Mechanisms by which botanical lipids affect inflammatory disorders\textsuperscript{1–4}

Floyd H Chilton, Lawrence L Rudel, John S Parks, Jonathan P Arm, and Michael C Seeds

ABSTRACT

Changes in diet over the past century have markedly altered the consumption of fatty acids. The dramatic increase in the ingestion of saturated and n−6 fatty acids and concomitant decrease in n−3 fatty acids are thought to be a major driver of the increase in the incidence of inflammatory diseases such as asthma, allergy, and atherosclerosis. The central objective of the Center for Botanical Lipids at Wake Forest University School of Medicine and Women’s Hospital is to delineate the mechanisms by which fatty acid–based dietary supplements inhibit inflammation leading to chronic human diseases such as cardiovascular disease and asthma. The key question that this center addresses is whether botanical n−6 and n−3 fatty acids directly block recognized biochemical pathways or the expression of critical genes that lead to asthma and atherosclerosis. Dietary supplementation with flaxseed oil, borage oil, and echium oil affects the biochemistry of fatty acid metabolism and thus the balance of proinflammatory mediators and atherogenic lipids. Supplementation studies have begun to identify key molecular and genetic mechanisms that regulate the production of lipid mediators involved in inflammatory and hyperlipidemic diseases. Echium oil and other oils containing stearidonic acid as well as botanical oil combinations (such as echium and borage oils) hold great promise for modulating inflammatory diseases. Am J Clin Nutr 2008; 87(suppl):498S–503S.

KEY WORDS n−6 Fatty acid, n−3 fatty acid, flax seed, echium oil, botanical oil, asthma, cardiovascular disease, atherosclerosis

THE EPIDEMIC OF INFLAMMATORY DISEASES IN DEVELOPED COUNTRIES

Within the next 2 decades, >1 in 3 US citizens will have an inflammatory disease such as asthma or atherosclerosis. For example, ≈20 million Americans have asthma today, twice as many as in 1980 (1). Asthma deaths among children increased 4% every year from 1980 to 1996. More than 50 million persons, 20% of the US population, have allergies; allergies are the 6th leading cause of chronic human disease. Cardiovascular disease is the number one killer of Americans; >71 million Americans have it in some form, and it killed almost 1 million persons in 2003 (2).

On the basis of many scientific publications, cardiac societies recommend the consumption of 1 g/d of the n−3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for cardiovascular disease prevention (3). Because many individuals cannot tolerate the taste or smell of fish oils, even when provided in capsules, and because of dwindling stocks of the cold-water fish that have a high content of EPA and DHA, interest in botanical sources of n−3 fatty acids has increased tremendously. Stearidonic acid (SDA), a precursor of EPA, is found in the seeds of several plants, including those of the boragenase family, such as Echium plantagineum.

DIETARY FATTY ACIDS

The major polyunsaturated fatty acids in human diets belong to the n−3 or n−6 fatty acids. This designation reflects the position of the first unsaturated carbon-carbon bond relative to the terminal methyl group of the molecule. The 2 primary n−6 and n−3 fatty acids in human diets are linoleic acid and α-linolenic acid (ALA), respectively. In most mammals, these 18-carbon fatty acids can be converted to longer-chain and more unsaturated fatty acids via a series of elongation and desaturation steps (Figure 1). Humans can obtain longer more unsaturated fatty acids, such as arachidonic acid (AA; 20:4n−6), directly from their diet and actually convert little of ingested linoleic acid or ALA to AA or EPA, respectively, because of limited Δ6 desaturase activity (the first biochemical step in the pathway). Mammals are unable to convert n−3 to n−6 fatty acids and rely on dietary sources of n−3 fatty acids, principally plants and fish. These facts are critically important when trying to use dietary fatty acids to block the production of inflammatory mediators.

