The oxygenated metabolites synthesized from arachidonic acid are important mediators in a variety of biological processes (1). The suggestion has been made that excessive biosynthesis of these compounds is associated with physiological dysfunctions, such as thrombosis (2), cancer metastasis (3), inflammatory disease, asthma (4), psoriasis (5), and autoimmune diseases such as rheumatoid arthritis (6). Lipoxigenases are enzymes that convert arachidonic acid into biologically-active compounds, such as leukotrienes, lipoxins, and an array of hydroxyl derivatives (1). In mammalian lungs, 12- and 15-lipoxygenases catalyze reactions that produce 12-hydroxyeicosatetraenoic (12-HETE) and 15-hydroxyeicosatetraenoic acid (15-HETE) from arachidonic acid (7,8). While the functions of some 5-lipoxygenase products have been well established, the physiological roles of the 12- and 15-lipoxygenases are not clearly understood. Although the association of several biological functions with 12- and 15-lipoxygenase pathways has been suggested (9), the involvement of the 12-lipoxygenase product 12-HETE in tumor cell metastasis is most striking (10). Studies have shown that 12-HETE promotes tumor-cell adhesion by increasing surface expression of alpha-v-beta-3 integrins on endothelial cells (11). Recently, a specific receptor for 12-HETE in Lewis-lung carcinoma cells was reported (12). Also, dietary maneuvers that enhanced arachidonic acid levels were associated with increased metastasis to the lung (13,14). These findings underline the potential importance of 12-lipoxygenase products in cellular activities, particularly in promoting the adhesion of tumor cells (10). Thus, it would be important to understand how the production of 12-lipoxygenase metabolites is regulated in vivo.

Considerable research has focused on understanding how dietary fatty acids are incorporated into cell membrane lipids and how this affects cell function. Changes in the fatty acid composition of tissue membrane lipids are routinely seen in response to dietary changes. Furthermore, biochemical studies show that specific changes in the fatty acyl composition of membrane phospholipids alter cellular and physiological processes, such as platelet aggregation, leukocyte chemotaxis, macrophage cytolysis, and tumor metastasis (15-17). Since these processes are frequently unbalanced in disease states, manipulation of membrane composition could result in important clinical benefits. Manipulation of fatty acids in membranes also alters synthesis of their oxygenated derivatives. Since different fatty acid oxygenation pathways show varying sensitivity to nutritional modulation, it is of interest to investigate how 12-lipoxygenase activity is regulated by dietary polyunsaturated fatty acids (PUFA).

Of particular interest are the n-3 family of PUFA and how they modulate tissue lipid composition, arachidonic acid metabolism, and eicosanoid production. Dietary supplementation with fish oil has been suggested to be beneficial in some

**ABSTRACT:** This study investigated the potential of dietary fats to modulate the arachidonic acid content of mouse lung phospholipids and the formation of lipoxygenase products from arachidonic and eicosapentaenoic acids. Prior to breeding, female mice were fed for five months diets with 10 wt% of either olive oil, safflower oil, fish oil, or linseed oil. The same diets were fed to the females during gestation and to the pups from day 18 to day 42 postpartum. On day 42, the phospholipids were extracted from fresh lung tissue and separated into classes [phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylcholine (PC), and phosphatidylinositol (PI)] by thin-layer chromatography. Methyl esters of phospholipid fatty acids and unesterified fatty acids were analyzed by gas chromatography. At comparable dietary n-3/n-6 ratios, arachidonic acid was reduced 85 and 75% in lungs from mice fed linseed oil and fish oil, respectively, compared to lungs of safflower oil-fed mice. Dietary fats affected the proportion of arachidonic acid in phospholipids in the order: PE > PC > PS > PI. Following incubation of homogenized lung tissue, the total amount of 12-lipoxygenase products was lowest in lungs from mice fed olive oil, and 12-hydroxyeicosatetraenoic acid was lowest in incubated lungs from mice fed linseed oil. Comparison of the amounts of lipoxygenase substrate fatty acids in the individual phospholipids with the lipoxygenase products suggested that the major substrate pool for the 12-lipoxygenase pathway in mouse lung homogenates was PC.

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Abbreviations: 12-HETE, 12-hydroxyeicosatetraenoic acid; 15-HETE, 15-hydroxyeicosatetraenoic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PUFA, polyunsaturated fatty acids.
disease states that involve arachidonic acid metabolism (18). Although the mechanism by which n-3 PUFA in fish oil exert their effects is not completely understood, the putative beneficial effects may be derived from either modification of the arachidonic acid pool through n-3 PUFA replacement or the competition of n-3 PUFA-derived oxygenated metabolites (19,20). However, data are lacking to evaluate the ability of shorter-chain n-3 PUFA to modify arachidonic acid content in lung membrane phospholipids and to alter eicosanoid production.

