

Atopy, eczema and breast milk fatty acids in a high-risk cohort of children followed from birth to 5 yr

Oddy WH, Pal S, Kusel MMH, Vine D, de Klerk NH, Hartmann P, Holt PG, Sly PD, Burton PR, Stanley FJ, Landau LI. Atopy, eczema and breast milk fatty acids in a high-risk cohort of children followed from birth to 5 yr.

Pediatr Allergy Immunol 2005. © 2005 Blackwell Munksgaard

Background: The incidence of atopic diseases such as eczema is increasing in westernized societies. The suggestion that there is a 'protective' association between the unique fatty acid composition of breast milk, particularly the omega-3 (n-3) and omega-6 (n-6) essential polyunsaturated fatty acid content, and the development of atopic disease in children was investigated in a cohort study of 263 infants born into families with a history of allergy (one or both parents had asthma, hayfever, eczema). The objectives of this study were to determine the lipid profile [specifically in relation to long-chain polyunsaturated fatty acid (LC-PUFA) composition] in maternal breast milk samples collected at 6 wk and at 6 months following birth, and to investigate the potential role of these fatty acids in modulating the phenotype of children at high genetic risk of developing atopic disease. **Method:** Breast milk samples were available from 91 atopic mothers at their child's ages of 6 wk and 6 months. These samples were analysed for the fatty acid spectrum. Analysis of variance was used to detect differences between groups of outcomes (no atopy or eczema, non-atopic eczema, atopy, atopic eczema) at ages 6 months and 5 yr, and a multiple comparisons procedure was conducted to isolate the parameters producing the different results (*F*-test, LSD test). For the exposure variables, n-3 and n-6 fatty acids are expressed as weight percentage and as a ratio (at both time-points). **Results:** The fatty acid profiles of maternal breast milk at 6 wk and 6 months were similar. An increased ratio of n-6: n-3 fatty acids in both 6 wk and 6 month milk samples was associated with non-atopic eczema ($p < 0.005$) but not atopy alone or atopic eczema. **Conclusion:** We found milk fatty acids were a significant modulator of non-atopic eczema but not atopy or atopic eczema in infants at 6 months. In mothers with a history of asthma, hayfever or eczema, their 6-month-old infants were more likely to develop non-atopic eczema if their milk had a higher ratio of n-6: n-3 LC-PUFA.

Wendy H. Oddy^{1,2}, Sebely Pal², Merci M. H. Kusel¹, Donna Vine², Nicholas H. de Klerk¹, Peter Hartmann³, Patrick G. Holt¹, Peter D. Sly¹, Paul R. Burton⁴, Fiona J. Stanley¹ and Louis I. Landau⁴

¹Telethon Institute for Child Health Research, Centre for Child Health Research, University of Western Australia, PO Box 855, West Perth, Perth, WA, Australia, ²School of Public Health, Curtin University of Technology, Perth, WA, Australia, ³Department of Biochemistry, University of Western Australia, Nedlands, Perth, WA, Australia, ⁴Department of Health Sciences and Institute of Genetics, University of Leicester, West Leicester, UK, ⁵Dean, Faculty of Medicine and Dentistry, University of Western Australia, Nedlands, Perth, WA, Australia

Key words: atopy; eczema; breast milk; fatty acids; children

Dr W.H. Oddy, Telethon Institute for Child Health Research, PO Box 855, West Perth, WA 6872, Australia
Tel.: +61 8 9489 7879
Fax: +61 8 9489 7700
E-mail: wendyo@icmr.uwa.edu.au

Accepted 10 September 2005

Asthma, hayfever, atopic eczema, food allergy and other related allergic syndromes (collectively known as the 'atopic diseases') are amongst the most common chronic conditions in the developed world and there is accumulating epidemiological evidence that the prevalence and severity of atopic disease is rising (1).

Early work raised the possibility that breastfeeding may reduce allergic manifestations in high-risk individuals (2). Prospective studies have often reported a prophylactic effect of breastfeeding in the high-risk groups (3). A Finnish study (4) that followed infants from birth to 17 yr showed that breastfeeding as preventive

against atopic diseases – including atopic eczema, food allergy and respiratory allergy throughout childhood and adolescence. However, the association is not conclusive. Some studies have not shown any benefit from breastfeeding (5) whilst others suggest breastfeeding may increase the risk of asthma in later life (6).

