Dietary Supplementation with γ-Linolenic Acid Alters Fatty Acid Content and Eicosanoid Production in Healthy Humans[1,2]

Margaret M. Johnson,* Dennis D. Swan,* Marc E. Surette,* Jane Stegner,* Tanya Chilton,* Alfred N. Fonteh* and Floyd H. Chilton*†[3]

Department of Internal Medicine, Section on Pulmonary and Critical Care Medicine, and †Department of Biochemistry, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27157–1054

ABSTRACT To understand the in vivo metabolism of dietary γ-linolenic acid (GLA), we supplemented the diets of 29 volunteers with GLA in doses of 1.5–6.0 g/d. Twenty-four subjects ate controlled eucaloric diets consisting of 25% fat; the remaining subjects maintained their typical Western diets. GLA and dihomo-γ-linolenic acid (DGLA) increased in serum lipids of subjects supplemented with 3.0 and 6.0 g/d; serum arachidonic acid increased in all subjects. GLA supplementation with 3.0 and 6.0 g/d also resulted in an enrichment of DGLA in neutrophil phospholipids but no change in GLA or AA levels. Before supplementation, DGLA was associated primarily with phosphatidylethanolamine (PE) of neutrophil glycerolipids, and DGLA increased significantly in PE and neutral lipids after GLA supplementation. Extending the supplementation to 12 wk did not consistently change the magnitude of increase in either serum or neutrophil lipids in subjects receiving 3.0 g/d. After GLA supplementation, A23187-stimulated neutrophils released significantly more DGLA, but AA release did not change. Neutrophils obtained from subjects after 3 wk of supplementation with 3.0 g/d GLA synthesized less leukotriene B_4 ($P < 0.05$) and platelet-activating factor. Together, these data reveal that DGLA, the elongase product of GLA, but not AA accumulates in neutrophil glycerolipids after GLA supplementation. The increase in DGLA relative to AA within inflammatory cells such as the neutrophil may attenuate the biosynthesis of AA metabolites and may represent a mechanism by which dietary GLA exerts an anti-inflammatory effect. J. Nutr. 127: 1435–1444, 1997.

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

It has become increasingly clear in the past two decades that oxygenated metabolites of arachidonic acid (AA),[4] prostaglandins and leukotrienes, play key roles in many inflammatory diseases (for a review see Chilton et al. 1996, Fischer 1989, Henderson 1994). It has also been demonstrated that manipulation of dietary fatty acids can alter the in vivo or ex vivo metabolism of AA within inflammatory cells. Epidemiological studies in the 1970s showed that diets composed of high levels of cold water fish, which are rich in (n-3) fatty acids, concomitant with lower quantities of (n-6) fatty acids were associated with a lower incidence of some chronic diseases and could perhaps affect AA metabolism (Bang and Dyerberg 1972, Bang et al. 1976, Dyerberg and Bang 1979, Dyerberg et al. 1978, Herxheimer and Schaefer 1974, Kromhout et al. 1985, Shahar et al. 1994). Since these pioneering studies, a variety of dietary approaches including supplementation with (n-3) fatty acids, reduction of dietary essential fatty acids or avoidance of dietary AA have been used in animal and human studies in an attempt to reduce eicosanoid generation and subsequently attenuate manifestations of inflammation (Arm et al. 1989, Fischer and Weber 1984, Gibney and Hunter 1993, Lee et al. 1985, Prescott 1984). The success of these different approaches has been variable. An alternative approach to modify eicosanoid generation has been to supplement diets with an 18-carbon polyunsaturated fatty acid of the (n-6) series, γ-linolenic acid (GLA). This fatty acid is found primarily in the oils of the evening primrose and borage plants and to a lesser extent in meats and eggs. Animal data as well as some clinical human studies suggest that dietary supplementation with GLA may attenuate the signs and symptoms of chronic inflammatory diseases such as rheumatoid arthritis and atopic dermatitis (Kunkel et al. 1981, Leventhal et al. 1993 and 1994, Lovell et al. 1981, Morse et al. 1989, Wright and Burton 1982). However, the mechanism by which dietary GLA may exert anti-inflammatory effects has not been fully elucidated.

GLA is a potential metabolic precursor of AA. For example,

[1] Supported in part by a grant from the National Institutes of Health, AI24985, and a grant from the General Clinical Research Center, MO1-RR07122, at Bowman Gray School of Medicine. M.E.S. is the recipient of a Centennial Fellowship from the Medical Research Council of Canada.

[2] The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

[3] To whom correspondence and reprint requests should be addressed.

[4] Abbreviations used: AA, arachidonic acid; DGLA, dihomo-γ-linolenic acid; DIPE, diisopropylethylamine; GLA, γ-linolenic acid; 2H8-AA, octadeuterated arachidonic acid; HBSS, Hanks' balanced salt solution; 15-HETrE, 15-hydroxy eicosatrienoic acid; 2H3-SA, trideuterated stearic acid; IL, interleukin; LTB_4, leukotriene B_4; NICI-GC/MS, negative ion chemical ionization-gas chromatography/mass spectrometry; 20-OH-LTB_4, 20-hydroxy-LTB_4; PAF, platelet-activating factor; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PFBBR, pentafluorobenzyl bromide; PGB_2, prostaglandin B_2; PGE_1, prostaglandin E_1; PI, phosphatidylinositol; PS, phosphatidylserine.

Manuscript received 7 October 1996. Initial review completed 19 November 1996. Revision accepted 9 April 1997.

TABLE 1

Menus of controlled diet[1,2]

	Fat grams		Fat grams
	Day 1		
Breakfast		Dinner	
Bagel	1.78	Beef (round, lean)	4.38
Cream cheese	8.72	Macaroni (dry)	0.63
Milk 2%	4.61	Tomato sauce (canned)	0.17
Cola	0.00	Whole wheat bread	0.65
Jelly	0.01	Margarine	4.03
Ham cured (extra lean)	4.96	Pear (canned with juice)	0.12
		Lettuce (iceburg)	0.19
Lunch		Dressing (nonfat Ranch)	0.90
Turkey (light, roasted)	3.22	Radish	0.05
Whole wheat bread	1.30	Oreo cookies	7.42
Mayonnaise	11.91		
Lettuce (iceberg)	0.03	Snack	
Apple	0.72	Milk 2%	4.61
Carrot	0.05	Peach (canned with juice)	0.05
Cola	0.00	Graham crackers	5.34
Milk 2%	1.92	American cheese	15.63
Fig bar	1.57	Whole wheat bread	1.30
Energy, *MJ*	12.623		
Total fat, *g*	86.27		
	Day 2		
Breakfast		Dinner	
Cheerios	1.92	Chicken breast meat	1.24
Raisin (seedless)	0.10	Rice (white, enriched)	0.42
Milk 2%	9.22	Broccoli frozen	0.22
Egg (hard cooked)	10.61	Margarine	16.91
Cola	0.00	Lettuce (iceberg)	0.19
Milk skim	0.22	Cucumber	0.03
		Dressing (nonfat Ranch)	0.90
		Apple	0.54
Lunch			
Ham (cured extra lean)	7.44	Snack	
Whole wheat bread	1.95	Milk skim	0.43
Mayonnaise	11.91	Peach (canned with juice)	0.07
Lettuce (iceburg)	0.04	Fig bar	3.14
Pineapple (canned with juice)	0.13	Chicken breast meat	1.24
Carrot	0.05	Mayonnaise	17.47
Cola	0.00	Celery	0.01
		Whole wheat bread	1.30
Energy, *MJ*	12.757		
Total fat, *g*	87.70		

(continued)

it can be elongated in vivo to dihomo-γ-linolenic acid (DGLA) and subsequently desaturated to AA (for a review see Fischer 1989, Sprecher 1989). Consequently, it is difficult to reconcile how dietary supplementation with a precursor to AA, a potent source of proinflammatory mediators, could attenuate inflammation. However, in vitro studies reveal that elongation of GLA to DGLA may occur in some cell types more rapidly than does the desaturation of DGLA to AA. Such a scenario in vivo could result in higher DGLA to AA ratios within certain cells after GLA supplementation (Chapkin and Ziboh 1984, Chapkin et al. 1987).

