

Breast-fed and formula-fed infants do not differ in immunocompetent cell cytokine production despite differences in cell membrane fatty acid composition¹⁻³

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ABSTRACT

Background: Breast-fed and formula-fed infants differ in the amount and type of polyunsaturated fatty acids consumed. The fatty acid composition of cell membranes is related to dietary fatty acids and, in adults, changes in membrane fatty acid composition are accompanied by changes in monocyte cytokine production and hence a modification of the immunologic response.

Objective: Our objective was to determine whether production by immunocompetent cells of the proinflammatory cytokines interleukin 1 (IL-1) and tumor necrosis factor (TNF) differs between breast-fed and formula-fed infants.

Design: Twenty-six healthy infants (13 breast-fed and 13 fed modified cow-milk formula) aged 2–4 mo were studied. The fatty acid composition of red blood cell (RBC) membrane phospholipids was measured by gas-liquid chromatography and IL-1 and TNF release were measured in whole blood culture in bacterial-endotoxin-stimulated and unstimulated cells.

Results: The infants' ages, weights, hemoglobin concentrations, and white blood cell counts did not differ significantly between groups. The percentage of n-3 fatty acids of total RBC phospholipid fatty acids was significantly higher in breast-fed than in formula-fed infants ($6.31 \pm 2.5\%$ compared with $2.98 \pm 0.97\%$); docosahexaenoic acid (22:6n-3) concentrations were also markedly higher in breast-fed infants ($5.1 \pm 1.2\%$ compared with $2.2 \pm 0.9\%$, $P < 0.001$), but eicosapentaenoic acid (20:5n-3) and docosapentaenoic acid (22:5n-3) concentrations did not differ significantly between groups. The percentage of n-6 fatty acids was not significantly different between groups. The percentage of oleic acid (18:1) was higher in formula-fed than in breast-fed infants ($16.2 \pm 0.7\%$ compared with $20.6 \pm 1.1\%$; $P < 0.001$). IL-1 and TNF release in whole blood culture did not differ significantly between groups.

Conclusion: The release of proinflammatory cytokines by immunocompetent cells does not differ significantly in breast-fed and formula-fed infants despite differences in cell membrane fatty acid composition. *Am J Clin Nutr* 2000;72:1202–5.

KEY WORDS Breast milk, cow-milk formula, polyunsaturated fatty acids, interleukin 1, tumor necrosis factor, infants, breast-feeding, fatty acid composition

See corresponding editorial on page 1071.

INTRODUCTION

Breast-feeding is believed to confer protection against infections during infancy. Breast-fed infants have an enhanced local humoral immune response, resulting in a lower prevalence of gastrointestinal and respiratory tract infections than in formula-fed infants (1, 2). Furthermore, exclusive breast-feeding for the first few months has been suggested to be protective against the development of atopic disease (3, 4). Immunoglobulins, lymphocytes, and lactoferrin, which are all present in human milk, play a specific immunologic role. The possible beneficial effects of breast-feeding on the immune response, which may be attributable to the fatty acid content of breast milk, have not been studied previously.

Infants fed breast milk and infants fed commercial formulas differ considerably in the fatty acid pattern of their diet. Human milk contains long-chain polyunsaturated fatty acids (20–22 carbons) of both the n-3 and n-6 class, which constitute $\approx 2\%$ of total fatty acids and which are undetectable in unsupplemented formulas prepared from vegetable oils (5–7).

The fatty acid composition of cell membranes is directly related to dietary fatty acids. Erythrocyte membranes of unsupplemented formula-fed infants are relatively enriched in α -linolenic acid (18:3n-3) and depleted in arachidonic acid (AA; 20:4n-6) (6). Red blood cell (RBC) membranes of breast-fed infants contain significantly more 20–22-carbon polyunsaturated fatty acids (PUFAs) both of the n-6 and n-3 classes than do RBC membranes of infants consuming vegetable fat (6, 7). The n-3 fatty acid eicosapentaenoic acid (EPA; 20:5 n-3) has been shown to compete with AA as the substrate for the cyclooxygenase and lipoxygenase enzymes. Leukotrienes synthesized from EPA differ in physiologic activity from the AA-derived com-