Some prospective studies show disturbances in the fatty acid composition between milk from atopic and non-atopic mothers with the composition of n-3 fatty acids related to atopic development in the children (7, 8). One study found no evidence that the fatty acid level of breast milk is likely to be responsible for the differential effects of breastfeeding by atopic and non-atopic mothers (9), and another study showed that higher n-3 levels in colostrum may be a risk factor for atopy (10). However, n-3 fatty acids may exert their effects by modulating signal transduction and/or gene expression within inflammatory and immune cells (11, 12). Infantile eczema is less likely to be associated with atopy (13), presenting in the first few months of life and resolving by 2–3 yr of age.

We hypothesized that the fatty acid composition of maternal breast milk was an important modulator of the atopy-associated inflammatory response in a birth cohort of high-risk breastfed infants at 6 months of age followed to 5 yr. The objectives of the study reported here were to measure the fatty acid composition of maternal breast milk samples collected from the cohort mothers at the 6 wk and 6 month checks following birth, and to determine if there were any associations with atopic status in the infants at 6 months and 5 yr as measured by doctor diagnosed eczema and skin prick test response.

Methods

The Childhood Asthma Study (CAS) commenced in 1996 and is based at the Telethon Institute for Child Health Research in Perth, Western Australia. It is a prospective birth cohort study of 263 infants at high risk of atopy. The CAS was established to recruit high-risk infants whose families had a history of atopy (one or both parents had asthma, hayfever or eczema); 93% of mothers had asthma, eczema or hayfever ($n = 84/91$). If the mother did not have any of the defined conditions, the father was selected to have one of the conditions mentioned. Therefore, each child in the study (100% of children) had a family history of asthma, hayfever, allergy or eczema.

Mothers attended the research centre at 6 wk and 6 months after birth for comprehensive

physical testing by the study doctor (MK), skin testing, questionnaire completion and breast milk collection. Annual checks were conducted to 5 yr of age on a variety of atopic endpoints as well as factors that may be on the aetiological pathway of atopy. The Ethics Committee at Princess Margaret Hospital for Children approved the study.

Breast milk collection

Comprehensive questionnaires on infant feeding methods were administered at the 6 wk and 6 month check-up after birth. At these check-ups breast milk samples were collected from each mother by the study doctor (two 1 ml samples of breast milk, one from each breast). Breast milk samples were obtained from 91 mothers at both 6 wk and 6 months. The samples were stored at -20°C for subsequent fatty acid analyses.

Total fatty acid determination

We used established, conventional methods (14, 15) in determining fatty acid composition. Lipid extraction and fatty acid analyses were conducted as described by Mitoulas et al. (16). Fatty acids (n-3, n-6) were presented as mean values of fatty acid composition (weight per cent of total fatty acids – wt%) of the total lipid content of the milk sample.

Atopy

Skin Prick testing was conducted at 6 months and 5 yr on all study children to determine atopic status. Testing was conducted for Alternaria, house dust mite, cat, ryegrass, Aspergillus, milk and egg. Histamine 10 mg/ml was used as a positive control and normal saline as the negative control. After 15 min the contours of the wheals were encircled by means of a fine filter tip pen. Transparent tape was placed on the site of the drawings and then transferred to a registration sheet. A positive test was a wheal size of 2 mm or greater at 6 months, and 3 mm or greater at 5 yr. The clinical significance of the positive skin prick tests was to define atopic status. No elimination or challenge tests were performed.

Eczema

Eczema was defined as an itchy, dry rash on the face, arms or legs and was confirmed by the study doctor (MK) at the time of the physical assessment, and following criteria previously described (17). The mother of the infant was asked at the

6 month check and on annual checks if their child had dry skin, or an itchy skin condition at the moment, or whether their child had ever had a skin condition which occurred in his/her skin creases (by skin creases we meant in front of the elbows, behind the knees, the fronts of the ankles, around the neck, or around the ears or eyes). The children in the study were seen at regular intervals at 6 wk, 6 months, 12 months, and then annually. Because low-grade sensitization is a normal phenomenon in early infancy, the definition of eczema required that three or more of the criteria were fulfilled by the study doctor (MK) at each interval.

