

Gamma-linolenic acid supplementation for prophylaxis of atopic dermatitis--a randomized controlled trial in infants at high familial risk

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γ -Linolenic acid supplementation for prophylaxis of atopic dermatitis—a randomized controlled trial in infants at high familial risk^{1–3}

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ABSTRACT

Background: Studies suggest that low concentrations of n–6 long-chain polyenes in early life are correlated to atopic disease in later life.

Objective: The purpose of the study was to investigate the possible preventive effect of γ -linolenic acid (GLA) supplementation on the development of atopic dermatitis in infants at risk.

Design: In a double-blind, randomized, placebo-controlled trial, formula-fed infants ($n = 118$) with a maternal history of atopic disease received borage oil supplement (containing 100 mg GLA) or sunflower oil supplement as a placebo daily for the first 6 mo of life. Main outcome measures were the incidence of atopic dermatitis in the first year of life (by UK Working Party criteria), the severity of atopic dermatitis (SCORing Atopic Dermatitis; SCORAD), and the total serum immunoglobulin E (IgE) concentration at the age of 1 y.

Results: The intention-to-treat analysis showed a favorable trend for severity of atopic dermatitis associated with GLA supplementation ($\bar{x} \pm SD$ SCORAD: 6.32 ± 5.32) in the GLA-supplemented group as compared with 8.28 ± 6.54 in the placebo group ($P = 0.09$; $P = 0.06$ after adjustment for total serum IgE at baseline, age 1 wk), but no significant effects on the other atopic outcomes. The increase in GLA concentrations in plasma phospholipids between baseline and 3 mo was negatively associated with the severity of atopic dermatitis at 1 y (Spearman's correlation coefficient = -0.233 , $P = 0.013$). There was no significant effect on total serum IgE concentration.

Conclusion: Early supplementation with GLA in children at high familial risk does not prevent the expression of atopy as reflected by total serum IgE, but it tends to alleviate the severity of atopic dermatitis in later infancy in these children. *Am J Clin Nutr* 2003;77:943–51.

KEY WORDS Fatty acids, essential fatty acids, atopy, atopic dermatitis, children, supplementation

INTRODUCTION

Essential fatty acids (EFAs) are believed to be involved in the etiology of atopic disease (1). In the n–6 EFA series, linoleic acid (LA, 18:2n–6), derived from food, is subsequently converted into γ -linolenic acid (GLA, 18:3n–6) and longer-chain polyenes (LCPs) such as dihomogamma-linolenic acid (DGLA, 20:3n–6) and arachidonic acid (AA, 20:4n–6). Although LCPs of the n–3 EFA series can be derived from α -linolenic acid (ALA, 18:3n–3), the

major source of n–3 LCPs is food. As early as 1937, lower concentrations of AA were reported in the serum of children with atopic dermatitis (AD; 2). More recent studies have shown higher concentrations of LA and substantially lower concentrations of its LCPs in the blood of these patients (3–5). In newborn infants with a family history of atopic disease, lower n–6 LCP concentrations in umbilical cord blood were found to precede the development of AD (6). One suggested explanation for these findings was a reduced conversion of LA into GLA and subsequent LCPs, possibly as a result of impaired activity of the enzyme linoleoyl-CoA desaturase (Δ^6 -desaturase; EC 1.14.19.3) (3, 7). Other studies showed that breast milk from mothers whose infants subsequently developed AD contained less n–6 LCP than did milk from mothers whose infants remained unaffected (8, 9). Unlike breast milk, infant formulas until recently contained only LA and ALA as EFAs. Only lately are some brands of formula being enriched with LCPs, including GLA.

Intervention studies with GLA supplementation in patients with AD have shown inconsistent results. Most trials were carried out in a mixed population of adults and children; only 2 trials were restricted to children (10, 11). All of these trials aimed at decreasing the severity of existing eczema; no preventive trials have been conducted.

A possible role of GLA in the prevention of atopy in early life has been postulated by Melnik and Plewig (12), on the basis of the following 3 observations. First, body composition with respect to EFAs in newborn infants is entirely dependent on intrauterine supply and the subsequent choice of breast- or bottle-feeding (13). Second, mothers of atopic infants have lower concentrations of n–6 LCP in their breast milk than do mothers of nonatopic infants (9). Third, infants who have atopic symptoms at the age of 1 y have consistently and significantly lower mean concentrations of

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TABLE 1
Fatty acid profiles of verum (borage oil) and placebo supplements¹

	Verum	Placebo
SFA (% by wt of total fatty acids)		
14:0	0.1	0.1
16:0	10.7	5.8
18:0	3.8	3.9
20:0	0.2	0.3
22:0	0.2	0.7
Total	15.0	10.8
MUFA (% by wt of total fatty acids)		
18:1n-9	15.4	50.7
20:1n-9	4.0	0.3
22:1n-9	2.7	0.0
Total	22.1	51.0
PUFA (% by wt of total fatty acids)		
18:2n-6, LA	36.8	36.6
18:3n-6, GLA	23.1	0.0
18:3n-3, ALA	0.2	0.2
Total	60.1	36.8
Other or unknown (% by wt of total fatty acids)		
Total	2.8	1.4
Total	100	100

¹SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

n-6 LCPs in umbilical cord blood and in serum at 1 and 3 mo of age than do infants who remain unaffected (6). Prostaglandins derived from n-6 LCPs are thought to play a role in the maturation of the immune system (12). Because the conversion of LA to GLA is thought to be the rate-limiting step in the total chain of conversions (14), supplementation with GLA in infancy might compensate for the lower n-6 LCP concentrations and prevent atopy or decrease its severity in infants, especially if the mother has an atopic constitution. The purpose of the present study was to investigate whether GLA supplementation protects against the development of atopy in high-risk, formula-fed infants.