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TABLE 1
Characteristics of the infants studied¹

	Breast-fed (n = 7 M, 6 F)	Formula-fed (n = 8 M, 5 F)
Age (mo)	2.8 ± 0.9	2.8 ± 0.8
Weight (% of 50th percentile)	106 ± 15.1	102.3 ± 12.2
Height (% of 50th percentile)	102.6 ± 7.2	99.5 ± 16.1
Hemoglobin (g/L)	105 ± 7	106 ± 9
WBC (×10 ⁹ cells/L)	11.1 ± 2.8	11.5 ± 4.4

¹ $\bar{x} \pm SD$. There were no significant differences between groups. WBC, white blood cells.

pounds (8–10). In adults receiving n–3-enriched diets, changes in mononuclear cell membrane fatty acid composition are accompanied by changes in leukotriene B₄ generation and endotoxin-stimulated cytokine production (11–13).

Epidemiologic data on the incidence of autoimmune and allergic diseases in populations with a high dietary intake of n–3 very-long-chain fatty acids further support the presumed role of these fatty acids in immune modulation (14). Interleukin 1 (IL-1) and tumor necrosis factor (TNF) are 2 of the principal polypeptide mediators synthesized in response to injury and infectious, inflammatory, and immunologic challenges. Because synthesis of IL-1 and TNF by mononuclear cells has been shown to be affected by both dietary n–3 and n–6 fatty acids (13, 15), we compared the production of IL-1 and TNF in breast-fed and formula-fed infants.

SUBJECTS AND METHODS

Subjects

Twenty-six healthy infants aged 2–4 mo were studied; 13 infants had been solely breast-fed since birth and 13 infants had received a modified cow-milk formula as their sole dietary intake since birth. The fat content of the formula was 36 g/L. The unsaturated fatty acids contained in the formula were oleic acid (18:1n–9), constituting 36–38% of total fatty acids; linoleic acid (18:2n–6), constituting 9–11% of total fatty acids; and α-linolenic acid, constituting 0.8–1.8% of total fatty acids.

Infants were studied during a routine visit to a “well-baby” clinic. Infants underwent a developmental assessment and physical examination by a pediatrician and weight, height, and head circumference were recorded and expressed as percentages of the 50th percentile for age. Parental consent was obtained before inclusion of the infant in the study, which was approved by the Hadassah University Hospital medical ethics committee.

Methods

The fatty acid composition of erythrocyte membrane phospholipids was determined by using the method of Dodge and Philips (16). In brief, erythrocytes and plasma were separated from EDTA-containing blood samples and the erythrocytes were hemolyzed. Total lipids were extracted with chloroform:methanol (1:1, by vol). Fatty acid methyl esters were prepared by transesterification with methanolic trimethylammonium hydroxide (Meth Prep II; Applied Science, Deerfield, IL) (17). During the separation procedures, 2,6-di-tert-butyl-P-cresol (50 mg/L) was used as an antioxidant. The fatty acid methyl esters were separated and quantified by gas–liquid chromatography with a Tracor 565 column (1.85 m × 4 mm internal diameter, 10% SP-2330 on 100/120 Chromosorb in

WAW 1-1851; Alltech, Deerfield, IL). Temperature programming was used from 185 to 220°C. Peaks were identified by comparing retention times with known standards (Supelco, Bellefonte, PA).

Mononuclear cell IL-1 and TNF release were measured by using a whole blood culture system as described by Wilson et al (18). In brief, 3.5 mL heparinized blood (10 × 10³ U/L) was diluted 1:1 with RPMI 1640 (containing 200 mmol glutamine/L, 200 × 10³ U penicillin/L, 200 mg streptomycin/L). A total of 0.5 mL diluted blood for each infant was divided among 12 wells in microtiter flat-bottomed plates (Nunc, Roskilde, Denmark) as follows: 3 wells [–lipopolysaccharide (LPS)] for measurement of IL-1, 3 wells (+LPS) for measurement of poststimulation IL-1 concentrations, 3 wells (–LPS) for measurement of TNF, and 3 wells (+LPS) for measurement of poststimulation TNF concentrations. In wells containing LPS (lipopolysaccharide *E. coli* 055 B₅; Sigma, St Louis), 20 μg LPS was added per well. Plates were incubated overnight at 37°C. Aliquots were centrifuged (5 min, 37°C, 121 × g) and supernates were removed and stored at –20°C. Supernates were not pooled and the supernate of each well was assayed separately. IL-1 and TNF concentrations were measured by immunoassay (Quantikine human IL-1β and human TNF-α; R&D Systems, Minneapolis)