Non-atopic and atopic eczema

A child diagnosed with eczema (as defined above) but who was not atopic was considered to have 'non-atopic eczema'. Children with both eczema (as defined above) and a positive skin prick test were considered to have atopic eczema. This was defined at 6 months and at 5 yr.

Statistical analysis

The general approach we took was first to perform an analysis of variance (multivariate ANOVA) to detect differences between groups of outcomes, and then use a multiple comparisons procedure (unpaired *t*-test) to explore the parameters producing the different results. For each outcome in ANOVA, mean, standard error of the mean (s.e.m.), *F*-test, least significant difference (LSD) test and *p*-values are given. The *F*-test detects differences between group means and is appropriate for any differences between means which arise naturally out of the structure of the investigation. The LSD test detects differences between pairs of means which are significant at the 5% level. For milk fatty acids the exposure variables, n-3 and n-6 fatty acids are expressed as weight% of the total fatty acids and as a ratio of n-6 to n-3 (from 6 wk and 6 month milk samples). Independent-sample *t*-tests were used to compare the maternal milk fatty acid means for infants with either no eczema or eczema at 6 months, for infants with positive atopic status or negative atopic status and for atopic eczema at 6 months and 5 yr. For milk fatty acids, comparisons between the two groups identified as different (eczema, no atopy or eczema) were performed with an unpaired *t*-test and mean, s.e.m., difference and *p*-value given. The correlations between milk fatty acid and atopic status were also measured. The level of significance was $p < 0.05$. Statistical analyses were performed

using the SPSS for Windows program (Version 11.0; SPSS, Inc., Chicago, IL, USA).

Results

Of the original 263 infants in the study, complete milk samples (at 6 wk and 6 months) and infant outcome data were available from 91 mother–infant pairs. Of the parents 93% of mothers and 86% of fathers had some form of atopy (asthma, eczema or hayfever). All children had one or both parents with atopy. All infants were breastfed from birth but 39% (36/91) received some infant formula within the first week after birth. By 6 months all were still breastfeeding even if they had received formula at any other time. The children in the cohort received other foods, with 6% receiving other foods before 4 months, 79% at 4 months, 13% at 5 or 6 months and 2% after 6 months. At 6 months 48% of the infants had been diagnosed with eczema by a doctor and 30% (13/44) of those children had a positive skin prick test. By 5 yr 41% of children had any eczema and 30% of these were atopic. Maternal and child characteristics are shown in Table 1.

At 6 wk there were more differences in the milk fatty acid content compared with that at

Table 1. Maternal and infant characteristics

	% (n = 91)
Outcomes at 6 months	
No atopy or eczema	38.5 (35)
Non-atopic eczema	34.1 (31)
Atopy (positive skin prick test one or more)*	13.2 (12)
Atopic eczema (Atopy and eczema)	14.3 (13)
Outcomes at 5 yr	
No atopy or eczema	57.1 (52)
Non-atopic eczema	15.4 (14)
Atopy (positive skin prick test one or more)*	4.4 (4)
Atopic eczema (Atopy and eczema)	23.1 (21)
Atopy at 6 months	
Egg	18.5 (17)
Cow's milk	12.0 (11)
House dust mite	6.5 (6)
Aspergillus	4.4 (4)
Cat	5.4 (5)
Rye grass	3.3 (3)
Alternaria	2.2 (2)
Child characteristics	
Maternal asthma, atopic eczema or hayfever (%)	93.3 (84)
Paternal asthma, atopic eczema or hayfever (%)	85.7 (78)
Gender of infant (% male)	71.4 (65)
Maternal smoking (%)	2.2 (2)
Mean gestational age at birth (mean weeks $\times$ s.d.)	39.00 $\pm$ 1.51
Birth weight of infant (mean kg $\pm$ s.d.)	3.43 $\pm$ 0.44
Exposure variables	
Still breastfeeding 6 months – also given formula	100 (91)

*Atopy is identified alone, without eczema.

