67 ± 0.19	0.75	0.72 ± 0.22	0.66 ± 0.19	0.40
20:5 EPA	0.06 ± 0.03	0.06 ± 0.02	0.59	0.06 ± 0.02	0.05 ± 0.02	0.27
22:5 DPA	0.31 ± 0.09	0.23 ± 0.06	0.001*	0.28 ± 0.08	0.23 ± 0.06	0.002*
22:6 DHA	0.52 ± 0.20	0.44 ± 0.15	0.08	0.52 ± 0.19	0.43 ± 0.14	0.007*
Total						
n-6	15.8 ± 3.9	14.3 ± 3.7	0.20	15.4 ± 4.6	14.2 ± 3.5	0.25
n-3	2.03 ± 0.37	1.80 ± 0.32	0.04	1.99 ± 0.38	1.78 ± 0.31	0.009*
n-6 : n-3	8.10 ± 3.1	8.24 ± 3.0	0.99	8.11 ± 3.9	8.25 ± 2.7	0.82

Data are mean ± SD, wt%.
*$P < 0.01$ was considered statistically significant, *P*-values have been adjusted for maternal atopic status. SPT data unavailable for $n = 48$ infants. SPT, skin prick test; LA, linoleic acid; GLA, γ-linolenic acid; DHGLA, dihomo γ-linolenic acid; AA, arachidonic acid; LNA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; SD, standard deviation.

immunological development and the genesis of atopy in high-risk infants.

The FA profile in colostrum in the present study is consistent with that reported previously in Australian women. Gibson and Kneebone [16] have previously shown that milk obtained at days 3–5 of lactation, from 120 healthy women volunteers on *ad libitum* diets, contained 1.83 wt% total n-3. Yu et al. [15] also examined the change in FA profile over the lactation period. The relative levels of LA and LNA increased up to 3 months after delivery, while the levels of their long-chain metabolites were higher in colostrum than mature milk. The distribution and relative proportioning of FA between colostrum and 3-month EBM are consistent with the current study.

In this study, no differences were found in the FA profile between atopic and non-atopic mothers for either colostrum or 3-month EBM. Other studies suggest that maternal breast milk FA profile may be influenced by maternal atopic status, as well as the sampling time-point during lactation. Longitudinal studies in recent years [15, 17] have reported lower total n-3 FA levels in atopic compared with non-atopic mothers in milk samples collected after 1 month of lactation. However, in agreement with our data, no differences were found in either colostrum or 3-month EBM. Thus, any effect of maternal atopy on breast milk FA levels appears to be transient, evident at 1 month but not with colostrum or during later periods of lactation.

The major findings of the current study were significantly *increased* levels of DPA and DHA and hence total long-chain n-3 FA in colostrum of food-sensitized infants at 6 months and aero-allergen-sensitized infants at 24 months. This pattern of sensitization is anticipated as infants with skin sensitization are more likely to be sensitized to food allergens first, due to the exposure to these allergens in the first year of life, followed by aero-allergen sensitization peaking at 2 years [18]. There was moderate longitudinal consistency in skin test responses by our cohort of infants (data not shown). There was also an increase in the long-chain n-6 FAs (C22:4 and C22:5) at 24 months only in food-sensitized infants, but not in the total n-6 FA level.

In the largest previous study of this nature, Duchén et al. [17] investigated the n-3 FA composition in maternal milk in relation to the development of allergic disease in children during the first 18 months of life. They studied 120 infants born to mothers, of whom approximately half were allergic on the basis of clinical symptoms. In contrast to our study, no difference in the PUFA profile of maternal colostrum was seen for atopic compared with non-atopic infants at 18 months. Furthermore, they found lower levels of long-chain n-3 FA in mature breast milk of mothers of atopic as compared with non-atopic infants. There are a number of differences between the studies, which may explain the differing results. We are the first, to our knowledge, to investigate this issue in Australia, which has a very high prevalence of atopy. Indeed, we selected a high-risk population group, in which inclusion at study entry was based on a family history of allergic disease. In contrast, Duchén et al. [17] investigated a Scandinavian population, which was selected to include roughly equal numbers of atopic and non-atopic women. Secondly, they investigated allergen sensitization only up to 18 months whereas our data include sensitization up to 24 months of age. Thirdly, their SPT allergen panels included three food allergens (hen's egg, milk, peanut extract) with only one aero-allergen (cat extract) and were analysed together. In contrast, our panel included equal numbers of food (cow's milk, egg and peanut) and aero-allergens (house dust mite, rye grass pollen and cat extract) with sensitization to each group analysed separately. Finally, atopy in the Duchén study was defined more restrictively by symptoms of allergic disease with a positive SPT, whereas

classification in the current study was based solely on SPT sensitization. These factors, together with the internally consistent nature of our data, suggest differing effects of maternal breast milk FA levels on atopy development in this high-risk vs. a lower-risk population.

Our study has some limitations. Only 31% of women enrolled in the MACS provided a colostrum specimen and 19% provided EBM at 3 months. However, the women included in this analysis were broadly representative of the cohort. Although the numbers in some subgroups were small, there was sufficient statistical power to detect clinically important differences in breast milk FA composition. Artefact due to the degradation of FAs is unlikely as the recent literature confirms the stability of lipids in cold storage over a long duration of time [19]. It is also unlikely that our findings have arisen by chance alone, as appropriate adjustments were made for multiple comparisons.

These findings are significant in the light of a recently published longitudinal cohort study investigating the risk of atopy in breastfed children [20]. This study found that breastfed children were twice as likely as children who were not breastfed to be SPT positive for any allergen at an age of 13 years, or to have asthma at an age of 9 years. Furthermore, the duration of breastfeeding needed to show an effect appeared to be short (as little as 4 weeks). The postulated mechanisms by which breastfeeding increased the risk of atopy include maternal transmission of immunological responses, the effects on feeding patterns on gut flora and factors related to the hygiene hypothesis [20]. The results of our study suggest higher n-3 FA levels in breast milk as one additional factor potentially responsible for the increased risk of atopy for breastfed infants.

Atopy is characterized by the production of IgE against common environmental allergens. The formation of IgE by B lymphocytes is regulated by a differential cytokine milieu according to the Th1/Th2 hypothesis. The influence of n-3 FAs on this paradigm and the subsequent development or modification of atopic disease is controversial. One postulated mechanism favouring protection against atopy by n-3 FAs is through decreased synthesis of pro-inflammatory lipid mediators such as prostaglandin E_2 (PGE_2). PGE_2 enhances the synthesis of Th2-like cytokines and IgE antibodies, and inhibits the differentiation of Th1-like lymphocytes. Hence, reduced synthesis of PGE_2 by dietary n-3 FAs may influence the polarization of the naïve Th cell towards a non-atopic Th1 immune response [4]. However, experimental data also suggest that dietary n-3 FAs may have a converse effect on Th cell function. In a murine model, dietary n-3 FAs down-regulated Th1 proliferative capacity directly by a reduction in IL-2 production, and indirectly through an enhancement of counter-regulatory, IL-4-driven Th2 cells [21]. Hence, an increased risk of atopy through exposure to elevated n-3 FA levels in maternal breast milk during the critical neonatal period of immunological development is biologically plausible. In this population of high-risk infants, such early life influences may be crucial to subsequent development of atopy and allergic disease. The differences between the results of this study and the others discussed above warrant further investigation and research in different populations. The implications for clinical practice and public health nutrition await further clarification.

Conflict of interest

None of the investigators have any conflict of interest with this manuscript.

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