

# Dietary Supplementation With Oils Rich in (n-3) and (n-6) Fatty Acids Influences In Vivo Levels of Epidermal Lipoxygenase Products in Guinea Pigs<sup>1</sup>

CRAIG C. MILLER, VINCENT A. ZIBOH,<sup>2</sup> TERESA WONG AND MARK P. FLETCHER\*

Departments of Dermatology and \*Internal Medicine, University of California, Davis 95616

**ABSTRACT** Certain dietary oils may have therapeutic potential in the treatment of inflammatory skin disorders. Presumably, the fatty acid constituents of these dietary oils exert their effects by altering the levels of cutaneous eicosanoids. Prompted by this possibility, we investigated whether supplementation of guinea pig diets with fish oil [rich in 20:5(n-3)] or borage oil [rich in 18:3(n-6)] could significantly alter epidermal levels of eicosanoids compared with control animals supplemented with olive oil. After feeding periods of 4, 8 or 12 wk, the epidermis from the animals was analyzed for: 1) fatty acid composition of individual epidermal phospholipids, 2) levels of lipoxygenase products, and 3) levels of cyclooxygenase products (prostaglandins). Our results demonstrated that the animals supplemented with dietary fish oil had elevated levels of 20:5(n-3) in epidermal phospholipids and elevated epidermal levels of 15-hydroxyeicosapentaenoic acid (15-HEPE) [the 15-lipoxygenase product of 20:5(n-3)] compared with guinea pigs fed olive oil or borage oil. Similarly, the animals supplemented with dietary borage oil had elevated levels of 20:3(n-6) [the epidermal elongase product of 18:3(n-6)] in epidermal phospholipids and elevated epidermal levels of 15-hydroxyeicosatrienoic acid [15-HETrE, the epidermal 15-lipoxygenase product of 20:3(n-6)] compared with guinea pigs fed olive oil or fish body oil. There were no significant changes in epidermal levels of prostaglandins. Both 15-HEPE and 15-HETrE have been identified as possible anti-inflammatory metabolites, and their elevated presence in the epidermis of animals fed oils rich in 20:5(n-3) or 18:3(n-6) may provide a mechanism for the beneficial effects of these oils on inflammatory conditions. *J. Nutr.* 120:36-44, 1990.

#### INDEXING KEY WORDS:

- cutaneous eicosanoids
- epidermal prostaglandins • olive oil
- borage oil • fish oil
- epidermal lipoxygenase products • guinea pigs

Arachidonic acid [20:4(n-6)] and other 20-carbon polyunsaturated fatty acids (PUFA) are metabolized by mammalian oxygenases to a group of biologically active

products collectively referred to as the eicosanoids. These autacoids play a major role in the regulation of several pathophysiological processes, notably inflammation and thrombosis. The involvement of eicosanoids in the pathogenesis of diseases had led to an increasing desire to modulate eicosanoid synthesis. The principal means of altering eicosanoid levels had traditionally involved pharmacological intervention, which has often been accompanied by deleterious side effects. To minimize these effects, recent efforts have focused on the ability of dietary manipulation to alter tissue levels of eicosanoid precursor fatty acids and thereby directly to regulate the source of tissue eicosanoids.

An example of this manipulation is the use of dietary fish oil supplements in the management of psoriasis (1-3), an inflammatory skin disorder characterized by an infiltration of neutrophils into the epidermis (4). Extracts from lesional areas of psoriasis are known to contain elevated levels of the neutrophil arachidonic acid product leukotriene B<sub>4</sub> (LTB<sub>4</sub>) (5, 6). This eicosanoid, a product of neutrophil 5-lipoxygenase, is a potent inflammatory mediator that can elicit psoriasis-like symptoms when applied to normal skin (7-9). Although fish oil has low levels of the (n-6) fatty acid arachidonic acid, it is rich in the (n-3) fatty acids eicosapentaenoic acid [20:5(n-3)] and docosahexaenoic acid [22:6(n-3)] (10). A possible mechanism for the beneficial effects of fish oil on psoriasis was presumed to be related to findings that the incorporation of 20:5(n-3) into neutrophils results in a competitive inhibition of neutrophil conversion of 20:4(n-6) into LTB<sub>4</sub> (11, 12). However, the clinical improvement in psoriatic patients fed fish oil reportedly does not correlate with the ability of peripheral neutrophils to synthesize LTB<sub>4</sub> (2), but it does correlate with elevated levels of 20:5(n-3) incorporated into the epider-

<sup>1</sup>Supported in part by National Institutes of Health Research Grants AM-30679 and AR 39040 of the U.S. Public Health Service.

