Addition of Eicosapentaenoic Acid to γ-Linolenic Acid–Supplemented Diets Prevents Serum Arachidonic Acid Accumulation in Humans[1]

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ABSTRACT Previous studies reveal that supplementation of human diets with γ-linolenic acid (GLA) reduces the generation of lipid mediators of inflammation and attenuates clinical symptoms of chronic inflammatory disorders such as rheumatoid arthritis. However, we have shown that supplementation with this same fatty acid also causes a marked increase in serum arachidonate (AA) levels, a potentially harmful side effect. The objective of this study was to design a supplementation strategy that maintained the capacity of GLA to reduce lipid mediators without causing elevations in serum AA levels. Initial in vitro studies utilizing HEP-G2 liver cells revealed that addition of eicosapentaenoic acid (EPA) blocked Δ-5-desaturase activity, the terminal enzymatic step in AA synthesis. To test the in vivo effects of a GLA and EPA combination in humans, adult volunteers consuming controlled diets supplemented these diets with 3.0 g/d of GLA and EPA. This supplementation strategy significantly increased serum levels of EPA, but did not increase AA levels. EPA and the elongation product of GLA, dihomo-γ-linolenic acid (DGLA) levels in neutrophil glycerolipids increased significantly during the 3-wk supplementation period. Neutrophils isolated from volunteers fed diets supplemented with GLA and EPA released similar quantities of AA, but synthesized significantly lower quantities of leukotrienes compared with their neutrophils before supplementation. This study revealed that a GLA and EPA supplement combination may be utilized to reduce the synthesis of proinflammatory AA metabolites, and importantly, not induce potentially harmful increases in serum AA levels. J. Nutr. 130: 1925–1931, 2000.

KEY WORDS: • *arachidonic acid* • *γ-linolenic acid* • *inflammation* • *leukotrienes* • *neutrophils* • *humans*

γ-Linolenic acid (GLA)[3] is an 18-carbon polyunsaturated fatty acid of the (n-6) series. When given as a dietary supplement, this fatty acid has been shown to relieve the signs and symptoms of chronic inflammatory diseases, including rheumatoid arthritis and atopic dermatitis (Andreassi et al. 1997, Kunkel et al. 1981, Leventhal et al. 1993 and 1994, Lovell et al. 1981, Morse et al. 1989, Tate et al. 1989, Zurier et al. 1996). Many of the clinical effects of GLA supplementation have been attributed to its capacity to block the metabolism of arachidonic acid (AA) to bioactive eicosanoids. However, this is a somewhat paradoxical finding because GLA, via its metabolism by elongase and Δ-5-desaturase activities, is a potential precursor of AA; thus, adding dietary GLA might be expected to increase AA levels with subsequent proinflammatory effects. Recent in vitro and in vivo studies have begun to resolve this paradox by demonstrating that inflammatory cells such as human neutrophils contain the elongase but not the Δ-5-desaturase activity, and thus dietary GLA supplementation leads to the accumulation of dihomo-γ-linolenic acid (DGLA) and not AA in cellular glycerolipids. Importantly, neutrophils from subjects supplemented with GLA produce less leukotriene B_4 (LTB_4) than they did before supplementation (Johnson et al. 1997, Ziboh and Fletcher 1992). Together, these studies reveal that the endogenous elongase activity in certain inflammatory cells can be utilized to synthesize close structural analogs of AA (i.e., DGLA) from appropriate dietary precursors, and these analogs may then affect AA metabolism (Chilton-Lopez et al. 1996, Johnson et al. 1997).

In contrast to neutrophils, GLA supplementation can markedly increase serum AA, suggesting that dietary GLA in circulation has the potential to be both elongated to DGLA and subsequently desaturated to AA. Thus, in vivo GLA supplementation in humans attenuates AA metabolism in certain inflammatory cells such as neutrophils, but can also

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[3] Abbreviations used: AA, arachidonic acid, DGLA, dihomo-γ-linolenic acid; DMEM, Dulbecco's modified Eagle's medium; EPA, eicospentaenoic acid; GCRC, General Clinical Research Center; GLA, γ-linolenic acid; HBSS, Hanks' balanced salt solution; oleic acid; LA, linoleic acid; LTB_4, leukotriene B_4; NICI-GC/MS, negative ion chemical ionization-gas chromatography/mass spectrometry; OA, xxxxx; 20-OH, 20-hydroxy; PGB_2, prostaglandin B_2.