SUBJECTS AND METHODS

Study design and population

The present study was a double-blind, randomized, placebo-controlled trial in infants at high risk for AD. It is part of a larger study of EFAs and atopy, called the EFAtop study. Subjects eligible for the study were atopic pregnant women and their infants born during the study period. They were recruited between October 1997 and April 2000 by midwives and via advertisements in local newspapers in the provinces of Limburg and Noord-Brabant, Netherlands. The atopic status of both parents of each infant was assessed with the use of a validated telephone questionnaire. Inclusion criteria for atopic mothers were a history of allergic asthma or allergic rhinoconjunctivitis related to aeroallergen exposure, atopic dermatitis, a positive allergen test, or improvement of asthma or rhinoconjunctivitis with the use of antihistamine or anti-asthma drugs. Exclusion criteria were diabetes treated with medication or diet or both, preeclampsia, and metabolic disease.

Infants were born between December 1997 and May 2000. Inclusion criteria for the infants were gestational age of ≥ 38 wk, birth weight > 2500 g, an uncomplicated perinatal period, and exclusive formula-feeding from 2 wk of age. All data were collected at the participants' homes except for the final visit when the

infant was aged 1 y, which took place at the University Hospital Maastricht. The study was approved by the Medical Ethics Committee of the University Hospital Maastricht, and written informed consent was obtained from both parents of each infant.

Randomization, intervention, and compliance

After inclusion, infants were categorized according to the atopic status of the father (as the main prognostic factor in addition to the mother's atopy) and then randomly assigned to the experimental or placebo group with the use of block randomization in blocks of 4. Intervention started as soon as possible after baseline blood sampling at the age of 7 ± 2 d, or 14 d at the latest. The supplementation period ended when the infant reached the age of 6 mo. The intervention comprised a daily supplement, given as 1 g powder and consisting of fish gelatin (135 mg), maltodextrin (397 mg), silicic acid (21 mg), and oil (446 mg). The oil was either borage oil (*Borago officinalis*, verum) or sunflower oil (placebo), which closely resembled borage oil with respect to fatty acid composition, except that the GLA in verum was replaced by oleic acid (18:1n-9) (Table 1). Both supplements contained vitamins C and E as antioxidants, and they were provided by Hoffmann-La Roche (Basel, Switzerland). The powders were packaged in low-oxygen sachets to blind the investigators and parents to possible differences in their smell and appearance. So that the supplement would be distributed evenly throughout the day, parents were instructed to put the supplement in the total amount of formula made for 1 d. The dose was chosen to reflect upper normal concentrations of GLA in human milk. Mean GLA concentrations reported for human milk [as percentages by weight (wt%) of total milk lipids] in Western populations vary from 0.07wt% (9) to 0.35wt% (15). Other studies reviewed by Jensen (1992) showed concentrations between these values (13). We aimed for the highest concentration, 0.35wt%, of GLA in human milk. At a total typical daily output of ≈ 750 g milk with 4wt% total fat, this amounts to < 100 mg GLA/d. The actual amount of GLA present in the GLA supplement was 23.1% of the 446 mg of borage oil in a daily dose, ie, 103 mg GLA/d.

Compliance was measured by counting the number of sachets returned. Mothers were instructed to keep a diary focusing on events that disturbed the infants' food or supplement intake. Compliance was considered to be complete if $\geq 85\%$ of the sachets had been used during the entire supplementation period. We expected that the rise in plasma GLA would reflect compliance on the basis of earlier supplementation studies (13), and therefore we measured the rise in plasma GLA from baseline (before supplementation) up to 3 and 6 mo.

Sampling and laboratory analyses

Blood was collected from the women by venipuncture of the median cubital vein at 34-36 wk of gestation and from the infants at the ages of 1 wk, 3 mo, and 6 mo by heel prick or finger prick and at the age of 1 y by venipuncture of the hand vein or of the median cubital vein. Blood was collected in tubes containing EDTA (Becton Dickinson, Orangeburg, NJ) and in serum-separator tubes (Sherwood-Davis & Geck, St Louis). The samples were transported on an ice-and-water mixture and, within 24 h after collection, centrifuged at $3000 \times g$ for 10 min at 4°C and stored at -20°C (serum) or -50°C (plasma) under nitrogen until analysis. If an insufficient amount of venous blood was acquired, finger prick blood was collected on blotting paper for total serum immunoglobulin E (IgE) analysis.

Fatty acids were analyzed as previously described (16). Lipid extracts were prepared from plasma samples, and phospholipid fractions separated using aminopropyl-bonded phase columns (17). Phospholipids were hydrolyzed and fatty acids were transmethylated with boron trifluoride (Sigma Chemical Co, St Louis) in methanol. The composition of the fatty acid methyl esters obtained was determined by capillary gas chromatography with the use of a polar capillary column (CPSil 88; Chrompack, Middelburg, Netherlands) and with helium as the carrier gas. The amount of each fatty acid was quantified by adding an internal standard [dinonadecanoyl (19:0)-phosphatidylcholine; Sigma Chemical Co]. Results are reported as wt% of total fatty acids, computed as previously described (18).

