

Impact of early dietary gamma-linolenic acid supplementation on atopic eczema in infancy

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Polyunsaturated fatty acids (PUFAs) are components of cell membranes and may play an immunomodulating role in the pathogenesis of atopic dermatitis (AD). The goal was to determine the impact of PUFAs on AD by dietary supplementation of infants. Based on the parents' decision on their babies' primary feeding, mothers and newborns were randomized to the supplementation with gamma-linolenic acid (GLA) or placebo for up to 6 months. Breastfed infants received GLA by supplementing their mothers. Formula diet was commercial whey hydrolysate unsupplemented with PUFAs. Of 131 eligible infants, 24 developed AD within the first year of life. Of these, nine belonged to the exclusively breastfed group ($n = 58$), 14 to the combined-fed group ($n = 53$), and one to the never breastfed group ($n = 20$). We could not find an influence of GLA on the development of AD. In subjects with AD, at 1 yr of age the serum-immunoglobulin E (IgE) was the lowest in the GLA-supplemented group A-subjects. In the GLA-supplemented group, GLA-levels in breast milk were similar in atopic and non-atopic infants. In the non-supplemented group the GLA-content of breast milk was 0.07% of total fatty acids in atopic infants vs. 0.17% in non-atopic infants ($p < 0.01$). Dietary GLA-supplementation could not prevent AD. Interestingly, the number of infants developing AD was the lowest in never breastfed children. In infants suffering from AD, GLA-supplementation seemed to reduce total IgE in the first year of life.

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Atopic dermatitis (AD) is a chronic inflammatory skin condition, often presenting in early infancy as the first clinical manifestation of atopy. Affecting about 20% of young children, primary prevention of atopy is a high public health priority (1). Exclusive breast feeding for the first few months has been suggested to prevent atopic disease (2, 3). The beneficial effect of breast-milk derived lactoferrin, lymphocytes, and immunoglobulins on infants' immunologic function is well established. Also polyunsaturated fatty acids (PUFAs) might play an additional role in the complex immunofunctions of infancy. The n-6 and n-3 series of PUFAs contribute for approximately 2% of the total fatty acids of human breast milk, whereas

commercial unsupplemented formula diet based on cow's milk protein has no PUFAs. Granot examined fatty acid compositions of healthy infants and found differences between breast and formula feeding for the n-3 series PUFAs in erythrocyte membrane phospholipids (4). Moreover, less content of n-6 series of PUFAs could be found in breast milk from mothers whose infants subsequently developed atopic eczema compared with milk from mothers whose infants remained unaffected (5, 6). Only lately some brands of formula are now enriched with PUFAs, including gamma-linolenic acid (GLA).

Gamma-linolenic acid is the precursor of inflammatory mediators such as prostaglandine E_1 (PgE_1), which influence AD mainly through

two pathways. First, PGE_1 is involved in the maturation and differentiation of T-lymphocytes, and thus might influence immunoglobulin E (IgE) production (7). Secondly, GLA supplementation might alleviate the inflammatory component of AD.

Gamma-linolenic acid is the primary substrate for arachidonic acid (AA). In patients with atopic eczema, a defect of delta-6-desaturase has been suggested, which is the rate limiting enzyme transforming linoleic acid (LA) to GLA. This hypothesis is based on animal experiments and could be confirmed in humans first by experiments of Manku et al. (8) who examined plasmaphospholipids in adolescents with AD and found high level of LA, while GLA was decreased. In another study the same group found no differences in the uptake of PUFAs from the diet and concluded from their observations a deficiency in the metabolism of LA, best interpreted by an insufficiency in the delta-6-desaturase enzyme (9).

In the metabolism of n-6-PUFAs such as GLA, AA maintains fluidity of phospholipid cell membranes and augments the barrier function of skin cells. Both GLA and AA are precursors of PEG_1 and PEG_2 , having a major impact on the pathogenesis of AD (10). Thus, a lack of AA potentially results in skin breakdown, as observed in AD. Additionally, PUFAs play a crucial role in the immune system, having a direct influence on the T-helper cell differentiation. Besides being precursors of anti- and proinflammatory mediators (e.g. cysteinyl-leucotriens), they also influence immune interactions directly (11).

