

Fatty acids in breast milk of allergic and non-allergic mothers: The PIAMA birth cohort study

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Fatty acid composition was studied in breast milk of allergic and non-allergic mothers, focusing in particular on concentrations of the n-6 and n-3 long-chain polyunsaturates (LCP) in relation to maternal allergy. Milk samples were obtained from 168 mothers with asthma or inhalant allergies and 107 mothers without asthma and inhalant allergies, between 2 and 35 weeks after delivery. Mean values of fatty acid concentration (weight% of total fatty acids) were estimated for individual fatty acids, for groups of fatty acids and for the metabolic index (the ratio between the sum of n-6 polyunsaturates and linoleic acid). For the most relevant fatty acids, the association with maternal allergy was subsequently analyzed in more detail using multiple regression analysis. The metabolic index in breast milk was significantly lower in the allergic than in the non-allergic mothers, but no significant differences were observed in the concentrations of any of the n-6 fatty acids. Also concentrations of the n-3 fatty acids and nearly all other fatty acids were similar in allergic and non-allergic mothers' breast milk. No differences in fatty acid concentrations were observed between mothers with asthma and mothers with single or with multiple inhalant allergies. Our data suggest that differences in fatty acid composition between breast milk of allergic and non-allergic mothers are small and are unlikely to be responsible for the differential effects of breastfeeding by allergic and non-allergic mothers that have been observed in some studies.

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Allergic mothers are commonly advised to breastfeed their babies in order to reduce the infants' risk of atopic disease. Literature reviews, however, show that the allergy preventing effect of breastfeeding has not been established beyond doubt (1, 2). Two recent papers by Wright et al. (3, 4) have provided evidence that breastfeeding by atopic asthmatic mothers may increase rather than reduce the risk of atopy and asthma in their children. This has renewed the interest in the question whether breast milk from allergic mothers may be different from breast milk from non-allergic mothers. If this really is the case, it might partly explain the observation that allergy in the mother more strongly increases the risk of allergy in the child than allergy in the father does (5).

It is unknown which of the many different components of breast milk might be responsible for the hypothesized differential effects of milk of allergic and of non-allergic mothers. Leukocytes, cytokines, immunoglobulins and fatty acids have all been suggested as potential candidates (6). In this study, the fatty acid composition has been investigated in milk samples obtained from allergic and non-allergic mothers. We have focused on assessing the evidence for the hypothesis (7, 8) of a disturbed metabolism of essential fatty acids in atopic persons.

In the n-6 group of fatty acids linoleic acid (LA, 18:2n-6) derived from food, is successively converted into gamma-linolenic acid (GLA, 18:3n-6), dihomo-gamma-linolenic acid (DGLA, 20:3n-6) and arachidonic acid (AA, 20:4n-6) (see

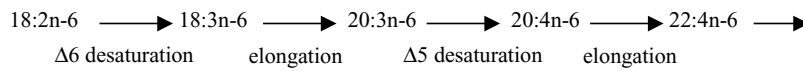


Fig. 1. Metabolic pathway of linoleic acid (18:2n-6).

Fig. 1). The precursors of prostaglandins and leukotrienes, 20:3n-6 and 20:4n-6, may be involved in the early maturation and differentiation of T-lymphocytes.

A number of studies have shown abnormal n-6 fatty acid levels in patients with atopic disorders: decreased concentrations of n-6 long chain polyunsaturates (LCP) in serum, plasma, red blood cells and mononuclear cells, and in adipose tissue, sometimes also with slightly increased concentrations of 18:2n-6 (7, 9–11). This has been interpreted to indicate that in atopic patients the conversion of 18:2n-6 to its LCP is reduced which might be caused by an impaired activity of the enzyme delta 6 desaturase that converts 18:2n-6 to 18:3n-6 (7, 8).

Breast milk, as opposed to infant formulas, contains certain amounts of long chain n-6 and n-3 fatty acids. The hypothesis that an abnormal n-6 fatty acid metabolism might play a role in the development of atopy has prompted a number of investigators to look for differences in the fatty acid composition of breast milk of atopic and non-atopic mothers (12–16). Most of these studies were small, comparing subgroups of between 15 and 30 mothers and the evidence obtained on breast milk fatty acid composition in relation to maternal atopy is still inconclusive. In the PIAMA (Prevention and Incidence of Asthma and Mite Allergy) study, we had the opportunity to collect milk samples from a relatively large group of mothers ($n = 275$). We were able to compare mothers with asthma and with single or multiple inhalant allergies and to assess the role of other factors besides maternal allergy that might influence breast milk fatty acid composition.

