

(n-6)-Fatty acids in plasma lipids of children with atopic bronchial asthma

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It has been suggested that atopy is associated with an impairment in the $\delta 6$ -desaturation of (n-6)-polyunsaturated fatty acids and subsequently low levels of eicosanoid precursors. To evaluate this hypothesis we analyzed the fatty acid composition of plasma phospholipids and plasma cholesteroles in a well-defined group of children with atopic bronchial asthma ($n=17$) and age-matched healthy controls ($n=10$). Atopic children showed significantly higher levels of linoleic acid and lower proportions of arachidonic acid in plasma lipids. No differences were observed with respect to γ -linolenic acid (GLA) and di-homo- γ -linolenic acid (DHLA). It is concluded that there is no biochemical evidence for a $\delta 6$ -desaturation defect in atopic children and therefore no justification for the supplementation of GLA and DHLA; e.g., by the use of evening primrose oil preparations.

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The essential fatty acid linoleic acid [LA, 18:2(n-6)] is metabolized in alternating steps of desaturation and elongation. Products are γ -linolenic acid [GLA, 18:3(n-6)], di-homo- γ -linolenic acid [DHLA, 20:3(n-6)], and arachidonic acid AA, 20:4(n-6)]. The last two substances are precursors of the eicosanoid biosynthesis. The key enzyme in LA metabolism is the $\delta 6$ -desaturase which catalyzes the formation of GLA from LA (1).

In 1947 the first study was published which suggested a relationship between disturbances in LA metabolism and the pathogenesis of atopic eczema (2). However, during the following years no further evidence for this hypothesis was established. In the last ten years the relationship between fatty acid (FA) metabolism and atopy regained scientific interest. Studies were published suggesting that an impairment of the activity of the $\delta 6$ -desaturase enzyme with subsequently low levels of DHLA and AA may be causative in the pathogenesis of atopy (3, 4). On the basis of these studies, it has been suggested that a dietary supplement of LA metabolites, e.g., GLA, might have a beneficial effect in atopic patients (5–

7). In some countries this has led to a marked increase in the use of oil preparations rich in GLA, mostly prepared from evening primrose oil.

Most of the clinical studies investigated patients with atopic dermatitis (review 8). Only a few investigators have studied patients with atopic bronchial asthma (9–11).

Atopic disease in children, however, is often characterized by a change of the clinical picture from dermatitis in early childhood to a later presentation of bronchial asthma. If there is – as has been proposed (3, 5, 6) – a causative relationship between an inborn metabolic defect, namely, an impairment of the $\delta 6$ -desaturase activity, and atopy, then one would also expect lower levels of DHLA in children with atopic dermatitis as well as in atopic bronchial asthma. On the basis of such a disturbance, a dietary supplement with $\delta 6$ -desaturase products (e.g., evening primrose oil preparations) might be indicated. For this reason we investigated the FA composition of plasma phospholipids (PL) and plasma cholesteroles (CE) from 17 children with allergic bronchial asthma.

Patients and methods

The criteria for the inclusion were as follows: typical clinical appearance and pathological pulmonary function tests; bronchial asthma grade 2 or more according to the classification by von der Hardt (12);

Abbreviations – AA = arachidonic acid 20:4(n-6); CE = plasma cholesteroles; DHLA = di-homo- γ -linolenic acid 20:3(n-6); FA = fatty acids; GLA = γ -linolenic acid 18:3(n-6); LA = linoleic acid 18:2(n-6); PL = plasma phospholipids.

multiple allergic sensitizations according to PRIST and RAST tests; total IgE > 1000 U/ml; no prior therapy with specific FA preparations; no continuous systemic therapy with corticosteroids; no dermal efflorescences at the time of the study. These criteria were met by 17 patients (15 boys, two girls; mean age 9 years). All patients received antiasthmatic therapy (disodium cromoglycate, theophylline, inhalation with steroids). None of them were malnourished or growth retarded. Ten age-matched children with no history or clinical signs of atopic disease served as controls.