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TABLE 1

Nutritional composition of the controlled diet[1]

Nutrient	d 1	d 2	d 3	d 4	d 5	Mean
Protein, *% total energy*	21.08	20.44	19.22	18.71	19.56	19.71
Carbohydrate, *% total energy*	55.73	55.73	56.35	57.79	56.74	56.46
Fat, *% total energy*	25.95	26.11	26.06	25.56	26.55	26.05
Cholesterol, *mg/d*	201.45	503.98	198.99	285.40	251.58	288.28
Total SFA, *g/d*	22.91	15.27	18.71	13.75	16.72	17.47
Total PUFA, *g/d*	12.89	16.82	10.40	15.42	15.15	14.14
Total MUFA, *g/d*	17.49	21.67	20.91	22.09	23.33	21.10
Linoleic acid, *g/d*	11.17	14.91	9.33	14.38	13.34	12.63
Linolenic acid, *g/d*	1.63	1.34	0.95	0.78	1.44	1.23

[1] Abbreviations: SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid.

lead to the potentially adverse effect of increasing serum AA levels. Previous studies have suggested that the accumulation of AA in serum can have important consequences in humans. For example, AA has been shown to enhance the formation of platelet-aggregating endoperoxides and thromboxanes (Hamberg et al. 1974 and 1975, Smith et al. 1974, Willis 1974). Moreover, high levels of AA in humans result in an increased tendency for the secondary irreversible phase of platelet aggregation (Seyberth et al. 1975). In most cases, an increase in sensitivity of platelets to aggregating stimuli is not desirable.

The observation that serum AA accumulates after GLA supplementation raises important concerns about the long-term effect of this dietary supplementation strategy (Johnson et al.1997). It also highlights the need to find dietary strategies that will produce natural inhibitors (such as DGLA) of AA within inflammatory cells, thereby reducing the synthesis of proinflammatory eicosanoids without increasing serum levels of AA. We tested the hypothesis that the addition of the (n-3) fatty acid product of the Δ-5-desaturase reaction, eicosapentaenoic acid (EPA), attenuates the in vitro and in vivo conversion of DGLA to AA by nonneutrophil sources, thereby reducing serum AA accumulation observed during GLA supplementation.

SUBJECTS AND METHODS

Materials. Prostaglandin B_2 (PGB_2), octadeuterated arachidonic acid and trideuterated stearic acid were obtained from Biomol Research Laboratories (Plymouth Meeting, MA). Leukotriene B_4 (LTB_4), 20-hydroxy-LTB_4 (20-OH-LTB_4) and all fatty acids (GLA, linoleic acid [LA], oleic acid [OA], DGLA, AA and EPA) were obtained from Cayman Chemical (Rockford, IL). Ficoll-Paque was obtained from Pharmacia (Uppsala, Sweden). Dextran 70 (6g/L) in 0.9g/L sodium chloride was purchased from Abbott Laboratories (North Chicago, IL). Bakerbond solid phase extraction octadecyl (C_{18}) disposable columns were obtained from J. T. Baker Chemical (Phillipsburg, NJ). Ionophore A23187 was purchased from Calbiochem (San Diego, CA). All solvents (HPLC grade) were obtained from Fisher Scientific (Norcross, GA). Hanks' balanced salt solution (HBSS) with and without calcium was purchased from Mediatech Cellgro (Herndon, VA). Pentafluorobenzyl bromide (20 mL/L in acetonitrile) and diisopropyl ethylamine (20 mL/L in acetonitrile) were obtained from Pierce (Rockford, IL). Dulbecco's modified Eagle's medium (DMEM), insulin-transferrin-selenium-X and fetal bovine serum were purchased from Life Technologies (Grand Island, NY). The penicillin + streptomycin mixture was obtained from Bio Whittaker (Walkersville, MD). BIO-EFA borage oil capsules were a generous gift from Health From the Sun (Sunapee, NH). Twin EPA extra-strength fish oil concentrate capsules were obtained from Twin Laboratories (Ronkonkoma, NY).

Dietary protocols. The protocols used were approved by the Institutional Review Board and written consent was obtained from each volunteer before starting the study. Healthy volunteers had baseline interviews with a nutritionist for diet history and a review of study procedures. Height, weight, activity levels and usual eating habits were assessed to determine energy needs and to eliminate potentially noncompliant subjects. Energy intake needs were established using the Harris Benedict equation with the addition of a factor of 1.3–1.7 for activity level. All food consumed by subjects for the 21-d outpatient period was prepared by the Metabolic Kitchen of the Wake Forest University School of Medicine General Clinical Research Center (GCRC) using a 5-d menu cycle prepared under controlled, constant conditions. The nutritional composition of the diet is given in **Table 1**. Subjects reported to the GCRC five times per week to be weighed and receive their meals. Subjects received daily checklists of foods to be consumed and returned them with notations of any deviations from the diet provided. Regular contact and communication with the GCRC nutritionists were maintained, and minor modifications to the menus were made as needed to ensure compliance. Weights were monitored and energy intakes adjusted (in increments of 418 kJ) if a weight change of >1 kg from baseline was observed for three consecutive visits or total weight change exceeded 1.5 kg. All subjects maintained body weight within 1.5 kg of baseline weight during the study as seen in **Table 2**; only one subject required adjustment of energy intake during the study (Table 2). **Table 3** shows the composition of several minor fatty acids consumed during the 5-d menu cycle as determined by negative ion chemical ionization gas chromatography/mass spectrometry (NICI-GC/MS; see below). There were no adverse effects reported by any of the volunteers.