It is difficult to predict the in vivo metabolism of GLA on the basis of in vitro experiments with individual cell types because there are numerous cellular sites where enzymes can metabolize GLA, DGLA and AA and there are many fatty acid pools where GLA or its metabolites can reside. Therefore, there are considerable deficits in our understanding of the in vivo metabolism of dietary GLA. To maximize any therapeutic effect of GLA, a more thorough understanding of the in vivo metabolism of dietary GLA must be gained. The following study was designed to elucidate the metabolism of dietary GLA in healthy human volunteers. Specifically, the relationship between the time and dose of the GLA supplement and the incorporation of GLA and its metabolites into serum and cellular compartments was examined. The in vivo sites of GLA and DGLA metabolism were also identified by examining changes in quantities of metabolites in different serum and cellular pools. Finally, the effect of diet-induced changes in the fatty acid composition of glycerolipids was determined by measuring the capacity of neutrophils obtained from subjects after GLA supplementation to produce eicosanoids and platelet-activating factor.

SUBJECTS AND METHODS

Materials. Prostaglandin B_2 (PGB_2), octadeuterated arachidonic acid ($^2H_8$-AA) and trideuterated stearic acid ($^2H_3$-SA) were obtained from Biomol Research Laboratories (Plymouth Meeting, MA). [^{3}H]-Acetate and [^{14}C]-phosphatidylcholine (PC) were purchased from New England Nuclear (Boston, MA). Phospholipid standards used

TABLE 1 *(continued)*

Menus of controlled diet[1,2]

	Fat grams		Fat grams
		Day 3	
Breakfast		Dinner	
Wheaties	0.65	Pork loin (lean, braised)	14.60
Milk 2%	9.22	Rice (brown)	1.34
Raisin (seedless)	0.07	Green bean (frozen)	0.14
Egg (hard cooked)	10.61	Margarine	6.44
Cola	0.00	Applesauce (unsweetened)	0.13
		Graham crackers	4.00
Lunch		Snack	
Turkey (light, roasted)	3.22	Milk 2%	3.46
Mayonnaise	14.29	Whole wheat bread	1.82
Whole wheat bread	1.30	Turkey (light, roasted)	2.42
Lettuce (iceberg)	0.03	Mayonnaise	7.94
Cola	0.00	Orange juice (diluted, frozen)	0.22
Fig bar	3.14	Lettuce (iceburg)	0.03
		Granola bar (chocolate chip)	3.50
Energy, *MJ*	12.648		
Total fat, *g*	88.57		
		Day 4	
Breakfast		Dinner	
Bagel	2.08	Beef (round, lean)	3.50
Milk 2%	4.61	Bean kidney (red, cooked)	0.25
Cream cheese	8.72	Tomato sauce (canned)	0.17
Jelly	0.01	Rice (white, enriched)	0.21
Cola	0.00	Lettuce (iceberg)	0.19
Ham	7.68	Carrot	0.05
		Dressing (nonfat, 1000 island)	0.90
		Margarine	8.05
Lunch		Whole wheat bread	0.78
Tuna (canned, water, salt)	0.80	Oreo cookies	7.42
Whole wheat bread	1.30		
Mayonnaise	15.88	Snack	
Lettuce (iceberg)	0.05	Milk 2%	4.61
Pear (canned with juice)	0.10	Whole wheat bread	1.30
Fig bar	1.57	Ham (cured, extra lean)	4.96
Cola	0.00	Mayonnaise	7.94
		Pineapple (canned with juice)	0.20
		Lettuce (iceberg)	0.03
		Graham crackers	2.67
		Milk skim	0.22
Energy, *MJ*	12.535		
Total fat, *g*	86.25		

[1] All cooked weight except when denoted.
[2] Subjects ate a controlled diet composed of four different menus served on a rotating basis. Serving sizes were adjusted to fulfill each subject's energy requirement.

in normal-phase HPLC were purchased from Avanti Polar Lipids (Birmingham, AL). Leukotriene B_4 (LTB_4), 20-hydroxy-LTB_4 (20-OH-LTB_4), 15-hydroxy eicosatrienoic acid (15-HETrE), prostaglandin E_1 (PGE_1), and all fatty acids (GLA, DGLA, AA, and linoleic acid) were obtained from Cayman Chemical (Ann Arbor, MI). Monobasic potassium phosphate was obtained from Aldrich Chemical (Milwaukee, WI). Bakerbond octadecyl (C-18) bound to silica gel columns was purchased from JT Baker Chemical. (Phillipsburg NJ). Ionophore A23187 was purchased from Calbiochem-Behring (San Diego, CA). Ficoll-Plaque was purchased from Pharmacia LKB Biotechnology (Piscataway, NJ). All solvents (HPLC grade) were purchased from Fisher Scientific (Norcross, GA). Pentafluorobenzyl bromide (20% in acetonitrile) and diisopropylethylamine (20% in acetonitrile) were purchased from Pierce (Rockford, IL). Ultra-GLA capsules were purchased from Twin Labs Specialty (Ronkonkoma, NY). BIO-EFA borage oil capsules were a generous gift from Health From the Sun (Sunapee, NH).

Supplementation protocols. The diets of 29 healthy volunteers were supplemented with GLA using four different protocols. Each protocol was approved by the Institutional Review Board and each volunteer gave informed consent before beginning the study. All control diets were prepared in the metabolic kitchen of the General Clinical Research Center at Bowman Gray School of Medicine. No adverse effects were reported by any of the subjects before or after supplementation for any of the supplementation protocols.

Protocol 1. Nine healthy adult volunteers (6 males and 3 females, ages 24–56 y) consumed a controlled eucaloric diet consisting of 25% fat, 55% carbohydrate and 20% protein prepared in a metabolic kitchen under the direction of a registered dietitian (JS) for 3 wk. Four menus were served on a rotating basis throughout the study period (see **Table 1**). Serving sizes were adjusted to provide each subject's energy needs as calculated by the Harris-Benedict equation. The total fatty acid content for each of the four menus of one subject's diet (37-y-old male, 12.5 MJ/d) was analyzed by negative ion chemical ionization-gas chromatography/mass spectrometry (NICI-GC/MS) (results shown in **Table 2**). Subjects supplemented this diet with 10 capsules/d (5 capsules in the morning and 5 capsules in the evening with meals) of either Ultra-GLA or BIO-EFA capsules. Ultra-GLA

and BIO-EFA capsules were analyzed by NICI-GC/MS and each was shown to contain ~300 mg of GLA, 475 mg of linoleic acid, and 200 mg of oleic acid. Blood was obtained and serum and neutrophils isolated twice in the week before beginning the supplementation, on the final day of supplementation and 2 wk after completing the dietary regimen (washout).

Protocol 2. Five healthy adult volunteers (3 males and 2 females; ages 28–33 y) supplemented their typical diets with 10 capsules/d (5 capsules in the morning and 5 in the evening) of Ultra-GLA for 3 wk. Blood was obtained and serum and neutrophils isolated twice in the week before beginning the supplementation, on the final day of supplementation and 2 wk after completing the dietary regimen (washout).

Protocol 3. Four groups of three volunteers each participated in a dose-response study. All participants ate the controlled diet described in Protocol 1 for 3 wk. Group 1 (all females, ages 34–56 y) received no GLA supplementation. Group 2 (all female, ages 22–38 y) received 5 capsules/d (3 capsules in the morning and 2 capsules in the evening) of BIO-EFA (1.5 g/d). Group 3 (2 females and 1 male, age 28–37 y) received 10 capsules (5 capsules in the morning and 5 capsules in the evening) of BIO-EFA (3.0 g/d). Group 4 (1 female and 2 males, age 30–37) received 20 capsules (10 capsules in the morning and 10 capsules in the evening) of BIO-EFA (6.0 g/d). Bleeding times were obtained twice during the supplementation period in the subjects receiving the highest dose of GLA. Blood was obtained and serum and neutrophils isolated twice in the week before beginning the supplementation, on the final day of supplementation and 2 wk after completing the dietary regimen (washout).

Protocol 4. Three healthy volunteers (2 males and 1 female, age 36–40 y) supplemented their typical diet with 10 capsules (5 capsules in the morning and 5 capsules in the evening) of BIO-EFA for 12 wk. Blood was obtained and serum and neutrophils isolated twice in the week before beginning supplementation, every 2 wk during supplementation and 4 wk after supplementation had ended (washout).