Screening tests for total IgE and for common aeroallergens and food allergens (Phadiatop and Fx5, respectively) were performed in serum from infants at age 1 y with the use of a new in vitro test system (UniCAP; Pharmacia Upjohn, Uppsala, Sweden) as described elsewhere (19). Measurement of total IgE in eluted blood spot material was performed in a sandwich assay, as previously described (20), with minor modifications: a mixture of anti-human IgE monoclonal antibodies was coupled to Sepharose 4B (Pharmacia Upjohn) to bind IgE; Sepharose-bound IgE was detected with the use of radiolabeled antibodies against human IgE, raised in sheep.

Clinical outcome variables and adverse events

At the follow-up hospital visit when the infants were age 1 y, a trained dermatologist (CH) made the clinical diagnosis of AD by using the criteria of the UK Working Party (21, 22). Briefly, the probability of the presence of AD was derived from the presence of 4 clinical symptoms: (1 the presence of itchy rash (PIR; coded as 1 = present, 0 = absent), (2 a history of flexural dermatitis (HFD; 1 = present, 0 = absent), (3 visible flexural dermatitis (VFD; 1 = present, 0 = absent), and (4 onset before 2 y of age (OB2; 1 = present, 0 = absent). HFD and VFD were modified to the extensor side of the limbs to match the typical clinical predilection sites in infants (21, 22). The UK Working Party probability score for AD was then computed as

$$\text{Probability (AD)} = \text{odds (AD)} / [\text{odds (AD)} + 1] \quad (1)$$

where $\text{odds (AD)} = \exp[4.86 + 1.84(\text{HFD}) + 3.46(\text{OB2}) + 2.09(\text{VFD}) + 1.71(\text{PIR})]$ (21, 22). In the present study, infants with a >0.50 probability of AD were considered to have AD.

Severity of dermatitis was scored by one dermatologist (CH) using clinical criteria (SCORing Atopic Dermatitis; SCORAD; 23, 24). These criteria include measuring the size of the affected area (A) and scoring the intensity of the dermatitis (B), with 5 criteria for scoring intensity: erythema, edema or papulation, oozing or crusting, excoriation, and lichenification (each with scores 0–2). The SCORAD index was computed as $A/5 + (7 \times B)/2$ (23, 24).

At the same time, the SCORAD index was obtained independently by one of the other trained investigators (CJAWvG or CT) to assess interobserver variability with the dermatologist. Parents were asked to report on the severity of itch and on sleeplessness as a result of itch, with the use of a visual analogue scale ranging from 0 (smiling baby) to 100 (crying baby). Parents were also asked to bring the infants' medication to the hospital, and the use of emollients and corticosteroids was carefully noted.

Size of the study population

The reported incidence of AD in the first year of life ranged from 22–33% (25) to 38% (26), with a mean of 35.5%, in breast-fed infants at risk and from 49% (25) to 57% (27), with a mean of

53%, in formula-fed infants. Several studies have reported a stronger maternal than paternal inheritance; the only study to report incidences separately for atopic mothers and atopic fathers found a 47% incidence of atopic eczema in children with atopic mothers and a 10% incidence in children with atopic fathers (28).

Assuming an average AD incidence of 53% in the placebo group, we aimed at a 41% risk reduction in the intervention group (which would equal an incidence of $\approx 31\%$, equivalent to the incidence in breast-fed infants). For a power of 80% (at $\alpha = 0.05$; one-sided), the study size needed to detect this risk reduction was calculated to be 122 infants.

Statistical analysis

Statistical analyses were performed with SPSS for WINDOWS software, version 10.0 (SPSS, Inc, Chicago). AD was the main outcome variable, with SCORAD index and total and specific IgE concentrations as secondary outcome measures. Total serum IgE was not normally distributed and was therefore transformed to its natural logarithm (log IgE). Spearman's rank correlations were calculated to assess the correlation between the different outcome variables (SCORAD, log IgE, and UK Working Party probability score).

In the intention-to-treat analysis comparing GLA and placebo groups for binary outcome measures (AD, reported itch, topical steroid use, emollient use, and positive Fx5), odds ratios were computed with the use of logistic regression analysis. Differences in mean SCORAD and log IgE between the GLA and placebo groups were tested for significance by the use of Student's *t* test ($\alpha = 0.05$, two-sided) or analysis of covariance when corrected for covariables; 95% CIs are reported throughout.

In the explanatory analysis with an increase in plasma GLA as a marker of compliance, logistic regression analysis was performed to test the association between the increase in the infant's plasma phospholipid GLA concentration from the age of 1 wk (baseline) to 3 mo and from the age of 1 wk to 6 mo and the incidence (or nonincidence) of AD. Because the SCORAD index was not distributed normally and log transformation did not overcome this problem, logistic regression analysis was also used to test the association between the higher and lower tertiles of the SCORAD index and the rise in GLA. Linear regression analysis was used to investigate the relation between log IgE at the age of 1 y and the rise in GLA; in addition, we performed logistic regression analysis with the higher and lower tertiles of IgE as the outcome.

Potential confounders included as covariates in the multivariate logistic regression models were variables that are known to be associated with atopy: the atopic status of the father, smoking during pregnancy, smoking by parents in the first year of the infant's life, the age of the mother at delivery, the infant's sex, the total serum IgE of the infant at 1 wk, the total serum IgE of the mother, the number of siblings, day-care attendance, the educational level of the father, the educational level of the mother, the presence of a dog in the household, the presence of a cat in the household, and the use of allergen-free bed covers.

