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Breast milk from mothers of children with newly developed atopic eczema has low levels of long chain polyunsaturated fatty acids

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Background: Infants at risk of atopic dermatitis have lower than normal levels of long chain polyunsaturated fatty acids. These fatty acids are normally present in substantial quantities in human breast milk.

Methods: Because of the equivocal evidence concerning the ability of breastfeeding to delay the onset or reduce the severity of atopic dermatitis, we have analyzed the fatty acid composition of breast milk from the mothers of children with newly developed disease with the use of gas chromatography.

Results: Breast milk lipids from mothers of children with newly developed atopic dermatitis had increased proportions of linoleic acid and significantly decreased proportions of its long chain polyunsaturated derivatives compared with a control group. The ratio of linoleic acid to the sum of its metabolites, γ -linolenic acid, dihomo- γ -linolenic acid, and arachidonic acid was 11.78 in the atopic group and 9.02 in the control group ($p < 0.01$).

Conclusions: These results are consistent with previous findings of an abnormal fatty acid status in atopic subjects and may account for some of the inconsistent results from studies of the effect of breastfeeding on the subsequent development of atopic dermatitis. We conclude that further studies to examine the effects of supplementation of the diet of breastfeeding mothers with long chain polyunsaturates should be done. (*J ALLERGY CLIN IMMUNOL* 1993;91:1134-9)

Key words: Breast milk, atopic eczema, essential fatty acids, γ -linolenic acid, dihomo- γ -linolenic acid, arachidonic acid, prostaglandins

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Accepted for publication Dec. 16, 1992.

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0091-6749/93 \$1.00 + .10 1/145024

Abbreviations used

LCPs:	Long chain polyunsaturated fatty acids
GLA:	γ -linolenic acid
DGLA:	Dihomo- γ -linolenic acid
PG:	Prostaglandin
IL:	Interleukin

There has been controversy surrounding the role of breastfeeding in the development of infantile atopic dermatitis since Grulee and Sanford¹ first proposed a protective effect. Although the majority of studies suggest that exclusive breastfeeding exerts a protective effect,^{2, 3} a number of others fail to show this. Several studies show breastfeeding to be associated with an increase in the subsequent risk of atopic disease.^{4, 5}

In general, the premise underlying these studies has been based on the assumption that any protective effect that breastfeeding may confer is likely to be attributable to avoidance of cow's milk antigens and perhaps to the provision of various immunoprotective substances within breast milk. Hence, it has also been proposed that mothers of children who are "at risk" for atopic dermatitis or atopic disease should avoid the ingestion of cow's milk while they are breastfeeding.⁶

A number of recent studies of lipid composition in children and infants with atopic dermatitis have shown them to have abnormally low levels of long chain polyunsaturated fatty acids (LCPs) derived from linoleic acid⁷⁻¹¹ (Fig. 1). These abnormalities are present in lymphocytes from umbilical cord blood of infants at risk for atopy⁹ and may be directly related to levels of IgE in cord blood.¹⁰

Low levels of these prostaglandin precursor fatty acids may be implicated in the development of some of the abnormalities of the immune system that are characteristic of atopic dermatitis.^{11,12} Normally, breastfed infants ingest these LCPs directly as triglycerides in human breast milk, but a recent study of breast milk lipid composition has shown that mothers of children with recently developed atopic dermatitis have lower than normal levels of dihomogamma-linolenic acid (DGLA), the PGE₁ precursor fatty acid, and higher than normal levels of linoleic acid.¹³

We have studied lipid fatty acid composition in breast milk from a group of mothers whose infants have newly developed atopic dermatitis.

PATIENTS AND METHODS

Patients

All mothers were attending a pediatric allergy clinic and gave informed consent to the collection of breast

milk samples. In all children, atopic dermatitis was diagnosed by an experienced pediatrician. All children were born at full term, and birth weight ranged from 2850 to 4000 gm (mean, 3360 gm). There were 6 female and 17 male children, all of whom had atopic dermatitis before 6 months of age. At the time of breast milk sampling, the infants were between 2.0 and 8.8 months of age (mean, 4.1 months).

In six cases there was a positive history of atopic dermatitis in at least one first-degree relative, and there was a history of respiratory atopy in 13 cases (56%). In all cases in which there was a family history of atopic dermatitis, there was also a history of atopic respiratory disease in the family. None of the mothers had active atopic dermatitis.

