

ORIGINAL PAPER

Serum, cheek cell and breast milk fatty acid compositions in infants with atopic and non-atopic eczema

K. Laitinen^{*†‡}, J. Sallinen[†], K. Linderborg[†] and E. Isolauri^{*§}

^{*}Department of Paediatrics, Turku University Central Hospital, Turku, Finland, [†]Department of Biochemistry and Food Chemistry, University of Turku, Turku, Finland,

[‡]Functional Foods Forum, University of Turku, Turku, Finland and [§]Department of Paediatrics, University of Turku, Turku, Finland

Clinical and Experimental Allergy

Summary

Background The major theory implicating diet with allergic diseases is associated with altered food consumption and subsequent changes in fatty acid composition.

Objective To investigate fatty acid compositions among infants with atopic and non-atopic eczema and healthy infants and to evaluate the expediency of non-invasive cheek cell phospholipid fatty acid composition as a marker in patients with eczema.

Methods Diagnosis of eczema in infants was confirmed clinically and by positive (atopic eczema, $n = 6$) or negative (non-atopic eczema, $n = 6$) skin prick testing in comparison with controls ($n = 19$). The fatty acid compositions of infant cheek cell and serum phospholipids and breast milk total lipids were analysed by gas chromatography.

Results The distinction between atopic and non-atopic eczema was manifested in cheek cell phospholipids as linoleic acid (14.69 (13.67–15.53)% of total fatty acids; the median (interquartile range)), the sum of n-6 fatty acids (19.94 (19.06–20.53)%) and the sum of polyunsaturated fatty acids (22.70 (21.31–23.28)%) were higher in infants with atopic eczema compared with non-atopic eczema (12.69 (10.87–13.93); 17.72 (15.63–18.91) and 19.90 (17.64–21.06), respectively; $P < 0.05$) and controls (12.50 (12.16–13.42); 18.19 (17.43–18.70) and 20.32 (19.32–21.03), respectively; $P < 0.05$). Serum phospholipid γ -linolenic acid was lower in both atopic and non-atopic eczema compared with controls ($P < 0.05$) and additionally eicosapentaenoic acid was higher in atopic eczema compared with controls ($P < 0.05$).

Conclusion These preliminary results suggest differences in fatty acid compositions between the two types of eczema, calling for further evaluation in a larger setting. The two types of eczema may be regulated by different immunological processes, and fatty acids may have a more profound role in the atopic type.

Keywords atopic eczema, breast milk, cheek cells, fatty acids, non-atopic eczema, serum

Submitted 6 May 2005; revised 23 August 2005; accepted 26 October 2005

Correspondence:

Kirsi Laitinen, University of Turku,
Itäinen Pitkäkatu 4A, 5th floor, 20520
Turku, Finland.
E-mail: kirsi.laitinen@utu.fi

Introduction

Causes have been sought for the rise in the prevalence of allergic disease [1], investigations ranging from nutrition to general environmental changes. The onset of allergic disease has been seen to result from exposure to dietary or environmental antigens and subsequent sensitization. However, the elimination of environmental factors, including certain elements of diet [2], has failed to effect a convincing long-term impact in reducing the risk of allergic disease.

Currently, the major theory implicating diet with allergic diseases is associated with altered dietary habits, mainly the consumption of margarine [3] or fish [4], modifying fatty acid intake. In particular, the increased intake of n-6 fatty acids results in the production of arachidonic acid (AA, 20:4n-6)-derived eicosanoid prostaglandin E₂ and thereby in elevated IgE synthesis, pro-inflammatory cytokine responses and hence allergic disease [5]. However, supplementation with single dietary components including fatty acids [6, 7], which have become popular in the search for the factors associated

with allergic disease overlooks the role of the overall dietary composition with a range of nutrients and other potentially active components.

Equally, it is recognized that allergic disorders form an inchoate group of diseases. Eczema, the first expression of atopic disease, may be classified according to the clinical manifestation and response to detection of antigen-specific IgE antibodies by serum or skin test in atopic (allergic) eczema in comparison with non-atopic (non-allergic) eczema [8]. In like manner, the onset of allergic disease may evolve via different mechanisms.

Previous studies exploring the role of fatty acids in allergic disease have for the most part focused on patients with manifest disease or the diagnosis of eczema has been obtained by non-clinical methods, including questionnaires not mentioning differentiation of type, and possibly explaining inconclusive results [9–11]. The opposite approach was adjusted in the present study. In this pilot study, we investigated whether differences in fatty acid compositions could be detected in well-defined groups of infants with atopic and non-atopic eczema. We further sought to establish whether a non-invasive cheek cell test for phospholipid fatty acid composition [12–14] reflecting the current dietary composition and closely connected with mucosa-associated immune effector cells [15] may be used as a marker in infants with eczema.

Methods

Subjects and study design

Breastfeeding mothers participating in an ongoing mother–infant follow-up study were asked to donate breast milk samples, and serum and cheek mucosal cell samples from their children for the present study. The study was approved by the Ethical Committee of the Hospital District of South–West Finland. Informed consent was obtained from the mothers.