<sup>2</sup>Author to whom requests for reprints should be addressed.

mis (1). This latter observation suggests that an additional effect of 20:5(n-3) or eicosanoids derived from this fish oil constituent may exert effects at the epidermal lesion.

Another example of the use of dietary oil is dietary supplementation with evening primrose oil, which is rich in  $\gamma$ -linolenic acid [18:3(n-6)] and has been reported to alleviate the clinical signs of atopic eczema (13, 14). Like psoriasis, this inflammatory skin disorder is associated with elevated lesional levels of LTB<sub>4</sub> (15). The supplementation of guinea pig diets with vegetable oils rich in  $\gamma$ -linolenic acid [18:3(n-6)] resulted in increased epidermal levels of dihomo- $\gamma$ -linolenic acid [20:3(n-6)] (16, 17), the epidermal elongase product of 18:3(n-6) (18). Epidermal extracts from these animals also showed increased levels of 15-lipoxygenase product derived from 20:3(n-6), 15-hydroxyeicosatrienoic acid (15-HETrE) (17). In *in vitro* experiments, 15-HETrE was shown to be a potent inhibitor of LTB<sub>4</sub> synthesis much like 15-hydroxyeicosapentaenoic acid (15-HEPE) (19, 20).

The successful use of dietary oils rich in 20:5(n-3) or 18:3(n-6) in treating inflammatory skin disorders suggests that the incorporation of specific PUFA into epidermal lipids followed by the conversion of these fatty acids into 15-lipoxygenase products may provide for the *in vivo* generation of anti-inflammatory mediators. Furthermore, it has been proposed that different classes of phospholipids may act as donors of precursor fatty acids for eicosanoid synthesis (21–23); however, no such efforts have been made to evaluate how dietary oils may influence the distribution of fatty acids in the individual epidermal phospholipids *in vivo* and how this would influence the levels of epidermal 15-lipoxygenase products that are generated. To answer these questions, guinea pigs were fed a basal diet supplemented with either 6% olive oil (control), 3% olive oil + 3% borage oil [containing approximately 25% 18:3(n-6)], or 3% olive oil + 3% fish oil [containing approximately 20% 20:5(n-3) and 17% 22:6(n-3)]. The epidermis from these animals was analyzed for the distribution of incorporated (n-6) and (n-3) fatty acids in individual phospholipids to determine whether the incorporation of specific PUFA into the epidermis resulted in the *in vivo* generation of significant levels of the corresponding 15-lipoxygenase products.

## MATERIALS AND METHODS

**Dietary treatment.** Male weanling Hartley guinea pigs ( $n = 27$ ) (Simonsen, Gilroy, CA) were fed a basal purified control diet that contained 6% olive oil by weight. The animals were housed individually in a cage at 22–24°C with a 12-h diurnal light cycle; deionized water (containing 0.05% ascorbic acid) and diet were provided *ad libitum*. After the animals had acclimated

TABLE 1  
Composition of the basal diet

Constituents	Amount
	g/100 g dry wt
Oil <sup>1</sup>	6
Casein (vitamin-free) <sup>2</sup>	30
Corn starch <sup>2</sup>	25.798
Sucrose <sup>2</sup>	10
Cellulose <sup>3</sup>	13
Mineral mix <sup>4</sup>	6
Vitamin mix <sup>5</sup>	4
Agar <sup>6</sup>	2
DL-methionine	0.2
Potassium acetate	2.5
Magnesium oxide	0.5
Zinc carbonate	0.002

<sup>1</sup>The oils added were: OLI (100% olive oil), BOR (50% olive oil, 50% borage oil) and FBO (50% olive oil, 50% fish body oils). Olive oil, borage oil and fish body oil were generous gifts from Sid Tracy (Traco Labs, Champaign, IL).