Methods

Design of the study

The study on fatty acids in breast milk in relation to maternal allergy is part of the PIAMA research project. This is a prospective birth cohort study on incidence, risk factors and prevention of asthma and inhalant allergy. At baseline, the PIAMA study population consisted of the children of 1327 allergic mothers and of 2819 non-allergic mothers who were recruited from the general population during pregnancy. At the time of recruitment, the pregnant mothers

filled in a validated short screening questionnaire on allergy. Mothers reporting at least one of the following: (a history of) asthma, current hay fever, current allergy for pets, or current allergy for house dust or house dust mite were defined as 'allergic' and mothers reporting that they had none of these complaints were defined as 'non-allergic'. A validation study of the screening questionnaire (17) has shown that its positive predictive value (number of atopic subjects correctly detected by the questionnaire/total number of subjects identified by the questionnaire as 'allergic') was 71.7% for having a positive test for specific IgE against house dust mite, cat, dog, birch or grass. The children were born in 1996/1997 and will be followed until the age of 8 years.

In the PIAMA study, data were collected mainly with the help of questionnaires. In addition, all children of allergic mothers and a random sample of about 600 children of non-allergic mothers were visited at home at one or more times during the study period. During the home visit that took place when the infants were about 3 months old, 275 mothers, 168 allergic and 107 non-allergic, who were still breastfeeding, were included in the breast milk study. Although the home visits were planned to take place when the child was about 3 months old, in practice the children were between 2 weeks and 35 weeks old when their mothers donated a breast milk sample.

Collection and analysis of the milk samples

Milk samples were collected in small plastic cups either by manual expression or using a breast pump, depending on the preference of the mother. The mothers were asked to record how and at what time of the day they had produced their milk sample. The samples were then put into tubes containing 2 μ l of butylated hydroxytoluene (BHT) solution in order to prevent oxidation. They were sent by mail to the National Institute of Public Health and the Environment (RIVM) and stored there at -70°C .

Fatty acid composition of the breast milk was determined by gas-liquid chromatography, according to Foreman-van Drongelen et al. (18). In short, for transesterification of fatty acids in human milk 100 μ l samples of milk were added to 2 ml of methanol-hydrochloric acid (5:1, v/v)

and heated at 90°C for 4 h. After cooling, the samples were extracted with 1 ml of iso-octane (19). The samples were analyzed using capillary gas-liquid chromatography with flame ionization detection on a 50 m × 0.25 mm CP-Sil 5 CB, film thickness 0.12 µm (Chrompack, Middelburg, the Netherlands) column, using helium as the carrier gas. The analyses were performed using temperature programming, which had been optimized with a reference mixture containing most of the fatty acid methyl esters (fames) of interest. The standard mixture was also used to identify the fames corresponding to the various peaks. Fatty acid amounts present in the total lipid fraction were quantified based on the amount of 19:0 internal standard fame recovered.

Data analysis

Mean values of fatty acid concentration (weight percent of total fatty acids) were estimated for the allergic mothers and for the non-allergic mothers for individual fatty acids, for groups of fatty acids and for the metabolic index. The metabolic index is the ratio between the content of total n-6 LCP and the content of linoleic acid (18:2n-6) and is used as a measure of the conversion of linoleic acid from food to its essential LCP metabolites. Differences between the mean values of allergic and non-allergic mothers were tested using the Student's *t*-test. *p*-values < 0.05 were considered statistically significant. For the fatty acids most relevant to our research question, the association with the mother's allergy was subsequently analyzed in more detail using multiple regression analysis. In this analysis effects were assessed of the duration of lactation, the number of older children the mother had, maternal fish consumption, age and education. Associations have been reported between essential fatty acid levels in maternal blood and duration of lactation (20), number of older children (21) and fish consumption (22). Because a relationship between essential fatty acid levels in blood and in human milk has also been described (22), these factors were included in the analyses. An association between duration of lactation and essential fatty acids has also been observed in breast milk (13, 14, 16). Maternal age was included because of its close association with the number of older children and maternal education because it is associated with (prolonged) breastfeeding and could be an indicator of aspects of life style that might influence essential fatty acid status.