RESULTS

One hundred twenty-one infants were included in the study. At the age of 1 y, 118 infants had completed follow-up, 58 in the GLA group and 60 in the placebo group (Figure 1). The other 3 infants did not complete the supplementation and were lost to follow-up.

Complete compliance ($>85\%$ of the sachets used) was observed in 85% of the infants (51 in the GLA group and 51 in the placebo

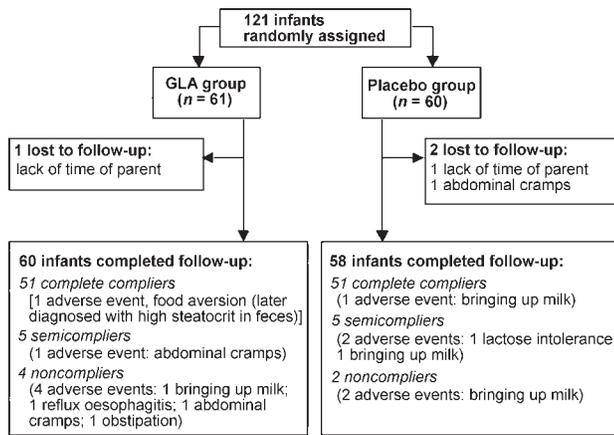


FIGURE 1. Trial profile: follow-up, compliance, and reported adverse events in the study (EFAtop study).

group). Five infants in each group had used 50–85% of the sachets (semicompliers), whereas 4 infants in the GLA group and 2 infants in the placebo group had used <50% (noncompliers) (Figure 1).

Adverse events were equally divided between the GLA and placebo groups all 121 infants, with abdominal cramps (3 infants) and bringing up milk (5 infants) being the most frequent symptoms (Figure 1). Both symptoms were usually temporary, but in most cases they resulted in the supplements being withheld from the infant by the parents for 2 d to 2 wk, to ensure that the symptoms were not caused by the supplements. When the symptoms subsided, parents were asked to reintroduce the supplement slowly and to report whether the problems recurred.

The GLA and placebo groups did not differ significantly with regard to baseline variables except for the presence of a carpet in the bedroom of more of the infants in the GLA group than of those in the placebo group (Table 2). In our population, the ratio of LA to ALA in infant formulas was between 10:1 and 6:1, and the formulas contained no LCPs. The course of GLA, DGLA, and AA concentrations in the infants' plasma phospholipids for the GLA and placebo groups is shown in Figure 2. At 3 mo, the concentrations of GLA, DGLA, and AA were, respectively, 70%, 24%, and 13% higher in the GLA group infants than in the placebo group infants. At 6 mo, the concentrations of GLA, DGLA, and AA were, respectively, 47%, 19%, and 15% higher in the GLA group infants than in the placebo group infants. When corrected for baseline concentrations (at 1 wk), all these differences remained significantly different. In the GLA group, compliers had distinctly higher GLA, DGLA, and AA concentrations at 3 and 6 mo than did compliers in the placebo group, whereas semicompliers and noncompliers in the GLA group had concentrations similar to those of semicompliers and noncompliers in the placebo group.

The Spearman's rank correlation between the UK Working Party probability score and SCORAD index was 0.56 ($P = 0.01$). There was no correlation between the UK Working Party probability score and log total serum IgE ($r = 0.12$, $P = 0.21$) or between the SCORAD index and log total serum IgE ($r = 0.09$, $P = 0.29$). We defined AD by dichotomizing the UK Working Party probability score into a low score (ie, 0.29, meaning "AD absent"; 67 infants) and high scores (probability 0.69–0.95, meaning "AD present"; 51 infants).

In the intention-to-treat analyses (Table 3), infants in the GLA group showed a trend toward lower SCORAD values ($P = 0.09$;

TABLE 2

Baseline variables and co-interventions between the γ -linolenic acid (GLA) and placebo groups¹

	GLA group (n = 60)	Placebo group (n = 58)
Family history		
Atopic history of mother		
Asthma (n)	27	26
Rhinitis (n)	51	43
Atopic eczema (n)	23	20
Positive allergen test (n)	47	41
Use of antihistamine drugs (n)	23	20
Total serum IgE of mother (n in tertiles) ²		
1–52 (kU/L)	21	18
53–209 (kU/L)	22	17
210–5506 (kU/L)	17	23
Positive aeroallergen screening test in mother (n)	44	46
Positive food allergen screening test in mother (n)	9	9
Atopic history of father (n)		
Positive	22	21
Uncertain or negative	38	37
Age of mother at time of delivery (y)	31.2 ± 4.0 ³	30.5 ± 3.9
Duration of pregnancy (wk)	40.1 ± 1.3	40.1 ± 1.1
Child		
Girls (%)	55	53
Birth weight (g)	3513 ± 483	3528 ± 463
Birth length (cm)	50.6 ± 2.5	49.9 ± 2.5
Head circumference at birth (cm)	34.9 ± 1.6	34.8 ± 1.4
Total serum IgE in infants at age 1 wk (n in tertiles) ²		
0.00–0.10 (kU/L)	23	16
0.11–0.73 (kU/L)	19	20
0.74–21.00 (kU/L)	16	21
Risk factors		
Highest educational level of either parent (n)		
Low	4	8
Middle	34	30
High	22	20
Number of siblings (n)		
0	27	30
1	22	19
2 or more	11	9
Day-care attendance (d/wk)	0.92 ± 1.06	1.04 ± 1.13
Pets present in the house (n)		
Cat	5	2
Dog	19	17
Rabbit	2	2
Carpet in living room (n)	6	5
Carpet in infant's bedroom (n)	14 ⁴	3
Allergen-free cover on infant's bed (n)	2	6

¹IgE, immunoglobulin E.