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1 From the Center for Botanical Lipids (FHC, LLR, JSP, JPA, and MCS) and the Departments of Physiology and Pharmacology (FHC), Pathology/Section on Lipid Sciences (LLR and JSP) and Internal Medicine/Section on Molecular Medicine (MCS) at Wake Forest University, Winston Salem, NC, and the Division of Rheumatology, Immunology and Allergy and the Partners Asthma Center (JPA) at Brigham and Women’s Hospital, Harvard Medical School, Boston, MA.
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4 Reprints not available. Address correspondence to FH Chilton, Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157. E-mail: schilton@wfubmc.edu.
PROPOSED MECHANISMS BY WHICH DIETARY FATTY ACIDS AFFECT INFLAMMATORY DISEASES

The Center for Botanical Lipids at Wake Forest University School of Medicine and the Brigham and Women’s Hospital is testing 3 primary botanicals to determine whether they affect surrogate biomarkers of inflammatory diseases—flax, echium, and borage seed oils. These seed oils contain, among other fatty acids, ALA (18:3n-3 in flaxseed oil), and γ-linolenic acid (18:3n-6 from borage oil), PG, prostaglandin; LT, leukotriene; COX, cyclooxygenase; LOX, lipoxygenase.

MECHANISMS BY WHICH FLAXSEED OIL AFFECTS ATHEROSCLEROSIS

To study the molecular mechanism by which oils containing ALA, such as flaxseed oil, induce alterations of hepatic lipid metabolism, we use a model of atherosclerosis, the B100-only, LDLr−/− mouse, to profile plasma lipid and lipoprotein metabolism. Many aspects of the plasma lipoprotein profile in this model, including lipoprotein particle composition, are similar to those seen in humans (4–6).

Groups of female mice were fed a diet containing 10% of energy as fat enriched with flaxseed oil for 20 wk (enough so that ALA was equal to 2% of energy, an achievable dose for humans). A comparable diet with isocaloric substitutions of palm oil, as a negative control with a similar background fatty acid composition to fish oil, or fish oil matched to have EPA plus DHA as 2% of energy (positive control) and n−6 polyunsaturated fat mostly as linoleic acid (positive control) were also fed to separate groups of mice. All diets contained 0.017% cholesterol (7). A surprisingly high percentage of fatty acids were present as saturated and monounsaturated fatty acids (>75%) in both cholesterol esters and triacylglycerols (Figure 2). The percentage of n−6 polyunsaturated fatty acids in cholesteryl esters was ≈5% in all diet groups except the linoleic acid group, where the value was over 15%. The triacylglycerol patterns were similar except that linoleic acid was >20% in mice fed n−6 polyunsaturated fatty acids. The percentage of n−3 fatty acids was highest in triacylglycerols with values ≤15% in the fish oil group. The percentage of n−3 fatty acids in the flaxseed oil group was only ≈5%, with about one-half of this being ALA in both cholesteryl esters and triacylglycerols. The fatty acid pattern in phospholipids was quite different. In the mice fed n−6 polyunsaturated and saturated fatty acids, ≈30% of the fatty acids were n−6 polyunsaturated. Conversely, in the mice fed fish oil or flaxseed oil, ≈30% of the fatty acids were n−3 fatty acids and the distribution in the groups was similar. This suggests that in these animals ALA was converted into EPA and DHA, which was incorporated selectively into phospholipids by the liver.

These fatty acid compositional changes did not translate into effects on plasma lipoprotein cholesteryl ester concentrations (Table 1). Compared with dietary saturated fat, each lipoprotein class was significantly lower when n−3 was from fish oil but was not lowered by the flaxseed oil. Both VLDL and LDL are believed to be proatherogenic lipoproteins, and the concentrations of these fractions were as high in the flaxseed oil group as in the saturated and n−6 polyunsaturated groups. HDL-cholesterol concentrations, likewise, were lower in the fish oil group but not in the flaxseed oil group. These data suggest that the flaxseed oil diet offered little atheroprotection. By contrast, both the n−6 polyunsaturated fat diet and the fish oil diet groups had less atherosclerosis than did the saturated fat group, whereas aortic cholesteryl ester for the flaxseed oil group was intermediate between that for the polyunsaturated fat and saturated fat groups and not significantly lower than in the saturated fat group.