Table 2. Analysis of variance comparing weight% of n-3 and n-6 fatty acids and the ratio of n-6: n-3 fatty acids from milk samples (6 wk and 6 months) and outcomes (no atopy or eczema, non-atopic eczema, atopic, atopic eczema) at 6 months and 5 yr

Fatty acids from milk sample (SE)	Outcome at 6 months (n = 91)						Outcome at 5 yr (n = 91)					
	No atopy or eczema	Non-atopic eczema	Atopy (SPT+)	Atopic eczema	F-Test	p-value	No atopy or eczema	Non-atopic eczema	Atopy (SPT+)	Atopic eczema	F-Test	p-value
Wt% milk n-3												
6 wk	2.44 (0.12)	2.12* (0.08)	2.14 (0.19)	2.18 (0.12)	1.76	0.161	2.28 (0.09)	2.25 (0.13)	2.49 (0.48)	2.26 (0.11)	0.393	0.758
6 mo	2.32 (0.11)	2.05 (0.08)	2.29 (0.21)	2.16 (0.15)	1.30	0.280	2.26 (0.09)	2.25 (0.13)	1.81 (0.82)	2.09 (0.86)	1.06	0.369
Wt% milk n-6												
6 wk	12.80 (0.56)	14.60* (0.47)	13.27 (0.56)	13.27 (0.56)	2.37	0.076	13.61 (0.35)	13.90 (0.59)	15.16 (0.56)	12.87 (0.88)	0.898	0.446
6 months	13.13 (0.47)	14.06 (0.59)	12.91 (0.95)	13.70 (1.12)	0.608	0.612	13.44 (0.41)	13.98 (0.83)	11.00 (0.43)	13.81 (0.86)	0.986	0.403
Milk n-6:n-3												
6 wk	5.54 (0.28)	7.21 (0.38)**	6.75 (0.69)	6.27 (0.39)	4.39	0.006	6.28 (0.23)	6.46 (0.46)	7.21 (2.04)	6.37 (0.55)	0.265	0.850
6 months	5.87 (0.19)	7.09 (0.38)***	5.79 (0.32)†	6.38 (0.64)	3.37	0.022	6.26 (0.26)	6.29 (0.31)	6.21 (0.82)	6.77 (0.43)	0.436	0.728

LSD tests, *p = 0.040 (n-3); p = 0.010 (n-6) compared with no atopy or eczema; **p = 0.001 compared with no atopy or eczema; ***p = 0.005 compared with no atopy or eczema;

†p = 0.028 compared with non-atopic eczema.

6 months in relation to the outcomes. Analysis of variance was conducted to determine where differences were significant (Table 2). The biggest differences observed using LSD tests were between infants with no atopy or eczema and infants with non-atopic eczema, for milk n-6 at 6 wk (p = 0.040) and for the ratio of n-6: n-3 in both the 6 wk (p = 0.001) and 6 month (p = 0.005) milk samples. A difference was also apparent between atopy compared to non-atopic eczema for n-6: n-3 at 6 months (p = 0.028). No effect was seen for any outcome at 5 yr.

Some of the fatty acids from the 6 wk milk sample were increased in infants with non-atopic eczema at 6 months (total polyunsaturated fatty acid, total monounsaturated and 18:1n-9, total n-6, 18:2n-6, and overall n6:n3) whereas a number were reduced [20:4n-6 (arachidonic acid, AA); total n-3, 18:4n-3, 20:5n-3 (eicosapentaenoic acid, EPA) 22:5n-3 (docosapentanoic acid, DPA) and 22:6n-3 (docosahexanoic acid, DHA)]. The ratio of n-6 to n-3 decreased from 6 wk to 6 months (7.21 at 6 wk; 7.09 at 6 months). The 6 month milk sample showed similar effects to the 6 wk milk sample with 18:4 n-3, 20:4 n-6, 20:5 n-3 22:5 n-3 and total n-3 decreased overall in infants with non-atopic eczema, and the ratio of n-6: n-3 at 6 months being significantly greater. The increased n-6: n-3 ratio observed in milk of mothers of infants with non-atopic eczema may be due to the reduced level of 20:5 n-3 and 22:6 n-3 and increased level of 18:2 n-6. In addition, a difference was observed between atopy alone and non-atopic eczema (Table 2) with 22:4 n-6 (p = 0.010) and n-6: n-3 (p = 0.050) being increased in the milk of children with atopy alone. No differences were apparent between the weight percentage fatty acid content of breast milk from

mothers with infants who were either positive or negative for atopy or atopic eczema.