Analysis of serum lipids. Serum was separated from 2 mL of whole blood as previously described (Chilton et al. 1993). Lipids were then extracted from a 100-μL aliquot of serum using a modified method of Bligh and Dyer (1959). Fatty acids were analyzed in a 1:100 dilution of the lipids extracted from 100 μL of serum as described below. Initially, fatty acyl chains were hydrolyzed from glycerolipids with 0.6 mol/L KOH in methanol/water (75:25, v/v) at 60°C for 30 min. Trideuterated stearic acid and octadeuterated arachidonic acid were then added as internal standards. After 30 min, water was added to samples and the reaction mixtures were acidified with 6 mol/L HCL. A fatty acid–enriched fraction was obtained from this mixture by a previously described column extraction procedure using a C-18 octadecyl column (Chilton et al. 1993). Fatty acids were then converted to pentafluorbenzyl esters by using 20% pentafluorobenzyl bromide (PFBBR) and 20% diisopropylethylamine (DIPE) in acetonitrile at 40°C for 40 min. Solvents were then evaporated under a stream of nitrogen and the samples were resuspended in hexane. Fatty acid quantities were then determined by NICI-GC/MS as described below.

In some experiments, serum lipids were further separated into neutral lipid classes by TLC. Lipids were isolated on silica gel G developed in hexane/ethyl ether/formic acid (90:60:6, v/v/v) as a mobile phase. Once separated, internal standards were added and fatty acids in lipid classes were analyzed after base hydrolysis by NICI-GC/MS as described below.

Analysis of neutrophil lipids. Neutrophils were isolated by dextran sedimentation followed by Ficoll-Paque density centrifugation (Chilton et al. 1993). Neutrophils were then suspended at 10^{10}/L in Hanks' balanced salt solution (HBSS), and aliquots were taken to determine the fatty acid composition of glycerolipid classes in resting cells and the release of fatty acids and the synthesis of leukotrienes during cell stimulation.

To determine the fatty acid composition of glycerolipids, total lipids were extracted by the method of Bligh and Dyer (1959). Octadeuterated arachidonic acid and trideuterated stearic acid were added to aliquots of the total extract as internal standards. Samples then underwent base hydrolysis, column extraction and derivatization with PFBBR and DIPE as described above for serum lipids. The quantities of fatty acids in the total lipid extracts were then determined by NICI-GC/MS.

In some experiments, neutrophil lipids were further separated into glycerolipid classes using normal-phase HPLC (Patton et al. 1982). Solvents were evaporated from lipid extracts under a stream of nitrogen and suspended in 500 μL of a normal-phase injection solvent of hexane/isopropanol/water (4:5.5:0.3, v/v/v). This mixture was then loaded onto a silica HPLC column (Ultrasphere, 4.6 × 250 mm; Rainin Instrument, Woburn, MA) and eluted with hexane/isopropanol/ethanol/phosphate buffer (pH 7.4)/acetic acid (490:367:100:30:0.6, v/v/v/v/v) for 5 min at a flow rate of 1 mL/min. The amount of phosphate buffer in the eluting solvent was then increased to 5% over a 10-min period, and this solvent composition was maintained until all of the major glycerolipid classes were eluted from the column. Appropriate peaks were collected on the basis of elution times of glycerolipid standards run in the same system. Internal standards were then added to the isolated glycerolipid classes; they were then subjected to base hydrolysis and analyzed for fatty acid content by NICI-GC/MS as described below.

Analysis of lipid products from stimulated neutrophils. Neutrophils, suspended at 10^{10} cells/L in HBSS containing calcium, were preincubated for 5 min at 37°C. Aliquots were then stimulated with ionophore A23187 (μmol/L) and maintained at 37°C for an additional 5 min. Reactions were terminated with methanol/chloroform (2:1, v/v) or methanol for fatty acid or leukotriene analysis, respectively. To determine the quantity of fatty acids released from glycerolipids during cell activation, octadeuterated arachidonic acid and trideuterated steric acid were added to the terminated reaction mixture and lipids were extracted by the method of Bligh and Dyer (1959). Samples were then converted to pentafluorobenzyl esters (described above) and analyzed by NICI-GC/MS as described below.

To determine the quantity of leukotrienes produced after cell stimulation, prostaglandin B_2 was added to each terminated reaction as an internal standard. Samples were then centrifuged (400 × *g* for 10 min) to remove protein debris. An aliquot of the supernatant was loaded onto a reverse-phase ODS HPLC column (Ultrasphere, 4.6 × 250 mm; Rainin Instrument) and eluted with methanol/water/phosphoric acid (550:450:0.2, v/v/v, pH 5.5) for 5 min at a flow rate of 1 mL/min (Chilton et al. 1993). At 5 min, the composition of methanol was increased to 100% over 30 min. Appropriate peaks for LTB_4, its *trans* isomers and its metabolite, 20-OH-LTB_4, were identified on the basis of the elution times of standards analyzed in the same system. Typically, 20-OH LTB_4 and LTB_4 eluted from the column 10–14 min and 19–23 min, respectively, after injection. Quantities of leukotrienes were determined by comparing their areas with that of prostaglandin B_2 added as an internal standard and using appropriate standard curves to normalize for recoveries.

To determine the quantity of platelet activating factor (PAF) synthesized during cell stimulation, neutrophils were incubated for 5 minutes at 37°C in HBSS containing 148 MBq/L of [^{3}H]-acetate and 2 μmol/L of ionophore A23187 (Mueller et al. 1984). Reactions were terminated by adding chloroform/methanol (2:1, v/v) to the reaction mixture. [^{14}C]-Phosphatidylcholine was added to each sample as an internal standard to monitor lipid extraction recovery. Lipids were then extracted by the method of Bligh and Dyer (1959) and PAF isolated by TLC on silica gel G developed in chloroform/methanol/acetic acid/water (50:25:8:4, v/v/v/v). PAF and metabolites were added as standards and visualized after chromatography by exposure to iodine vapors. Radioactivity on TLC plates was detected using a BioScan System 200 Imaging Scanner (BioScan, Washington, DC). The amount of radioactivity migrating with PAF was determined by zonal scraping followed by liquid scintillation counting.

Analysis of the fatty acid content of foods in the diet. The total foods provided to one subject on each of the 4 d was homogenized into a liquid preparation. Total lipids were extracted by the method of Bligh and Dyer (1959). Octadeuterated arachidonic acid and trideuterated steric acid were added to aliquots of the total extract as internal standards. Samples then underwent base hydrolysis, column extraction and derivatization with PFBBR and DIPE as described above for serum lipids. The quantities of fatty acids in the total lipid extracts were then determined by NICI-GC/MS as described below.

Analysis of GLA capsules. The fatty acid compositions of the Ultra-GLA and BIO-EFA capsules were assessed by dividing a capsule

TABLE 2

Fatty acid composition of controlled diet[1]

	GLA[2]	DGLA	AA
		mg	
Day 1	21.6	19.8	42.7
Day 2	20.7	19.2	43.4
Day 3	23.6	7.6	47.7
Day 4	13.5	5.5	26.8

[1] The total daily food amount for one subject (37-y-old male) was combined and lipids extracted by the method of Bligh and Dyer (1959). After base hydrolysis and column extraction, fatty acid quantities were determined by negative ion chemical ionization-gas chromatography/mass spectrometry.

[2] GLA, γ-linolenic acid; DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid.

in methanol to separate the fatty acids from the outer capsule shell. Total lipids were then extracted by the method of Bligh and Dyer (1959). Octadeuterated arachidonic acid and trideuterated steric acid were added to aliquots of the total extract as internal standards. Samples then underwent base hydrolysis, column extraction and derivatization with PFBBR and DIPE as described above for serum lipids. The quantities of fatty acids in the total lipid extracts were then determined by NICI-GC/MS as described below.

Negative ion chemical ionization-gas chromatography/mass spectrometry of fatty acids. NICI-GC/MS analysis was conducted on a Hewlett-Packard (Wilmington, DE) 5989A single-stage quadrapole mass spectrometer (Chilton et al. 1993). The gas chromatography was performed using a 30-m DB-17 fused silica column (SPB-5; 0.25-mm i.d., 0.25-mm film thickness; Supelco, Bellafonte, PA) on a Hewlett-Packard 5890 GC. The initial column temperature was 60°C. The column was heated to 220°C at a rate of 40°C/min with subsequent increase in temperature to 280°C at a rate of 5.0°C/min. The injector temperature was maintained at 250°C. Each injection was performed in the splitless mode. A volume of 1 μL of the 200 μL of recovered material dissolved in hexane was injected. Helium was used as the carrier gas. The pentafluorobenzyl esters were analyzed using selected ion-recording techniques to monitor for AA (*m/z* 303), GLA (*m/z* 277), DGLA (*m/z* 305), trideuterated stearic acid (*m/z* 286) and octadeuterated arachidonic acid (*m/z* 311). Quantities of each fatty acid were determined by obtaining ratios of the areas of the signals for a given fatty acid and the internal standards. These ratios were then used to calculate actual quantities of fatty acids by applying them to standard curves previously generated by analyzing ratios of a given fatty acid to the internal standards at a variety of concentrations of the fatty acid. 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.