ECHIUM OIL AND ATHEROSCLEROSIS

The molecular mechanism by which SDA-containing oils, such as echium oil, affect plasma lipids, hepatic gene expression, and VLDL particle composition is of interest as a substitute for fish oil for atherosclerosis-protective effects. Echium oil supplementation reduces plasma triacylglycerol concentrations in mildly hypertriglyceridemic patients, but the mechanism for the reduction is unknown (8). Echium oil is enriched in 18:4n-3 (SDA), the immediate product of Δ6 desaturation of 18:3n-6. A key hypothesis is that SDA is efficiently elongated and desaturated to 20:5n-3 (EPA), which results in reduced plasma triacylglycerol concentrations similar to the effects reported for fish oil supplementation. Echium oil may reduce plasma triacylglycerol concentrations by several mechanisms, including decreased hepatic synthesis and secretion or increased lipolysis and catabolism of triacylglycerol-enriched lipoproteins in plasma. Male B100 only LDLr−/− mice are mildly hypertriglyceridemic and have elevated plasma cholesterol concentrations that result in the development of atherosclerosis. These mice were fed a basal diet consisting of 0.2% cholesterol and 10% palm oil for 4 wk and were then switched to diets containing 0.2% cholesterol and 20% of energy.
as palm oil, 10% as palm oil and 10% as echium oil, or 10% as palm oil and 10% as fish oil for 8 wk. Echium oil and fish oil resulted in a decrease in total plasma cholesterol compared with the basal diet (10% of energy as fat), whereas palm oil resulted in a slight increase (Figure 3). Total plasma cholesterol concentrations were similar for the echium oil and fish oil groups and were significantly lower than in the palm oil group. Triacylglycerol concentrations were similar and slightly reduced for the fish oil and echium oil groups but increased for the palm oil group compared with the basal diet, a significant difference between the palm oil and the other groups.

We next examined the effect of supplementation on genes involved in hepatic triacylglycerol synthesis. Results of quantitative real-time polymerase chain reaction showed significantly less mRNA for sterol regulatory element binding protein 1c, fatty acid synthase, and stearoyl coenzyme A desaturase 1 in the livers of mice supplemented with echium oil and fish oil compared with palm oil (Figure 4). This finding suggests that one mechanism for the reduction in plasma triacylglycerol concentrations was through decreased transcription of genes important in hepatic lipid synthesis.

Enrichment of hepatic phospholipid, triacylglycerol, and cholesteryl ester fractions with EPA in the echium oil group was significantly greater than in the palm oil group and significantly less than in the fish oil group. The decrease in expression of genes involved in triacylglycerol synthesis suggested that secreted VLDL particles may be smaller in the echium oil group. Dynamic laser light scatter as well as compositional analyses supported this hypothesis; VLDL particle size was in the order palm oil > echium oil > fish oil. Analysis of plasma apolipoprotein B concentration by Western blot revealed no consistent difference among groups, which suggests that the number of VLDL particles in plasma was similar. These data suggest that echium oil and fish oil reduce hepatic triacylglycerols but not apolipoprotein B synthesis.

**TABLE 1**

<table>
<thead>
<tr>
<th>Diet</th>
<th>VLDL</th>
<th>LDL</th>
<th>HDL</th>
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<tbody>
<tr>
<td>Fish oil</td>
<td>2 ± 11</td>
<td>170 ± 63</td>
<td>15 ± 11</td>
</tr>
<tr>
<td>PUFA (n-6, linoleic acid)</td>
<td>45 ± 18</td>
<td>415 ± 39</td>
<td>40 ± 1</td>
</tr>
<tr>
<td>Flaxseed oil</td>
<td>76 ± 5</td>
<td>545 ± 26</td>
<td>28 ± 8</td>
</tr>
<tr>
<td>Saturated (palm oil)</td>
<td>140 ± 70</td>
<td>494 ± 29</td>
<td>36 ± 8</td>
</tr>
</tbody>
</table>

1 All values are \( \bar{x} \pm \text{SEM} \); \( n = 3 \). PUFA, polyunsaturated fatty acid.
and secretion, resulting in a similar number of smaller VLDL particles in plasma relative to palm oil.