²Tertiles based on total cohort (GLA and placebo groups), with one subject missing in the placebo group.

³ $\bar{x} \pm SD$.

⁴Significantly different from placebo group, $P < 0.007$ (Fisher's exact test).

$P = 0.06$ after adjustment for total serum IgE at baseline). In contrast, the GLA group showed a tendency toward higher total serum IgE values than did the placebo group. When the analyses were restricted to the compliers, the differences for SCORAD values remained roughly the same (Table 4). IgE or positivity on the food

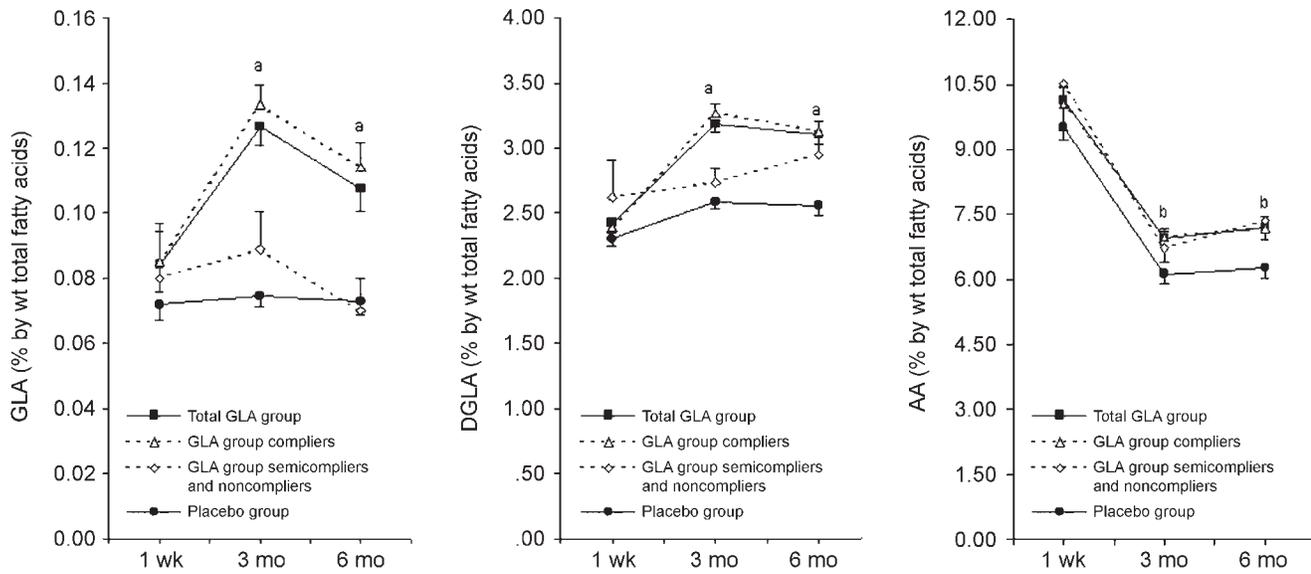


FIGURE 2. Mean (±SEM) γ-linolenic acid (GLA), dihomo-γ-linolenic acid (DGLA), and arachidonic acid (AA) concentrations in infants' plasma phospholipids in the GLA group and the placebo group. Total GLA group, all infants randomly assigned to receive GLA supplementation (*n* = 58); placebo group (*n* = 60); GLA compliers, infants in the GLA group who complied with the treatment (*n* = 51); GLA semicompliers and noncompliers, infants in the GLA group who partially complied (semicompliers; *n* = 5) or did not comply (noncompliers; *n* = 4). Values were missing for 3 GLA compliers at 1 wk and for 2 infants in the placebo group at 3 mo. Significance of differences between the total GLA group and the placebo group by analysis of covariance: ^a*P* < 0.001 after adjustment for baseline concentrations at 1 wk; ^b*P* < 0.01 after adjustment for baseline concentrations at 1 wk.

allergens screening test did not differ significantly between the GLA and the placebo groups. None of the infants had a positive Phadiatop aeroallergen screening test.

In the explanatory analysis with the increase in plasma GLA as a marker of compliance, that from baseline to 3 mo of age was negatively and significantly associated with the SCORAD index at 1 y of age (Tables 5 and 6). The odds ratios for this association were insensitive for the cutoff value of SCORAD (between 0.36 and 0.39) and were even slightly lower (0.27 and 0.29) after adjustment for possible confounding factors. No such associations

were found for AD or total serum IgE (Table 5). The increase in plasma GLA concentration from baseline to 6 mo was not related to any of the atopic outcomes.

