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Phospholipid fatty acids in cord blood: family history and development of allergy

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The fatty acid composition of umbilical cord serum phospholipids was investigated by gas chromatography in 33 infants with allergic and 35 babies of non-allergic mothers. The relative levels of the linoleic acid metabolites C20:3, arachidonic acid (AA, C20:4) and C22:4n-6, and two α-linolenic acid metabolites, i.e. eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, C22:6) were significantly higher in infants of allergic mothers than in non-allergic mothers (all $p < 0.05$). Furthermore, an altered proportional relationship between the various fatty acids in n-6 series fatty acids and between n-3 and n-6 series fatty acids was present already at birth in infants who developed allergic disease during their first 6 years of life. These observations cannot be employed for the prediction of allergy, however, as the individual variations were considerable. □ *Allergic disease, cord blood, fatty acids, prediction*

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Long-chain polyunsaturated fatty acids (LCP) are structured components of all biomembranes, affecting membrane fluidity and thereby the function of cell and intracellular plasma membranes such as cell signal transduction and plasma membrane-associated enzymes (1, 2). Two families of LCP are derived from the essential fatty acids linoleic acid (LA, C18:2 n-6) and (-linolenic acid (LNA, C18:3 n-3), i.e. the n-6 and n-3 series (Fig. 1). Eicosanoids, derived from the n-6 series of fatty acids are more potent inflammatory mediators than the corresponding compounds derived from the n-3 series (3, 4). The possibility to attenuate inflammatory responses related to n-6 fatty acid metabolites by the administration of n-3 fatty acids has been suggested (4–6).

An altered fatty acid composition has been observed in subjects with atopic diseases including eczema, allergic asthma, allergic rhinitis and atopic dermatitis (7–11). Manku et al. (7, 8) reported slightly elevated LA levels in plasma phospholipids of patients with atopic eczema, whereas the levels of all LA metabolites were substantially below normal. The alteration of fatty acid composition in atopic individuals was even more obvious in red cell and white cell phospholipids (9). Griese et al. (11) reported significantly elevated eicosapentaenoic acid (EPA, C20:5 n-3) in 11 allergic asthmatic children and that the levels of EPA correlated with total serum-IgE levels.

Only few studies have addressed the question whether an abnormality in essential fatty acid composition is a primary defect in atopic individuals or merely a consequence of disease. Stranneård et al. reported that the levels of LA in umbilical cord serum lecithin were significantly higher in infants with elevated serum IgE than in those with normal levels (12). It was not analysed, however, whether fatty acid composition in cord blood could be used to predict allergic diseases in children. Thus, no studies have been reported so far in which the fatty acid composition in clinically healthy infants was related to the development of allergic manifestations later in life nor to the heredity for allergic disease.

The aim of this study was to investigate whether an abnormal composition of the n-3 and n-6 fatty acid is a primary defect in atopy and to assess whether an analysis of the fatty acid composition of cord blood could predict the development of atopic disease.

Patients and methods

Patients

Umbilical venous blood samples were obtained from 33 infants with allergic mothers and 35 infants of non-allergic mothers after clamping and very careful cleaning of the cord. In addition, venous blood samples were obtained from 47 of their mothers. The details of the relationship between maternal and foetal fatty acid composition will be the subject of another report. The maternal and paternal histories of allergy were obtained during the last trimester of pregnancy and included allergic asthma, seasonal rhino conjunctivitis and/or atopic dermatitis. None of the mothers had food allergy and they were all on an unrestricted diet.

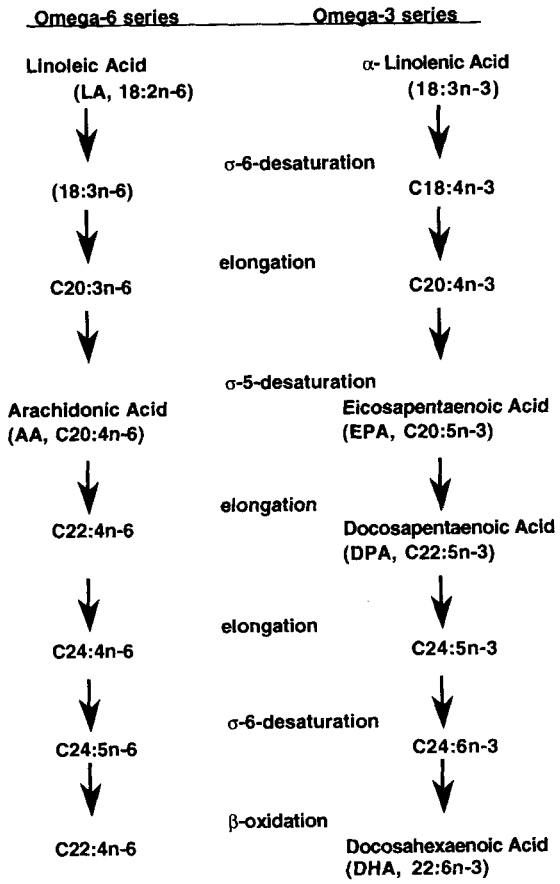

Fig. 1. Schematic depiction of the metabolic pathways of n-6 and n-3 fatty acids.