Table 1. Characteristics of the study population

	Allergic mothers n = 168	Non-allergic mothers n = 107
Mother's age (years, mean ± SD)	31.1 ± 3.6	30.8 ± 4.1
No. of older children (%)		
0	53.3	53.3
1	31.5	35.5
2	13.3	5.6
>2	1.8	5.6
Time since delivery when milk was collected (days, mean ± SD)	107 ± 29	110 ± 30
Habitual fish consumption during pregnancy (%)		
<1 × p. month	16.5	23.4
1–3 × p. month	53.1	52.3
1 × p. week	25.0	22.4
>1 × p. week	5.5	1.9
Recent fish consumption (freq. in week preceding milk collection, %)		
0 x	54.5	57.9
1 x	30.5	28.0
2 x	7.8	10.3
>2 x	7.2	3.7
Mother's educational level*		
Low (%)	16.5	11.7
Intermediate (%)	35.4	45.6
High (%)	48.1	42.7

*Low: primary, lower vocational and lower general; intermediate: senior high school and intermediate vocational; high: higher vocational and university.

Results

Table 1 shows characteristics of the allergic and non-allergic mothers participating in the breast milk study. The mothers in the breast milk study were more highly educated than those in the PIAMA study as a whole (35.4% higher education and 23.5% low education), but were similar in other respects. There were no statistically significant differences between the allergic and non-allergic mothers in the breast milk study with respect to these characteristics.

Of the 168 allergic mothers in the study, 47 (28.0%) had (a history of) asthma, 88 (52.4%) were allergic to pets, 103 (61.3%) were allergic to house dust and/or house dust mite and 114 (67.9%) had hay fever. Of those reporting these complaints, between 76% (of those with pet allergy) and 98% (of those with asthma) also reported that a doctor had diagnosed their disease. One hundred and two mothers (60.7%) reported having two or more of these complaints. Of the 47 mothers with asthma, 41 also reported one or more inhalant allergies and six reported having only asthma. Of those with hay fever, about one-third reported only hay fever and no other complaints.

The mothers were free to produce a breast milk sample how and when it suited them best, but

they were asked to record what they had done. The way in which the mother had produced the milk sample (by manual expression or using a breast pump, before or after feeding the child) and the time of the day when this was done, were found to influence the total fat content of the milk, but not the concentrations of individual fatty acids.

In Table 2, mean concentrations of breast milk fatty acids are shown for allergic and non-allergic mothers. No statistically significant differences were observed in mean breast milk concentrations of the n-6 fatty acids between allergic and non-allergic mothers. The mean concentrations of 20:4n-6 and total n-6 LCP were lower and the mean concentration of 18:2n-6 was higher in the milk of allergic mothers, but differences were small and not statistically significant. The metabolic index (the ratio between the content of total of n-6 LCP and the content of 18:2n-6), however, was significantly lower in the milk of the allergic mothers.

In the n-3 series of fatty acids, there were no statistically significant differences between allergic and non-allergic mothers in any of the individual fatty acids or in the sum of n-3 LCP. Of all other fatty acids determined, except 22:00 and 16:1n-7, the concentrations were similar in the milk of allergic and non-allergic mothers.

Mean breast milk fatty acid concentrations were also estimated for subgroups within the group of allergic mothers. For the most relevant fatty acids, the results are shown in Table 3. Both the mean concentration of total n-6 LCP and the mean metabolic index were slightly lower in milk from mothers who had at least two of the following symptoms – hay fever, pet allergy and house dust (mite) allergy – as compared with mothers with only one of these complaints. However, there were no statistically significant differences between the two groups. In milk from mothers with asthma, the mean concentrations of n-6 LCP and also the mean metabolic index were not significantly different from those in the milk of non-allergic mothers.