Because the association between GLA increase and SCORAD value could be biased (being based on an explanatory analysis), it may be subjected to protopathic bias. This bias exists when an early (protopathic) stage of disease or an underlying etiologic condition leads to changes in the risk factor, which produce a spurious association between the risk factor and the disease (29). In the EFAtop study, this bias may have occurred for the following reasons:

TABLE 3

Atopic outcomes at age 1 y in infants randomly assigned to receive γ-linolenic acid (GLA) or placebo supplementation in intention-to-treat analysis¹

	GLA group (<i>n</i> = 60)	Placebo group (<i>n</i> = 58)	OR (95% CI)	<i>P</i>	Adjusted OR ² (95% CI)	<i>P</i>
Atopic dermatitis, UK Working Party probability score > 0.50 (<i>n</i>)	23	28	0.67 (0.32, 1.39)	0.28	0.69 (0.33, 1.45)	0.33
Parent reported itch (<i>n</i>)	3	7	0.38 (0.09 – 1.56)	0.18	0.33 (0.08, 1.39)	0.13
Severity of atopic dermatitis (SCORAD index) ^{3,4}	6.32 ± 5.32	8.28 ± 6.54		0.09		0.06
Topical steroid use, class 1 or 2 (<i>n</i>)	2 (1, 1)	5 (4, 1)	0.37 (0.68, 1.96)	0.24	0.41 (0.08, 2.23)	0.30
Emollient use (<i>n</i>)	27	21	1.44 (0.39, 3.02)	0.33	1.37 (0.64, 2.92)	0.42
Total serum IgE at age 1 y ^{5,6}	9.31 ± 4.45	6.87 ± 3.56		0.24		0.10
Positive food allergen test (<i>n</i>)	10 (2 missing)	5 (4 missing)	2.04 (0.65, 6.92)	0.22	2.32 (0.72, 7.46)	0.16

¹OR, odds ratio; SCORAD, SCORing Atopic Dermatitis; IgE, immunoglobulin E.

²Adjusted for total serum IgE at baseline (age 1 wk).

³Differences of the means between GLA group and placebo group, -1.90 (95% CIs: -4.07, 0.27); *P* = 0.09 (Student's *t* test). Differences of the means between GLA group and placebo group corrected for baseline IgE (at 1 wk), -2.16 (95% CIs: -4.38, 0.06); *P* = 0.06 (analysis of covariance).

⁴ $\bar{x} \pm SD$.

⁵Differences of the geometric means of total serum IgE between GLA and placebo groups, 1.36 (95% CIs: 0.82, 2.25); *P* = 0.24 (Student's *t* test). Differences of the geometric means of total serum IgE between GLA and placebo groups, corrected for baseline IgE (at 1 wk), -1.54 (95% CIs: 0.92, 2.56); *P* = 0.10 (analysis of covariance).

⁶Geometric $\bar{x} \pm SD$.

TABLE 4Atopic outcomes at age 1 y in infants randomly assigned to receive γ -linolenic acid (GLA) or placebo supplementation (compliers only)¹

	GLA group (n = 51)	Placebo group (n = 51)	OR (95% CI)	P	Adjusted OR ² (95% CI)	P
Atopic dermatitis, UK Working Party probability score > 0.50 (n)	19	22	0.78 (0.35, 1.73)	0.54	0.81 (0.36, 1.82)	0.61
Parent reported itch (n)	3	5	0.58 (0.13, 2.55)	0.47	0.50 (0.11, 2.30)	0.37
Severity of atopic dermatitis (SCORAD index) ^{3,4}	6.07 ± 5.16	7.92 ± 6.26		0.11		0.06
Topical steroid use, class 1 or 2 (n)	1 (1, 0)	4 (4, 0)	0.24 (0.03, 2.18)	0.20	0.24 (0.03, 2.24)	0.21
Emollient use (n)	24	17	1.78 (0.80, 3.96)	0.16	1.68 (0.73, 3.86)	0.22
Total serum IgE at age 1 y ^{5,6}	7.9 ± 4.3	6.9 ± 3.7		0.67		0.34
Positive food allergen test (n)	8 (0 missing)	4 (4 missing)	2.05 (0.57, 7.32)	0.27	2.48 (0.67, 9.16)	0.87

¹OR, odds ratio; SCORAD, SCORing Atopic Dermatitis; IgE, immunoglobulin E.²Adjusted for total serum IgE at baseline (age 1 wk).³Differences of the means between GLA group and placebo group, -1.84 (95% CIs: -4.09, 0.41); P = 0.11 (Student's *t* test). Differences of the means between GLA group and placebo group corrected for baseline IgE (at 1 wk), -2.25 (95% CIs: -4.57, 0.08); P = 0.06 (analysis of covariance).⁴ $\bar{x} \pm$ SD.⁵Differences of the geometric means of total serum IgE between GLA and placebo groups, 1.12 (95% CIs: 0.65, 1.93); P = 0.67 (Student's *t* test). Differences of the geometric means of total serum IgE between GLA and placebo groups, corrected for baseline IgE (at 1 wk), 1.31 (95% CIs: 0.99, 1.25); P = 0.34 (analysis of covariance).⁶Geometric $\bar{x} \pm$ SD.