In the last two decades, many studies investigated the role of imbalances in the metabolism of PUFAs in the pathogenesis of inflammatory and atopic diseases. Both epidemiologic studies and intervention trials showed preventive and therapeutic effects of dietary PUFAs on the development and severity of AD. Differences in fatty acid composition between later atopic and non-atopic infants were found in umbilical cord blood (12, 13), colostrum, and mature breast milk (14, 15) of atopic mothers. Intervention studies with GLA supplementation solely of children are scarce, only one study of preventive supplementation during the first months of life (16). Thus, our study investigates early GLA supplementation of infants either via their lactating mothers or via their formula diet. Our approach was not to perform a dose-finding study, but to supplement physiologic amounts of GLA as found in healthy non-atopic individuals.

Materials and methods

This study is a double-blind placebo-controlled trial in mothers and their infants at increased risk for atopy. Our prospective controlled multicenter study encompassed 148 infants, who were screened by study doctors using a questionnaire. We found 137 cases eligible. Of those, six infants had to be excluded during the first year because of non-compliance. Inclusion criteria specified full-term healthy newborns with at least one first-line relative suffering from atopic disease. Exclusion criteria specified newborns unable to be fed orally, severe concurrent disease until time of enrollment, non-compliance to the diet in the first five months, and considerable absence from study visits. Infants were screened within the first 2 days of life, and grouped in accordance with their mother's decision on the form of feeding. Group A comprised infants, who were exclusively breastfed for 4 months, followed by feeding of whey hydrolysate formula. Group B comprised initial breastfeeding for a maximum of 4 wk followed by whey hydrolysate formula. Group C comprised an exclusive feeding of whey hydrolysate formula feeding from the beginning (Fig. 1). The mother's decision whether to breast feed or not was not influenced by the study staff. Each group was randomly assigned to supplementation of GLA or placebo for the first 5 months of life. Breast-fed infants received GLA by supplementing their mothers. This study used GLA concentrations equalized to the content of term breast milk (5). The commercial whey formula (Beba HA®; Nestlé, Munich, Germany) contains 0.14% GLA/total fat, resulting in a daily intake of 40 mg GLA/day, based on an estimated daily oral intake of 800 ml. To reach the breast milk contents the whey formula for the infant had to be supplemented with 0.60%/total fat. Whey hydrolysate for the breastfeeding mothers was enriched with GLA to the lowest therapeutic dose of 100 mg GLA/day

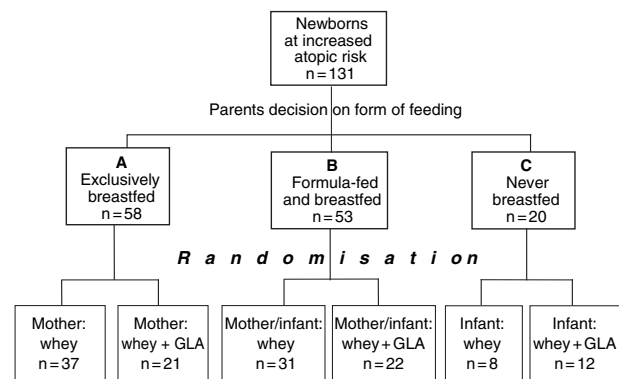


Fig. 1. Stratification of feeding situation.

(0.89%/total fat). Non-enriched dietary whey for mothers contained 0.02% GLA/total fat.

A diary was kept by the parents of each patient with details on the feeding frequencies, daily maternal diet for breast-fed infants, weekly body weight of the infant, vaccinations, concurrent diseases like infections and any signs of AD (e.g. eczema with localization). Beyond normal nutrition and whey supplementation, breast-feeding mothers were instructed to avoid the intake of allergens such as hen's egg, cow's milk, fish, nuts, soy-products as well as citrus fruits while breast-feeding. Study subjects were seen at the age of 1 wk (visit 1), 4 months (visit 2), and 12 months (visit 3). At each visit the skin atopy score of atopic dermatitis (SCORAD) was risen and diagnosis of atopic eczema was made by the criteria of Hanifen (17). This entailed the compilation of major criteria (e.g. positive family history, itching, and eczema at typical locations for at least 3 wk) and increased IgE as minor criteria. During visits 1 and 2, breast milk was collected by a hand pump and stored frozen (-20°C) until analyzed for fatty acid content. Total serum IgE of infants was determined at birth, at the age of 4 and 12 months.

Informed consent was obtained from all patients' parents or guardians. Human experimentation guidelines of Good Clinical Practice, the German Drug Act and the Declaration of Helsinki/Hong Kong were followed in the conduct of clinical research. Local ethical committee approval was obtained.