Blood was drawn by venous puncture for routine clinical purposes (total and specific IgE levels; Phadebas, PRIST and RAST, Pharmacia, Uppsala). The remaining plasma was separated and immediately stored at -20°C until further analysis. With immediate separation and freezing no relevant changes in the fatty acid composition of plasma lipids occur (13). Extraction, separation and processing of plasma lipid fractions were performed according to methods published earlier (14). PL and CE were separated by unidimensional thin layer chromatography. Transesterification was achieved in all fractions through heating with methanolic HCl at 85°C for 60 min. After neutralization the samples were dried under a stream of nitrogen and stored at -18°C . The FA composition of the lipid fractions was determined by capillary gas-chromatography using a VEGA 6000, Series 2 GC (Fisons Instruments, Mainz, Germany) equipped with a CP Sil 88 capillary column (Chrompack, Germany; length 50 m, diameter 0.32 mm) and a flame ionization detector. The oven temperature was raised to between 140°C and 220°C with $5^{\circ}\text{C}/\text{min}$, and then kept isothermic for 15 min (injector temperature 200°C , detector temperature 240°C). Helium was used as carrier gas. Identification of substances was achieved by comparison with commercially available standards (Nu Chek, Elysian, USA). Values were calculated as weight percentages of all FA determined.

Statistical evaluation of differences between patients and controls was done by nonparametric tests (Mann-Whitney-Wilcoxon). $p < 0.05$ was chosen as minimum level of significance.

Results

The proportions of the most important (n-6)-FA in the different lipid fractions are shown in Tables 1 and 2. When compared with controls, atopic children showed higher levels of LA and decreased AA. This led to a highly significant difference in the ratio LA/AA. However, in both lipid fractions analyzed, there were no differences in the proportions of GLA and DHLA between patients and controls. Despite the observed differences for the mean values of LA

Table 1. Selected (n-6)-fatty acids in plasma phospholipids of patients with atopic bronchial asthma (values in weight percentages of all fatty acids analyzed; n.s.: not significant)

	Patients			Controls	
	Mean	SD	p <	Mean	SD
LA	23.44	3.38	0.001	18.70	2.63
GLA	0.05	0.04	n.s.	0.08	0.08
DHLA	2.86	0.52	n.s.	2.91	0.52
AA	8.62	1.55	n.s.	9.65	1.56
LA/DHLA	8.82	2.93	0.05	6.84	2.40
DHLA/AA	0.34	0.08	n.s.	0.30	0.07
LA/AA	2.82	0.71	0.005	1.99	0.44

Table 2. Selected (n-6)-fatty acids in plasma cholesteroles of patients with atopic bronchial asthma (values in weight percentages of all fatty acids analyzed; n.s.: not significant)

	Patients			Controls	
	Mean	SD	p <	Mean	SD
LA	53.87	4.93	0.001	45.77	2.83
GLA	0.66	0.29	n.s.	0.75	0.33
DHLA	0.58	0.11	n.s.	0.67	0.07
AA	5.46	0.99	0.05	6.76	1.34
LA/DHLA	98.06	24.22	0.001	66.78	8.72
DHLA/AA	0.11	0.02	n.s.	0.10	0.02
LA/AA	10.22	2.17	0.005	7.06	1.74

and AA, it has to be emphasized that 13 of 17 atopic patients had LA and AA values in the normal range (mean of the controls ± 2 standard deviations). A high correlation between the FA values in PL and CE was found. For LA, the correlation coefficient was 0.86 ($p < 0.001$); for AA, 0.94 ($p < 0.001$).

Discussion

The results from our study disagree with the hypothesis that an inborn defect of the $\delta 6$ -desaturase is causative in the pathogenesis of atopy. Furthermore, the results do not provide a biochemical justification for the use of GLA- or DHLA-enriched oils for the treatment of atopic bronchial asthma.