RAST against a battery of antigens (cow's milk, egg, wheat, soy protein, house dust mite, and codfish), prick tests, a radioimmunosorbent test for total IgE, or a combination of these investigations was done in 20 infants. Of 17 infants investigated by RAST, 15 had positive results for at least one antigen-specific IgE, and 11 of these 15 had more than one positive RAST result to the battery of antigens used. Eight of eleven infants tested had positive results for at least one skin prick test. Total IgE levels ranged from 0 to 388 IU/ml (mean, 49.8 IU/ml) and were measured in 14 infants. One infant had negative RAST and skin prick test results and undetectable levels of plasma total IgE. In the other infant with negative RAST and skin prick test results, total IgE was 13 IU/ml.

The duration of atopic dermatitis in the infant at the time that maternal breast milk was sampled ranged between 3 days and 6 months (mean, 2.5 months).

Control samples ($n = 18$) were collected from mothers with no history of atopic disease in a first-degree relative, and in particular, no personal history of atopic disease. In this group the infants were between the ages of 0.5 months and 7 months at the time of sampling (mean, 2.5 months). There were 7 male and 11 female infants with a mean birth weight of 3390 gm (range, 3080 to 3970 gm). Both control group mothers and mothers of children with newly developed atopic dermatitis had a typical Italian-Mediterranean diet, with olive oil as the main source of dietary fat.

Breast milk sampling

Five-milliliter samples of midstream breast milk were collected in triplicate over 3 successive days at the same time of day, (between 10:00 AM and 12:00 PM) into sterile plastic collecting jars, and immediately stored at -20° C until analysis. The methods of analysis have been described previously.¹⁴ Briefly, a 0.5 ml sample of milk was mixed with 9.5 ml of chloroform:methanol:water (12:6:1). After shaking, the mixture was centrifuged, and the upper phase was removed and discarded. The lower phase was removed and evaporated to dryness under nitrogen, and the tubes were sealed and stored under nitrogen until the batch was ready for analysis.

Milk lipids were methylated by the method of Christo-

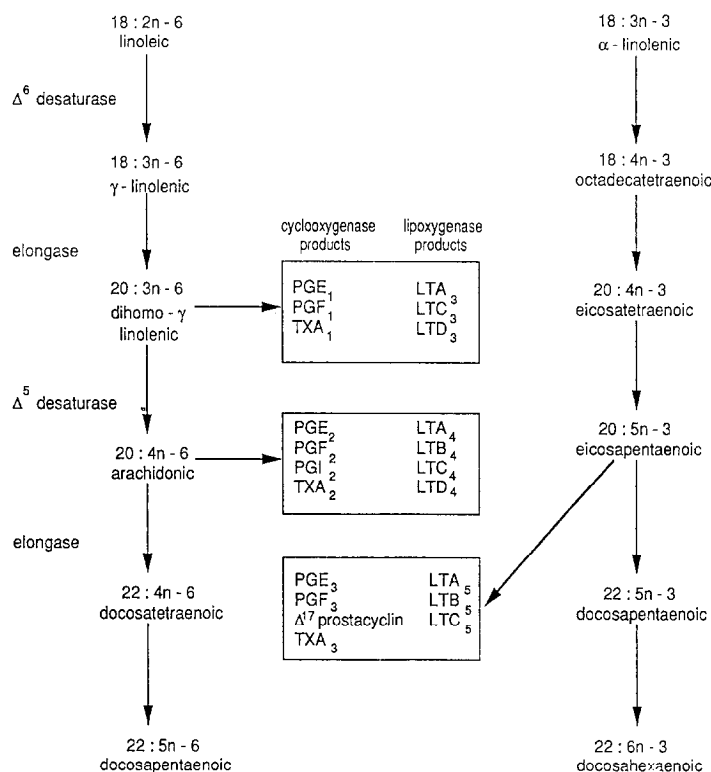


FIG. 1. The pathways of metabolism of the n-6 and n-3 series of fatty acids and their prostanoid derivatives.

pherson and Glass,¹⁵ as modified by Gibson and Kneebone,¹⁴ and the resultant fatty acid methyl esters were analyzed by gas chromatography on a Hewlett Packard 5890 chromatograph (Hewlett-Packard Co., Avondale Div., Avondale, Pa.) with a 2-meter glass column packed with Silar 10c (3M Dental Products, St. Paul, Minn.) on Chromosorb WAW (Johns-Manville). Fatty-acid methyl esters were identified by comparison with commercially available standards (Nuchek Prep Inc., Minneapolis, Minn.). The level of each fatty acid was expressed as weight percent of total lipid, and comparison between groups was done with Student's *t* test (two-tailed).