Statistical analysis. Analysis of fatty acid or eicosanoid content was performed in duplicate or triplicate for each subject at each time point studied. Results are expressed as the mean of each subject's mean ± SEM. Pre- and postsupplementation values were analyzed using two-tailed paired Student's *t* tests. One-way ANOVA was used to compare differences between time points. Post-hoc comparisons were made between pre- and postsupplementation values and values during supplementation using Fisher's protected least significant difference multiple comparison text.

RESULTS

Incorporation of the GLA supplement into serum lipids. Initial studies examined the effect of dietary supplementation with GLA on the fatty acid concentration of serum lipids. **Figure 1** demonstrates the effect of GLA supplementation at three different doses (Protocol 1) on serum levels of GLA, DGLA and AA. In all three groups of subjects, AA significantly increased in serum lipids at the end of the 3-wk dietary period compared

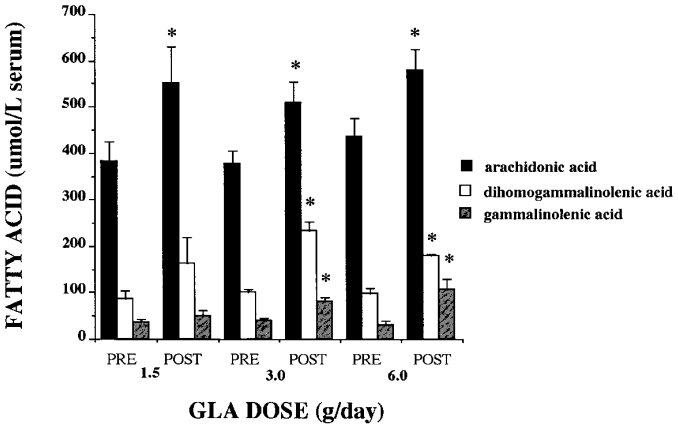

FIGURE 1 Effect of γ-linolenic acid (GLA) supplementation on serum concentrations of fatty acids after 3 wk of supplementation. Volunteers supplemented a controlled 25% fat diet with 1.5, 3.0 or 6.0 g/d of GLA for 3 wk. PRE and POST indicate levels of fatty acids found before and after supplementation, respectively. These data are means ± SEM, $n = 3$. *Denotes significant difference ($P < 0.05$) when a given fatty acid is compared before and after supplementation.

with base-line values. Both GLA and DGLA increased significantly in the groups receiving 3.0 and 6.0 g/d. In the two highest dose groups, DGLA levels increased ~100% and AA levels increased ~30% compared with base-line values of these fatty acids in the same subjects. There was no significant change in serum fatty acid concentrations of volunteers eating control (25% energy as fat) diets, but not receiving the GLA supplement (data not shown).

An important difference between these studies and most clinical trials in the literature is the length of time of supplementation. Therefore, a long-term supplementation study (Protocol 4, 3.0 g/d) was performed over a 12-wk period to assess whether fatty acid ratios and distribution would change in a manner that was not observed at 3 wk. **Figure 2** shows the concentrations of GLA, DGLA and AA in serum at 2-wk intervals during and after supplementation. This study demonstrated that serum GLA, DGLA and AA increased over base-line values within 2

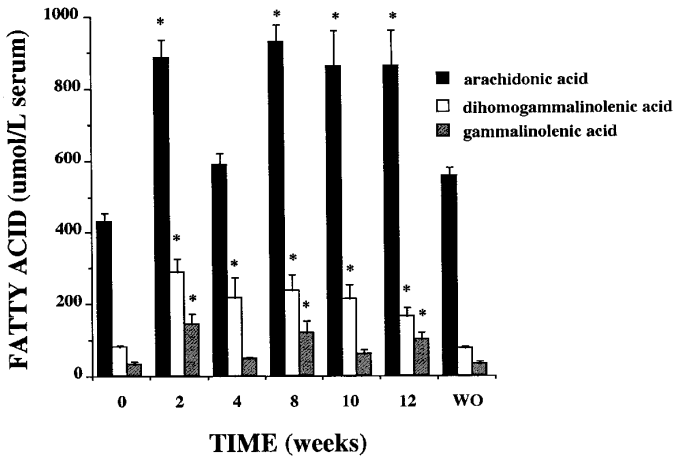

FIGURE 2 The time course of the incorporation of dietary fatty acids into serum lipids of volunteers receiving γ-linolenic acid (GLA) supplementation. Volunteers supplemented a typical Western diet with 3.0 g/d of GLA for 12 wk. Serum lipids were extracted and total fatty acid quantities determined. These data are means ± SEM, $n = 3$. *Denotes significance difference ($P < 0.05$) when a given fatty acid at one time point is compared with presupplementation values.

wk of initiation of supplementation. Extension of the supplementation period did not consistently alter the magnitude of change compared with 2-wk supplementation values. Postsupplementation serum concentrations were not significantly different than base-line (before supplementation) concentrations. Taken together, these data suggest that although some dietary GLA remains in the serum unchanged, substantial quantities of the elongation product, DGLA, and the elongation Δ-5-desaturase product, AA, accumulate in serum after GLA supplementation.

The next set of experiments was designed to determine the distribution of supplemented fatty acids or their metabolites within individual glycerolipid classes of serum. Serum was collected from volunteers before and after they received 6.0 g/d of GLA. **Figure 3**A–C show the distribution of GLA, DGLA and AA, respectively, in lipid classes of serum. GLA was located predominately in triglycerides (36–38% of total), phospholipids (26–33% of total) and cholesterol esters (17–21% of total). After supplementation, GLA increased significantly in both phospholipids and cholesterol esters. In contrast, DGLA and AA were located almost exclusively in serum phospholipids, with very little of these fatty acids found in other serum lipid pools. After supplementation, both DGLA and AA increased only in serum phospholipids.

Incorporation of the GLA supplement into neutrophil lipids. The fatty acid composition of the neutrophil lipids in subjects eating a controlled diet supplemented with 1.5, 3.0 or 6.0 g/d of GLA was also analyzed. No consistently detectable amounts of GLA were found in the glycerolipids of neutrophils before or after supplementation. Although relatively large quantities of AA were found in neutrophils of unsupplemented subjects, there was no significant change in AA within glycerolipids after GLA supplementation at any of the doses given (**Fig. 4**). In contrast, DGLA levels increased by 72% in those receiving 3.0 g/d and by 130% in those receiving 6.0 g/d of GLA. The AA/DGLA ratio decreased from ~5.4:1 before supplementation to 2.3:1 3 wk after 6.0 g/d of GLA supplementation. There was no significant change in fatty acid levels in control subjects eating the study diet without supplementation (data not shown). Subjects supplementing their typical diets with 3.0 g/d of GLA also showed significant increases in DGLA after 3 wk of supplementation without concomitant changes in AA within the neutrophil glycerolipids (data not shown). These findings suggest that neutrophils rapidly elongate GLA to DGLA but lack the ability to desaturate DGLA to AA. Taken together with the changes demonstrated in serum lipid composition, these data support the hypothesis that the elongase activity is limited to specific cell types.

To better determine the distribution of fatty acids within different glycerolipid classes, neutrophils were obtained from subjects before and after supplementation with 6.0 g/d of GLA. The majority of AA (>60%) within the neutrophil lipids was located in phosphatidylethanoamine (PE), and neither the absolute amount nor its relative distribution changed significantly after dietary supplementation with GLA (**Fig. 5**A). Similarly, the bulk of DGLA in the neutrophil was associated with PE (40%) (Fig. 5B). There were significant increases in the amount of DGLA associated with both PE and neutral lipids after supplementation. The AA/DGLA ratio in PE decreased from 8.3:1 before supplementation to 4:1 after supplementation for 3 wk with 6.0 g/d of GLA. These data illustrate that AA and DGLA reside in similar glycerolipid pools both before and after GLA supplementation.