Infants were recruited into the study in the order of enrolment at study visits at the age of 1 or 6 months (controls) or at additional visits for evaluation of symptoms of allergic disease. At the time of sample collection the median age of the infants was 5.6 months (interquartile range (IQR) 1.3–6.1). Diagnosis of atopic eczema was confirmed if the following features were detected: pruritus, facial and/or extensor involvement and chronic course of at least 1 month [16]. The median age at onset of symptoms in children with eczema was 2.5 (IQR 1.6–3.8) months.

Sensitization to common antigens was measured by skin prick testing as described previously [17] in infants at the time of sample collection or, in children younger than 4 months, at 6 or 12 months (one child) of age and in mothers during the last trimester of pregnancy. The antigens tested included cow's milk, hen's egg white,

wheat and rice flour both diluted 1/10 (w/v) with 0.9% (w/v) sodium chloride, gliadin diluted 1 mg/mL with ethanol/glyceroleum/ALK-diluent (Allergologisk Laboratorium A/S, Horsholm, Denmark) mixture, cod, soya bean, birch, six grasses, cat, dog, dermatophagoides pteronyssinus allergen (Allergologisk Laboratorium A/S), latex (Stallergenes S.A., Antony Cedex, France) and banana, potato and carrot by the prick–prick technique and for mothers also peanut, hazelnut, alder and mugwort (Allergologisk Laboratorium A/S).

Three infants with eczema yielded a positive skin prick test (SPT) for milk, one for egg, one for milk and egg and one for egg and potato. Infants with no clinical symptoms of eczema and negative SPT result were included in the control group ($n = 19$). Infants with eczema were divided into two groups according to positive ($n = 6$, atopic infants) or negative ($n = 6$, non-atopic infants) SPT results. There were no differences between the groups in age at the time of skin prick testing (median (IQR); atopic eczema 6.4 (6.0–8.6), non-atopic eczema 5.0 (2.9–6.5) and controls 6.1 (6.0–6.3) months, $P = 0.286$, Kruskal–Wallis test). Allergic disease in mothers was defined as the presence of self-reported atopic eczema, allergic rhinitis, asthma or food intolerance and a positive SPT result ($n = 16$) and healthy mothers with no allergic disease and negative SPT reaction ($n = 12$).

Weights and heights of the children were measured at study visits, background data on family history were obtained by interview and hospital records were examined for birth data on the children. The birth and clinical characteristics of the infants and mothers were comparable and are presented in Table 1.

Definitions: Here, atopic eczema comprises atopic eczema according to Hanifin's criteria [16] and positive SPT result [8].

Non-atopic eczema comprises atopic eczema according to Hanifin's criteria [16] and negative SPT result [8].

Controls comprise no symptoms of atopic eczema and negative SPT result.

Dietary evaluation

Dietary intake of the mothers was evaluated by 3-day food diaries with household measures and the frequency of fish consumption during the 2 weeks prior to the sampling was ascertained by interview. Energy and nutrient intakes were calculated with a Micro-Nutrica[®] computerized program (version 2.0, Research Centre for Social Insurance Institution, Turku, Finland).

Sampling

Breast milk collection was standardized for all mothers. First thing in the morning, infants were allowed to suckle for a few minutes and then a milk sample was collected by manual expression, whereafter feeding continued. Blood

Table 1. Birth, clinical and maternal characteristics of infants with atopic and non-atopic eczema and control infants*

	Atopic eczema (<i>n</i> = 6)		Non-atopic eczema (<i>n</i> = 6)		Controls (<i>n</i> = 19)	
Birth characteristics						
Weight (g)	3510	(3450–4165)	3560	(3560–3730)	3570	(3570–4460)
Length (cm)	51	(50–53)	51	(50–55)	51	(47–54)
Head circumference (cm)	36	(34–37)	36	(36–37)	35	(33–37)
Duration of gestation (weeks)	40	(38–42)	41	(39–41)	40	(38–42)
Clinical characteristics at sampling						
Weight for height (%)	− 0.50	(− 4 to 17)	1.50	(− 1 to 11)	− 5.0	(− 16 to 23)
Height (SD score)	− 0.15	(− 1.0 to 1.0)	0.40	(− 1.0 to 1.5)	0.20	(− 1.8 to 2.1)
Maternal characteristics [†]						
Maternal allergic disease	5	(80)	6	(100)	14	(73)
Positive skin prick test in mother	4	(67)	6	(100)	11	(61)

*Data are shown as median and (range) unless otherwise stated. Differences amongst groups not significant (Kruskal–Wallis test). [†]*n* and (%). Differences between combined group of atopic patients and controls not significant (Fisher's exact test).

samples were drawn from the antecubital vein by a laboratory technician. Several sampling devices were tested with adults to ensure efficient cheek cell collection. Gynobrush (Heintz Herenz, Hamburg, Germany) was selected because it could easily be bent into a circular shape, the sample obtained was of reasonable size for analysis, the brush was easy to use, and it was economical. To isolate the cheek cells, the mouth of the infant was first rinsed with a few sips of water. Mucosal cells were then scraped with a circular-shaped Gynobrush (Heintz Herenz). Both sides of the inner cheeks were stroked back and forth 25 times. The cells were suspended in saline and isolated by centrifugation (3030 *g*, 10 min). All samples were frozen and stored at -70°C until analysis of fatty acid compositions of infant cheek cell and serum phospholipids and breast milk total lipids.