Statistical analysis

The aim of our intervention was to reduce the atopy risk of the offspring from atopic parents (38.8%) to a risk lower than the one of the normal population (16.6%) (18). Thus, sample size calculation was based on an estimated 35% effect of our intervention, an alpha-error of 0.05, a power of 0.80, and a sample size of at least 43 subjects per group. Data were analyzed using the software package SPSS for Windows® 11.0 (SPSS Inc., Chicago, Illinois, USA). All values are expressed as medians plus ranges. Since we could not estimate normal distribution of data, non-parametric tests (Mann-Whitney *U* and Kruskal-Wallis) were performed. Statistical significance was defined as $p < 0.05$.

Results

During the course of the study 24 of 131 (18.3%) eligible infants developed AD. When stratifying for feeding, 15.5% (9/58) of the exclusively breast-

fed group developed this characteristic, 26.4% (14/53) of group B, and 5.0% (1/20) of the never breast-fed group C. There was no detectable influence of GLA supplementation on the development of AD. For subgroup classification depending on GLA supplementation, see Table 1.

As expected, all groups showed an increase of IgE-concentrations within one year (Table 2). As there were no GLA-supplemented subjects with eczema in group C, no IgE-values can be provided for this subgroup. In subjects with AD, the IgE increase was the lowest in the breast-fed group A with GLA supplementation after 12 months. There was a significant impact of GLA supplementation on IgE levels. In GLA-supplemented atopic subjects, median values of IgE were 10.0 IU/ml in group A vs. 25.5 IU/ml in group B ($p < 0.05$). In contrast, non-GLA supplemented atopic subjects presented IgE levels of 125 IU/ml and 173 IU/ml in group A and group B, respectively ($p < 0.05$). In groups A and B, atopic subjects under GLA supplementation had significant lower IgE levels than non-supplemented subjects ($p < 0.01$). In non-atopic subjects, there were no significant differences in IgE-values between the groups A, B, and C and no impact of GLA supplementation was detected. The only atopic group C individual had an IgE of 366 IU/ml and was not GLA supplemented.

Gamma-linolenic acid supplementation did not change the PUFA levels in erythrocytes of infants. There was neither a significant difference between the concentrations of GLA, di-homo-gamma linolenic acid (DGLA) and AA between the intervention groups, nor between subjects developing AD or not.

The levels of GLA and AA of breast milk were independent of the maternal atopy status and could not be influenced by GLA supplementation over a period of 4 months of lactation. Only when stratifying for the infant's atopy status, an impact of GLA-supplementation on the GLA-content of breast milk could be detected. At birth, GLA-values were similar in atopic and

Table 1. Number of infants developing atopic dermatitis in the first year of life depending on feeding modalities and gamma-linolenic acid (GLA) supplementation

Intervention	Group A (n = 58; exclusively breastfed)	Group B (n = 53; formula-fed and breastfed)	Group C (n = 20; never breastfed)
All	9/58 (15.5)	14/53 (26.4)	1/20 (5.0)
GLA+	3/21 (14.3)	7/22 (31.8)	0/12 (0.0)
GLA-	6/37 (16.2)	7/31 (22.6)	1/8 (12.5)

Values in parentheses are expressed in percentage.

Table 2. Median values (range) of serum immunoglobulin E levels depending on intervention and development of atopic dermatitis

Intervention	Group A (n = 58; exclusively breastfed)			Group B (n = 53; formula fed and breastfed)			Group C (n = 20; never breastfed)		
	Birth	4 months	12 months	Birth	4 months	12 months	Birth	4 months	12 months
GLA+									
AD	1.4 (0.1–9.0)	2.95 (1.2–7.0)	10.0 (7.0–13.0)*	0.3 (0.1–4)	8.5 (3–48)	25.5 (12–302)*	None	None	None
NAD	0.2 (0.0–5.0)	2.2 (0.0–36)	7.0 (0.6–156)†	0.3 (0.1–1.2)	3 (0.5–46)	11 (4.3–149)†	0.31 (0.03–0.87)	2.2 (0.5–8)	4.0 (1.8–14.0)†
GLA–									
AD	0.7 (0.2–6.0)	14.0 (2.9–84.0)	125 (10–1405)†‡	2.0 (0.2–86)	92 (3–664)	173 (4.5–712)†‡	5.2 (n = 1)	72 (n = 1)	366 (n = 1)§
NAD	0.3 (0.0–5.0)	1.8 (0.0–36)	6.8 (1.1–163)†	0.4 (0–5)	2.3 (0.4–21)	4.9 (1.4–50)†	0.95 (0.3–2.04)	11.4 (0.3–16)	42.5 (1.2–81)†

AD, atopic dermatitis; NA, non-atopic dermatitis.