Statistical analysis

Statistical analysis was performed by using the two-tailed Student’s *t* test and the Mann-Whitney *U* test for comparisons between groups. Analyses were performed with INSTAT (GraphPad software Inc, San Diego).

RESULTS

Characteristics of the infants studied are shown in **Table 1**. The mean age, weight and height (expressed as a percentage of the 50th percentile for age), hemoglobin concentration, and white blood cell (WBC) count did not differ significantly between breast-fed and formula-fed infants.

The fatty acid composition of RBC membrane phospholipids is shown in **Table 2**. The concentration of n–3 fatty acids with 5 unsaturated carbon bonds [EPA and docosapentaenoic acid (DPA; 22:5n–3)] did not differ significantly between breast-fed and formula-fed infants (technically, the peaks were inseparable with gas–liquid chromatography). In contrast, docosahexaenoic acid (DHA; 22:6n–3) was >2-fold higher in breast-fed infants than in formula-fed infants. The percentage of oleic acid was higher in

TABLE 2
Fatty acid composition of red blood cell membrane phospholipids¹

	Breast-fed (n = 13)	Formula-fed (n = 13)
	<i>% of total fatty acids</i>	
18:0	15.3 ± 1.3	13.4 ± 1.5
18:1	16.2 ± 0.7	20.6 ± 1.1 ²
18:2n–6	13.7 ± 0.9	13.7 ± 1.3
18:3n–3	0.58 ± 0.8	0.32 ± 0.2
20:3n–6	1.4 ± 0.3	1.6 ± 0.4
20:4n–6	13.5 ± 1.8	12.3 ± 1.3
20:5n–3 + 22:5n–3	0.56 ± 0.2	0.44 ± 0.1
22:6n–3	5.14 ± 1.3	2.2 ± 0.9 ²

¹ $\bar{x} \pm SD$. Values are percentage of total fatty acids by weight.

²Significantly different from breast-fed infants, *P* < 0.0001.

TABLE 3
Stimulated [+ lipopolysaccharide (LPS)] and unstimulated (–LPS) release of interleukin 1 (IL-1) and tumor necrosis factor (TNF) in a whole blood cell culture system¹

	Breast-fed (n = 13)	Formula-fed (n = 13)
	ng/L	
IL-1		
–LPS	632 ± 162	823 ± 152
+LPS	1326 ± 45	1401 ± 52
TNF		
–LPS	40 ± 9	27 ± 8
+LPS	731 ± 130	740 ± 116

¹ $\bar{x} \pm$ SE of 3 measurements for each infant. There were no significant differences between groups.

formula-fed infants than in breast-fed infants. No significant differences were observed in the relative percentages of stearic acid (18:0), linoleic acid, α -linolenic acid, or AA between groups.

The percentage of n–6 fatty acids in RBC membrane phospholipids did not differ significantly between breast-fed and formula-fed infants (28.9 ± 2.16% compared with 27.7 ± 1.24%, respectively) but the percentage of n–3 fatty acids was significantly higher in breast-fed infants (6.31 ± 2.5% compared with 2.98 ± 0.97%; $P < 0.001$). Thus, the ratio of n–6 to n–3 fatty acids was significantly lower in breast-fed infants than in formula-fed infants: 4.8 ± 1.3 compared with 10.5 ± 1.5 ($P < 0.001$).

Shown in **Table 3** are values for IL-1 and TNF production in whole blood culture, under stimulated and unstimulated conditions. There were no significant differences between breast-fed and formula-fed infants.