Fatty acid analysis

Total lipids in the samples were extracted with chloroform : methanol 2 : 1 v/v [18]. Polar lipids of cheek cell and plasma samples were then separated from neutral lipids by solid-phase extraction with silica columns [19]. As samples were assumed not to contain significant amounts of free fatty acids or mono or diacylglycerols, the polar lipid fraction was taken to consist of phospholipids.

Fatty acid methyl esters were prepared from the isolated polar lipid fraction of cheek cells and plasma, and total lipids of the breast milk with boron trifluoride, and analysed by gas chromatography (PerkinElmer Auto-System, Norwalk, CT, USA) with a DB-23 column (60 m $\times$ 0.25 mm i.d., 0.25 μm film thickness; Agilent Technologies, Palo Alto, CA, USA). Diheptadecanoylphosphatidylcholine (Sigma-Aldrich, St Louis, MO, USA) was used as an internal standard in cheek cell and plasma samples and triheptacosanoin (Sigma-Aldrich) in breast milk samples.

The small amount of lipids in the infant cheek cells constituted a challenge for fatty acid composition analysis. All glassware and equipment used were new and

disposable. The lipid class fractionation process was scaled down from that of Hamilton and Comai [19] and the gas chromatographic analysis was run from a volume of 50 μL in vial inserts. Boron trifluoride was used as a catalyst for the methylation reaction because it did not leave a precipitate at the bottom of the inserts. Splitless/split injection was used to ensure the passage of the sample to the column without overloading the column with the solvent.

Statistical methods

Values are reported as the median and IQR, unless otherwise stated. The differences among the three groups were analysed by a non-parametric Kruskal–Wallis test and by the Mann–Whitney *U*-test between the two groups. Fisher's exact test was used for dichotomous variables. Correlations between lipid classes were analysed by Pearson's correlation coefficient (*r*) and by Spearman's rank correlation coefficient (ρ) for analysis including only the exclusively breastfed infants. All statistical analyses were performed on SPSS for Windows (version 12.0.1; SPSS Inc., Chicago, IL, USA).

Results

Association of maternal diet and allergic disease with fatty acid compositions

Mothers' (*n* = 30) mean ($\pm$ standard deviation (SD)) daily intake of energy was 8.5 ($\pm$ 1.5) MJ, protein 85.1 ($\pm$ 18.3) g, carbohydrates 246 ($\pm$ 57) g, fat 74.8 ($\pm$ 19.4) g, saturated fatty acids (SFA) 28.3 ($\pm$ 8.3) g, monounsaturated fatty acids (MUFA) 25.8 ($\pm$ 7.5) g and polyunsaturated fatty acids (PUFA) 13.9 ($\pm$ 5.3) g. There were no differences in the intakes of energy and energy-yielding nutrients among the three groups (all NS, Kruskal–Wallis test, data not shown). The maternal intake of PUFA correlated with linoleic acid (LA, 18:2n-6 r = 0.392, P = 0.039), sum of n-6

fatty acids ($r=0.392$, $P=0.039$) and sum of PUFA ($r=0.425$, $P=0.024$) in breast milk. Intake of SAFA correlated inversely with breast milk AA ($r=-0.432$, $P=0.022$) and sum of n-6 fatty acids (-0.394 , $P=0.038$).

Both the serum and cheek cell phospholipid eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) were higher in infants whose mothers had consumed fish during the 2-week period prior to sample collection (83% of the mothers) compared with those whose mothers had not consumed fish (Fig. 1). The proportion of breast milk total lipid DHA was higher in mothers who had consumed fish compared with those who had not, but no difference was observed in the proportion of EPA. The proportion of mothers consuming fish was not different between controls and infants with eczema ($P=0.682$).