<sup>2</sup>ICN Nutritional Biochemicals, Cleveland, OH.

<sup>3</sup>Alphacel non-nutritive bulk, ICN.

<sup>4</sup>Briggs chick salt mixture A, ICN (g/kg diet mix): CaCO<sub>3</sub>, 250; Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>·5H<sub>2</sub>O, 233; CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.3; Ferric citrate·5H<sub>2</sub>O, 6.7; MgSO<sub>4</sub>·7H<sub>2</sub>O, 83.3; MnSO<sub>4</sub>·4H<sub>2</sub>O, 7.0; KI, 0.7; K<sub>2</sub>HPO<sub>4</sub>, 150; NaCl, 146.7; Na<sub>2</sub>HPO<sub>4</sub>, 121.7; and ZnCO<sub>3</sub>, 0.3; U.S. Biochemicals, Cleveland, OH.

<sup>5</sup>Vitamin mix provided the following (mg/kg) complete diet except as noted:  $\alpha$ -tocopherol, 200 IU/kg; L-ascorbic acid, 1800; choline chloride, 3000; D-calcium pantothenate, 120; inositol, 200; menadione, 90; niacin, 180; *p*-aminobenzoic acid, 200; pyridoxine-HCl, 40; riboflavin, 40; thiamin-HCl, 40; retinyl acetate, 36,000 IU/kg; ergocalciferol, 4000 IU/kg; biotin, 0.8; folic acid, 3.6, and cyanocobalamin, 0.054.

<sup>6</sup>Difco Labs, Detroit, MI.

for 2 wk to the diet based on olive oil, the animals were randomly assigned to three groups, varying only in the type of oil used in the diet: either 6% olive oil (OLI) control, 3% olive oil + 3% borage oil (BOR) containing  $\gamma$ -linolenic acid [18:3(n-6)] or 3% olive oil + 3% fish body oil (FBO) containing [20:5(n-3)] and [22:6(n-3)]. The oils were generous gifts from Sid Tracy, Traco Labs, Champaign, IL. The composition of the basal diet is shown in Table 1 and the fatty acid composition of the dietary oils is shown in Table 2. The diets were initially mixed in a powder form and stored under N<sub>2</sub> at -20°C. A purified diet in gel form was prepared with agar as described previously (22). The gel-type diets were flushed with N<sub>2</sub> and stored at 4°C. Diets were periodically analyzed for autoxidation by checking fatty acid profiles. Fresh diet was provided daily to each group to minimize autoxidation. Body weights were recorded three times per week throughout the study. There were no significant differences in the growth curves of the three groups (Fig. 1) and no macroscopic differences in the appearance of the animals were discernible. Because there were no symp-

TABLE 2  
Fatty acid composition of dietary oils<sup>1</sup>

Fatty acid	OLI	BOR	FBO
	wt %		
16:0 <sup>2</sup>	12.55	11.00	13.45
16:1(n-7)	0.03	0.07	4.01
18:0	0.05	1.58	1.25
18:1(n-9)	69.89	41.89	40.98
18:2(n-6)	[11.78] <sup>3</sup>	[25.98]	[6.58]
18:3(n-6)	—	[12.15]	—
20:4(n-6)	—	—	[0.52]
20:5(n-3)	—	—	[9.82]
22:6(n-3)	—	—	[7.20]

<sup>1</sup>Values are expressed as mg/100 mg total fatty acids. OLI, 100% olive oil; BOR, 50% olive oil + 50% borage oil; FBO, 50% olive oil + 50% fish body oil.

<sup>2</sup>Only the major fatty acids are listed.