TABLE 2

Subject data

Subject	Baseline weight	Baseline BMI[1]	Energy intake	Final weight
	kg	*kg/m²*	*kJ/d*	*kg*
1	84.17	24.0	12,970	84.17
2	69.21	25.0	10,042	68.36
3	68.48	22.6	8786	67.36
4	62.86	24.2	10,460	62.91
5	65.94	25.8	9205	65.91
6	80.45	23.8	12,134	79.27
7	82.00	25.9	12,552	82.54
8	52.52	20.5	8368	53.54
9	56.42	24.7	7113	55.45
10	57.96	19.6	10,042	57.64
11	81.18	23.3	11,297	81.09
12	53.24	21.0	7950	54.72

[1] BMI, body mass index.

TABLE 3

The fatty acid composition of the 5-d rotating menu[1,2]

	AA	EPA	DGLA	GLA
		mg		
d 1	49.7	3.1	18.4	45.8
d 2	53.6	16.6	15.7	35.2
d 3	92.2	3.5	23.5	37.4
d 4	51.8	1.0	10.6	35.1
d 5	59.2	6.8	16.8	31.6

[1] The total amount of food for a 42-y-old male was combined and homogenized. Lipids were extracted, hydrolyzed and quantities of fatty acids were determined by negative ion chemical ionization-gas chromatography/mass spectrometry as described in Subjects and Methods. These data are the total fatty acids (mg) for a 5-d rotating menu.

[2] Abbreviations: AA, arachidonic acid; EPA, eicosapentaenoic acid; DGLA, dihomo-γ-linolenic acid; GLA, γ-linolenic acid.

Protocol A. Healthy volunteers (n = 4; 2 men and 2 women; ages ranging from 25 to 37 y) consumed the controlled diet (described above). They took 10 capsules (5 capsules in the morning and 5 capsules in the evening) of borage oil (BIO-EFA) containing ~3.0 g GLA/d. Blood was obtained, and serum and neutrophils were isolated after an overnight fast, the morning before starting the supplementation and each week of the supplementation.

Protocol B. Healthy volunteers (n = 12; 5 women and 7 men; ages ranging from 23 to 42 y) consumed the controlled diet (described above) and were supplemented with oils enriched in GLA (~3 g/d) and EPA (~3 g/d). Specifically, they consumed 10 capsules/d (5 capsules in the morning and 5 capsules at night) of BIO-EFA and 5 capsules/d (3 capsules in the morning and 2 capsules at night) of concentrated fish oil (Twin EPA), for 21 d. NICI-GC/MS (see below) revealed that the Twin EPA capsule contained ~600 mg of EPA and ~280 mg of docosahexaenoic acid (DHA). Blood was obtained, and serum and neutrophils isolated after an overnight fast, the morning before supplementation, each week during the supplementation and 2 wk after ending the supplementation (washout).

Analysis of serum lipids. Venous blood (~2 mL) was taken from each volunteer at each time point described above, and serum was isolated as previously described (Chilton et al. 1993). The lipids from a 100-μL aliquot of the serum were extracted by the method of Bligh and Dyer (1959). Trideuterated stearic acid (100 ng) and octadeuterated arachidonic acid (100 ng) were added as internal standards to the samples. Fatty acids were cleaved from glycerolipids by base hydrolysis [0.5 mol/L potassium hydroxide in methanol/water (3:1) for 30 min at 60°C]. Reactions were stopped by neutralizing the mixture using 0.5 mL of 6 mol/L HCl. Samples were then loaded onto Bakerbond octadecyl columns and fatty acid–enriched fractions were extracted as previously described (Chilton et al. 1993). Fatty acids were then converted to pentafluorobenzyl esters using 20% pentafluorobenzyl bromide and 20% diisopropylethylamine for 30 min at 40°C. Quantities of fatty acids were then determined by NICI-GC/MS as described below.

Analysis of fatty acid composition of neutrophil glycerolipids. Neutrophils were isolated from whole blood of each volunteer at each time point and were suspended at 10×10^9 cells/L in HBSS containing calcium. Mole quantities of fatty acids were determined as previously described (Chilton et al.1993). Briefly, total lipids were extracted by the method of Bligh and Dyer (1959). Octadeuterated arachidonic acid and trideuterated stearic acid (100 ng each) were added to samples as internal standards. Fatty acids were hydrolyzed from glycerolipids utilizing base hydrolysis, and fatty acids extracted and derivatized as described above. Quantities of fatty acids were then determined by NICI-GC/MS as described below.