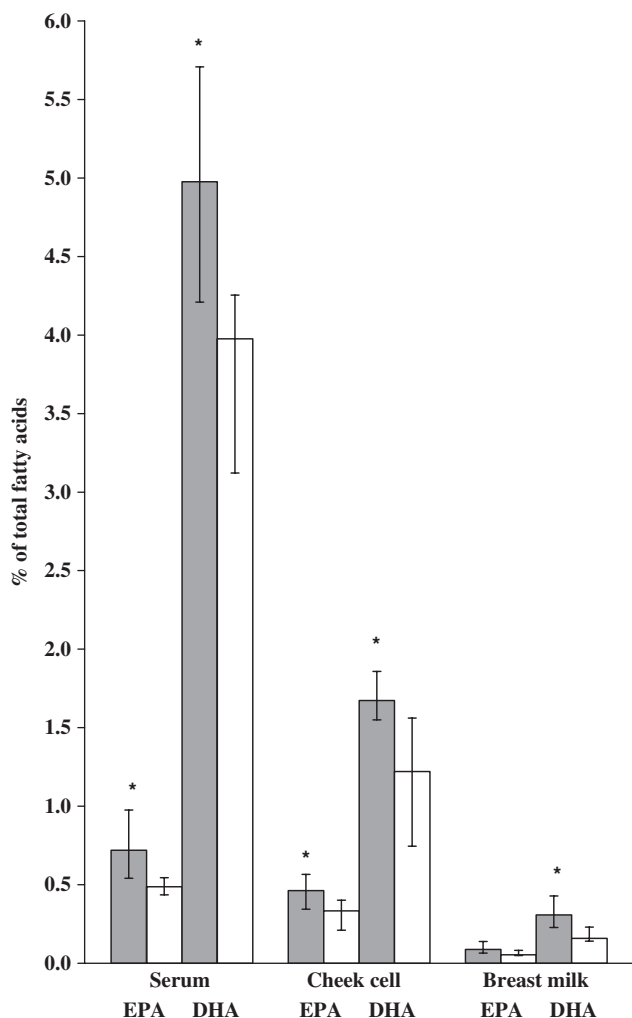


Fig. 1. Proportions of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of total fatty acids in serum and cheek cell phospholipids from infants whose mothers had consumed fish (■, $n=24$), compared with those who had not (□, $n=5$) and in breast milk total lipids according to mothers' fish consumption. The bars represent median and the error bars interquartile range. Significant difference between the groups. * $P < 0.05$, Mann-Whitney U-test.

Breast milk dihomo- γ -linolenic acid (DHGLA, 20:3n-6) (0.28 (0.25–0.34)%) and the ratio of n-6 to n-3 fatty acids (5.25 (4.59–6.02)%) were higher in mothers with allergic disease compared with mothers without allergic disease (DHGLA 0.24 (0.19–0.27)%, $P=0.033$; ratio of n-6 to n-3 fatty acids 4.54 (4.04–4.84)%, $P=0.020$). Cheek cell phospholipid AA (3.55 (3.00–4.05)%) and sum of PUFA (19.99 (18.54–21.10)%) were lower in infants whose mothers had allergic disease ($n=18$) compared with those infants whose mothers were healthy ($n=12$; AA 3.94 (3.63–4.67)%, $P=0.035$; sum of PUFA 21.37 (20.34–22.53)%, $P=0.043$). No differences were found in infants' serum phospholipid fatty acid composition according to maternal allergic disease status.

Correlations between cheek cell and serum phospholipid and breast milk total lipid fatty acid compositions

The proportions of cheek cell phospholipid α -linolenic acid (ALA, 18:3n-3), EPA, DHA, DHGLA and AA as well as the sum of n-3 fatty acids and ratio of LA/ALA and ratio of n-6 to n-3 fatty acids correlated with those of serum phospholipid fatty acids (Table 2). Equal significant correlations were found for breast milk and serum phospholipid fatty acid compositions, except that no correlation was observed for ALA and the ratio of n-6 to n-3 fatty acids and a significant correlation was found for the ratio of AA/EPA. The cheek cell phospholipid ALA, EPA, DHA, 20:2n-6, sum of n-3 fatty acids and ratio of AA/EPA correlated with those of breast milk fatty acids.

Fatty acid compositions among serum and cheek cell phospholipids and breast milk total lipids in infants with atopic and non-atopic eczema and controls

The proportion of serum phospholipid γ -linolenic acid (GLA, 18:3n-6) was lower in both atopic and non-atopic eczema subjects compared with controls, and additionally EPA was higher in atopic eczema but not in non-atopic eczema compared with controls (Table 3). Cheek cell phospholipids LA, sum of n-6 fatty acids and sum of PUFA were higher in infants with atopic eczema compared with controls and compared with infants with non-atopic eczema.

Cheek cell phospholipid 22:1n-9 was lower in infants with non-atopic eczema (0.14, 0.10–0.22) compared with controls (0.29, 0.23–0.44; $P=0.003$), but comparable with that in infants with atopic eczema (0.22, 0.11–0.40, $P=0.394$; Kruskal-Wallis test, $P=0.031$). No differences were found among the groups in the compositions of other MUFA or SFA in serum or cheek cell phospholipid (data not shown) or breast milk total lipid fatty acid compositions (Table 3.)

Table 2. Correlations between breast milk total lipid and cheek cell and serum phospholipid fatty acid compositions (FA)*

Fatty acid	Cheek cell FA with serum FA (<i>n</i> = 18)		Cheek cell FA with breast milk FA (<i>n</i> = 18)		Breast milk FA with serum FA (<i>n</i> = 17)	
	<i>R</i> [†]	<i>P</i>	<i>R</i> [†]	<i>P</i>	<i>R</i> [†]	<i>P</i>
Total saturated	−0.05	NS	−0.16	NS	0.05	NS
Total monounsaturated	0.42	NS	−0.12	NS	0.26	NS
18:3n-3	0.55	0.01	0.60	0.008	0.27	NS
20:5n-3	0.56	0.01	0.53	0.02	0.87	< 0.01
22:6n-3	0.72	0.001	0.49	0.04	0.75	0.001
Sum n-3	0.78	< 0.001	0.52	0.02	0.72	0.001
18:2n-6	0.25	NS	−0.40	NS	−0.12	NS
18:3n-6	−0.41	NS	0.02	NS	−0.16	NS
20:2n-6	0.01	NS	−0.49	0.04	0.20	NS
20:3n-6	0.49	0.03	0.25	NS	0.73	0.001
20:4n-6	0.61	0.007	0.02	NS	0.60	0.01
Sum n-6	−0.11	NS	−0.43	NS	0.04	NS
Total polyunsaturated	−0.08	NS	−0.37	NS	0.34	NS
18:2n-6/18:3n-3	0.60	0.01	0.36	NS	0.67	0.003
20:4n-6/20:5n-3	0.42	NS	0.56	0.02	0.78	0.001
Sum n-6/sum n-3	0.86	< 0.001	0.39	NS	0.48	NS

*Only control infants are included in the analysis. [†]Pearson's correlation coefficient.