We further investigated whether factors other than maternal allergy influenced breast milk fatty acid composition and whether these factors influenced the relationship between maternal allergy and concentrations of 18:2n-6, 20:3n-6, 20:4n-6, total n-6 LCP, total n-3 LCP and the metabolic index. Effects of maternal age, maternal education, the number of older children she had, the number of days between delivery and production of the milk sample and the mother's reported habitual frequency of fish consumption during pregnancy were first tested univariately

Table 2. Fatty acid concentrations (weight percentages of total fatty acids) in breast milk of allergic and non-allergic mothers

Fatty acid	Allergic mothers (n = 168) Mean ± SD	Non allergic mothers (n = 107) Mean ± SD	p-value for difference
Total (g/l) wt%	32.9 ± 21.0	32.5 ± 18.2	0.880
n-6 polyunsaturated			
18:2n-6	15.53 ± 4.41	14.86 ± 3.75	0.197
18:3n-6	0.09 ± 0.05	0.10 ± 0.05	0.340
20:2n-6	0.24 ± 0.07	0.24 ± 0.07	0.752
20:3n-6	0.30 ± 0.11	0.30 ± 0.10	0.534
20:4n-6	0.37 ± 0.09	0.39 ± 0.10	0.051
22:2n-6	0.02 ± 0.02	0.03 ± 0.13	0.227
22:4n-6	0.07 ± 0.02	0.07 ± 0.04	0.161
22:5n-6	0.02 ± 0.02	0.03 ± 0.02	0.168
n-3 polyunsaturated			
18:3n-3	1.03 ± 0.39	1.01 ± 0.33	0.751
18:4n-3	0.13 ± 0.04	0.12 ± 0.04	0.317
20:5n-3	0.05 ± 0.04	0.06 ± 0.06	0.454
22:5n-3	0.14 ± 0.05	0.15 ± 0.07	0.645
22:6n-3	0.20 ± 0.12	0.22 ± 0.22	0.530
Monounsaturated			
16:1n-7	2.13 ± 0.64	2.31 ± 0.69	0.028
17:1	0.16 ± 0.04	0.17 ± 0.05	0.081
18:1n-7	1.66 ± 0.27	1.73 ± 0.36	0.099
18:1n-9	28.93 ± 2.85	29.30 ± 2.68	0.286
20:1n-9	0.30 ± 0.07	0.32 ± 0.13	0.206
24:1n-9	0.04 ± 0.02	0.04 ± 0.04	0.799
Saturated			
10:00	1.26 ± 0.27	1.24 ± 0.24	0.676
12:00	5.74 ± 1.72	5.50 ± 1.52	0.250
14:00	6.74 ± 1.85	6.57 ± 1.61	0.453
15:00	0.30 ± 0.10	0.32 ± 0.09	0.242
16:00	21.49 ± 2.17	21.79 ± 2.09	0.264
17:00	0.32 ± 0.06	0.33 ± 0.06	0.191
18:00	7.48 ± 1.22	7.34 ± 1.12	0.361
20:00	0.21 ± 0.05	0.20 ± 0.05	0.107
22:00	0.10 ± 0.06	0.09 ± 0.05	0.030
24:00	0.07 ± 0.03	0.07 ± 0.03	0.400
Totals			
Σn-6	16.64 ± 4.54	16.03 ± 3.82	0.250
Σn-6LCP	1.11 ± 0.26	1.17 ± 0.28	0.087
Σn-3	1.55 ± 0.44	1.55 ± 0.47	0.963
Σn-3LCP	0.52 ± 0.20	0.54 ± 0.33	0.632
Σn-7+n-9	33.29 ± 3.23	33.92 ± 3.03	0.104
Σn-9LCP	0.19 ± 0.07	0.19 ± 0.06	0.484
Σpufa	18.38 ± 4.67	17.77 ± 3.80	0.236
Σmufa	33.25 ± 3.24	33.90 ± 3.04	0.097
Σsat	43.73 ± 4.68	43.48 ± 3.97	0.650
Σtrans	2.73 ± 1.08	2.88 ± 1.36	0.338
Met index	0.075 ± 0.021	0.083 ± 0.032	0.025

LCP: long-chain polyunsaturates.