Influence of GLA supplementation on the release of fatty acids or the production of lipid mediators by stimulated neutrophils. These experiments revealed that in vivo GLA supplementation causes marked changes in the fatty acid composition of circulating neutrophils. However, it was not apparent from these studies whether changes in neutrophil composition would affect the quantities of fatty acids released or lipid medi-

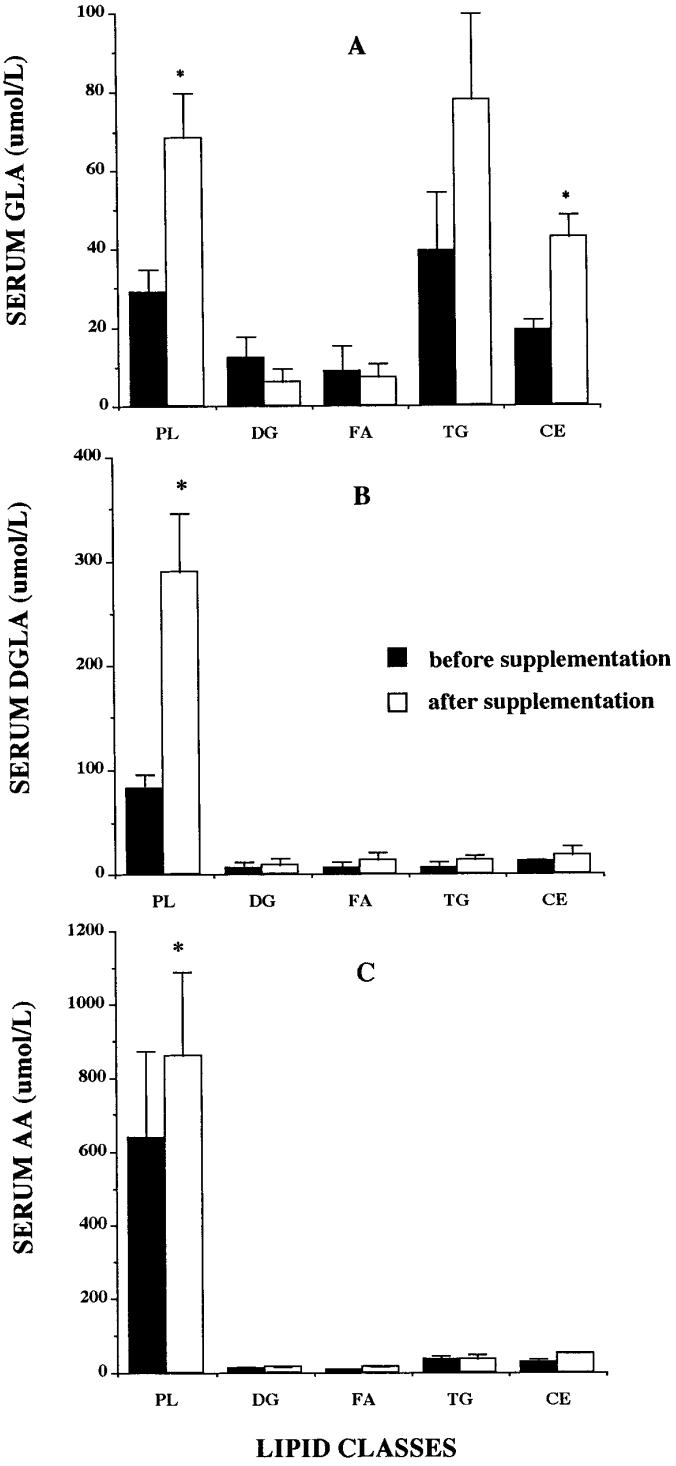

FIGURE 3 Distribution of γ-linolenic acid (GLA) (*Panel A*), dihomo-γ-linolenic acid (DGLA) (*Panel B*) and arachidonic acid (AA) (*Panel C*) in lipid classes of serum before and after GLA supplementation. Volunteers supplemented a controlled diet with 6.0 g/d of GLA for 3 wk. Serum glycerolipids were isolated and fatty acids were analyzed. PL, DG, FA, TG and CE represent fatty acids in phospholipids, diglycerides, free fatty acids, triglycerides and cholesterol esters, respectively. These data are means ± SEM, $n = 3$. *Denotes significant difference ($P < 0.05$) when a given fatty acid in a lipid class is compared before and after supplement.

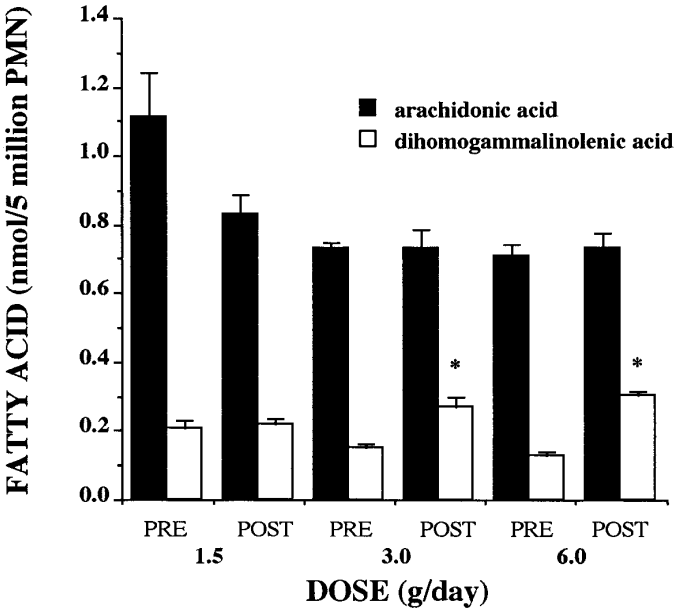

FIGURE 4 Effect of γ-linolenic acid (GLA) supplementation of human volunteers on the fatty acid composition of glycerolipids of neutrophil membranes. Volunteers supplemented a controlled diet with 1.5, 3.0 or 6.0 g/d of GLA for 3 wk. PRE and POST indicate levels of fatty acids found before and after supplementation, respectively. These data are means ± SEM of three individuals in each of the three dosage groups. *Denotes significant difference ($P < 0.05$) when a given fatty acid is compared before and after supplementation.

ators produced by these cells upon stimulation. Neutrophils were obtained from subjects before and after supplementation and stimulated with ionophore A23187. The release of AA from the neutrophil glycerolipids after stimulation did not change after GLA supplementation (**Fig. 6**). In contrast, the release of DGLA increased by 180, 185 and 200% in those volunteers receiving 1.5, 3.0 and 6.0 g/d GLA, respectively (Fig. 6). Similarly, neutrophils isolated from subjects consuming typical Western diets supplemented with GLA for 3 wk (Protocol 2) released 180% more DGLA upon stimulation than neutrophils from subjects consuming the same diet without GLA. In contrast, the amount of AA did not change (data not shown). During long-term (12 wk) supplementation with GLA, the release of DGLA from neutrophil phospholipids upon stimulation increased the first 2 wk after initiation of supplementation but did not increase further during the next 10 wk of supplementation (data not shown). These data support the hypothesis that the fatty acid composition of the neutrophil glycerolipids affects the fatty acids released upon cellular stimulation. They also suggest that the enzyme responsible for mobilizing fatty acids, phospholipase A_2, hydrolyzes DGLA in addition to AA.

Although these experiments demonstrated that GLA supplementation did not influence the ex vivo release of AA from neutrophil glycerolipids, it was unclear whether GLA supplementation would alter leukotriene biosynthesis. To examine this question, neutrophils were stimulated as described in Subjects and Methods and the amounts of LTB_4, 20-OH-LTB_4 and the six *trans* isomers of LTB_4 were measured. In contrast to fatty acid release, neutrophils from subjects supplementing their control diets with 3.0 g/d GLA produced 58% less LTB_4 than the same subjects before supplementation (**Fig. 7**). Levels of 20-OH-LTB_4, 6-*trans* LTB_4 and 6-*trans* 12-epi LTB_4were decreased to a similar degree after supplementation (data not shown).

A final set of experiments measured changes in the capacity of neutrophils to generate PAF ex vivo before and after GLA supplementation. Neutrophils of subjects receiving 3.0 g/d of GLA produced 36% less PAF after supplementation than neutrophil obtained from those same subjects before supplementation (**Fig. 8**). Taken together, these data reveal that GLA supplementation can alter the capacity of neutrophils to generate lipid mediators. This inhibition appears to occur at some step distal to the phospholipase-catalyzed cleavage of AA from membrane phospholipids.