Discussion

Fatty acids are evidently important immune regulators, but their significance in allergic disease remains to be elucidated. We have provided preliminary evidence of a discrepancy in the fatty acid composition of infants with atopic as opposed to non-atopic eczema and healthy infants by applying cheek cell fatty acid composition, thus offering a tool for larger studies.

The cheek cell fatty acid composition may provide a particularly useful marker to reflect current dietary fatty acid composition. Although some studies have observed differences in the fatty acid composition of breast milk received by infants with allergic disease compared with controls [9, 20], also opposing results [11], supported by those in the present study, have also been reported. This may reflect a variability in breast milk composition depending on factors such as the duration or stage of feeding and the method of expression, although variability may be reduced by collecting samples in a systematic manner and presenting data as proportional fatty acid composition rather than concentrations, as was done in our study. Cheek cells are available non-invasively and they have a rapid turnover rate, which may better reflect the infant's current diet [12–14] than serum fatty acid composition, even if the fatty acid analysis involved considerable effort because of the small cell material obtainable from the infants. The additional advantage of using cheek cells is that they may be used to reflect the site of mucosal immunity, the first line of protection in the gastrointestinal tract [15], wherein fatty acids may contribute [21].

The most prevalent explanation for the altered fatty acid composition in allergic disease is an impaired capacity to metabolize n-6 PUFA and a deficient δ -6-desaturase activity, which view has not, however, received consistent support [10, 22]. Our results on high cheek cell LA and sum of n-6 fatty acids in atopic eczema compared with controls might by the same token support the conception whereby atopic patients evince elevated IgE synthesis deriving ultimately from preponderant n-6 fatty acids. Also, similar to Biagi *et al.* [23], low serum GLA was observed in both types of eczema. However, the high serum EPA in atopic eczema compared with controls observed in the present study, in accord with reports on allergic asthma [24], but not eczema [22, 23] and the absence of differences among the groups in the ratios of the product to precursor fatty acids or n-6 to n-3 fatty acids, appear to challenge this theory. Another explanation may be that there are differences in the incorporation of PUFA into cell membranes.

In harmony with our finding of elevated EPA in atopic eczema, plasma EPA has been seen to correlate with IgE concentration [24]. This and recent clinical observations have provided evidence for the possible deleterious effects of n-3 fatty acids in allergic disease, as total n-3 fatty acids, docosapentaenoic acid and DHA were higher in colostrum received by infants sensitized to foods at 6 months [25] and the risk of asthma was increased in relation to increased dietary intake of EPA and DHA and alternatively reduced in relation to erythrocyte membrane LA levels [26]. The possible adverse effects of n-3 fatty acids may be explained by their direct effect on T helper 1 cell activation because of the suppression of IL-2 and by

Table 3. Fatty acid composition of serum and cheek cell phospholipids and breast milk total lipids in infants with atopic and non-atopic eczema and control infants (% of total fatty acids)