All the infants were delivered vaginally at term (38–42 post-menstrual weeks) and the perinatal period was uncomplicated. The children were followed up at 18 months and 6 years. On these occasions, a questionnaire was completed and a physical examination was carried out, focusing on symptoms and signs of allergy. Skin prick tests were also performed in single, with low fat milk, hen's egg and extracts of birch and timothy pollen, animal dander and house dust mite (ALK, Hørsholm, Denmark).

The diagnostic criteria for allergy in the parents were a convincing clinical history of either asthma, hay fever and/or atopic dermatitis, supported by the demonstration of circulating IgE antibodies against a mixture of common allergens (PhadiatopTH, Pharmacia, Uppsala, Sweden). The diagnostic criteria in the children have been described previously (13). Briefly, asthma was defined as obstructive bronchitis appearing at least three times and verified by a physician. The diagnosis of eczema was based on the criteria by Hanifin and Rajka (14), as adopted for young children (15). Allergic rhino conjunctivitis was defined as seasonal nasal watery discharge. The presence of allergy was verified by at least one positive skin prick test.

In addition to the collection of cord blood, a venous blood sample was drawn from a peripheral vein of the mother at the time of delivery. The blood samples were centrifuged, and the serum was frozen and stored at −20°C until analysis. The clinical assessments and laboratory analyses were performed by different persons, none of whom had any information about the results of the other determinations.

Extraction and determination of fatty acids in serum phospholipids

The serum samples were thawed at room temperature and analysis was begun within 10 min of thawing. Total lipids were extracted by chloroform and methanol (1/1, v/v) with 5 mg/100 ml butylated hydroxytoluene (BHT) (16) from 0.2 ml serum. Total phospholipids were separated by thin-layer chromatography (TLC) on silica gel plates (silica gel 60 F254 (17). Twenty μl chloroform extracts were applied in alternate lanes onto the plate and were allowed to air dry under the hood.

The plates were first developed in methanol with 5% BHT to approximately 1.5 cm above the preadsorbent zone and then, after evaporating the methanol, further developed (1.5 cm) in chloroform/methanol (1/1, v/v). The plates were developed to 15 cm above the preadsorbent zone using hexane/diethyl ether/acetic acid (80/20/1, v/v/v). The resulting lipid bands were visualized by spraying the plate with rhodamin 6G (0.02% in 95% ethanol) and viewing the plate under UV light. Phospholipid bands located 1 cm above the preadsorbent zone were identified by comparing Rf values with known standards on each plate and then scraped into glass tubes.

Methyl esters of fatty acids were prepared by triflouboron-ethylether and methanol (2/1, v/v) in water at 90–100°C for half an hour (18). The methyl esters were extracted with 2 ml hexane and 4 ml distilled water and stored at −20°C for GC analysis within 3 days. Before injection, samples were dried under pure N2 and dissolved in 200 ml hexane, of which a 0.5 μl volume was injected.

Fatty acid methyl esters were separated and quantitated using 5790A GC, equipped with a capillary column (25 m × 0.2 mm × 0.33 μm) cross-linked 5% phenyl methyl silicone gum phase and with 3390A integrator (Hewlett-Packard Co, USA). The splitless injection mode was used. The following temperature gradients were used for the GC analysis: Temp. 1, 160°C; time 1, 4 min; temp. 2, 250°C; time 2, 15 min; rate, 2.7°C/min; injection temp. 260°C, detector temp. 280°C. Helium gas was used as carrier gas (20 cm/s). Fatty acids of carbon chain length C14–22 were identified by comparing the retention time with those of known standards (Sigma Chemical Co, St Louis, MO, USA). The levels of the individual fatty acids were expressed as weight percentage (wt%) of total fatty acids.

Table 1. Phospholipid fatty acid composition (weight %, mean ± SD) of umbilical cord blood in 35 infants with allergic and 33 with non-allergic mothers (independent of paternal disease). The composition is also shown for the 25 infants who developed and 43 infants who did not develop allergic diseases during the first 6 years of life. MUFA = monounsaturated fatty acids; SFA = saturated fatty acids.