(1 a genetic atopic constitution is truly associated with a later high SCORAD index and forms the underlying condition; (2 an atopic constitution increases the risk of food allergy in the first few months of life, and this is associated with feeding problems; and (3 these feeding problems may lead to difficulty with the use of the supplement, as indicated by noncompliance or suboptimal compliance. The resulting lower intake of GLA is reflected in failure to show an increase in plasma concentrations of GLA. In this way, a negative association between the increase in GLA and the SCORAD index is produced, even if there is no true effect on the SCORAD index. We scrutinized the data in search of typical cases of infants who fulfilled these conditions for protopathic bias. As indicative of feeding problems related to food allergy, we selected infants who were switched to hypoallergenic formula, were suspected by the parents of having a food allergy, or had a positive food allergen test and infants who had feeding problems or were known as noncompliers. We evaluated in these infants whether the

SCORAD index was particularly high (as a consequence of the underlying atopic condition) and, if so, whether the GLA increase was indeed low (as a consequence of possible noncompliance due to early atopic symptoms). This was done only for infants in the group receiving verum, because only in this group does supplementation lead to GLA increase. We identified only 2 infants who fulfilled the conditions for protopathic bias. Even so, the SCORAD index in those infants was in the middle range (4.3–7.4), and thus the impact on the results of the group as a whole is quite limited, which leads us to conclude that protopathic bias is not likely.

DISCUSSION

As far as we know, this is the first published preventive trial of GLA supplementation on AD; earlier trials have been therapeutic, aiming at the improvement of existing AD. Results of these therapeutic trials have been inconsistent (30, 31). Some of the trials

TABLE 5Association between the increase in plasma phospholipid γ -linolenic acid (GLA) concentration and atopic outcomes at age 1 y¹

	Increase in GLA between 1 wk and 3 mo ²		Increase in GLA between 1 wk and 6 mo ³	
	Crude OR (95% CI)	Adjusted OR (95% CI) ⁴	Crude OR (95% CI)	Adjusted OR (95% CI) ⁴
Atopic dermatitis ⁵				
Present (n = 51) compared with absent (n = 67)	0.81 (0.40, 1.65)	1.11 (0.47, 2.65)	0.94 (0.57, 1.55)	1.25 (0.66, 2.40)
SCORAD index ⁶				
High+middle (n = 81) compared with low (n = 37) tertile	0.36 (0.15, 0.87)	0.27 (0.09, 0.86)	0.78 (0.44, 1.38)	0.70 (0.31, 1.54)
High (n = 38) compared with low+middle (n = 80) tertile	0.39 (0.17, 0.89)	0.29 (0.09, 0.90)	0.68 (0.39, 1.17)	0.71 (0.34, 1.47)
Total serum IgE ⁷				
Middle+high (n = 79) compared with low (n = 39) tertile	1.29 (0.62, 2.70)	1.44 (0.57, 3.60)	1.18 (0.70, 2.00)	1.20 (0.63, 2.29)
High (n = 40) compared with low+middle (n = 78) tertile	1.02 (0.49, 2.14)	1.36 (0.52, 3.54)	1.18 (0.68, 2.06)	1.14 (0.58, 2.24)

¹SCORAD, SCORing Atopic Dermatitis; IgE, immunoglobulin E.²Five values missing (SCORAD index: 4 in middle category and 1 in high category; IgE: 2 in low category, 1 in middle category, and 2 in high category).³Three values missing (SCORAD index: 2 in middle category and 1 in high category; IgE: 1 in low category and 2 in high category).⁴Covariables included in the multivariate logistic regression model were atopic status of father, smoking during pregnancy, smoking by parents in the infants' first year, age of mother at time of delivery, sex of infant, IgE of infant at 1 wk, IgE of the mother, number of siblings, day-care attendance, educational level of the father, educational level of the mother, presence of dog, presence of cat, and use of allergen-free bed covers.⁵Present if UK Working Party probability score was > 0.50.⁶SCORAD index range, 0–30.3 divided into tertiles (cutoffs were 4.1 and 8.7).⁷Range, 1.00–371 kU/L divided into tertiles (cutoffs were 4.00 and 12.40 kU/L).

TABLE 6Association between the increase in plasma phospholipid γ -linolenic acid (GLA) concentration between age 1 wk and 3 mo and SCORAD index at age 1 y¹

SCORAD index at age 1 y (tertiles)	GLA increase between age 1 wk and 3 mo (tertiles)			Total
	Low tertile (-0.216 to 0.004 wt%)	Middle tertile (0.005-0.040 wt%)	High tertile (0.041-0.179 wt%)	
Low tertile (0-4.1)	7	10	17	34
Middle tertile (4.2-8.6)	12	17	13	42
High tertile (8.7-30.3)	18	12	7	37
Total	37	39	37	11

¹SCORAD, SCORing Atopic Dermatitis. Spearman's correlation coefficient, -0.233; $P = 0.013$. Linear-by-linear association, $P = 0.002$.

had methodologic drawbacks such as heterogeneity of the study population in terms of age (32) or the unblinded status of either the subjects or the investigators (33). Among 4 trials in children with AD, 3 showed no greater effect of GLA than of placebo (11, 34, 35), and only 1 (10) showed a favorable effect on the severity of eczema of GLA compared with placebo. Our study was designed to investigate the possible protective role of GLA in the development of atopic dermatitis in infants with atopic mothers.

On the basis of an intention-to-treat analysis, we observed a trend for severity of dermatitis (as measured by the SCORAD index) in the GLA group that was favorable but not significantly different from that in the placebo group. The effects of GLA supplementation on another clinical atopic outcome (ie, itch) trended similarly but were not significant.