DISCUSSION

It has been suggested that dietary supplementation with GLA may modulate the manifestations of some chronic inflammatory diseases in animals and humans including rheumatoid arthritis, eczema, Sjogren's syndrome, ulcerative colitis and systemic sclerosis (Horrobin 1992, Kunkel et al. 1981,

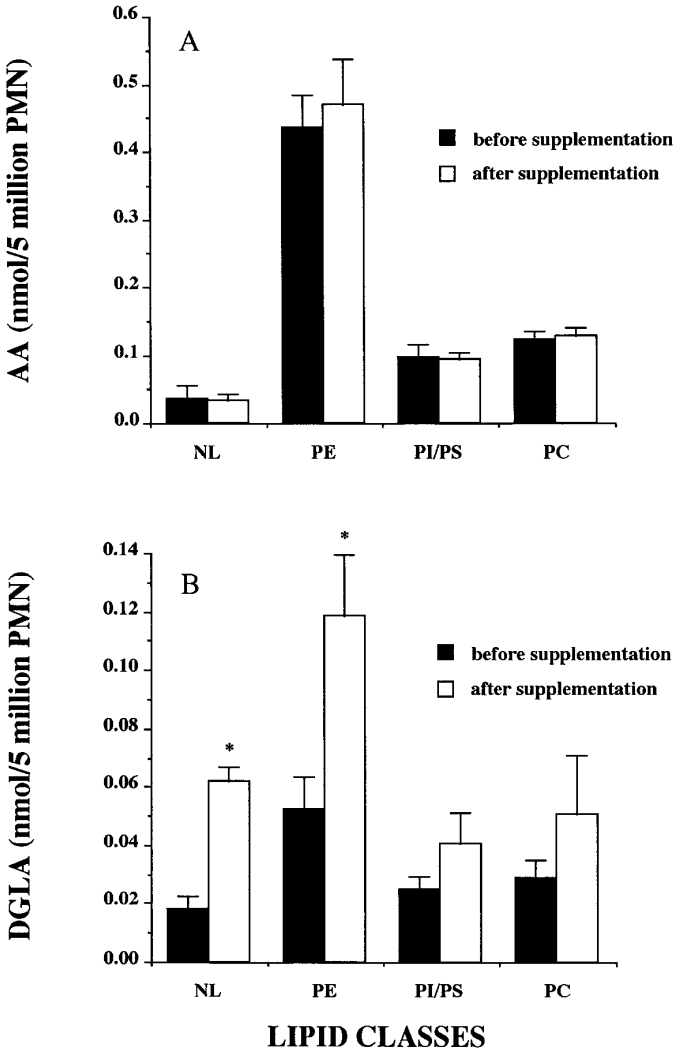

FIGURE 5 Effect of γ-linolenic acid (GLA) supplementation on arachidonic (AA) (*Panel A*) and dihomo-γ-linolenic acid (DGLA) (*Panel B*) distribution in neutrophil glycerolipid classes. Volunteers supplemented a controlled diet with 6.0 g/d of GLA for 3 wk. NL, PE, PI/PS and PC represent fatty acids in neutral lipids, phosphatidylethanolamine, phosphatidylinositol/phosphatidylserine and phosphatidylcholine, respectively. These data are means ± SEM, $n = 3$. *Denotes significance ($P < 0.05$) when a given fatty acid in a lipid class is compared before and after supplementation.

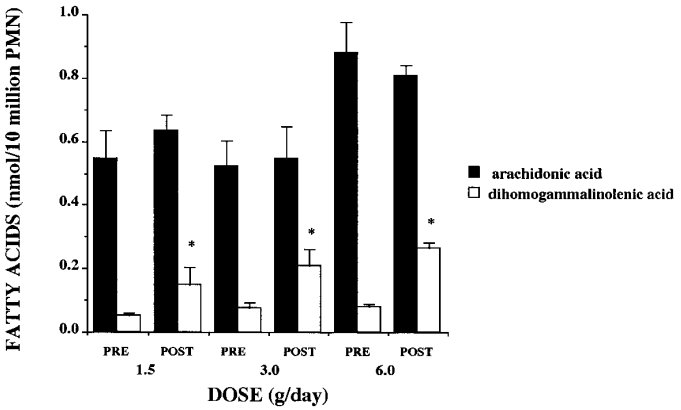

FIGURE 6 Effect of γ-linolenic acid (GLA) supplementation on the release of fatty acids from glycerolipids of neutrophils stimulated with ionophore A23187. Volunteers supplemented a controlled diet with 1.5, 3.0 or 6.0 g/d of GLA for 3 wk. PRE and POST indicate the levels of fatty acids found before and after supplementation, respectively. These data are means ± SEM of three individuals in each of the three dosage groups. *Denotes significant difference ($P < 0.05$) when a given fatty acid is compared before and after supplementation.

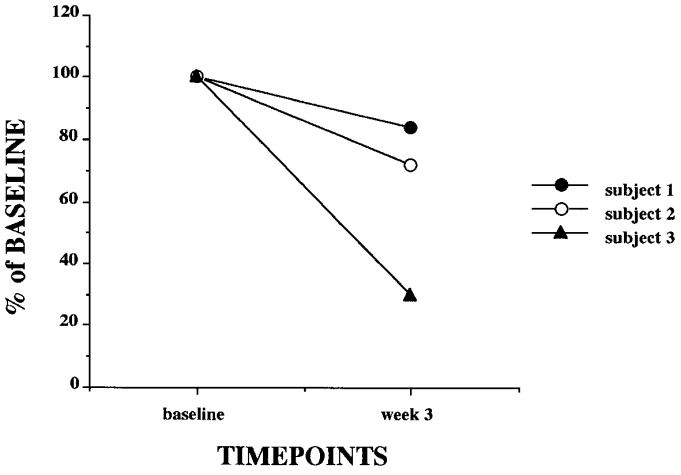

FIGURE 8 Effect of γ-linolenic acid (GLA) supplementation on the biosynthesis of platelet-activating factor (PAF) by A23187-stimulated neutrophils. Volunteers supplemented their controlled diet with 3.0 g/d of GLA for 3 wk. These data are results of three individuals analyzed in duplicate at each time point.

Leventhal et al. 1993 and 1994, Lovell et al. 1981, Morse et al. 1989, Wright and Burton 1982). Although it has been suggested that immune modulation by GLA may occur as a result of inhibition of eicosanoid biosynthesis, little is known about a number of important variables such as the kinetics and dose dependency of in vivo GLA supplementation (Ziboh and Fletcher 1992). In light of the overwhelming evidence that oxygenated metabolites of AA are central mediators of inflammation, it has also been particularly difficult to explain how a putative precursor of AA, GLA, attenuates eicosanoid generation and inflammation. The current study has addressed these questions by examining the in vivo conversion of GLA to potential elongation and desaturation products and then determining the effect of GLA supplementation on the capacity of neutrophils to release AA and synthesize eicosanoids and PAF under a variety of dietary conditions.

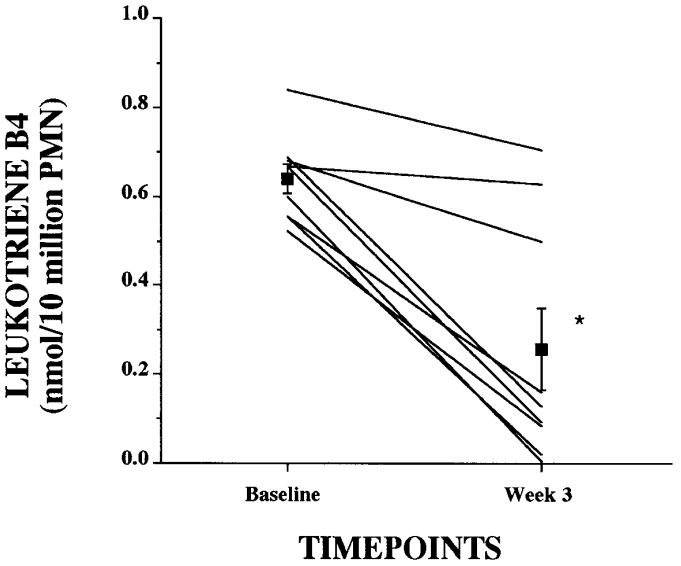

FIGURE 7 Effect of γ-linolenic acid (GLA) supplementation on the biosynthesis of LTB_4 by A23187-stimulated neutrophils. Volunteers supplemented their controlled diet with 3.0 g/d of GLA for 3 wk. Quantities of leukotrienes were determined by comparing their area under the curve to that of prostaglandin B_2 added as an internal standard. These data are results of nine individuals analyzed in duplicate and represent the mean ± SEM. *Denotes significance ($P < 0.05$) when LTB_4 is compared before and after supplementation.