RESULTS

Table I compares the breast milk fatty acid composition in 23 mothers of children with atopic dermatitis to that in 18 control subjects.

Among the saturated fats, palmitic acid (16:0) ($p < 0.05$) and stearic acid (18:0) ($p < 0.01$) are both significantly decreased in the atopic group. The monounsaturate, oleic acid (18:1n-9), is present in significantly greater proportions in the atopic group ($p < 0.01$).

Of the n-6 series of polyunsaturates, the parent fatty acid, linoleic acid (18:2n-6) is slightly higher in the group of mothers with atopic children, whereas its long chain derivatives, DGLA (20:

3n-6) and adrenic acid (22:4n-6) are significantly lower; and arachidonic acid (20:4n-6) and γ -linolenic acid (GLA) (18:3n-6) are present in slightly lower quantities. Thus the ratio of linoleic acid to its δ -6-desaturated derivatives is 11.78 in the atopic group and 9.02 in the control group ($p < 0.01$).

Among the n-3 series of polyunsaturated fatty acids, the pattern is similar (Table I). Although the parent fatty acid, α -linolenic acid, is elevated in the atopic group, its derived fatty acids are present at levels equal to or lower than those of the control group. Thus the ratio of α -linolenic acid to its derived fatty acids is 3.3 in the atopic group and 2.1 in the control group. This difference is statistically significant at $p < 0.05$.

DISCUSSION

Our study shows the breast milk from mothers of children with newly developed atopic dermatitis to have abnormalities of fatty acid composition. In particular, they have significantly low levels of the highly unsaturated fatty acids DGLA (20:3n-6) and adrenic acid (22:4n-6) of the n-6 series and of docosahexaenoic acid (22:6n-3) of the n-3 series, despite raised levels of the parent

TABLE 1. Breast milk total lipid fatty acid composition in mothers of children with atopic dermatitis and control subjects

Fatty acid	Mothers of atopic children (n = 23)	Control subjects (n = 18)
12:0	5.4 ± 1.6	5.7 ± 2.1
14:0	6.2 ± 2.3	7.0 ± 2.2
16:0	20.6 ± 3.1	22.6 ± 2.0*
16:1n-7	3.1 ± 0.7	3.0 ± 0.4
18:0	5.0 ± 1.0	5.8 ± 0.6†
18:1n-9	44.0 ± 5.5	40.1 ± 3.3†
18:2n-6	10.4 ± 3.9	9.6 ± 2.0
18:3n-6	0.06 ± 0.05	0.07 ± 0.05
20:3n-6	0.3 ± 0.1	0.4 ± 0.10*
20:4n-6	0.5 ± 0.1	0.6 ± 0.14
22:4n-6	0.1 ± 0.04	0.2 ± 0.10†
18:3n-3	0.9 ± 0.5	0.7 ± 0.1
20:5n-3	0.03 ± 0.03	0.03 ± 0.04
22:5n-3	0.1 ± 0.05	0.1 ± 0.04
22:6n-3	0.2 ± 0.10	0.3 ± 0.06†
18:2n-6	11.78 ± 3.17	9.02 ± 1.65†
18:3n-6 + 22:3n-6 + 22:4n-6		
18:3n-3	3.30 ± 1.80	2.14 ± 1.30*
20:5n-3 + 22:5n-3 + 22:6n-3		

Data are expressed as percentage of total lipid.