<sup>3</sup>Brackets represent polyunsaturated fatty acids.

toms of essential fatty acid deficiency, the energy percentage of 18:2(n-6) in the three diets (OLI, 1.7%; BOR, 3.8%; and FBO, 1.0%) appeared to be adequate. At 4-wk intervals starting from the initiation of feeding the experimental diets (wk 4, 8 and 12), three animals from each group were killed for analyses of epidermal lipids and eicosanoids.

**Lipid analysis.** To isolate the epidermis from the guinea pigs, the animals were shaved, their hair was removed by depilatory and they were killed. Epidermal slices from areas of the dorsum (0.2 mm thick) were quickly removed by a motor-driven keratome (Storz, St. Louis, MO) over ice as previously described (16). The epidermal strips were rapidly placed in ice-cold 0.1 M KCl and homogenized over ice using a Polytron (Brinkmann Instruments, Westbury, NY). A portion of the crude epidermal homogenate was used to quantitate protein concentration by a modified Lowry method (24).

**Isolation and separation of individual phospholipids.** Lipids from a portion of the crude epidermal homogenate were extracted with CHCl<sub>3</sub>:MeOH (2:1, v/v) as described previously (25). To isolate individual phospholipids, the extracted total lipids were fractionated by thin layer chromatography on 0.25-mm silica gel G plates (Merck, Darmstadt, West Germany) using CHCl<sub>3</sub>:methanol acetic acid:H<sub>2</sub>O (50:37.5:3.5:2, v/v/v/v) (26). The individual phospholipids were visualized under ultraviolet light after spraying with 0.2% 2',7'-dichlorofluorescein in ethanol. The phospholipid fractions were scraped off the plates, eluted from the silica gel and then dried under N<sub>2</sub> gas; diheptadecanoyl-L- $\alpha$ -phosphatidylcholine was added as an internal standard to determine the relative contribution of fatty acids from each phospholipid class. After transmethylation of the individual phospholipids in 6% methanolic-HCl at

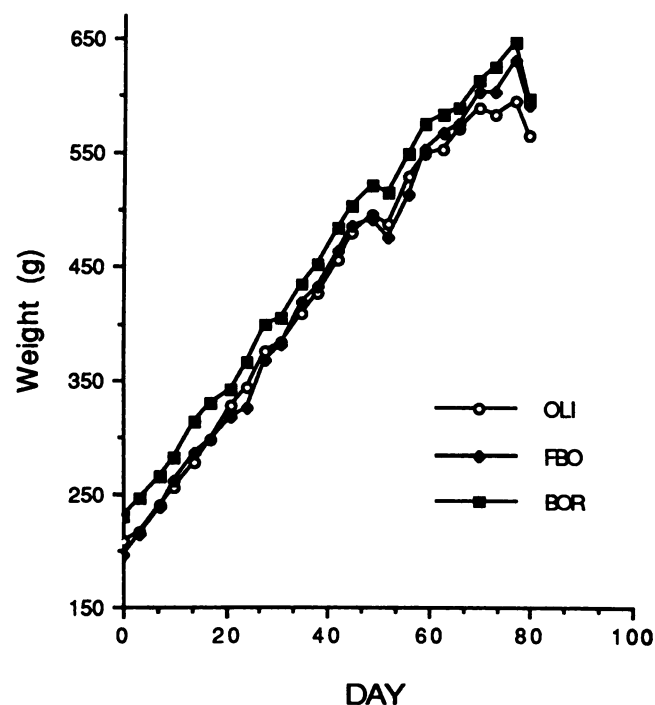
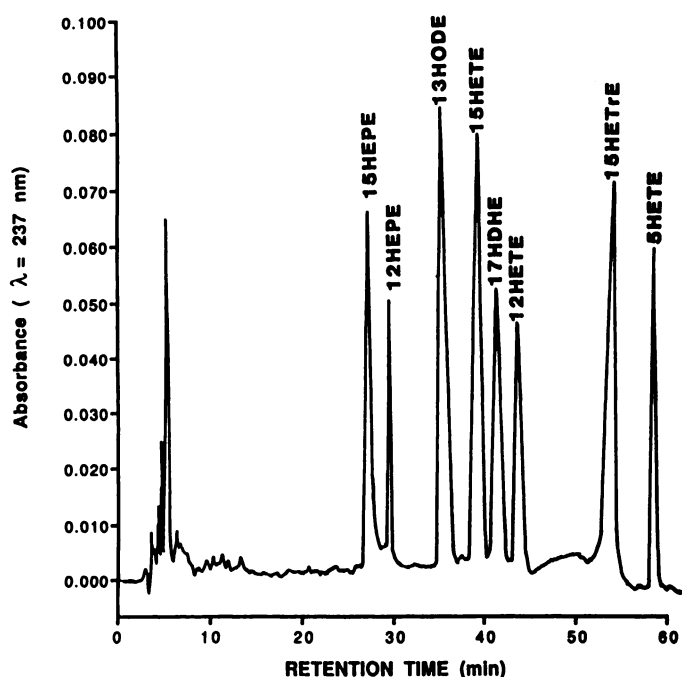