DISCUSSION

Despite the significant differences in cell membrane fatty acid composition between breast-fed and formula-fed infants, release from WBCs of the major mediators of inflammation, IL-1 and TNF, was not significantly different between groups under both unstimulated and stimulated (in response to bacterial endotoxin) conditions. In breast-fed infants, total n–3 fatty acids in RBC membrane phospholipids were 2-fold higher than in infants fed a modified cow-milk formula that was not supplemented with long-chain fatty acids. Accordingly, the ratio of n–6 to n–3 fatty acids was 2-fold higher in formula-fed infants.

Although the formula-fed infants consumed a formula that was not supplemented with AA, percentages of AA in cell membrane phospholipids did not differ significantly between breast-fed and formula-fed infants. Linoleic acid, the principal polyunsaturated fatty acid in plant seed oil, can undergo elongation and desaturation to yield AA. Although studies in which stable isotopes were used indicated that the capacity for endogenous AA synthesis is limited in early life (19), it is noteworthy that percentages in our formula-fed group were not significantly lower than those in the breast-fed group. As for the α -linolenic acid–derived n–3 PUFAs, EPA and DHA, only DHA percentages were markedly higher in the breast-fed group; no other significant differences were noted for n–3 fatty acids.

In this study, cytokine production was assayed in whole blood cell culture, a method that is considered superior to isolated peripheral blood mononuclear cell culture. The separation procedure may stress or damage the WBCs, thereby resulting in selective depletion


or enrichment of certain subpopulations of lymphocytes or monocytes and can even lead to preactivation. Furthermore, whole blood cell culture requires only small amounts of blood and may allow for better reproducibility of results (18, 20, 21).

The small differences between means and the large SDs for the measurements of IL-1 and TNF indicate that the statistical power of the study was low. To increase the power of the study, a very large sample size would be required, which is not feasible in a study of healthy infants. Indeed, our study may have missed a small difference between groups that would become apparent if a large enough sample was studied. However, although this small difference may then be found to be statistically significant, this would not necessarily indicate medical relevance.

Because of the limited amount of blood drawn from each infant, only the fatty acid composition of RBC membrane phospholipids was determined. However, it may be assumed that the changes observed occur also in WBC membranes. In adults, a diet supplemented with EPA was shown to result in modification of WBC membrane fatty acid compositions (10, 13). In animal studies, variations in dietary fat were shown to result in parallel changes in the fatty acid composition of both RBC and small-intestinal microvillus membranes (22). Furthermore, Makrides et al (23) noted that changes in dietary fat result in a similar fatty acid composition pattern in the RBCs, neurons, and retinal cells of breast-fed and formula-fed infants.

In adults, dietary supplementation with n–3 PUFAs was shown to modify the membrane fatty acid composition of platelets, neutrophils, and monocytes, resulting in a decrease in the ratio of AA to EPA (9, 10). In mononuclear cells these changes in membrane fatty acid composition are accompanied by changes in cytokine production: a decrease in endotoxin-stimulated production of 2 of the principal polypeptide mediators of inflammation, IL-1 and TNF (13). A diet enriched in n–3 fatty acids was also shown to decrease monocyte and neutrophil leukotriene B₄ generation and neutrophil chemotaxis (11, 12). A possible mechanism for suppression of IL-1 production is decreased synthesis of leukotriene B₄ and generation of the biologically less active leukotriene B₅ from EPA. In cell culture, adding leukotriene B₄ to mononuclear phagocytes in the presence of endotoxin results in an increase in synthesis of IL-1.

The above observations were noted in adults fed a diet enriched with 2–3 g EPA and DHA/d. This diet resulted in a 5–7-fold increase in the cell membrane content of EPA and DPA (10, 11). In contrast, in the present study, the content of EPA and DPA in RBC membranes did not differ significantly between breast-fed and formula-fed infants, whereas the content of DHA was only 2-fold higher in breast-fed infants. Thus, the differences observed in cell membrane fatty acid composition between breast-fed and formula-fed infants in our population are probably not sufficient to affect cytokine production.

In breast-fed infants, bacterial endotoxin-stimulated and unstimulated mononuclear cell production of mediators of inflammation was similar to that observed in formula-fed infants. This suggests that differences in cytokine production do not account for the presumed immunomodulatory effects of breast milk. 

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