Increased lipoprotein lipase or hepatic lipase activity or increased efficiency of lipolysis of VLDL may be responsible for the decreased plasma triacylglycerol concentrations. Studies with plasma after heparin treatment did not show significant differences in the activity of these lipases among the 3 groups (data not shown). However, preliminary data suggested that release of free fatty acids is significantly greater after incubation of VLDL with purified lipoprotein lipase from mice supplemented with echium oil and fish oil compared with palm oil, suggesting more efficient hydrolysis.

Our data suggest that echium oil and fish oil supplementation result in decreased plasma triacylglycerol concentrations in these mice by decreased hepatic synthesis and increased efficiency of lipolysis in plasma. Future studies will focus on the molecular details of echium oil regulation of hepatic gene expression and will include attempts to correlate such changes with atherosclerosis. Botanical oils enriched in ALA versus SDA may differentially affect atherosclerosis outcome by the extent to which these fatty acids are metabolized to EPA and docosahexaenoic acid and incorporated into specific hepatic lipid pools.

MECHANISMS BY WHICH BOTANICAL OILS AFFECT INFLAMMATION IN HUMANS

Substantial evidence implicates leukotrienes in the pathogenesis of asthma, and leukotriene-modifying drugs are now an established treatment for the disease. Because leukotrienes are derived from the n-6 fatty acids, much research effort over the past 20 y has focused on how AA metabolism can be controlled by dietary manipulation. Humans have very little Δ⁶ desaturase activity. Researchers have attempted to inhibit leukotrienes by providing botanical oils, such as borage oil, containing the metabolic intermediate, GLA, which is not a usual constituent of human diets (9, 10). Because GLA is a product of the Δ⁶ desaturase, providing dietary GLA bypasses the Δ⁶ desaturase regulatory step. This GLA is elongated to dihomo-γ-linolenic acid (DGLA), which is then converted to AA by Δ⁵ desaturase. However, key inflammatory cells lack Δ⁵ desaturase activity, resulting in an accumulation of DGLA relative to AA (11). DGLA can then bind to 5-lipoxygenase and compete with AA, leading to a reduction in leukotrienes. DGLA released from stimulated polymorphonuclear granulocytes (PMNs) can be metabolized to the 15-lipoxygenase product 15-hydroxytrienoic acid; providing either this or DGLA to PMNs immediately before stimulation almost completely inhibited leukotriene B₄ biosynthesis (11). Thus, in addition to direct inhibition of critical enzymes regulating lipid mediator production, DGLA can be converted by lipoxygenases and cyclooxygenases to products that act as modulators of the conversion of AA to leukotrienes (11, 12). Therefore, supplementation of the diet with borage oil containing GLA leads to the accumulation of natural inhibitors of leukotrienes within inflammatory cells.

To test this in humans, we fed healthy subjects diets supplemented daily with concentrated borage oil (containing 1.5 g/d GLA) for 3 wk (13). We measured plasma fatty acids and ex vivo stimulated whole-blood leukotriene production in blood samples collected at baseline, weekly for 3 wk, and after a 2-wk washout period. Leukotriene synthesis significantly decreased within 2 wk compared with baseline levels (Figure 5). After a 2-wk

![FIGURE 3](https://academic.oup.com/ajcn/article-abstract/87/2/498S/4633415)

**FIGURE 3.** Total plasma cholesterol (TPC) and triacylglycerol (TG) concentrations in mice (mean ± SEM, n = 8–9 per diet) after a 4-wk basal diet (ie, 0 wk) consisting of 0.2% cholesterol and 10% of energy as palm oil (PO) and after 8 wk of supplementation with diets consisting of 0.2% cholesterol and 20% PO, 10% PO + 10% echium oil (EO), or 10% PO + 10% fish oil (FO).