* $p < 0.05$.† $p < 0.01$ (by two-tailed t test).

fatty acids of those two series—linoleic acid (18:2n-6) and α -linolenic acid (18:3n-3). Interestingly, the 18-carbon monounsaturate oleic acid (18:1n-9) was also significantly elevated in the breast milk of the mothers of children with atopic dermatitis. The decreased levels of stearic acid (18:0) are likely to be a consequence of the increased levels of these 18-carbon-containing polyunsaturates. Our control samples were taken somewhat earlier than in those from mothers with children who had atopic dermatitis. We do not believe that this is likely to account for the differences we found, because all samples were of “mature” breast milk in which the differences in fatty acid composition with the progression of lactation are much smaller than the differences we found.¹⁴

These abnormalities are likely to be reflected in the fatty acid composition of infant plasma and blood cells, because this closely reflects the dietary fatty acid intake.¹⁶ Although we have not examined infant blood in this study, there are several reports of abnormal fatty acid composition in infants with atopic dermatitis. Bordoni et al.¹⁷ found raised levels of linoleic acid and decreased levels of its long chain derivatives in plasma and white cells from children with atopic dermatitis.

Strannegard et al.¹⁸ reported similar results and also found a positive correlation between the fatty acid abnormality and the level of IgE in umbilical cord blood. Umbilical cord blood IgE is a predictor for the subsequent development of atopic disease.¹⁹ Galli et al.⁹ reported that the cord blood lymphocyte phospholipids in neonates at risk for atopy also had raised levels of linoleic acid and low levels of its long chain polyunsaturated derivatives.

These studies have been interpreted as indicative of a defect in the conversion of linoleic acid, probably at the level of the enzyme, δ -6-desaturase.²⁰ Direct measurements of enzyme activity are not available at present, but the ratio of substrates to product may be regarded as an indication of the rate of conversion of substrate. In the study we report here, this ratio was significantly different between the mothers of infants with atopic dermatitis and the control group for the n-6 and the n-3 families of essential fatty acids. Another possible interpretation of these findings would be a selective incorporation of 18:2n-6 and 18:3n-3 into lipids at the expense of their metabolites. In our view this is less likely.

Atopic dermatitis is characterized by a number of abnormalities of the immune system. In partic-

ular, suppressor T lymphocyte numbers and function appear to be depressed.^{21, 22} The relatively uncontrolled synthesis of IgE by cultured atopic peripheral blood mononuclear cells may be a consequence of the unsuppressed activity of CD4-positive T lymphocytes.^{23, 24} Histologically, the condition is characterized by a cutaneous infiltrate, which comprises CD4-positive lymphocytes.

The consequences for the infant immune system of an abnormally low supply of these prostaglandin precursor fatty acids may be substantial. DGLA is the precursor of PGE₁, and arachidonic acid is the precursor of PGE₂. Both these prostaglandins are likely to be crucial to the development of the infant immune system. PGE₁ is found in particularly large amounts in the thymus gland and has thymic hormone-like effects in the process of T lymphocyte maturation.²⁵ The influence that PGE₁ and PGE₂ are able to exert on the immune system may be mediated in part by their ability to stimulate cyclic adenosine monophosphate formation, and subjects with atopic disease have an abnormally low cyclic adenosine monophosphate response to stimulation.²⁶

In adults and older children, supplementation of the diet with GLA in the form of evening primrose oil results in return of DGLA levels toward normal,²⁷ with increased capacity to synthesize PGE₁.²⁸ Evening primrose oil contains 9% to 10% GLA in the triglyceride form. The administration of evening primrose oil also results in symptomatic improvement in the condition.²⁹ Supplementation of the maternal diet with preformed long chain polyunsaturates does result in an increase in these LCPs in the breast milk.³⁰ The impact of such supplementation on the subsequent development of atopic disease in the offspring should be the subject of further study.

There are a number of other interactions between these prostaglandin precursor fatty acids, prostaglandins themselves, and the immune system, which may be of relevance to atopic disease. DGLA is able to reduce the production of IL-2 by a prostaglandin-independent mechanism³¹ and is able to inhibit IL-2-driven T cell proliferation,³² effects which may be of relevance to atopic dermatitis. IL-4-driven IgE synthesis is inhibited by PGE₁ and PGE₂,²³ and lack of either of these controlling factors may allow for uninhibited production of IgE in the subject with atopy. We were not able to find any relationship between the concentration of prostaglandin precursor fatty acids in maternal breast milk and the level of total IgE or antigen-specific IgE in the infants.

We have previously shown that the development of atopic disease in a group of infants at risk for atopy is associated with persistently low levels of these prostaglandin precursor fatty acids in their blood.³³ The present study suggests that the abnormally low levels of these LCPs found in infants at risk for atopy is unlikely to be corrected by breastfeeding and emphasizes the need for a study of the effects of early dietary supplementation in these "at risk" infants with GLA.

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