Analysis of products after neutrophil stimulation. Isolated neutrophils were suspended in HBSS containing calcium at a concentration of 10×10^9 cells/L. Neutrophils were then stimulated by the addition of ionophore A23187 (1 μmol/L) and reactions allowed to proceed for 5 min. When analyzing the capacity of neutrophils to release fatty acids, reactions were terminated with methanol/chloroform (2:1, v/v). Trideuterated stearic acid (100 ng) and octadeuterated arachidonic (100 ng) acid were added as internal standards. Mole quantities of fatty acids released were determined utilizing NICI-GC/MS as described below. When analyzing the capacity of neutrophils to synthesize leukotrienes, reactions were terminated by removing cells from supernatant fluids utilizing centrifugation (400 × g, 5 min, 4°C). Supernatant fluids were removed and acidified with 9% formic acid. PGB_2 (250 ng) was added to each sample as an internal standard before the fatty acids and eicosanoids were extracted with four volumes of ethyl acetate (2X). This extract was then loaded onto an LC-18 reverse-phase narrowbore HPLC column (25 cm × 2.1 mm) purchased from Supelco (Bellefonte, PA); the leukotrienes were eluted with a mobile phase of methanol/water/phosphoric acid (55:45:0.02, v/v/v, pH 5.6) at a flow rate of 0.3 mL/min. After 5 min, the methanol composition of the mobile phase was increased to 100% over 30 min. The areas under the UV peaks (at 270 nm) corresponding to LTB_4, LTB_5, 6-*trans* isomers and 20-OH-LTB_4 were identified and compared with the peak area of PGB_2 that was added as an internal standard. Mole quantities of leukotrienes were determined utilizing standard curves.

Analysis of the fatty acid composition of the food samples from the 5-d rotating menu. A total day's food from each day of the menu cycle was homogenized using a blender. Lipids were extracted from the 5-d liquefied preparation by the method of Bligh and Dyer (1959). Octadeuterated arachidonic acid (100 ng) and trideuterated stearic acid (100 ng) were added as internal standards. Fatty acids were hydrolyzed from glycerolipids by base hydrolysis, extracted and derivatized as described above. Quantities of fatty acids were determined by NICI-GC/MS as described below.

Analysis of the fatty acid composition of borage oil and fish oil capsules. The contents of the capsules were suspended in methanol/chloroform (1:1, v/v). Octadeuterated arachidonic acid and trideuterated stearic acid (100 ng each) were added as internal standards. Fatty acids were hydrolyzed from glycerolipids by base hydrolysis, and fatty acids extracted and derivatized as described above. Mole quantities of fatty acids were determined by NICI-GC/MS.

In vitro fatty acid metabolism in HEP-G2 cells. HEP-G2 cells (10^6) were cultured in 6 mL of DMEM culture medium supplemented with 1 mL/L penicillin + streptomycin, 1 mL/L fetal bovine serum and 1 mL/L insulin + transferrin at 37°C in 5% CO_2. Solvents were removed from EPA and DGLA under a stream of nitrogen, and these fatty acids were resuspended in DMEM containing 1% fetal bovine serum. This buffer solution was incubated with HEP-G2 cells for 24 h at fatty acids concentrations ranging from 0 to 50 μmol/L. After 24 h, the media were removed and adherent cells washed (2X) with HBSS containing human serum albumin (0.25g/L). HEP-G2 cells were then removed (rubber policeman) from flasks and suspended in HBSS/methanol/chloroform (1:2:1, v/v/v). Lipids were extracted by the method of Bligh and Dyer (1959) as described above. Octadeuterated arachidonic acid (100 ng) and trideuterated stearic acid (100 ng) were added as internal standards. Fatty acids were removed from glycerolipids by base hydrolysis, and fatty acids extracted and derivatized as described above. Quantities of fatty acids were determined by NICI-GC/MS.

Negative ion chemical ionization-gas chromatography/mass spectrometry (NICI-GC/MS). NICI-GC/MS analysis was conducted on a single-stage quadrapole mass spectrometer (Hewlett-Packard 5989; Greensboro, NC) as previously described (Chilton et al. 1993). The gas chromatography was performed on a Hewlett-Packard 5890 GC using a 30-m DB-17 fused silica column (SPB-5; 0.25-mm film thickness; Supelco). The initial column temperature was 60°C. The column was heated to 220°C at a rate of 40°C/min with a subsequent increase in temperature to 280°C at a rate of 5°C/min. The injector temperature was maintained at 250°C. Each injection was performed in the splitless mode. A volume of 1 μL from 200 μL of recovered material dissolved in hexane was injected. Helium was used as a carrier gas. The pentafluorobenzyl esters were analyzed using selected ion-recording techniques to monitor GLA (m/z 277), LA (m/z 279), OA (m/z 281), EPA (m/z 301), DGLA (m/z 305), AA (m/z 303), trideuterated stearic acid (m/z 286) and octadeuterioarachidonate