FIGURE 1 Average weight of guinea pigs fed diets supplemented with 6% olive oil (OLI) ( $n = 9$ ), 3% olive oil + 3% fish body oil (FBO) ( $n = 9$ ) or 3% olive oil + 3% borage oil (BOR). Each group included nine animals. The three growth curves did not significantly differ ( $P > 0.05$ ).

70°C for 14 h as described previously (16), the fatty acid profiles of these fractions were determined by gas chromatography. The gas chromatograph (Hewlett-Packard model 5730A, Avondale, PA) was equipped with a DB-225 fused silica capillary column (50% cyanopropylphenyl, 0.15- $\mu$ m film thickness, 30 m  $\times$  0.25 mm i.d.; J&W Scientific, Rancho Cordova, CA). Hydrogen (36 cm/s) was used as the carrier gas, the oven was run isothermally at 200°C, and detection was performed by flame ionization.

**Isolation and identification of lipoxygenase products.** The eicosanoids from the epidermal homogenates were extracted with ice-cold CHCl<sub>3</sub>:MeOH (2:1, v/v) after acidification to pH 3.0. The profiles of the hydroxy fatty acids of the epidermal extracts were determined by reverse phase high performance liquid chromatography (RP-HPLC) using a Beckman 5- $\mu$ m octadecylsilica (ODS) column (25 cm  $\times$  4.6 mm i.d., Beckman, Palo Alto, CA). The chromatographic system was run isocratically at a flow rate of 1.0 mL/min on a Beckman system equipped with Model 100A/110A pumps and a 421 Controller. The mobile phase solvents were methanol (74%) and water acidified to pH 3.0 with acetic acid (27). The hydroxy fatty acids were monitored at 237 nm with a Beckman Model 165 Variable Wavelength Detector. Quantitation was performed with external standards of 13-hydroxyoctadecadienoic acid (13-HODE), 15-HETrE,



**FIGURE 2** A typical chromatogram of hydroxy fatty acid standards separated using a system of MeOH (74%) and H<sub>2</sub>O (acidified to pH 3.0 with acetic acid) run isocratically from 0–60 min at a flow rate of 1.0 mL/min.

15- or 12-hydroxyeicosatetraenoic acid (15-HETE or 12-HETE) and 15-HETE (Cayman Chemicals, Philadelphia, PA). A representative chromatogram of the hydroxy fatty acid standards as separated by this system is depicted in Figure 2.