	Maternal allergy		Allergic disease	
Fatty acids	Yes, $n = 33$	No, $n = 35$	Yes, $n = 25$	No, $n = 43$
18:2 n-6	8.2 ± 1.6[a]	8.7 ± 3.0[a]	8.4 ± 1.5	8.5 ± 2.8
20:2 n-6	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.1	0.5 ± 0.2
20:3 n-6	5.7 ± 0.7*	5.2 ± 1.2	5.7 ± 0.9	5.3 ± 1.1
20:4 n-6	14.7 ± 1.7*	13.3 ± 2.4	14.2 ± 1.8	13.8 ± 2.4
22:4 n-6	0.6 ± 0.1*	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1
20:5 n-3	1.0 ± 0.4*	0.8 ± 0.2	0.9 ± 0.3	1.0 ± 0.4
22:5 n-3	0.4 ± 0.2	0.4 ± 0.2	0.5 ± 0.2	0.4 ± 0.2
22:6 n-3	4.8 ± 1.2*	4.2 ± 1.2	4.8 ± 1.4	4.4 ± 1.2
Total n-6	29.6 ± 32.8	28.0 ± 4.0	29.2 ± 2.7	28.6 ± 3.4
Total n-3	6.3 ± 1.4**	5.4 ± 1.3	5.9 ± 1.3	6.0 ± 1.6
Total MUFA	12.7 ± 1.7	13.2 ± 2.9	12.7 ± 1.9	13.4 ± 2.7
Total SFA	51.5 ± 3.6	53.4 ± 4.6	52.2 ± 3.4	52.1 ± 4.6

[a]The level was 8.0 ± 2.0 in 46 infants with maternal and/or paternal allergy vs 9.3 ± 3.0 in 22 babies without any FHA ($p < 0.01$). Significant difference *$p < 0.05$ and **$p < 0.01$ (two-tailed t-test).

Statistical analysis

Unpaired Student's t-test was used to compare the differences of fatty acid composition, since the data were normally distributed. The relations between fatty acids were analysed by linear regression, and Parson's correlation coefficients were calculated using StatView™ SE + Graphics (Abacus Concepts, Inc).

Ethical considerations

The study was approved by the Local Ethics Committee.

Results

The levels of C20:3, arachidonic acid (AA, C20:4) and C22:4 of the n-6 series of fatty acids and of EPA and docosahexaenoic acid (DHA, C22:6) of the n-3 series, as well as the total amount of omega-3 fatty acids were slightly higher in cord blood of the 33 infants with allergic mothers than in the 35 babies of non-allergic mothers (Table 1). This was independent of paternal allergy. Thus, the differences were smaller, although statistically significant, when comparing the cord blood levels in the 46 infants with either maternal or paternal allergy with those in the 22 babies without a family history of allergy (FHA, data not shown). In the former group, however, the LA (C18:2, n-6) levels were significantly lower than in the babies lacking a FHA (Table 1). As also shown in Table 1, the levels were similar in babies who did and did not develop allergic diseases during the first 6 years of life. Possible differences in children with mild or severe atopic disease could not be assessed as the severity of atopic manifestations was not recorded. There were no differences in the fatty acid composition in children with different atopic manifestations, i.e., dermatitis ($n = 14$), asthma ($n = 6$) or both ($n = 5$). The levels of phospholipid fatty acids were similar in the allergic and non-allergic mothers (data not shown).

The levels of the n-6 fatty acids LA, C20:2, C20:3, AA and 22:4 correlated with each other to a large extent in the 43 babies who did not develop any allergic manifestations, as well as in the 22 infants without either a maternal or paternal history of allergy (Table 2). In these infants, also the levels of the n-3 fatty acids docosapentaenoic acid (DPA, C22:5) and DHA (C22:6) correlated with the n-6 fatty acid levels.

Table 2. Correlations between fatty acids of the n-3 and n-6 series of cord serum phospholipids in relation to the presence of family history of allergy (FHA) and the appearance of allergic manifestations in 25 babies who developed allergic disease during the first 6 years of life and 43 infants who did not (linear regressive analysis).

	FHA		Allergic disease	
Correlations (*r* values): N =	Yes 46	No 22	Yes 25	No 43
Within n-6				
LA/C20:2	0.59**§	0.70**	0.52*	0.66**
AA/C20:3	–	0.61**	–	0.53**
AA/C22:4	0.59**§	0.65**	–	0.67**
n–3 vs. n–6				
DHA/ C20:3	–	0.56*	–	0.50**
DHA/AA	–	0.77**	–	0.75**
DHA/C22:4	0.58**	0.77**	0.53*	0.74**
DPA/C20:3	–	–	−0.52*	–
DPA/C22:4	–	0.78**	–	0.65**

LA = linoleic acid (C18:2n-6); AA = arachidonic acid (C20:4n-6); DHA = docosahexaenoic acid (C22:6n-3); DPA = docosapentaenoic acid (C22:5n-3). *$p < 0.01$ significant correlation coefficient; **$p < 0.001$ significant correlation coefficient. § = no significant correlation in children with maternal history of allergy.