When the increase in plasma phospholipid GLA was used as a marker of compliance, the severity of AD as measured by the SCORAD index had a strongly negative association with the increase in GLA in infants aged 1 wk-3 mo. Apart from being a marker of compliance, the increase in GLA also incorporates the effect of intestinal uptake of GLA. Because this was not an intention-to-treat analysis, it is important to check for biases. First, selective follow-up was not likely to occur because all but 3 children completed the follow-up. Second, information bias was unlikely because the investigator, the patient, and the outcome assessor remained blinded for the allocation of the supplements and for GLA concentrations in the infants. Third, data were checked for protopathic bias, which was ruled out. Fourth, when we controlled for possible confounding factors in the analyses of plasma GLA increase and SCORAD, the association remained as strong and significant. We therefore think that the association is real. However, our results are not definite proof of a causal effect of supplementation. They could also be explained by a metabolic difference in EFA metabolism between infants with and without an atopic constitution, which occurs only after GLA supplementation, but this explanation does not fit any of the current hypotheses. No associations were observed between the increase in GLA and the presence or absence of AD or the total serum IgE concentration.

If there is a causal effect of GLA supplementation on the severity of AD, the observation that the clearest results are related to the GLA concentrations in the plasma of infants at age 3 mo would suggest that the constant dose of GLA given over time in this study might not be sufficient with progressing age and increasing body weight. It is therefore possible that the effect of GLA would be more pronounced with a higher dose of GLA at a later age. Another explanation for these findings might be that this early period represents the time frame within which the immune system is most susceptible to GLA. That would be consistent with the results of Galli et al (6), who showed that the concentrations

of n-6 LCPs were consistently and significantly lower in umbilical cord blood and in serum at 1 and 3 mo of age in infants who developed atopy at the age of 1 y than in infants who remained unaffected, and that, in infants aged 1 y, these differences in n-6 LCP concentrations were no longer present.

Our study shows an effect of GLA on the severity of AD but not on the development of IgE at the age of 1 y. This indicates that GLA supplementation has a beneficial effect on the inflammatory component of AD, rather than on its IgE-mediated component. The absence of a correlation between clinical outcome measures (incidence of AD and SCORAD index) and total and specific IgE in the present study also indicates that different components are implicated in the pathogenesis of AD. For a similar reason, it has recently been proposed to revise the nomenclature of AD and to refer to the condition as atopic eczema and dermatitis syndrome (AEDS) (36), which would include IgE-mediated and non-IgE-mediated pathogenesis. The heterogeneity of patients in terms of AEDS between the previous therapeutic studies might partially explain the inconsistent results of GLA supplementation in those studies.

The beneficial effect of GLA supplementation on the severity of AEDS can be explained by the results of in vitro studies on skin epidermis (37, 38). Normal skin epidermis is unable to convert LA into GLA. Dietary GLA is actively converted into DGLA in guinea pig epidermis (39), a model believed to resemble human epidermis. Because Δ^5 -desaturase (EC 1.14.99.-), which converts DGLA into AA, is absent from skin epidermis, the increase in DGLA in the skin as result of, for instance, GLA supplementation will not result in an increase in AA and prostaglandin E_2 in the skin. As a result, feeding humans a diet high in GLA raises prostaglandin E_1 and 15-hydroxy-eicosatrienoic acid concentrations (40). Both prostaglandin E_1 and 15-hydroxy-eicosatrienoic acid have antiinflammatory properties (37).

We found a slightly but significantly higher concentration of AA at 3 and 6 mo of age in the GLA group infants than in the placebo group infants. Because metabolites of AA are known to exert potent proinflammatory effects, a higher concentration can enhance inflammation (1). However, the results suggest that this is not the case, given the positive effects on the association of the SCORAD index with the increase in the plasma GLA concentration. The metabolic products of GLA and DGLA, prostaglandin E_1 and 15-hydroxy-eicosatrienoic acid, have apparently been produced in sufficient amounts to inhibit the formation of these AA metabolites (40).

Other studies provide additional explanations for the possible beneficial effects of EFAs on skin epidermis. Dry skin and itch are typical of patients with AD, and dry skin correlates with a disturbed epidermal barrier function (41). It has been shown that LA in particular is required for the formation and maintenance of the

epidermal barrier (42, 43). In addition, topically applied evening primrose oil has yielded positive results in stabilizing the stratum corneum barrier (44), and the most pronounced positive effect attributed to GLA in supplementation studies has been the reduction of itch (45–48). Besides the antiinflammatory effects of GLA on skin epidermis, it might play a physical structural role in the stability of the skin. Many of the immunologic changes associated with GLA supplementation have been attributed to alterations in GLA metabolites that in turn down-regulate the production of AA-derived leukotrienes (1). For instance, Ziboh and Fletcher (40) showed that GLA inhibits leukotriene B₄ in a dose-dependent way. The highest GLA dose in that study was quite similar to the dose used in our study (1500 mg GLA/d approximates 21 mg · kg⁻¹ · d⁻¹ for adults weighing ≈70 kg; 103 mg GLA/d in our study approximates 19 mg · kg⁻¹ · d⁻¹ for infants weighing 5.5 kg at age 3 mo). We found a similar dose-response relation. Therefore, we think that the results of Ziboh and Fletcher support our findings. However, to assess a cause-and-effect relation, future preventive trials with GLA supplementation should also focus on defining changes in the metabolites involved in AD.

In conclusion, the results show that early supplementation with GLA does not prevent the expression of atopy as reflected by total serum IgE, but that it does tend to alleviate the severity of AD in later infancy in children at high familial risk. Future studies should distinguish between atopic (IgE-mediated) and inflammatory components of AEDS. 

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