Comparison of groups after 12 months: * $p < 0.05$ (comparing groups A and B; Mann–Whitney U -test); †not significant (comparing groups A, B, and C; Kruskal–Wallis test); ‡ $p < 0.01$ (comparing atopic subjects depending on gamma-linolenic acid supplementation); §no statistic calculated for small sample size.

non-atopic infants (0.13–0.20% of total fatty acids). After a period of 4 months of lactation, in the not GLA-supplemented group, breast-milk contained 0.07% GLA of total fatty acids in atopic infants and 0.17% in non-atopic infants ($p < 0.01$). In the supplemented group, GLA content remained similar (0.22% of total fatty acids in atopic infants and 0.16% in non-atopic infants).

Discussion

Our study detected different impacts of GLA supplementation on the development of AD. At 1 year of age, never breast-fed infants demonstrated the lowest frequency of eczema, followed by the group of exclusively breast-fed infants, with no significant impact of GLA supplementation. Interestingly, results of the development of total serum IgE levels were different. As expected, all groups showed an increase of serum IgE concentrations in the first year of life. In subjects suffering from AD, breast-feeding in association with GLA supplementation resulted in a smaller increase of IgE concentrations after 12 months, with the best outcome for exclusively breast-fed study subjects. In reverse, the highest increase of total IgE was observed in atopic study subjects without GLA supplementation. Thus, GLA supplementation extenuated the natural increase of IgE in infancy, resulting in atopic study subjects with IgE values as moderate as in non-atopic individuals. This effect was independent of the route of GLA supplementation. Recent studies could not confirm an association between atopic eczema and serum IgE levels. In 345 children (mean age 2.9 yr), the correlation of the SCORAD and serum IgE levels was only weak ($r = 0.31$) and the standard deviation large (19, 20). Thus, and due to our small sample sizes, we did not analyze our data for interdependencies of these features. Our protocol foresaw not to influence the parents' decision whether to breast

feed or not, resulting in the majority of mothers trying to lactate, and in different group sizes. Also in other large studies the majority of mothers (92%) decided to breastfeed their babies (21).

Gamma-linolenic acid supplementation also did not influence the PUFA levels in erythrocytes of infants. There was neither a significant difference between the concentrations of GLA, DGLA, and AA between the intervention groups, nor between subjects developing AD or not, nor after 4 or 12 months. Overall, we could not detect differences of PUFAs in breast milk, as also observed by Thijs et al. (22).

Our results contribute to the controversy on preventive effects of breast feeding on the incidence of AD. Although many studies demonstrated at least a moderate protective effect of breast feeding against atopic disorders (23), some studies suggested an increase of atopic disorders in breast-fed children (24, 25). In our study infants, who were never breastfed had the lowest incidence of AD. Our findings support in part the observation of a large cohort study, which described an increase of AD with the duration of breast feeding (21). Interestingly, in our cohort AD was more often in mixed fed than in exclusively breast-fed infants, which goes with the established concept of an allergy preventive effect of breast-feeding. Nonetheless, our observation might be explained in part by the small sample sizes of our groups.

As to eczema, GLA supplementation of breast-feeding mothers had no impact in our cohort. Besides breast-feeding, also PUFA supplementation remains a matter of debate. A recent meta-analysis examined the impact of GLA supplementation on AD and also did not find a detectable influence on the overall result (26). The concept of therapeutical GLA supplementation is based on the theory of an insufficiency of the delta-6-desaturase, resulting in decreased levels of AA in umbilical cord blood

(13) and in the serum of atopic children (14, 27). Differences in the outcome of preceding intervention studies can be explained partially by the late onset of supplementation in children affected by or at risk for AD.

An Italian study also determined levels of long-chain PUFAs in the breast milk of mothers, whose offspring newly developed AD. The ratio of LA to the sum of its metabolites (GLA, DGLA, and AA) in mature breast milk was 11.8 in the atopic compared with 9.02 in the control group. The results from this study implicate a potential benefit from a maternal PUFA-enriched diet (6). Obviously, atopic mothers themselves suffer from a deficiency of delta-6-desaturase, resulting in an increased amount of LA and a lack of GLA in their breast milk. By breast-feeding, they render this imbalance to their offspring, who may then suffer from this inherited deficiency as well. Our study did not find differences in fatty acid composition of breast milk after GLA supplementation. This suggests that the increased LA-content might not have an impact on the development of atopic eczema, but expresses the genetically determined atopic coherence between mother and child.

In conclusion, GLA supplementation seems to have no impact on the development of AD. However, our data show a modulatory effect of GLA on IgE levels in atopic subjects.

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