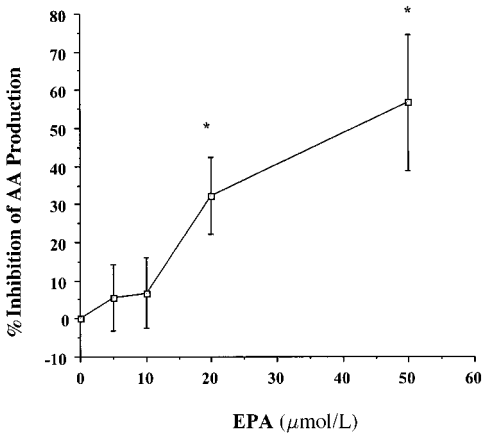

FIGURE 1 Percentage inhibition of arachidonic acid (AA) production induced by eicosapentaenoic acid (EPA) addition in HEP-G2 cells. HEP-G2 cells were maintained in culture supplemented with 20 μmol/L dihomo-γ-linolenic acid (DGLA) and varying concentrations of EPA. After 24 h, cellular AA was determined by negative ion chemical ionization-gas chromatography/mass spectrometry (NICI-GC/MS) as described in Subjects and Methods. These data are expressed as the percentage inhibition of AA biosynthesis by EPA and are means ± SEM, $n = 4$. *$P \leq 0.05$ vs. 0 μmol/L. Regression equation: $y = 1.9170 + 36.808x$, $r^2 = 0.993$

(*m*/*z* 311). A standard mixture of the aforementioned fatty acids was injected and analyzed by NICI-GC/MS before each biological sample to obtain precise retention times.

Data analysis. The data are presented as means ± SEM or as percentages of baseline values (LTB_4 production and influence of EPA on HEP-G2 cells). Differences were tested for significance ($P < 0.05$) using a Student's *t* test for paired samples.

RESULTS

Influence of EPA on Δ-5-desaturase activity in HEP-G2 cells. Initial experiments in this study examined whether EPA, a Δ-5-desaturase product, could inhibit the conversion of DGLA to AA in a human hepatocarcinoma cell line, HEP-G2. These HEP-G2 cells exhibit morphological features of mature hepatocytes (Aden et al. 1979). Moreover, this cell line is a relevant experimental model for investigating fatty acid metabolism of the human liver (Angeletti and Tacconi de Alaniz 1995). The addition of DGLA to HEP-G2 cells resulted in the conversion of DGLA to AA. For example, addition of 20 μmol/L DGLA to the HEP-G2 cells markedly increased cellular AA levels. Concomitant addition of EPA with a constant amount of DGLA caused a dose-dependent attenuation in the conversion of DGLA to AA (**Fig. 1**). EPA at 50 μmol/L inhibited AA formation from DGLA (20 μmol/L) by 50%. These data demonstrate the capacity of EPA to block Δ-5-desaturase activity in isolated hepatocytes.

Influence of the combination of GLA and EPA on the fatty acid compositions of serum lipids. A concern with long-term GLA supplementation is that a marked increase in serum levels of AA may occur. Therefore, dietary strategies that allow the accumulation of potential inhibitors of AA metabolism without increasing serum AA would be valuable. Because EPA inhibited Δ-5-desaturase in HEP-G2 cells, we determined whether EPA could similarly suppress hepatic conversion of DGLA to AA in humans. Two groups of volunteers consumed control diets (see Subjects and Methods) and were supplemented with either GLA (3.0 g/d) alone or a combination of GLA (3.0 g/d) and EPA (3.0 g/d). **Figure 2** (*upper panel*) illustrates that GLA alone markedly increased serum AA and DGLA levels within 3 wk of the initiation of GLA ingestion. In contrast, the combination of EPA with GLA did not increase serum AA levels (Fig. 2, *low panel*), suggesting that it is possible to block Δ-5-desaturase in humans with EPA. However, the GLA and EPA combination markedly increased serum EPA levels. After a 2-wk washout period, EPA levels returned to baseline levels. A previous study in our laboratory demonstrated that AA and DGLA levels increase in response to GLA supplementation and return to baseline values after 2 wk (Johnson et al. 1997). Together, these data suggest that the addition of EPA to human diets containing high levels of GLA provides a means to block increases in serum AA.