Initial experiments in this study focused on in vivo GLA metabolism in serum and neutrophils. Dietary supplementation with GLA increased the levels of both DGLA and AA in the serum, suggesting that once dietary GLA has reached the serum compartment, it has the potential to be both elongated to DGLA and subsequently desaturated to AA. The majority of GLA found in serum is associated with triglycerides, followed by phospholipids and cholesterol esters. The GLA levels of all three of these lipid pools are increased after supplementation. In contrast to GLA, DGLA and AA are almost exclusively localized in phospholipid pools. GLA supplementation enriches the phospholipid pool in both DGLA and AA but does not cause any appreciable change in other serum lipid classes. It is interesting that the 20-carbon fatty acids (DGLA and AA) are distributed in different serum classes than the 18-carbon fatty acid (GLA). The in vivo relevance of the differential distribution of the fatty acids in various serum lipid classes is not apparent at this time. In fact, little is known about how, or in what form, inflammatory cells incorporate a particular fatty acid into membrane glycerolipids. Studies by Habenicht and colleagues (1990) demonstrate that AA enters fibroblasts as part of an LDL particle and if LDL receptors are blocked, the capacity of the cells to produce prostaglandins is blocked. Future studies will be necessary to determine how GLA, DGLA and AA enter inflammatory cells such as neutrophils from the serum.

In contrast to the serum, GLA supplementation did not increase AA levels in neutrophil glycerolipids. This is consistent with previous in vitro studies in our laboratory, which demonstrated that human neutrophils contain high quantities of an elongase activity but lack the Δ-5-desaturase activity necessary to convert DGLA to AA (Chilton-Lopez et al 1996). This study again emphasizes that there is a distinction between many cells and tissues including inflammatory cells regarding their capacity to metabolize (n-6) fatty acids. For example, skin, murine peritoneal macrophages, platelets and epidermal cell lines appear to have large high elongase activity relative to Δ-5-desaturase activ-

ity (Chapkin and Coble 1991, Chapkin et al. 1988, de Bravo et al. 1985). In contrast, several tissues including liver, kidney, testes, brain and intestine contain both activities (Bernert and Sprecher 1975, Blond and Bezard 1991, Hurtado de Catalfo et al. 1992, Irazu et al. 1993, Luthria and Sprecher 1994, Pawlosky et al. 1994). Our results, which demonstrate an enhancement of AA in serum but not in neutrophils, are consistent with a hepatic location of Δ-5-desaturase.

Elongation of GLA to DGLA without further desaturation to AA could explain why GLA supplementation may attenuate AA metabolism in some cells. For example, the current study indicates that dietary supplementation with GLA leads to a marked increase in the DGLA to AA ratio in glycerolipids of circulating neutrophils. Concomitantly, the ratio of DGLA to AA released from membrane phospholipids increases after stimulation. These data suggest that DGLA is located in membrane phospholipids that are accessible to hydrolysis. Moreover, they indicate that the phospholipase A_2 that are responsible for fatty acid release will hydrolyze both DGLA and AA.

Finally, dietary supplementation with GLA attenuates the ex vivo capacity of neutrophils to produce both LTB_4 and PAF. The decrease in either or both of these very potent inflammatory mediators may represent a mechanism by which GLA exerts anti-inflammatory effects. Because GLA supplementation does not inhibit AA release, it is unlikely that the inhibition of lipid mediators is due to diminished phospholipase A_2 activity. However, DGLA release is markedly increased after GLA supplementation. DGLA can be converted to a 15-lipoxygenase product, 15-HETrE, upon stimulation of a variety of cells. 15-HETrE inhibits the formation of LTB_4 in human neutrophils and monocytes (Chilton-Lopez et al. 1996, Iversen et al. 1991 and 1992). Studies by Vanderhoek and colleagues (1980) suggest that inhibition of leukotriene biosynthesis by 15-lipoxygenase products occurs as a result of direct inhibition of the enzyme 5-lipoxygenase. A novel observation of this study is that dietary GLA can inhibit the production of PAF. In the case of PAF, 5-lipoxygenase products have been shown to potentiate PAF biosynthesis in human neutrophils (Billah et al. 1985). Consequently, it may be that blocking the synthesis of 5-lipoxygenase products during supplementation also attenuates PAF generation.

The mechanism by which GLA supplementation exerts an anti-inflammatory effect may not be limited to inhibition of LTB_4 or PAF production. For example, PGE_1, a cyclooxygenase product of DGLA, has potent anti-inflammatory properties. Dietary GLA supplementation has been shown to significantly enhance PGE_1 synthesis by mouse peritoneal macrophages (Fan and Chapkin 1992). Alternatively, dietary GLA may diminish the production of PGE_2, a potent inflammatory mediator (Pullman-Moar et al. 1990). Other studies have shown that DGLA suppresses the proliferation of interleukin-2–dependent lymphocytes from synovial fluid of patients with rheumatoid arthritis and interleukin-2–dependent proliferation of Ctl-2 cells (Borofsky et al. 1992, DeMarco et al. 1994). This effect was blocked by phorbol 12-myristate 13-acetate, an activator of protein kinase C, suggesting that dietary GLA may modify this enzyme's activity.

Although data from both animal studies and human trials support the hypothesis that dietary supplementation with GLA can modulate inflammation, an optimum feeding strategy to maximize its potential benefits has not yet been defined. This study demonstrates that dietary GLA increases the content of its elongase product, DGLA, within neutrophil cell membranes without concomitant changes in AA. The magnitude of this increase is similar in those who maintain a control diet (25% of total energy from fat) and those who maintain a more typical Western diet. Enrichment of membrane glycerolipids with DGLA occurs within the first 2 wk of GLA supplementation and is not further enhanced with prolonged supplementation. The profile of fatty acids released from neutrophils after dietary supplementation changes concomitantly with changes in the neutrophil membrane content. Taken together, these data describe the incorporation of dietary GLA into serum and neutrophil lipids and suggest mechanisms by which this supplement may modulate inflammation.

ACKNOWLEDGMENT

We thank Beverly Cox in the General Clinical Research Center for her help in the planning and preparation of the participants' controlled diets.

LITERATURE CITED

Arm, J. P., Horton, C. E., Spur, B. W., Mencia-Huerta, J. M. & Lee, T. H. (1989) The effects of dietary supplementation with fish oil lipids on the airways response to inhaled allergen in bronchial asthma. Am. Rev. Respir. Dis. 139: 1395–1400.

Bang, H. O. & Dyerberg, J. (1972) Plasma lipids and lipoproteins in Greenlandic west coast Eskimos. Acta Med. Scand. 192: 85–94.

Bang, H. O., Dyerberg, J. & Hjorne, N. (1976) The composition of food consumed by Greenland Eskimos. Acta Med. Scand. 200: 69–73.

Bernert, J. T. & Sprecher, H. (1975) Studies to determine the role rates of chain elongation and desaturation play in regulating the unsaturated fatty acid composition of rat liver lipids. Biochim. Biophys. Acta 398: 354–363.

Billah, M. M., Bryant, R. R. & Siegel, M. I. (1985) Lipoxygenase products of arachidonic acid modulate the biosynthesis of platelet-activating factor (1-0-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) by human neutrophils via phospholipase A_2. J. Biol. Chem. 260: 6899–6906.

Bligh, E. G. & Dyer, W. J. (1959) A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911–917.

Blond, J. P. & Bezard, J. (1991) Delta 5-desaturation of dihomogammalinolenic acid (20:3(n-6)) into arachidonic acid (20:4(n-6)) by rat liver microsomes and incorporation of fatty acids in microsome phospholipids. Biochim. Biophys. Acta 1084: 255–260.

Borofsky, M. A., Zurier, R. B., Rosenbaum, H., Weiner, D. B. & Williams, W. V. (1992) Effects of polyunsaturated fatty acids on interleukin-2-dependent T cell growth. Immunol. Res. 11: 154–164.

Chapkin, R. S. & Coble, K. J. (1991) Utilization of gammalinolenic acid by mouse peritoneal macrophages. Biochim. Biophys. Acta 1085: 365–370.

Chapkin, R. S., Somers, S. D. & Erickson, K. L. (1988) Inability of murine peritoneal macrophages to convert linolenic acid into arachidonic acid. Evidence of chain elongation. J. Immunol. 140: 2350–2355.

Chapkin, R.S. & Ziboh, V. A. (1984) Inability of skin enzyme preparations to biosynthesize arachidonic acid from linoleic acid. Biochem. Biophys. Res. Commun. 124: 784–792.

Chapkin, R. S., Ziboh, V. A. & McCullough, J. L. (1987) Dietary influences of evening primrose oil and fish oil on the skin of essential fatty acid-deficient guinea pigs. J. Nutr. 117: 1360–1370.

