Dietary Supplementation with \( \gamma \)-Linolenic Acid Alters Fatty Acid Content and Eicosanoid Production in Healthy Humans\(^1,2 \)

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ABSTRACT To understand the in vivo metabolism of dietary \( \gamma \)-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-\( \gamma \)-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 • \( \gamma \)-linolenic acid • arachidonic acid • neutrophils • leukotrienes • humans

It has become increasingly clear in the past two decades that oxygenated metabolites of arachidonic acid (AA),\(^*\) 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, \( \gamma \)-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. 1985, Shahar et al. 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,
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 B2 (PGB2), octadeuterated arachidonic acid (2H8-AA) and trideuterated stearic acid (2H3-SA) were obtained from Biomol Research Laboratories (Plymouth Meeting, MA). [3H]-Acetate and [14C]-phosphatidylcholine (PC) were purchased from New England Nuclear (Boston, MA). Phospholipid standards used for the metabolic studies were obtained from American Type Culture Collection (Rockville, MA).

**Table 1. Menus of controlled diet**

<table>
<thead>
<tr>
<th></th>
<th>Fat grams</th>
<th>Fat grams</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Breakfast</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bagel</td>
<td>1.78</td>
<td></td>
</tr>
<tr>
<td>Cream cheese</td>
<td>8.72</td>
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<tr>
<td>Milk 2%</td>
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<td></td>
</tr>
<tr>
<td>Cola</td>
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<td></td>
</tr>
<tr>
<td>Jelly</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Ham cured (extra lean)</td>
<td>4.96</td>
<td></td>
</tr>
<tr>
<td>Lunch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turkey (light, roasted)</td>
<td>3.22</td>
<td></td>
</tr>
<tr>
<td>Whole wheat bread</td>
<td>1.30</td>
<td></td>
</tr>
<tr>
<td>Mayonnaise</td>
<td>11.91</td>
<td></td>
</tr>
<tr>
<td>Lettuce (iceberg)</td>
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<td></td>
</tr>
<tr>
<td>Apple</td>
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<td></td>
</tr>
<tr>
<td>Carrot</td>
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</tr>
<tr>
<td>Cola</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
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<td>Fig bar</td>
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<tr>
<td><strong>Dinner</strong></td>
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<tr>
<td>Beef (round, lean)</td>
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<tr>
<td>Margarine</td>
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<tr>
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<tr>
<td>Lettuce (iceburg)</td>
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<tr>
<td>Dressing (nonfat Ranch)</td>
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<tr>
<td>Radish</td>
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<tr>
<td>Oreo cookies</td>
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<tr>
<td><strong>Snack</strong></td>
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</tr>
<tr>
<td>Milk 2%</td>
<td>4.61</td>
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<td>Peach (canned with juice)</td>
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<tr>
<td>Graham crackers</td>
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<td>Whole wheat bread</td>
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<td><strong>Energy, MJ</strong></td>
<td>12.623</td>
<td>12.757</td>
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<tr>
<td><strong>Total fat, g</strong></td>
<td>86.27</td>
<td>87.70</td>
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**Day 2**

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<tr>
<td>Cheerios</td>
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<tr>
<td>Raisin (seedless)</td>
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<tr>
<td>Milk 2%</td>
<td>0.10</td>
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<tr>
<td>Egg (hard cooked)</td>
<td>10.61</td>
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</tr>
<tr>
<td>Cola</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Milk skim</td>
<td>0.22</td>
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</tr>
<tr>
<td>Lunch</td>
<td></td>
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<tr>
<td>Ham (cured extra lean)</td>
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<td>Mayonnaise</td>
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<td>Lettuce (iceburg)</td>
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<td>Pineapple (canned with juice)</td>
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<td>Cola</td>
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<tr>
<td><strong>Dinner</strong></td>
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<tr>
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<td>Rice (white, enriched)</td>
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<td>Broccoli frozen</td>
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<td>Margarine</td>
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<tr>
<td>Lettuce (iceburg)</td>
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<td>Cucumber</td>
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<tr>
<td>Dressing (nonfat Ranch)</td>
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<td>Apple</td>
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<tr>
<td>Milk skim</td>
<td>0.43</td>
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<tr>
<td>Peach (canned with juice)</td>
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<tr>
<td>Chicken breast meat</td>
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<tr>
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</tr>
<tr>
<td><strong>Total fat, g</strong></td>
<td>87.70</td>
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(continued)
### TABLE 1 (continued)