	Atopic eczema (<i>n</i> = 6)		Non-atopic eczema (<i>n</i> = 6)		Controls (<i>n</i> = 19*)		
Fatty acid	Median	IQR	Median	IQR	Median	IQR	<i>P</i> -value [†]
Cheek cell phospholipids							
Total saturated	36.41	35.51–37.27	35.66	35.27–42.11	38.08	36.12–39.19	NS
Total monounsaturated	41.51	39.78–42.54	43.26	39.17–45.42	41.78	40.11–43.64	NS
18:3n-3	0.15	0.10–0.18	0.11	0.06–0.16	0.11	0.09–0.15	NS
20:5n-3	0.54	0.46–0.74	0.39	0.27–0.59	0.39	0.33–0.49	NS
22:6n-3	1.78	1.56–1.89	1.57	1.17–1.72	1.65	1.33–1.79	NS
Sum n-3	2.53	2.23–3.04	2.21	1.52–2.41	2.10	1.81–2.37	NS
18:2n-6	14.69	13.67–15.53 ^{‡,§}	12.69	10.87–13.93	12.50	12.16–13.42	0.02
18:3n-6	0.06	0.03–0.11	0.07	0.04–0.08	0.09	0.08–0.12	NS
20:2n-6	0.21	0.15–0.33	0.30	0.16–0.71	0.23	0.20–0.32	NS
20:3n-6	0.96	0.85–1.10	1.01	0.92–1.20	1.15	0.92–1.48	NS
20:4n-6	3.94	3.63–4.31	3.43	3.00–3.77	3.69	3.16–4.15	NS
Sum-6	19.94	19.06–20.53 ^{‡,§}	17.72	15.63–18.91	18.19	17.43–18.70	0.02
Total polyunsaturated	22.70	21.31–23.28 ^{‡,§}	19.90	17.64–21.06	20.32	19.32–21.03	0.03
Sum n-6/sum n-3	7.87	6.89–8.81	8.60	6.86–11.39	8.55	7.59–9.70	NS
Serum phospholipids							
Total saturated	46.53	45.99–47.14	46.91	46.41–47.27	46.58	46.41–46.97	NS
Total monounsaturated	14.02	13.30–14.62	14.40	13.76–15.77	14.73	14.41–16.17	NS
18:3n-3	0.22	0.14–0.26	0.19	0.15–0.22	0.19	0.17–0.22	NS
20:3n-3	0.13	0.10–0.16	0.12	0.07–0.16	0.11	0.09–0.14	NS
20:5n-3	0.90	0.76–1.18 [‡]	0.71	0.47–1.10	0.56	0.50–0.68	0.04
22:6n-3	5.62	5.04–6.03	4.61	3.94–5.87	4.57	3.97–5.30	NS
Sum n-3	6.86	6.43–7.31	5.53	4.72–7.38	5.50	4.58–6.06	NS
18:2n-6	20.98	19.37–21.73	19.43	17.20–21.54	20.04	18.66–22.23	NS
18:3n-6	0.02	0–0.03 [‡]	0.01	0–0.03 [‡]	0.04	0.03–0.06	0.006
20:2n-6	0.37	0.34–0.38	0.41	0.36–0.45	0.37	0.33–0.43	NS
20:3n-6	2.30	1.96–2.43	2.97	2.45–3.26	2.84	2.11–3.36	NS
20:4n-6	9.57	8.61–9.80	9.28	8.25–11.68	8.90	7.73–10.45	NS
Sum-6	32.79	31.63–33.55	32.78	31.76–33.55	32.62	31.61–33.23	NS
Total polyunsaturated	39.16	38.53–40.51	38.59	37.91–39.37	38.22	37.39–38.85	NS
Sum n-6/sum n-3	4.79	4.50–5.11	6.02	4.33–7.11	5.95	5.41–7.18	NS
Breast milk lipids							
Total saturated	43.73	42.60–47.25	43.35	40.54–42.67	44.73	40.05–49.27	NS
Total monounsaturated	41.60	40.40–42.85	42.92	40.09–46.23	42.90	38.63–44.36	NS
18:3n-3	2.16	1.24–2.56	1.88	1.25–2.25	1.70	1.31–2.14	NS
20:3n-3	0.06	0–0.12	0.04	0–0.08	0.06	0.05–0.07	NS
20:5n-3	0.12	0.10–0.17	0.09	0.05–0.13	0.06	0.05–0.10	NS
22:6n-3	0.36	0.31–0.52	0.23	0.19–0.34	0.25	0.19–0.42	NS
Sum n-3	2.58	1.83–3.24	2.31	1.51–2.73	2.01	1.70–2.69	NS
18:2n-6	10.61	8.74–11.73	10.72	8.89–11.68	9.43	8.40–11.04	NS
18:3n-6	0.09	0.07–0.09	0.08	0.07–0.09	0.07	0.05–0.10	NS
20:2n-6	0.19	0.16–0.23	0.20	0.17–0.23	0.20	0.18–0.23	NS
20:3n-6	0.25	0.20–0.27	0.27	0.22–0.29	0.26	0.20–0.33	NS
20:4n-6	0.32	0.30–0.36	0.29	0.27–0.31	0.32	0.27–0.37	NS
Sum-6	11.53	9.51–12.60	11.61	9.68–12.53	10.34	9.44–11.97	NS
Total polyunsaturated	13.55	11.62–15.84	14.07	11.08–15.05	12.75	11.19–15.38	NS
Sum n-6/sum n-3	4.26	3.69–5.69	5.11	4.61–6.31	4.76	4.50–5.75	NS

*One serum sample is missing from the control group because of difficulty in sample withdrawal. [†]Kruskal–Wallis test. [‡]Different from controls and [§]from infants with non-atopic eczema, *P* < 0.05, Mann–Whitney *U*-test.

indirect suppression of T helper 1 cells by the enhanced cross-regulatory function of T helper 2 cells observed in mice [27]. Alternatively, the effects of EPA may ensue via its regulatory role vis-à-vis n-6 fatty acids by means of inhibiting n-6 PUFA-derived eicosanoid production [28]. This effect of EPA would be particularly unfavourable in allergic disease, as both GLA and AA induce the activity of T regulatory cells [29].

Another explanation for the altered fatty acid composition in allergic disease may be simply the resulting immune responses after the synthesis of eicosanoids [30]. One explanation for the low serum GLA in both types of eczema compared with controls in the present study may be its rapid metabolism to its successor fatty acids and an attempt at compensatory production of n-6 PUFA-derived eicosanoids. n-6 fatty acids may exhibit a beneficial role in allergic disease via the effects of T helper 3 or T regulatory cells and thus via anti-inflammatory transforming growth factor- β_2 and IL-10 production [21].