Chilton, F. H., Fonteh, A. N., Surette, M. E., Triggiani, M. & Winkler, J. D. (1996) Control of arachidonate levels within inflammatory cells. Biochim. Biophys. Acta 1299: 1–15.

Chilton, F. H., Patel, M., Fonteh, A. N., Hubbard, W. C. & Triggiani, M. (1993) Dietary n-3 fatty acid effects on neutrophil lipid composition and mediator production: influence of duration and dosage. J. Clin. Invest. 91: 115–122.

Chilton-Lopez, T., Surette, M. E., Swan, D. D., Fonteh, A. N., Johnson, M. M. & Chilton, F. H. (1996) Metabolism of gamma-linolenic acid in human neutrophils. J. Immunol. 156: 2941–2947.

deBravo, M. M., DeTomas, M. E. & Mercuri, O. (1985) Metabolism of gamma-linolenic acid by human blood platelet microsomes. Biochem. Int. 10: 889–896.

DeMarco, D. M., Santoli, D. & Zurier, R. B. (1994) Effects of fatty acids on proliferation and activation of human synovial compartment lymphocytes. J. Leuk. Biol. 56: 612–615.

Dyerberg, J. & Bang, H. O. (1979) Hemostatic function and platelet polyunsaturated fatty acids in Eskimos. Lancet 2: 433–435.

Dyerberg, J., Bang, H. O., Stofferson, E., Moncada, S. & Vane, J. R. (1978) Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? Lancet 2: 117–119.

Fan, Y. Y. & Chapkin, R. S. (1992) Mouse peritoneal macrophage prostaglandin E_1 synthesis is altered by dietary γ-linolenic acid. J. Nutr. 122: 1600–1606.

Fischer, S. (1989) Dietary polyunsaturated fatty acids and eicosanoid formation in humans. Adv. Lipid Res. 23: 169–198.

Fischer, S. & Weber, P. C. (1984) Prostaglandin I_3 is formed in vivo in man after dietary eicosapentaenoic acid. Nature (Lond.) 307: 165–168.

Gibney, M. J. & Hunter, B. (1993) The effects of short- and long-term supple-

mentation with fish oil on the incorporation of n-3 polyunsaturated fatty acids into cells of the immune system in healthy volunteers. Eur. J. Clin. Nutr. 47: 255–259.
Habenicht, A.J.R., Salbach, P., Goerig, M., Zeh, W., Janssen-Timmen, U., Blattner, C., King, W. C. & Glomset, J. A. (1990) The LDL receptor pathway delivers arachidonic acid for eicosanoid formation in cells stimulated by platelet-derived growth factor. Nature (Lond.) 345: 634–636.
Henderson, W. R., Jr. (1994) The role of leukotrienes in inflammation. Ann. Intern. Med. 121: 684–697.
Herxheimer, H. & Schaefer, O. Asthma in Canadian Eskimos. (1974) N. Engl. J. Med. 291: 1419.
Horrobin, D. F. (1992) Nutritional and medical importance of gammalinolenic acid. Prog. Lipid Res. 31: 163–194.
Hurtado de Catalfo, G. E., Mandon, E. C. & de Gomez Dumm, I. N. (1992) Arachidonic acid biosynthesis in non-stimulated and adrenocorticotropin-stimulated Sertoli and Leydig cells. Lipids 27: 593–598.
Irazu, C. E., Gonzalez-Rodreiquez, S. & Brenner, R. R. (1993) Delta 5 desaturase activity in rat kidney microsomes. Mol. Cell Biochem. 129: 31–37.
Iversen, L., Fogh, K, Bojesen, G. & Kragballe, K. (1991) Linoleic acid and dihomogammalinolenic acid inhibit leukotriene B_4 formation and stimulate the formation of their 15-lipoxygenase products by human neutrophils in vitro. Evidence of formation of antiinflammatory compounds Agents Actions 33: 286–291.
Iversen, L., Fogh, K. & Kragballe, K. (1992) Effect of dihomogammalinolenic acid and its 15-lipoxygenase metabolite on eicosanoid metabolism by human mononuclear leukocytes in vitro: selective inhibition of the 5-lipoxygenase pathway. Arch. Derm. Res. 284: 222–226.
Kromhout, D., Bosschieter, E. B. & deLezenne Coulander, C. (1985) The inverse relationship between fish consumption and 20-year mortality from coronary heart disease. N. Engl. J. Med. 312: 1205–1209.
Kunkel, S. L., Ogawa, H., Ward, P. A. & Zurier, R. B. (1981) Suppression of chronic inflammation by evening primrose. Prog. Lipid Res. 20: 885–888.
Lee, T. H., Hoover, R. L., Williams, J. D., Sperling, R. I., Ravalese, J., Spur, B. W., Robinson, D. R., Corey. E. J., Lewis, R. A. & Austen, K. F. (1985) Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. N. Engl. J. Med. 312: 1217–1224.
Leventhal, L. J., Boyce, E. G. & Zurier, R. B. (1993) Treatment of rheumatoid arthritis with gammalinolenic acid. Ann. Int. Med. 119: 867–873.
Leventhal, L. J., Boyce, E. G. & Zurier, R. B. (1994) Treatment of rheumatoid arthritis with blackcurrant seed oil. Br. J. Rheumatol. 33: 847–852.
Lovell, C. R., Burton, J. L. & Horrobin, D. F. (1981) Treatment of atopic eczema with evening primrose oil. Lancet 1: 278.
Luthria, D. L. & Sprecher, H. (1994) A comparison of the specific activities of linoleate and arachidonate in liver, heart and kidney phospholipids after feeding rats ethyl linoleate-9, 10, 12, 13-d_4. Biochim. Biophys. Acta 1213: 1–4.
Morse, P. F., Horrobin, D. F., Manku, M. S., Stewart, J. C., Allen, R., Littlewood, S., Wright, S., Burton, J., Gould, D. J., Holt, P. J., Jansen, C. T., Mattila, L., Meigel, W., Dettke, T. H., Wexler, D. J., Guenther, L., Bordon, A. & Patrizzi, A. (1989) Meta-analysis of the placebo-controlled studies of the efficacy of Epogam in the treatment of atopic eczema: relationship between plasma essential fatty acid changes and clinical response. Br. J. Dermatol. 121: 75–90.
Mueller, H. W., O'Flaherty, J. T. & Wykle, R. L. (1984) The molecular species distribution of platelet-activating factor synthesized by rabbit and human neutrophils. J. Biol. Chem. 259: 14554–14559.
Patton, G. M., Fasuto, J. M. & Robins, S. J. (1982) Separation of phospholipids and individual molecular species of phospholipids by high performance liquid chromatography. J. Lipid Res. 23: 190–196.
Pawlosky, R., Barnes, A. & Salem, N., Jr. (1994) Essential fatty acid metabolism in the feline: relationship between liver and brain production of long-chain polyunsaturated fatty acids. J. Lipid Res. 35: 2032–2040.
Prescott, S. M. (1984) The effect of eicosapentaenoic acid on leukotriene B production by human neutrophils. J. Biol. Chem. 259: 7615–7621.
Pullman-Moar, S., Laposata, M., Lem, D., Holman, R. T., Leventhal, L. J., DeMarco, D. & Zurier, R. B. (1990) Alteration of the cellular fatty acid profile and the production of eicosanoids in human monocytes by gamma-linolenic acid. Arthritis Rheum. 33: 1526–1533.
Shahar, E., Folsom, A. R., Melnick, S. L., Tockman, M. S., Comstock, G. W., Gennaro, V., Higgins, M. W., Sorlie, P. D., Ko, W. J. & Szklo, M. (1994) Dietary n-3 polyunsaturated fatty acids and smoking-related chronic obstructive pulmonary disease. N. Engl. J. Med. 331: 228–233.
Sprecher, H. (1989) Interaction between the metabolism of n-3 and n-6 fatty acids. J. Intern. Med. 225 (suppl.): 5–9.
Vanderhoek, J. Y., Bryant, J. W. & Bailey, J. M. (1980) Inhibition of leukotriene biosynthesis by the leukocyte product 15-hydroxy-5,8,11,13-eicosatetraenoic acid. J. Biol. Chem. 255: 10064–10066.
Wright, S. & Burton, J. L. (1982) Oral evening-primrose-seed oil improves atopic eczema. Lancet 2: 1120–1122.
Ziboh, V. A. & Fletcher, M. P. (1992) Dose-response effects of dietary gamma-linolenic acid-enriched oils on human polymorphonuclear-neutrophil biosynthesis of leukotriene B_4. Am. J. Clin. Nutr. 55: 39–45.