Influence of the combination of GLA and EPA on the fatty acid composition of human neutrophils. When subjects were supplemented with a GLA and EPA combination, both DGLA and EPA were significantly increased at wk 3 compared with the baseline values (**Fig. 3**). AA levels in neutrophil lipids did not change. GLA supplementation alone did not increase AA or EPA levels in neutrophil glycerolipids (not shown), but did result in an increase in DGLA levels from 0.15 ± 0.02 to 0.27 ± 0.03 nmol/5 × 10^6 cells after supplementation.(Johnson et al. 1997). In addition, these data are consistent with previous in vitro observations that show human

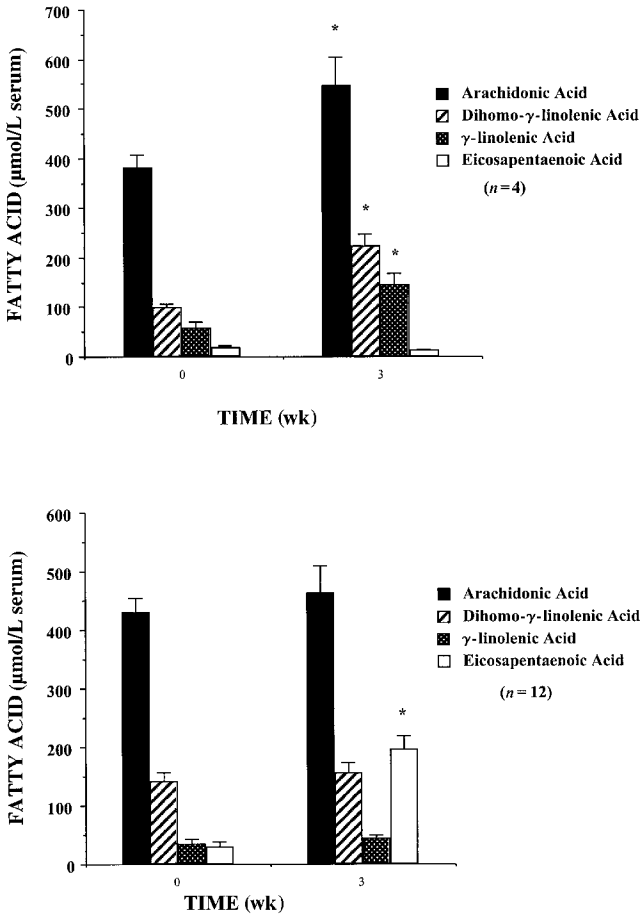

FIGURE 2 Effects of γ-linolenic acid (GLA; *upper panel*) and a combination of GLA and eicospentaenoic acid (EPA) supplementation (*lower panel*) on serum concentrations of fatty acids. Serum fatty acid compositions were determined before (wk 0) or after 3 wk of supplementation by negative ion chemical ionization-gas chromatography/mass spectrometry (NICI-GC/MS). Values are means ± SEM. *Significantly different from wk 0, $P < 0.05$.

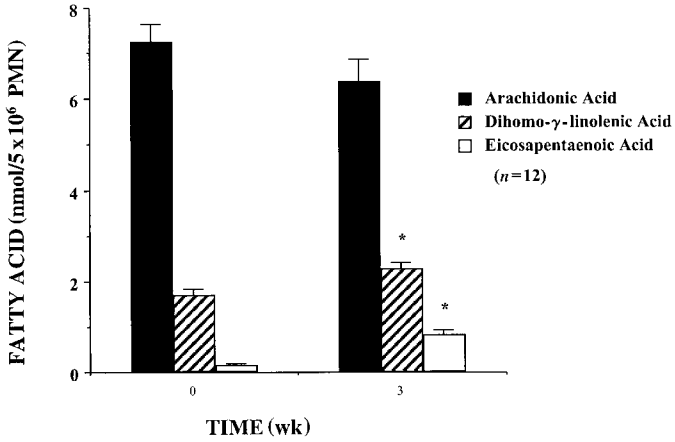

FIGURE 3 Effects of γ-linolenic acid (GLA) and eicospentaenoic acid (EPA) supplementation on the fatty acid composition of glycerolipids in neutrophil membranes. Values are means ± SEM. *Significantly different from wk 0, $P < 0.05$. Abbreviation: PMN, polymorphonuclear leukocytes.

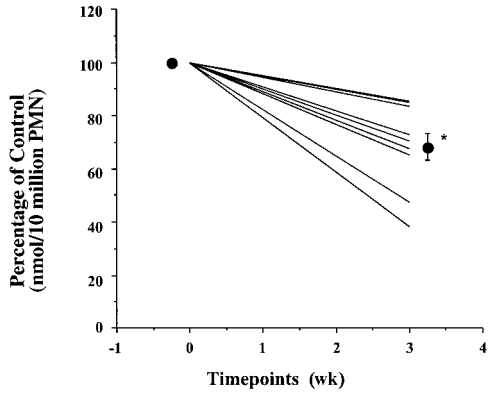

FIGURE 5 Effects of γ-linolenic acid (GLA) and eicospentaenoic acid (EPA) supplementation on the biosynthesis of leukotriene B_4 (LTB_4), 20-hydroxy (20-OH)-LTB_4 and 6-*trans*-isomers-LTB_4 by A23187-stimulated neutrophils. Neutrophils, isolated before (wk 0) and 3 wk after supplementation were stimulated with 1 μmol/L A23187. The lines represent the percentage of control values for each subject that participated in study and (●) represents the mean ± SEM, $n = 10$. *Significantly different from wk 0, $P < 0.05$. Abbreviation, PMN, polymorphonuclear leukocytes.

neutrophils contain elongase, but lack Δ-5-desaturase activity (Chilton-Lopez et al. 1996).