Whether the determining characteristic of fatty acids in allergic disease is their quantity or relative amount remains to be elucidated. In the rat, the balance between n-6 and n-3 fatty acids in the maternal diet has been shown to influence the ontogeny of the intestine in the offspring [31], and recently, the induction of immunological tolerance to ovalbumin in the offspring [32], which highlights the importance of the dietary fatty acid balance. This also calls in question the use of artificial supplementation of high doses of fatty acids with possible deleterious (or ineffective) influences in allergic disease. Modification of the maternal diet instead may provide a safe and moreover a natural mode of nourishment [33], which we will further evaluate in the ongoing study. Such a view is supported by our results showing differences in fatty acid composition according to mothers' habitual fish consumption (not supplementation) and associations between maternal diet and breast milk or infant fatty acid compositions, and a previous study showing an association between the lactating mother's dietary fatty acid composition and atopic sensitization of the infant [34]. Allergic disease in the mother imposes a further risk of allergic disease in the infant by influencing the fatty acid composition of the breast milk as observed in the present and previous studies [35], which, however, we were previously not able to observe, possibly because of a less precise definition of allergic disease, which did not take into account IgE concentration or SPT reactivity [30].

The present study highlights differences in fatty acid composition between the two types of eczema and in comparison with controls. This may result from different immunological processes regulating the onset of the atopic and non-atopic types of eczema, in the former of which fatty acids may have a more profound role. The importance of understanding the differences between the two types of eczema is thus emphasized. However, being

the earliest of allergic conditions, eczema may act as a portal for the development of IgE-mediated atopic manifestations and children with eczema who are initially negative to skin prick testing may become positive [36]. We intend to follow-up these children in the ongoing mother-infant study and to further explore the issue as there is a risk of underestimating the differences because of small number of subjects in each group.

As the differences in fatty acid composition were observed at an early age, maternal dietary modification commencing during pregnancy, complemented with infant dietary modification, may provide a tool for both risk reduction and the prevention of disease progression. The route for the first allergic responses arises from the gastrointestinal tract and thus early modification of the diet towards a balanced intake of nutrients and taking into account interactions among nutrients and microbes [33] may offer a preventive tool in addressing the increasing problem of eczema.

Acknowledgements

We thank Ms Ulla-Maija Eriksson and Johanna Hvitfelt-Koskelainen for their contribution towards establishing contacts with study subjects, and Mr Robert MacGilleon for revising the English text. We are grateful for the financial support provided by the Academy of Finland and the Juho Vainio Foundation.

References

- 1 Williams HC, Robertson C, Stewart A *et al*. Worldwide variations in the prevalence of symptoms of atopic eczema in the international study of asthma and allergies in childhood. *J Allergy Clin Immunol* 1999; 103:25–38.
- 2 Zeiger RS. Food allergen avoidance in the prevention of food allergy in infants and children. *Pediatrics* 2003; 111:1662–71.
- 3 Bolte G, Frye C, Hoelscher B, Meyer I, Wjst M, Heinrich J. Margarine consumption and allergy in children. *Am J Respir Crit Care Med* 2001; 163:277–9.
- 4 Nafstad P, Nystad W, Magnus P, Jaakkola JJK. Asthma and allergic rhinitis at 4 years of age in relation to fish consumption in infancy. *J Asthma* 2003; 40:343–8.
- 5 Black PN, Sharpe S. Dietary fat and asthma: is there a connection? *Eur Respir J* 1997; 10:6–12.
- 6 Dunstan JA, Mori TA, Barden A *et al*. Fish oil supplementation in pregnancy modifies neonatal allergen-specific immune responses and clinical outcomes in infants at high risk of atopy: a randomized, controlled trial. *J Allergy Clin Immunol* 2003; 112:1178–84.
- 7 Takwale A, Tan E, Agarwal S *et al*. Efficacy and tolerability of borage oil in adults and children with atopic eczema: randomised, double blind, placebo controlled, parallel group trial. *BMJ* 2003; 327:1385.
- 8 Johansson SGO, Bieber T, Dahl R *et al*. Revised nomenclature for allergy for global use: report of the nomenclature review