A defect of $\delta 6$ -desaturase in atopic patients would lead to low levels of LA metabolites; i.e., GLA, DHLA and AA. We did not find any significant difference between atopic patients and controls with respect to plasma GLA and DHLA levels. In favor of the proposed hypothesis one might argue that the $\delta 6$ -desaturase defect might only lead to diminished proportions of GLA and DHLA in certain cell systems; e.g., leukocytes. It is generally accepted (15) and has been repeatedly shown in malnourished children (16–18) that impairments of the $\delta 6$ -desaturase activity are well reflected by changes in the FA composition of plasma PL.

Significant alterations in the plasma FA composition were detected in the study group, as the levels

of LA were found to be higher and the levels of AA were found to be lower in patients than in controls. Rocklin et al. (9, 19) found increased LA levels and decreased AA levels in the phospholipids of the buffy coat of patients with allergic rhinitis and/or asthma. As in our results, no differences were observed with respect to GLA and DHLA. In that study, however, the plasma PL showed no differences between atopic patients and controls, leading the authors to suggest that alterations in the FA metabolism in atopic asthma may be restricted to white blood cells (18). A study by Griese et al. (10) did not show differences between atopic patients and controls in plasma PL. In both studies patient groups were not as sharply defined and homogeneous as was our study group. In the study group of Griese et al., for example, only three patients showed IgE levels over 1000 U/ml (10). For this reason other influences on the plasma FA status, such as recent dietary intake, may have masked existing alterations in the FA metabolism of atopic patients. This may be especially true for the study by Rocklin et al. (9, 19) where alterations were found in white blood cells but not in plasma. Ebdon et al. (11) analyzed the FA composition of plasma PL in 12 patients with mild atopic bronchial asthma before and after oral therapy with evening primrose oil. Although they did not statistically evaluate and discuss pretherapy differences between patients and controls it is interesting to note that they also found increased levels of LA, decreased levels of AA, and normal levels of GLA and DHLA in the plasma PL of patients. This pattern is remarkably similar to that found in the present study.

Two further aspects of the study by Ebdon et al. (11) should be pointed out. First, there was no clinical effect of the evening primrose oil therapy. Secondly, the application of evening primrose oil led to an increase of GLA and DHLA but did not elevate the lowered levels of AA. Other frequently cited papers (4, 20, 21) also do not provide evidence that AA levels can be restored by an increase in the dietary GLA supply. It is concluded that oral supply with large amounts of GLA and/or DHLA is not appropriate to increase plasma AA levels. On the contrary, it may as well be speculated that this approach may lead to a down-regulation of the $\delta 5$ -desaturase activity.

Our data show that atopy is not necessarily associated with diminished proportions of GLA and/or DHLA in plasma lipids. As in previous studies there were altered proportions of LA and AA in plasma lipids of atopic children. The fact that these alterations occurred in a similar way in both lipid fractions analyzed and the high correlations between both fractions may point at disturbances of FA metabolism in atopy. However, it cannot be excluded

that differences in the dietary PUFA intake have influenced these findings in our patients. The data do not provide any evidence that there is a specific impairment of the $\delta 6$ -desaturase enzyme.

Our study aimed to investigate whether or not supplementation with evening primrose oil is justified in children with atopic bronchial asthma for biochemical reasons. We conclude from our data and the results of previous studies that in the highly atopic subjects studied substitution therapy for an inborn error of metabolism by GLA is not indicated. If one would consider the supplementation of GLA as a pharmacological approach, one should be aware of the fact that DHLA is a precursor of biologically highly active substances. Any iatrogenically induced imbalance may have profound, as yet unobserved, side effects via eicosanoid-mediated functions and may lead to an increased susceptibility of PUFA-enriched membranes to oxidative damage. The possible negative consequences of long-term use of evening primrose oil are, as yet, not known. In our opinion there is at present no scientific basis for the view that the use of evening primrose preparations is essential (5, 7) in the therapy of atopic children.

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