**Menus of controlled diet**

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<th>Day 3</th>
<th>Fat grams</th>
<th>Day 4</th>
<th>Fat grams</th>
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<tr>
<td>Wheaties</td>
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<td>Beef (round, lean)</td>
<td>3.50</td>
</tr>
<tr>
<td>Milk 2%</td>
<td>9.22</td>
<td></td>
<td>Bean kidney (red, cooked)</td>
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</tr>
<tr>
<td>Raisin (seedless)</td>
<td>0.07</td>
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<td>Tomato sauce (canned)</td>
<td>0.17</td>
</tr>
<tr>
<td>Egg (hard cooked)</td>
<td>10.61</td>
<td></td>
<td>Rice (white, enriched)</td>
<td>0.21</td>
</tr>
<tr>
<td>Cola</td>
<td>0.00</td>
<td></td>
<td>Lettuce (iceberg)</td>
<td>0.19</td>
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<tr>
<td>Lunch</td>
<td></td>
<td></td>
<td>Carrot</td>
<td>0.05</td>
</tr>
<tr>
<td>Turkey (light, roasted)</td>
<td>3.22</td>
<td></td>
<td>Dressing (nonfat, 1000 island)</td>
<td>0.90</td>
</tr>
<tr>
<td>Mayonnaise</td>
<td>14.29</td>
<td></td>
<td>Margarine</td>
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<td>Oreo cookies</td>
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<tr>
<td>Cola</td>
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<td>Snack</td>
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<tr>
<td>Fig bar</td>
<td>3.14</td>
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<tr>
<td><strong>Total fat, g</strong></td>
<td>88.57</td>
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<table>
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<tr>
<td>Total fat, g</td>
<td>86.25</td>
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</table>

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 B4 (LTB4), 20-hydroxy-LTB4 (20-OH-LTB4), 15-hydroxy eicosatetraenoic acid (15-HETE), prostaglandin E1 (PGE1), 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 (Narcox, GA). Pentfluorobenzyl bromide (20% in acetonitrile) and disopropylethylamine (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 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 3. 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 octadecanoyl arachidonic acid standards were then added as internal standards. After 14 min, water was added to samples and the reaction mixtures were acidified with 0.6 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 perfluorobenzyl esters by using 20% perfluorobenzyl bromide (PFBBR) and 20% disopropylethylamine (DPE) 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 NCI-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) or acetic acid/water (50:25:8:4, v/v/v/v). Fatty acids were then analyzed on a normal-phase HPLC column (Ultrastrange, 4.6 × 250 mm; Rainin Instrument) and eluted with hexane/isopropanol/water (4:5:5.5:0.3, v/v/v/v). This mixture was then loaded onto a silica HPLC column (Ultrastrange, 4.6 × 250 mm; Rainin Instrument) and eluted with hexane/isopropanol/ethanol/phosphate buffer (pH 7.4)/acetic acid (490:367:100:30:0.6, 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 NCI-GC/MS as described below.

Analysis of lipid products from stimulated neutrophils. Neutrophils, suspended at 1010 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, intact cell activation, or oxidation of arachidonic acid and trideuterated stearic 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 perfluorobenzyl esters (described above) and analyzed by NCI-GC/MS as described below.

To determine the quantity of leukotrienes produced after cell stimulation, prostaglandin B2 was added to each terminated reaction as an internal standard. Samples were then centrifuged (400 x g for 10 min) to remove protein debris. An aliquot of the supernatant was loaded onto a reverse-phase ODS HPLC column (Ultrastrange, 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 LTBA, its trans isomers and its metabolite, 20-OH-LTBA, were identified on the basis of the elution times of standards analyzed in the same system. Typically, 20-OH-LTBA and LTBA eluted from the column at 19–23 min, respectively, after injection. Quantities of leukotrienes were determined by comparing their areas with that of prostaglandin B2 as 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 [14C]-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. [14C]-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 scanning 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). Octadecanoyl 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 DPE as described above for serum lipids. The quantities of fatty acids in the total lipid extracts were then determined by NCI-GC/MS as described below.
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 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

| Table 2: Fatty acid composition of controlled diet1 |
|---------|---------|---------|
|         | GLA2    | DGLA    | AA      |
| Day 1   | 21.6 mg | 19.8 mg | 42.7 mg |
| Day 2   | 20.7 mg | 19.2 mg | 43.4 mg |
| Day 3   | 23.8 mg | 7.8 mg  | 47.7 mg |
| Day 4   | 13.5 mg | 5.5 mg  | 26.8 mg |

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.
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 3A–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 volunteers before and after supplementation with 6.0 g/d of GLA. The majority of AA (>60%) within the neutrophil lipids was located in phosphatidylethanolamine (PE), and neither the absolute amount nor its relative distribution changed significantly after dietary supplementation with GLA (Fig. 5A). 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 sup-
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 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.

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.

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-

FIGURE 7  Effect of γ-linolenic acid (GLA) supplementation on the biosynthesis of LTB4 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 B2 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 LTB4 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.

TIMEPOINTS

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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 (Bennett 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 A2 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 LTB4 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 A2 activity. However, DGLA release is markedly increased after GLA supplementation. DGLA can be converted to a 15-lipoxygenase product, 15-HETE, upon stimulation of a variety of cells. 15-HETE inhibits the formation of LTB4 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 LTB4 or PAF production. For example, PGE2, a cyclooxygenase product of DGLA, has potent anti-inflammatory properties. Dietary GLA supplementation has been shown to significantly enhance PGE2 synthesis by mouse peritoneal macrophages (Fan and Chapkin 1992). Alternatively, dietary GLA may diminish the production of PGE2, 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 elongate 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.

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LITERATURE CITED