In contrast, these relationships within the n-6 series and between n-3 and n-6 series of fatty acids were largely absent in infants with either a family history of allergy or who developed allergic disease (Table 2). As shown in the table, the only statistically significant relationship in these two groups was identified between DHA and C22:4 and between LA and C20:2. Similarly, no significant correlations between any of the FAs of the n-6 series were recognized in the children of allergic mothers. As discussed in detail in another paper (Yu & Björkstén, manuscript) the recorded differences in fatty acid levels between atopic and non-atopic mothers did not explain the levels in cord serum.

Discussion

The fatty acid composition in umbilical cord blood phospholipids differed between infants with and without FHA, particularly maternal allergy. This was true both for the relative levels of several LA and LNA metabolites and for the ratios between some of LCP fatty acids. It is not likely that the differences in fatty acid levels were explained by dietary differences between the allergic and non-allergic mothers, as they were all on an unrestricted diet and the LCP levels (but not the relationships between individual fatty acids) in sera between the allergic and non-allergic mothers were similar (Yu & Björkstén, in manuscript).

Strannegård et al. observed slightly higher levels of LA in umbilical cord serum of infants with elevated serum IgE (12). We could not confirm this finding in atopic babies in our study, in which the children were subject to a 6-year follow-up and in which efforts were taken to obtain a reliable family history from both parents. The LA levels in cord blood are about three times lower than those in the maternal sera, while AA levels are three times higher in cord blood (19). As the levels of LA are much higher in maternal serum than in cord blood and the same is true for the IgE antibody levels, it is possible that the findings reported by Strannegård et al. (12) were influenced by maternal contamination of the cord blood.

The slightly higher levels of several LA and LNA metabolites in cord serum of infants with allergic mothers as compared to non-allergic mothers cannot be explained. Possibly, they represent a protective mechanism. Thus, it is conceivable that the higher levels of LA metabolites could be a consequence of suppressed AA metabolism by the cyclooxygenase and 5-lipoxygenase pathways. As eicosanoids derived from the n-3 series fatty acids are less potent mediators of inflammation than the n-6 derived compounds, even down-regulating the inflammatory response (1, 3–5, 20), the higher levels of LNA metabolites could be suggested to represent a protective response to elevated levels of AA. This notion would be supported by the correlations between the n-3 and the n-6 series fatty acids in the non-allergic children, but not in those who developed allergic manifestations.

The observed differences in the levels of LA and LNA metabolites in infants of allergic and non-allergic mothers had no predictive value with regard to manifestations of allergic disease, as the levels were similar in infants who did and did not develop allergic disease during their first 6 years of life. These findings may appear to contradict the results of some previous studies. Galli et al. (22) found decreased levels of AA in the cord blood of children who subsequently develop atopic disease. As the follow-up period was only 1 year they would have suffered from atopic dermatitis rather than respiratory allergy, which becomes the dominating atopic manifestation after 3 years of life. Similarly, the different results between our study and the recent report by Ioppi et al. (23) could possibly be explained by the length of the follow-up period and the fact that in the Italian study infants with and without atopic dermatitis were compared. Determination of fatty acid levels in cord blood does not appear to be useful for the prediction of allergic disease.

In the non-atopic infants, the levels of AA correlated with those of the precursor C20:3 and the product C22:4. These relationships were not observed in the allergic children, nor in babies with a biparental family history of allergy. The findings indicate that a metabolic disturbance of AA may be relevant for the development of allergic disease. It is possible that such disturbance could be the forerunner of the low levels of AA and other fatty acids of the n-6 series that have been reported in children with manifest allergic disease, particularly atopic dermatitis (7, 8, 12).

In the babies without a FHA, as well as in the infants who did not manifest allergic disease, there was a balance between n-3 and n-6 series fatty acid metabolites. Both DHA and DPA have been reported to inhibit delta-6 and delta-5 desaturation of AA metabolism by the 5-lipoxygenase pathway (1, 4, 21). These conclusions were based on in vitro experiments, however. Our findings in vivo do not support the results of the previous in vitro studies. Further studies are needed to clarify these differences. Interestingly, however, these relationships were largely absent in the atopic infants. The reasons for the difference between atopic and non-atopic newborns are unknown.

Taken together, our study supports and extends previous observations that allergic disease may be associated with a primary abnormality in the n-6 and n-3 fatty acid metabolism of yet unknown origin.

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