![FIGURE 4](https://academic.oup.com/ajcn/article-abstract/87/2/498S/4633415)

**FIGURE 4.** Quantitative real-time polymerase chain reaction measure of hepatic mRNA for sterol regulatory element binding protein 1c (SREBP1c), fatty acid synthase (FAS), and stearoyl CoA desaturase 1 (SCD-1). Each data point is the mean from 1 mouse, measured in triplicate; the horizontal line denotes the mean mRNA expression for each group. mRNA data were normalized to glyceraldehyde-3-phosphate dehydrogenase expression and are expressed relative to 1 PO sample. PO, palm oil; EO, echium oil; FO, fish oil.
Calcium ionophore A23187. Leukotriene B4 production decreased significantly after 2–3 wk of GLA supplements but returned to baseline after the washout period (no GLA), leukotriene synthesis returned to baseline levels.

GLA supplementation also resulted in increased circulating AA concentrations because Δ⁵ desaturase activity in other tissues (such as liver) converts GLA to AA. Thus, with time, consumption of dietary GLA leads to an elevation of circulating AA (Table 2) that can potentially reverse the ability of DGLA to interfere with leukotriene synthesis (13). Hence, when the diet is supplemented with GLA from a botanical oil such as borage oil, the GLA is efficiently converted to DGLA and AA, leading to significant rises in serum concentrations of these fatty acids. Circulating DGLA is efficiently incorporated in the PMN lipids, but because PMNs lack Δ⁵ desaturase, the PMN content of AA does not increase. Leukotriene B₄ and platelet-activating factor synthesis are markedly reduced. These biochemical changes revert to presupplementation values 2 wk after supplementation stops.

Although the effectiveness of borage oil for reducing lipid mediators of inflammation from PMNs is exciting, elevated serum AA concentrations have proinflammatory potential for enhanced platelet aggregation through increased thromboxane formation. Consequently, it was important to normalize circulating AA concentrations. Because EPA is the n–3 fatty acid product of Δ⁵ desaturase, its ability to inhibit the conversion of GLA to AA was tested (14). EPA inhibited the formation of AA from DGLA in the HEP-G2 hepatocarcinoma cell line in vitro, establishing proof of principal. When healthy individuals supplemented their diet with 3.0 g/d of GLA, the bioconversion of GLA to AA was prevented by the addition of 3.0 g/d of EPA to their diet. Therefore, when consumed in the correct amounts with GLA (from borage oil), EPA (from fish oil) prevents the unwanted increase in circulating AA observed with intake of GLA alone (Table 2). Potential mechanisms for this synergy are shown in Figure 6.

**BOTANICAL OIL COMBINATIONS AND LEUKOTRIENE GENERATION**

Echium seed oil contains ≈12.5% of its fatty acids as SDA and 11% as GLA (Table 3). SDA is metabolized to EPA in humans, and EPA or some intermediate prevents the rise in serum AA that would otherwise occur with GLA supplementation (15, 16). We are testing the hypothesis that the combination of echium and borage oils as sources of SDA and GLA will optimally inhibit leukotriene generation without the side effect of increasing circulating AA. The first test of this hypothesis is a dose-range study to determine the optimal dose of SDA required to prevent the bioconversion of GLA to AA without affecting the inhibitory action of GLA on leukotriene biosynthesis. Preliminary data show that a 3-wk dietary supplementation of GLA with SDA increases circulating and PMN fatty acid content of DGLA without increasing circulating AA. It is too early to determine the extent of inhibition of leukotriene generation or differences between the different study groups. Nevertheless, the preliminary data indicate that this combination of fatty acids significantly inhibits the generation of leukotriene B₄ and its precursors from PMNs and of cysteinyl leukotriene generation from basophils, thus confirming the potential utility of this approach for inhibiting leukotriene generation in asthma patients.