Met index: metabolic index: n-6LCP/18:2n-6.

and then in a multiple linear regression model including maternal allergy. The following effects were observed. The duration of lactation, which ranged from 60 days to 200 days in 270 of the 275 mothers, was significantly related to the concentration 20:3n-6, both in the univariate

Table 3. Fatty acid concentrations (weight percentages of total fatty acids) in breast milk of mothers with a single inhalant allergy, with multiple inhalant allergies and with asthma

Fatty acid	Mean wt% $\pm$ SD and p-value for difference with non-allergic mothers		
	Single allergy* (n = 66)	Multiple allergy† (n = 96)	Asthma‡ (n = 47)
18:2n-6	15.84 $\pm$ 4.10 p = 0.111	15.54 $\pm$ 4.59 p = 0.255	14.47 $\pm$ 3.84 p = 0.554
20:3n-6	0.31 $\pm$ 0.09 p = 0.590	0.29 $\pm$ 0.11 p = 0.301	0.29 $\pm$ 0.10 p = 0.551
20:4n-6	0.37 $\pm$ 0.08 p = 0.096	0.37 $\pm$ 0.10 p = 0.165	0.36 $\pm$ 0.10 p = 0.144
Total n-6 LCP	1.14 $\pm$ 0.25 p = 0.487	1.10 $\pm$ 0.28 p = 0.092	1.09 $\pm$ 0.26 p = 0.112
Metabolic index	0.075 $\pm$ 0.020 p = 0.046	0.074 $\pm$ 0.021 p = 0.022	0.079 $\pm$ 0.022 p = 0.388
Total n-3 LCP	0.56 $\pm$ 0.24 p = 0.659	0.50 $\pm$ 0.18 p = 0.322	0.52 $\pm$ 0.24 p = 0.720

*Mothers with only one of the following: pet allergy, house dust (mite) allergy, hay fever.

†Mothers with two or more of the following: pet allergy, house dust (mite) allergy, hay fever.

‡Mothers with asthma, with (n = 41) or without inhalant allergies (n = 6).

and in the multiple regression model. The effect was stronger in the allergic than in the non-allergic mothers. The concentration of 20:3n-6 decreased significantly with the duration of lactation in the allergic mothers (with 0.00081wt% per day of lactation, $p = 0.006$), but not in the non-allergic mothers (0.00027wt% per day of lactation, $p = 0.403$). The number of older children was significantly associated with the 18:2n-6 concentration in the univariate analysis ($p = 0.027$), but not in the multiple regression model. No other associations were observed between any of the factors studied and concentrations of n-6 fatty acids. The strength of the associations between maternal allergy and concentrations of n-6 fatty acids was unaffected by inclusion of the covariates in the regression model. The concentration of n-3 LCP, however, was associated with a number of factors. In univariate analyses, the n-3 LCP concentration increased significantly with maternal age ($p = 0.010$), with maternal education ($p = 0.007$) and with maternal fish consumption ($p < 0.001$), whereas it decreased significantly with the number of older children ($p = 0.024$). In the multiple regression analysis, the association with maternal education lost statistical significance, but the effects of maternal age, fish consumption and the number of older children remained the same. Maternal allergy was not related to the concentration of n-3 LCP either in crude or in adjusted regression analysis.

Discussion

We studied breast milk fatty acid composition in relation to maternal allergy in a relatively large study population. We compared subgroups of mothers with asthma and with single or multiple inhalant allergies and assessed the effects of potential confounders.

Based on the hypothesis of an impaired activity of delta 6 desaturase in atopic patients, one would expect a relatively high concentration of 18:2n-6, relatively low concentrations of 18:3n-6 and of the n-6 LCP derived from it and, as a consequence, a relatively low metabolic index in the milk of allergic mothers. Our data showed a significantly lower metabolic index in the allergic mothers' milk, but no statistically significant differences between allergic and non-allergic mothers in the mean concentrations of 18:2n-6 or any of its metabolites or in the total of n-6 LCP. Adjustment for potential confounders in the regression analysis did not change our results with respect to the n-6 fatty acids. Concentrations of n-3 fatty acids and of nearly all other fatty acids determined were similar in allergic and non-allergic mothers. No significant differences were observed in concentrations of n-6 fatty acids or in the metabolic index between specific subgroups of allergic mothers: mothers with only one type of allergy, mothers with multiple allergies and mothers with asthma.