- committee of the world allergy organization, October 2003. *J Allergy Clin Immunol* 2004; 113:832–6.
- 9 Duchén K, Casas R, Fagerås-Böttcher M, Yu G, Björkstén B. Human milk polyunsaturated long-chain fatty acids and secretory immunoglobulin a antibodies and early childhood allergy. *Pediatr Allergy Immunol* 2000; 11:29–39.
 - 10 Newson RB, Shaheen SO, the ALSPAC Study Team *et al.* Umbilical cord and maternal blood red cell fatty acids and early childhood wheezing and eczema. *J Allergy Clin Immunol* 2004; 114:531–7.
 - 11 Reichardt P, Muller D, Posselt U *et al.* Fatty acids in colostrum from mothers of children at high risk of atopy in relation to clinical and laboratory signs of allergy in the first year of life. *Allergy* 2004; 59:394–400.
 - 12 McMurchie EJ, Margetts BM, Beilin LJ, Croft KD, Vandongen R, Armstrong BK. Dietary-induced changes in the fatty acid composition of human cheek cell phospholipids: correlation with changes in the dietary polyunsaturated/saturated fat ratio. *Am J Clin Nutr* 1984; 39:975–80.
 - 13 Hoffman DR, Birch EE, Birch DG, Uauy R. Fatty acid profile of buccal cheek cell phospholipids as an index for dietary intake of docosahexaenoic acid in preterm infants. *Lipids* 1999; 34:337–42.
 - 14 Connor SL, Zhu N, Anderson GJ *et al.* Cheek cell phospholipids in human infants: a marker of docosahexaenoic and arachidonic acids in the diet, plasma, and red blood cells. *Am J Clin Nutr* 2000; 71:21–7.
 - 15 Cummings JH, Antoine J-M, Azpiroz F *et al.* PASSCLAIM – gut health and immunity. *Eur J Nutr* 2004; 43 (Suppl.):118–73.
 - 16 Hanifin JM. Atopic dermatitis in infants and children. *Pediatr Clin North Am* 1991; 38:763–89.
 - 17 Kalliomäki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised, placebo-controlled trial. *Lancet* 2001; 357:1076–9.
 - 18 Folch J, Lees M, Stanley GHS. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 1957; 226:497–509.
 - 19 Hamilton JG, Comai K. Rapid separation of neutral lipids, free fatty acids and polar lipids using prepacked silica Sep-Pak columns. *Lipids* 1988; 23:1146–9.
 - 20 Kankaanpää P, Nurmela K, Erkkilä A, Kalliomäki M, Holmberg-Marttila D, Salminen S. Polyunsaturated fatty acids in maternal diet, breast milk and serum lipid fatty acids of infants with relation to atopy. *Allergy* 2001; 56:633–8.
 - 21 Newberry RD, Stenson WF, Lorenz RG. Cyclooxygenase-2-dependent arachidonic acid metabolites are essential modulators of the intestinal immune response to dietary antigen. *Nat Med* 1999; 5:900–6.
 - 22 Yu G, Björkstén B. Polyunsaturated fatty acids in school children in relation to allergy and serum IgE levels. *Pediatr Allergy Immunol* 1998; 9:133–8.
 - 23 Biagi PL, Hrelia S, Celadon M *et al.* Erythrocyte membrane fatty acid composition in children with atopic dermatitis compared to age-matched controls. *Acta Paediatr* 1993; 82:789–90.
 - 24 Griesse M, Schur N, Laryea MD, Bremer HJ, Reinhardt D, Biggemann B. Fatty acid composition of phospholipids of plasma and of mononuclear blood cells in children with allergic asthma and the influence of glucocorticoids. *Eur J Pediatr* 1990; 149: 508–12.
 - 25 Stoney RM, Woods RK, Hosking CS, Hill DJ, Abramson MJ, Thien FCK. Maternal breast milk long-chain n-3 fatty acids are associated with increased risk of atopy in breastfed infants. *Clin Exp Allergy* 2004; 34:194–200.
 - 26 Broadbied EC, McKeever TM, Whitehurst A *et al.* A case-control study of dietary and erythrocyte membrane fatty acids in asthma. *Clin Exp Allergy* 2004; 34:1232–36.
 - 27 Arrington JL, Chapkin RS, Switzer KC, Morris JS, McMurray DN. Dietary n-3 polyunsaturated fatty acids modulate purified murine T-cell subset activation. *Clin Exp Allergy* 2001; 125:499–507.
 - 28 Lee TH, Hoover RL, Williams JD *et al.* Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. *N Engl J Med* 1985; 312:1217–24.
 - 29 Harbige LS. Fatty acids, the immune response, and autoimmunity: a question of n-6 essentiality and the balance between n-6 and n-3. *Lipids* 2003; 38:323–41.
 - 30 Laiho K, Lampi A-M, Moilanen E, Arvola T, Ruuska T, Isolauri E. Breast milk fatty acids, eicosanoids and cytokines in mothers with and without allergic disease. *Pediatr Res* 2003; 53: 642–7.
 - 31 Jarocka-Cyrta E, Perin N, Keelan M *et al.* Early dietary experience influences ontogeny of intestine in response to dietary lipid changes in later life. *Am J Physiol* 1998; 275:G250–8.
 - 32 Korotkova M, Telemo E, Yamashiro Y, Hanson LÅ, Strandvik B. The ratio of n-6 to n-3 fatty acids in maternal diet influences the induction of neonatal immunological tolerance to ovalbumin. *Clin Exp Immunol* 2004; 137:237–44.
 - 33 Laiho K, Hoppu U, Ouwehand A, Salminen S, Isolauri E. Probiotics- on-going research on atopic individuals. *Br J Nutr* 2002; 88 (Suppl.):19–27.
 - 34 Hoppu U, Kalliomäki M, Isolauri E. Maternal diet rich in saturated fat during breastfeeding is associated with atopic sensitization of the infant. *Eur J Clin Nutr* 2000; 54:702–5.
 - 35 Yu G, Duchén K, Björkstén B. Fatty acid composition in colostrum and mature milk from non-atopic and atopic mothers during the first 6 months of lactation. *Acta Paediatr* 1998; 87:729–36.
 - 36 Novembre E, Cianferoni A, Lombardi E, Bernardini R, Pucci N, Vierucci A. Natural history of 'intrinsic' atopic dermatitis. *Allergy* 2001; 56:452–63.