There were no differences in the fatty acid composition of maternal breast milk at 6 wk and 6 months, although there were significant differences between n-3 and n-6 from milk at 6 wk, and the development of non-atopic eczema in infants at 6 months, with more of the n-3 being protective (p = 0.044) and more of the n-6 being a risk (p = 0.018), a result not seen from the 6 month milk sample.

In multivariate logistic regression analyses the effects of milk n-3 and n-6 on all outcomes were modelled for both time-points. The higher the ratio of milk n-6: n-3 at either time-point, the greater the risk for non-atopic eczema after adjustment for maternal smoking during pregnancy, gestational age, birth-weight and gender. The introduction of other foods or other milks, or any other identified confounding variables did not show any effect on the development of non-atopic eczema at 6 months or 5 yr. No effect of n-3, n-6 or n-6: n-3 fatty acids was seen for skin prick test positive status overall, although skin prick test wheal size to *Alternaria* was significantly protected by a lower milk n-6: n-3 ratio at 6 wk (p = 0.034) and 6 months (p = 0.022), and to cat at 6 months (p = 0.019). The small numbers of infants atopic to these allergens precludes any conclusions from these results. There was no observed association between maternal asthma or atopy and milk fatty acids, most likely because the majority of mothers were either asthmatic or atopic.

Discussion

We have shown in a selected sample of high-risk infants who were breastfed that the ratio in milk

of n-6: n-3 fatty acids is associated with the risk of non-atopic eczema at 6 months. We found that in infants with non-atopic eczema as diagnosed by a doctor, that the milk from their mothers had less n-3, more n-6 and a higher ratio of n-6: n-3 than breastfed infants with no symptoms. These findings suggest that it is current dietary fatty acid intake that is important in the manifestation and modulation of inflammatory symptoms but not atopic symptoms at an early age.

This was a high risk cohort of mothers enrolled in mid-pregnancy (before any foetal outcomes were known). In consequence, the study population was globally enriched for both genetic and environmental determinants that increase the risk of atopy. Such ascertainment will greatly reduce the power to detect the effect of atopy but will have a much lesser effect on the power to detect the effect of non-atopic eczema (18). This was a high socioeconomic group, therefore, the generalizability of this study may be compromised. The introduction of other foods into the infant diet before 6 months could have affected the prevalence of non-atopic inflammatory symptoms, although we did not identify this as such. We did not comprehensively assess maternal dietary effects on n-3 or n-6 in the milk samples. Breastfeeding history was collected and verified by nurse interview when the child was 6 wk and 6 months of age, and there was a high rate of breastfeeding to at least 6 months. Although we could not ensure that the cohort was exclusively breastfed, they were predominantly breastfed.

Collection of outcome data was prospective and at frequent intervals and was based on validated questionnaires and methodologies. In relation to the inclusion of other tests for food allergens the observations would not change with inclusion of other allergen tests as these are rarely positive in early life. The most common allergens at 6 months in our data were milk (12%) and egg (18.5%). The chosen allergen panel was used at 6 months and 5 yr, and atopy was defined not only at 6 months but also at 5 yr.

Duch n hypothesized that variations in the lipid composition of milk could in part explain some of the controversies regarding the protective effects of breastfeeding against allergy (19) and concluded that fatty acid composition of human milk is disturbed in atopic mothers having an effect on atopic sensitization in the first 12 months of life (20, 21). Yu (21) showed that disturbances in the composition of the n-6 and n-3 fatty acids in milk may be related to atopic disease in infancy (7). Relatively

lower long-chain polyunsaturated fatty acid (LC-PUFA), especially EPA in relation to AA were found in milk from mothers of atopic babies at 1 month (8) and the n-6: n-3 ratio was higher in the milk from mothers with atopic babies. In a randomized controlled trial the gamma-linoleic acid concentration in plasma phospholipid between baseline and 3 months was negatively associated with atopic dermatitis severity at 1 yr ($p = 0.013$) but did not affect total serum immunoglobulin E (IgE) (22). Our data showed that total n-3, particularly the very LC-PUFA were reduced in the milk consumed by infants who manifested non-atopic eczema ($p < 0.05$) indicating that lower levels of the very long chain carbon chains are related to inflammatory infantile eczema but not atopy.