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**TABLE 2**

Fatty acid concentrations measured in plasma isolated from healthy subjects at baseline and 3 wk after daily consumption of α-linolenic acid (GLA, 1.5 g/d, n = 5) or GLA (1.5 g/d) + eicosapentaenoic acid (EPA, 1 g/d, n = 10).¹

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>GLA</th>
<th>GLA + EPA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Week 3</td>
</tr>
<tr>
<td>AA</td>
<td>378 ± 48</td>
<td>572 ± 66²</td>
</tr>
<tr>
<td>GLA</td>
<td>35 ± 5</td>
<td>47 ± 4</td>
</tr>
<tr>
<td>DGLA</td>
<td>115 ± 12</td>
<td>227 ± 14²</td>
</tr>
<tr>
<td>EPA</td>
<td>22 ± 11</td>
<td>15 ± 3</td>
</tr>
</tbody>
</table>

¹ All values are x ± SEM. AA, arachidonic acid; DGLA, dihomo-γ-linolenic acid. Baseline values were not significantly different between groups. Data adapted with permission from Excerpta Medica (13).

² Significantly different from baseline, P < 0.05 (one-way ANOVA).

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**FIGURE 5.** Biosynthesis of leukotriene B₄ in stimulated whole blood from healthy human subjects consuming γ-linolenic acid (GLA); n = 5. Whole blood was obtained at baseline, after 3 wk of dietary supplementation with 1.5 g GLA/d, and after an additional 2-wk washout without GLA. Whole-blood leukotrienes were measured after ex vivo stimulation with the calcium ionophore A23187. Leukotriene B₄ production decreased significantly after 2–3 wk of GLA supplements but returned to baseline after the washout period. Values are mean ± SEM. ²Significantly different from baseline, P < 0.05 (one-way ANOVA). Data were adapted with permission from Excerpta Medica (13).

**FIGURE 6.** Potential mechanisms by which eicosapentaenoic acid (EPA) and γ-linolenic acid (GLA) inhibit lipid mediator production. PGE₁, prostaglandin E₁; 15-HETre, 15-hydroxyeicosatrienoic acid; DGLA, dihomo-γ-linolenic acid; LT, leukotriene; FLAP, 5-lipoxygenase activating protein.
CONCLUSIONS

There is tremendous interest in botanical oils and supplements—as an alternative to fish oil—as a source of ω-3 fatty acids and combinations of ω-3 and ω-6 fatty acids for their health protection against chronic diseases in which elevated triacylglycerols and inflammation are major issues: cardiovascular diseases, asthma, diabetes, obesity, and arthritis. The Center for Botanical Lipids has shown that echium oil and other SDA-containing oils as well as botanical oil combinations (such as echium and borage oils) hold particular promise for modulating inflammatory responses.

FHC serves on the Board of Directors and is a shareholder of Pilot Therapeutics Inc. JPA has received honoraria from Critical Therapeutics, GlaxoSmithKline, and Merck for lectures. LLR, JSP, and MCS have no affiliations related to this work. FHC is Director of the Center for Botanical Lipids and PI of the study of mechanisms by which botanical oils affect inflammation in humans, LLR is PI of the study of the mechanisms by which flaxseed oil affects atherosclerosis, JSP is PI of the study of the mechanisms by which echium oil affects atherosclerosis, JPA is PI of the study of the mechanisms by which botanical oil combinations affect leukotriene generation in asthma, and MCS is the compiling author of this work.

REFERENCES


TABLE 3

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Percent</th>
</tr>
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<tbody>
<tr>
<td>Palmitic acid (16:0)</td>
<td>7.1</td>
</tr>
<tr>
<td>18:0</td>
<td>3.7</td>
</tr>
<tr>
<td>Oleic acid (18:1n−9)</td>
<td>15.4</td>
</tr>
<tr>
<td>Linoleic acid (18:2n−6)</td>
<td>18.8</td>
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<tr>
<td>γ-Linolenic acid (GLA; 18:3n−6)</td>
<td>11.0</td>
</tr>
<tr>
<td>α-Linolenic acid (ALA; 18:3n−3)</td>
<td>28.4</td>
</tr>
<tr>
<td>Stearidonic acid (SDA; 18:3n−3)</td>
<td>12.5</td>
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<td>Other</td>
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