Influence of the combination of GLA and EPA on the release of fatty acids and the production of LTB_4 and its metabolites by stimulated neutrophils. A final set of experiments was designed to investigate the influence of the GLA + EPA combination on the release of AA and the production of leukotrienes by neutrophils after stimulation with ionophore A23187. **Figure 4** shows the amounts of AA, DGLA and EPA released from stimulated neutrophil glycerolipids at baseline and 3 wk after the GLA + EPA combination. Release of DGLA and EPA from neutrophil glycerolipids was significantly increased after GLA/EPA supplementation. However, the quantity of AA released from neutrophils did not change.

Quantities of LTB_4, 20-OH-LTB_4 and the 6-*trans* isomers of LTB_4 were determined by HPLC and are expressed as percentage of control in **Figure 5**. There was a significant drop in total leukotriene production from neutrophils 3 wk after dietary GLA + EPA supplementation. Concomitant with a decrease in leukotrienes of the 4 series, there was an increase in leukotriene B_5 derived from released EPA (**Fig. 6**).

DISCUSSION

Studies by several investigators have demonstrated that dietary supplementation with GLA has the potential to reduce inflammation. This reduction in inflammation has been attributed to the capacity of the elongation product of GLA, DGLA, to block the synthesis of AA products and the capacity of DGLA to be converted to oxidized products that have anti-inflammatory activities (Billah et al. 1985, Chilton-Lopez et al. 1996, DeLuca et al. 1999, Iversen et al. 1991 and 1992, Vanderhoek et al. 1980). Our previous studies (Chilton et al.

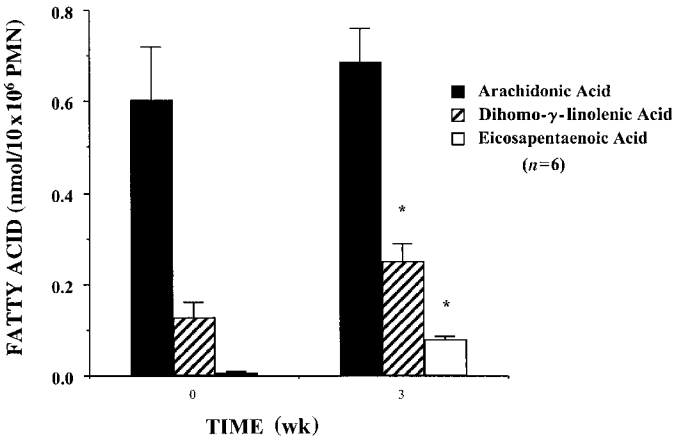

FIGURE 4 Effects of γ-linolenic acid (GLA) and eicospentaenoic acid (EPA) supplementation on the release of fatty acids from glycerolipids of neutrophils stimulated with ionophore A23187. Neutrophils, isolated before (wk 0) and 3 wk after supplementation were stimulated with 1 μmol/L A23187. Values are means ± SEM. *Significantly different from wk 0, $P < 0.05$. Abbreviation: PMN, polymorphonuclear leukocytes.

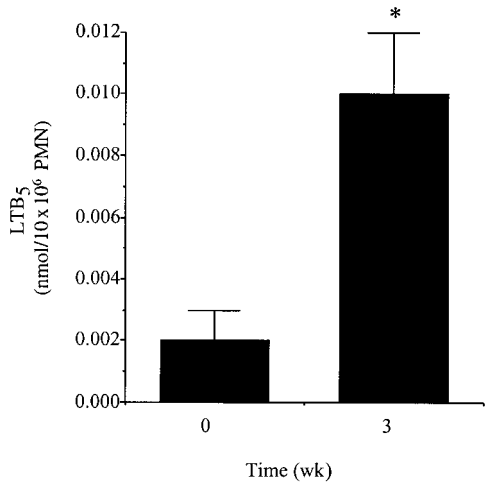

FIGURE 6 Effects of γ-linolenic acid (GLA) and eicospentaenoic acid (EPA) supplementation on the biosynthesis of leukotriene B_5 (LTB_5) by A23187-stimulated neutrophils. Neutrophils were isolated before supplementation (wk 0) and after 3 wk. After supplementation neutrophils were stimulated with 1 μmol/L A23187. Values are means ± SEM, $n = 12$. *Significantly different from wk 0, $P < 0.05$. Abbreviation: PMN, polymorphonuclear leukocytes.