Eicosapentaenoic acid has been shown to reduce inflammation and improve symptoms of inflammatory skin diseases (23, 24). In the skin, the highly active epidermal 15-lipoxygenase converts EPA into 15-hydroxyeicosapentaenoic acid (15-HEPE) and DHA into 17-hydroxydocosahexaenoic acid (17-HdoHE) (25). These monohydroxy acids exhibit anti-inflammatory properties, possibly by inhibiting the activity of 5-lipoxygenase in mononuclear cells, thus inhibiting the synthesis of leukotrienes (26). The predominant fatty acid in cells is AA. However, the fatty acid composition of diet can affect the ratio of fatty acids in immune cells, thus influencing their functions (27). This is supported by studies showing supplementation of EPA can improve inflammatory skin problems such as itching, scaling and erythema (23, 24). It is still controversial as to which level of EPA intake is required for the beneficial effects, and the requirements may differ according to individuals or age groups. In our study, we suggest that the differences were sufficient between groups to show a relationship with non-atopic eczema.

As the mechanism of non-atopic eczema is complex and not completely known, the pathways through which EPA may prevent its development are not completely understood. For example, while it is known that eczema is mainly IgE-mediated, recent evidence suggests that a non-IgE-mediated mechanism may be responsible for food-induced eczema (28). Future studies exploring the development of eczema should consider the role of LC-PUFA in its development.

Conclusion

Prophylactic measures are required to prevent the inflammatory skin condition of eczema in

infants who are breastfed. The present report highlights the potential importance of an increased n-6: n-3 breast milk fatty acid ratio as a risk factor for non-atopic eczema in infancy. Although this association does not mean a causal relationship, it is feasible that administration of n-3 to correct the balance either as a supplement or as a food source for mothers during pregnancy and lactation may decrease the ratio of n-6: n-3 in maternal milk, thereby protecting against eczema in breastfed infants.

Acknowledgments

Sincere thanks are extended to the study mothers for their contribution of the breast milk samples to the study without whose participation this research could not have been conducted. Full acknowledgments are extended to the study nurses, responsible for the collection of the data presented here. We acknowledge the work on the fatty acid analyses conducted by third year nutrition students at the School of Public Health, Curtin University of Technology in 2002.

Funding

W.H. Oddy is supported by a Population Health Research Fellowship from the National Health and Medical Research Council of Australia. The CAS is funded by project and programme grants from the National Health and Medical Research Council of Australia. Funding was obtained for this breast milk study from a Curtin University New Investigator Research Grant.

Author contributions

W.H. Oddy developed the hypothesis, undertook statistical analyses, wrote the main drafts of the paper and is correspondent for this manuscript and requests for reprints. S. Pal was the lipid biochemist on the study and was instrumental in securing funds for the fatty acid analyses, and with D. Vine was responsible for their analyses. M. Kusel (the medical doctor on the study) conducted the follow-ups and infant allergy testing. P. Hartmann initiated the breast milk collection and assisted with data interpretation; P.D. Sly and P.G. Holt were responsible for the key follow-ups and assisted with the interpretation of the data; P.R. Burton, F.J. Stanley and L.I. Landau established the cohort study and follow-ups and assisted in interpretation. N. de Klerk directly supervised the statistical analyses.

No competing interests are declared.