Other studies conducted so far on fatty acids in breast milk in relation to maternal allergy have found no evidence (12) or, like our study, only little evidence of effects in breast milk of an impaired delta 6 desaturase activity. Thijs et al. (15) found a significantly lower 20:4n-6 concentration in milk of atopic mothers, but no significant differences in the other n-6 LCP and the same metabolic index in the two groups. Yu et al. (13) and Duchen et al. (14) both reported a significantly lower 20:3n-6 concentration in milk of atopic mothers collected 1 month after delivery, but no significant differences in the milk collected 3 months after delivery. They found no differences in the other n-6 LCP or in 18:2n-6, either at 1 month or at 3 months of lactation. In a later study, Duchen et al. (16) found significantly lower levels of total n-3 LCP, of total n-6 LCP and of individual n-6 LCP in the milk of atopic mothers, but also of 18:2n-6, which is contrary to what would be expected in case of an impaired delta 6 desaturase activity. Again, these differences were only seen in milk collected 1 month after delivery and not in the milk collected from the same mothers 3 months after delivery.

We considered the possibility that differences in the fatty acid concentrations in breast milk between allergic and nonallergic mothers exist, but that we failed to detect these due to methodological limitations. This could have been caused by selection bias in the recruitment of mothers into the breast milk study, by misclassification of allergic or non-allergic mothers and/or by the timing of the collection of milk samples. The mothers in the breast milk study were found to be more highly educated than the mothers in the PIAMA study as a whole. This can be explained by the association between maternal education and (prolonged) breastfeeding in the study population (23). In the multiple regression analysis, maternal education did not influence the associations between maternal allergy and fatty acid concentrations. We are therefore not concerned that the selection of mothers with higher than average levels of education has influenced our results. Our classification of allergy is based on self-reported asthma, hay fever, allergy for pets, allergy for house dust or allergy for house dust mite, using a validated screening questionnaire (17). Between 76% and 98% of the individual complaints reported were, according to the respondents, diagnosed by a doctor. In the other studies discussed here, the definition of atopy was usually based on clinically defined bronchial asthma, allergic rhino conjunctivitis, atopic eczema and sometimes food allergy and IgE levels. Only in the study by Thijs et al. (15) were interview data used. Our definition did not include atopic eczema or food allergy. These conditions are not very common in adults and even less so if none of the other complaints covered by our questionnaire are present. However, the possibility that our group of non-allergic mothers included some mothers with food allergy or atopic eczema cannot be excluded. Nevertheless, we expect that when differences in breast milk fatty acid composition between allergic and non allergic mothers were substantial, they would have been detected in our study.

The timing of milk collection in our study may also have contributed to our negative findings. Yu and Duchon (13, 14, 16) found differences in breast milk fatty acid concentration between atopic and non-atopic mothers in milk collected 1 month after delivery, but not in the milk collected from the same mothers 3 months after delivery. No such differences were observed, however, in the studies by Schroten et al. (12) and Thijs et al. (15) who collected milk samples relatively early. Schroten used milk samples collected between 13 and 16 days after delivery and found no differences at all between atopic

and non-atopic mothers. Thijs used milk samples collected between 2 and 12 weeks (mean: 5 weeks) after delivery and only found a significant difference in the concentration of 20:4n-6. In our sample, only two mothers donated milk about 1 month after delivery, so we could not assess differences at that stage of lactation. During the period covered by our study (60–200 days after delivery), duration of lactation was not associated with the concentrations of any of the n-6 fatty acids except 20:3n-6.

In conclusion, we found very similar breast milk fatty acid concentrations in mothers with asthma or inhalant allergies and in mothers without asthma and inhalant allergies. We feel confident that this observation reflects reality and was not caused by selection bias, misclassification or the timing of lactation. With respect to the n-3 LCP we observed no association with maternal allergy, but significant associations with maternal age, fish consumption and the number of older children. We suggest that in studies on n-3 essential fatty acids these factors should be controlled for and that results of studies that failed to do so should be interpreted with caution. Our data do not support the hypothesis that an impaired activity of the enzyme delta 6 desaturase in atopic patients affects the breast milk of atopic mothers. We found no evidence that fatty acid composition of breast milk is likely to be responsible for the differential effects of breastfeeding by atopic and non-atopic mothers that were observed in the studies by Wright et al. (3, 4). We would expect that investigation of other components of breast milk might turn out to be more fruitful.

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