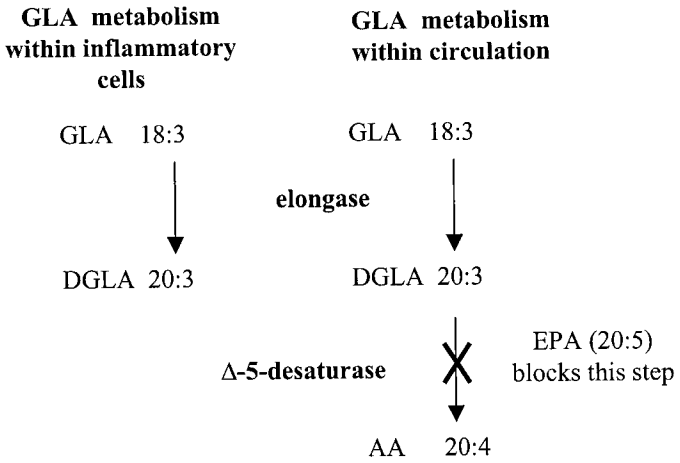

FIGURE 7 Mechanism of inhibition of Δ-5-desaturase by dietary eicospentaenoic acid (EPA). Abbreviations: GLA, γ-linolenic acid; DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid.

1993, Chilton-Lopez et al. 1996, Johnson et al.1996) and those of Ziboh and Fletcher (1992) have demonstrated that supplementation of low-to-moderate fat diets with GLA markedly decreases the capacity of inflammatory cells such as human neutrophils to produce leukotrienes (Johnson et al.1997). We further demonstrated that the likely mechanism of inhibition by GLA stems from its capacity to be elongated by an endogenous elongase activity within the neutrophil to a close structural analog of AA, DGLA (**Fig. 7**). However, neutrophils cannot further desaturate DGLA to AA because they lack Δ-5-desaturase activity (Chilton-Lopez et al.1996). Thus, the endogenous elongase activity within inflammatory cells can be utilized to synthesize close structural analogs of AA (i.e., DGLA) from appropriate dietary precursors. It is postulated that these analogs affect AA metabolism, thereby mitigating clinical manifestations induced by AA metabolites.

A potentially important side effect of GLA supplementation is elongation of GLA to DGLA and further desaturation via Δ-5-desaturase to AA by enzymes in the liver. This causes a marked increase in serum AA levels. In a previous study of AA supplementation in humans, similar increases in serum AA levels were associated with an increase in the in vivo aggregation of platelets (Seyberth et al.1975). This increase in platelet sensitivity raised concerns about potentially harmful cardiovascular side effects and the long-term safety of any dietary strategy that increases serum AA levels, including those current formulations being sold in nutraceutical markets.

The current study was designed to determine whether dietary strategies could be designed that have the anti-inflammatory potential of GLA without leading to increases in serum AA. This was accomplished by the addition of the Δ-5-desaturase product of the (n-3) pathway, EPA. Initial in vitro experiments demonstrated that EPA had the capacity to block Δ-5-desaturase activity in an isolated hepatocarcinoma cell line. These experiments were followed by in vivo studies that showed EPA supplementation of human diets prevented the accumulation of serum AA in response to GLA without inhibiting accumulation of DGLA in neutrophils. Thus, both in vivo and in vitro studies revealed that EPA may act as an end product inhibitor of the Δ-5-desaturase.

We further examined the influence of the GLA + EPA combination on leukotriene generation. The capacity of human neutrophils to release AA was not influenced by the GLA + EPA supplementation. In contrast, their capacity to generate leukotrienes (LTB_4, 20-OH-LTB_4 and related isomers) was inhibited significantly (~40%) compared with neutrophils from these same volunteers before supplementation. The inhibition observed in this study was greater than what has been observed before for EPA alone (Chilton et al.1993). In contrast, the inhibition with EPA/GLA was somewhat less than that seen in a previous study in our laboratory with GLA alone (Johnson et al. 1997). However, the differences in leukotriene generation in these studies were not powered sufficiently to detect statistically significant differences and may be a function of biologic variability among the volunteers.

Clinicians, patients, pharmaceutical and nutraceutical companies are all increasingly utilizing natural products for the treatment of clinical disorders. As this trend continues, it is important that these products be both safe and effective. Overall, little attention has been paid to the potentially adverse effects of dietary supplements and specifically, dietary fatty acid supplementation strategies. This study shows how a potentially important complication, arising from supplementation with a simple fatty acid, can be avoided by using appropriate fatty acid combinations. As the nutraceutical industry continues to experience explosive growth, it will be increasingly important to understand the safety profiles of dietary supplements and avoid complications that arise from such supplements.

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