The present study investigated differences in metabolism and incorporation of n-3 PUFA from two sources, linseed oil, which is rich in 18:3n-3, and menhaden fish oil, which is rich in 20:5n-3 and 22:6n-3, in comparison with oils rich in n-6 PUFA (safflower oil) and n-9 monounsaturates (olive oil). Previous studies with diets containing low n-3/n-6 ratios found that 18:3n-3 is a relatively inferior source in respect to the net accumulation of 20:5n-3 in tissues and the modulation of eicosanoid synthesis (21). In the present study, unsaturated fats were fed as both 18:3n-3, and 20:5n-3 plus 22:6n-3, at high n-3/n-6 ratio during the developmental period of mice. The mouse lung was chosen because: (i) previous work documents the differential incorporation of 18:3n-3 and fish oil n-3 PUFA (22), (ii) large quantities of this tissue are available for lipid analysis, and (iii) the organ releases a variety of lipid-derived bioactive compounds. The lung may be a good model for the study of lipoxygenase activities and functions (7,9,23).

MATERIALS AND METHODS

Animal and dietary protocols. Experimental animals were 42-day-old, pathogen-free Swiss Webster mice (Bantin & Kingman, Fremont, CA). The initial treatment, grouping, diet protocol, and breeding schemes of dams were previously described in detail (22). In brief, dams were divided into four groups and fed a nonpurified diet (Rodent Laboratory Chow no. 5001; Purina Mills, St. Louis, MO) for two weeks. The groups of mice were then fed one of four synthetic diets for a five-month period. Litters from each group were culled to ten pups/litter on day 3 postpartum. On day 18, the dams were removed from the cages. The pups were fed the synthetic diet continuously until day 42, when they were killed by CO2 intoxication. Lipid analysis and eicosanoid production assays were conducted on lungs of the 42-day-old pups.

Synthetic diets consisted of fat-free AIN-76A powdered diets (Dyets, Bethlehem, PA) modified by addition of the following dietary fats at 10 g/100 g oil: safflower oil (Dyets), linseed oil (Spectrum Naturals, Petaluma, CA), olive oil (G. Sensat, Extra Virgin no. 5; Specialty Food and Beverage Sales, West Milford, NJ), or a 9:1 (w/w) mixture of menhaden oil (NIH Biomedical Test Materials Program, Bethesda, MD) and safflower oil. Safflower oil was added to the fish oil diet to ensure that the linoleic acid requirement was met and to achieve a 3:1 ratio of n-3/n-6. The fatty acid composition of each oil and ratios of n-3/n-6 are summarized in Table 1. To achieve a 3:1 ratio of n-3/n-6, the fatty acid composition of each oil and ratios of n-3/n-6 are summarized in Table 1. To

### Table 1

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Safflower Oil</th>
<th>Olive Oil</th>
<th>Linseed Oil</th>
<th>Fish Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>61</td>
<td>13</td>
<td>32</td>
<td>23</td>
</tr>
<tr>
<td>18:0</td>
<td>6</td>
<td>17</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td>18:1n-9</td>
<td>17</td>
<td>6</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>13</td>
<td>3</td>
<td>15</td>
<td>24</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

aDiets contained 10 wt% of either safflower oil, olive oil, linseed oil, or fish/safflower at 9:1 (w/w) ratio.
bOnly key fatty acids are shown. Values are means of two determinations per oil and are expressed as mol%.
cFatty acid concentrations were below 0.01 mol% of total fat.

Tissue lipid analysis. Tissue lipids were extracted in the presence of sodium dodecyl sulfate (24) with >90% recovery of total phospholipids. They were separated into classes by thin-layer chromatography on 10 x 10 cm pre-coated silica gel 60 plates (E. Merck, Darmstadt, Germany) developed in a solvent system consisting of chloroform/methanol/acetic acid/water (50:0:37.5:3.5:2.0, by vol) (25). Individual lipid classes were visualized with 8-hydroxy-1,3,6-pyrenesulfonic acid trisodium salt (Eastman Kodak Co., Rochester, NY) (26). The bands containing phosphatidyldicholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), and phosphatidylethanolamine (PE) were scraped, and the fatty acids were directly transesterified to methyl esters with 3 N methanolic HCl (Supelco, Bellefonte, PA) at 60°C overnight. Dipentadecanoyl PC was used as an internal standard to quantify recovery and yields and was added into each sample vial prior to methylation. Fatty acid methyl esters were extracted, and fatty acid composition of each phospholipid class was determined by gas chromatography on a Hewlett-Packard model 5890 gas chromatograph (Hewlett-Packard Co., Palo Alto, CA) equipped with a flame-ionization detector and a DB-23 25 m x 0.25 mm capillary column (J&W Scientific, Folsom, CA). Fatty acid methyl esters were identified by their retention time relative to known standards (Nu-Chek-Prep, Elysian, MN).

Unesterified fatty acids in fresh lung homogenates were extracted after 10 min incubation at 37°C in the same buffer as described above, in the presence of the 12-lipoxygenase specific inhibitor esculetin (10 µM) (27), methylated with ethereal diazomethane (27), separated from other lipid classes using solid-phase extraction columns (28) (Analytichem International, Harbor City, CA), and analyzed by gas chromatography as described (21,29).

Eicosanoid synthesis by lung tissue. To determine the effect of dietary modifications on the biosynthesis of eicosanoids, the lipoxygenase products generated by homogenized lung tissue were characterized. Mice were anesthetized and