References

- ROBERTSON CF, DALTON MF, PEAT JK, et al. Asthma and other atopic diseases in Australian children: Australian arm of the International Study of Asthma and Allergy in Childhood. *Med J Aust* 1998; 168: 434–f8.
- GLASER J, JOHNSTONE DE. Prophylaxis of allergic disease in newborn. *JAMA* 1953; 00: 620.
- CHANDRA RK. Prospective studies on the effect of breastfeeding on incidence of infection and allergy. *Acta Paediatr Scand* 1979; 68: 691.
- SAARINEN UM, KAJOSAARI M. Breastfeeding as prophylaxis against atopic disease: prospective follow-up study until 17 years old. *Lancet* 1995; 346: 1065–9.
- WRIGHT AL, HOLBERG CJ, TAUSSIG LM, MARTINEZ FD. Factors influencing the relation of infant feeding to asthma and recurrent wheeze in childhood. *Thorax* 2001; 56: 192–7.
- SEARS MR, GREENE JM, WILLAN AR, et al. Long-term relation between breastfeeding and development of atopy and asthma in children and young adults: a longitudinal study. *Lancet* 2002; 360: 901–7.
- DUCHÉN K, BJÖRKSTÉN B. Polyunsaturated n-3 fatty acids and the development of atopic disease. *Lipids* 2001; 36: 1033–42.
- 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.
- WIJGA A, HOUWELINGEN AC, SMIT HA, et al. Fatty acids in breast milk of allergic and non-allergic mothers: the PIAMA birth cohort study. *Pediatr Allergy Immunol* 2003; 14: 156–62.
- STONEY RM, WOODS RK, HOSKING CS, HILL DJ, ABRAMSON MJ, THIEN FC. 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.
- CALDER PC. Immunoregulatory and anti-inflammatory effects of n-3 polyunsaturated fatty acids. *Brazilian Journal of Medical and Biological Research* 1998; 31: 467–90.
- DE CATERINA R, BASTA G. n-3 Fatty acids and the inflammatory response – biological background. *Eur Heart J Suppl* 2001; 3 (Suppl. D): D42–D49.
- PAJNO G, PERONI D, BARBERIO G, PIETROBELLI A, BONER A. Predictive features for persistence of atopic dermatitis in children. *Pediatr Allergy Immunol* 2003; 14: 292–5.
- FOLCH J, LEES M, SOANE GH. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 1957; 226: 497–509.
- GIBSON RA, KNEEBONE GM. Fatty acid composition of human colostrum and mature breast milk. *Am J Clin Nutr* 1981; 34: 252–7.
- MITOULAS LR, GURRIN LC, DOHERTY DA, SHERRIFF JL, HARTMANN PE. Infant intake of fatty acids from human milk over the first year of lactation. *Brit J Nutr* 2003; 90: 979–86.
- HANIFIN J, RAJKA G. Diagnostic features of atopic dermatitis. *Acta Dermatol Venereol* 1980; 92: 44–47.
- BURTON PR, PALMER LJ, JACOBS K, KEEN KJ, OLSON JM, ELSTON RC. Ascertainment Adjustment: Where Does It Take Us. *Am J Hum Genet* 2000; 67: 1505–14.
- DUCHÉN K. Are human milk polyunsaturated fatty acids (PUFA) related to atopy in the mother and her child? *Allergy* 2001; 56: 587.
- DUCHÉN K, YU G, BJÖRKSTÉN B. Atopic sensitization during the first year of life in relation to long chain polyunsaturated fatty acid levels in human milk. *Pediatr Res* 1998; 44: 478–84.
- 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.
- van GOOL CJ, THIJIS C, HENQUET CJ, et al. Gamma-Linolenic acid supplementation for prophylaxis of atopic dermatitis-a randomized controlled trial in

- infants at high familial risk. *Am J Clin Nutr* 2003; 77: 943–51.
23. BITTNER SB, TUCKER WF, CARTWRIGHT I, BLEEHEN SS. A double-blind, randomised, placebo-controlled trial of fish oil in psoriasis. *Lancet* 1988; 1: 378–80.
24. LASSUS A, DAHLGREN AL, HALPERN MJ, SANTALAHTI J, HAPPONEN HP. Effects of dietary supplementation with polyunsaturated ethyl ester lipids (Angiosan) in patients with psoriasis and psoriatic arthritis. *J Intern Med Res* 1990; 18: 68–73.
25. ZIBOH VA. The significance of polyunsaturated fatty acids in cutaneous biology. *Lipids* 1996; 31 (Suppl.): S249–53.
26. ZIBOH VA, MILLER CC, YUNHI C. Metabolism of polyunsaturated fatty acids by skin epidermal enzymes: generation of anti-inflammatory and antiproliferative metabolites. *Am J Clin Nutr* 2000; 71 (Suppl): 361S–66S.
27. CALDER PC. Polyunsaturated fatty acids, inflammation and immunity. *Lipids* 2001; 36: 1007–24.
28. BROCKOW K, HAUTMANN C, FOTISCH K, RAKOSKI J, BORELLI S, VIETHS S, et al. Orange-induced skin lesions in patients with atopic eczema: evidence for a non-IgE-mediated mechanism. *Acta Derm Venereol* 2003; 83: 44–8.