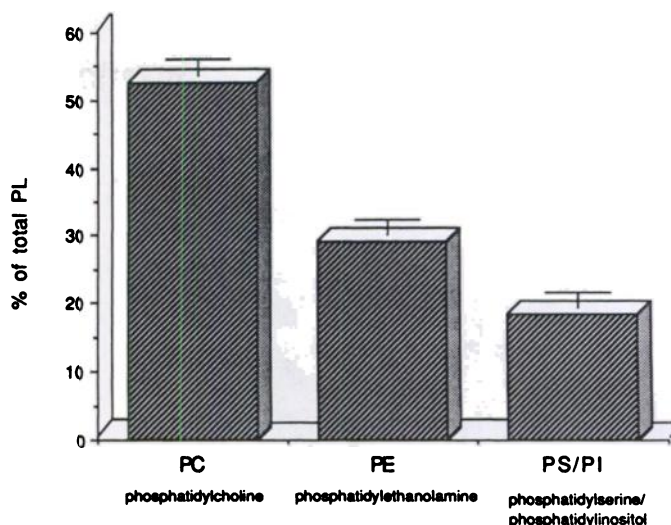
**Isolation and identification of the cyclooxygenase products.** Prostaglandins were analyzed using RP-HPLC and a mobile phase of acetonitrile (ACN) and water containing 0.02% H<sub>3</sub>PO<sub>3</sub> (17). The system was run at a constant flow rate of 1.0 mL/min with a successive gradient of 34% ACN (0–15 min) and 65% ACN (15–30 min). The prostaglandins were monitored at 205 nm and were quantitated against external standards for prostaglandins PGF<sub>2α</sub>, PGE<sub>2</sub>, PGD<sub>2</sub> and PGE<sub>1</sub> (Cayman Chemicals).

**Statistical analysis.** Data were assessed by one-way analysis of variance (ANOVA) comparing the means of the dietary groups within 4-wk periods. Significant differences were determined by the Student-Newman-Keul's (SNK) range test (28). The upper level of significance was chosen as  $P < 0.05$ .

## RESULTS

### Fatty acids in individual epidermal phospholipids.

Analysis of the individual epidermal glycerophospholipids (PL) revealed that phosphatidylcholine (PC), phosphatidylethanolamine (PE) and combined



**FIGURE 3** Data representing relative contributions of phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylserine/phosphatidylinositol (PS/PI) fractions of total glycerophospholipids (PL) isolated from guinea pig epidermal extracts. Values are means  $\pm$  SEM from guinea pigs ( $n = 27$ ) of all three dietary groups combined (OLI, FBO and BOR).

phosphatidylserine/phosphatidylinositol (PS/PI) represented 52.6%, 29.1% and 18.2% of total glycerophospholipids, respectively (Fig. 3). There were no significant differences in the epidermal phospholipid compositions of the three dietary groups at any time point.

The effect of diet on individual phospholipid fatty acids is presented in Table 3. Only data for the PUFA, the fatty acids that are probable substrates for enzymatic oxygenation, are listed. Statistical analysis was performed for values within a PL class for each time point (wk 4, 8 or 12). **These results clearly indicate the significant effects of the dietary oils on the fatty acid composition of the epidermal phospholipids. The observed differences generally reflected the differences in the fatty acid compositions of the diets (Table 2).** The epidermis of the animals fed the FBO diet [rich in 20:5(n-3) and 22:6(n-3)] had elevated levels of 20:5(n-3) (wk 4, 8 and 12) in PC and PE and elevated levels of 22:6(n-3) (wk 4, 8 and 1) in PE but interestingly, not in PC. The highest absolute gains for both 20:5(n-3) and 22:6(n-3) were in PE. Incorporation of the (n-3) fatty acids into the PS/PI fraction was less extensive. The epidermis of the animals fed the BOR diet [rich in 18:3(n-6)] had significantly elevated levels of 20:3(n-6) (wk 4, 8 and 12). This epidermal elongase product of 18:3(n-6) incorporated into PC, PE and PS/PI, with highest absolute gains of 20:3(n-6) occurring in PC. Notably, 20:3(n-6) was sequestered only in the PS/PI fraction of animals fed the OLI and FBO diets.

**Epidermal lipoxygenase products.** The analysis of epidermal lipoxygenase products (hydroxy fatty acids) revealed that the hydroxy fatty acid content of the

TABLE 3  
Effects of diets enriched in (n-3) and (n-6) fatty acids on distribution of polyunsaturated fatty acids (PUFA) in epidermal phospholipids in guinea pigs<sup>1</sup>

PL	PUFA	Week 4			Week 8			Week 12		
		OLI <sup>2</sup>	BOR <sup>2</sup>	FBO <sup>2</sup>	OLI	BOR	FBO	OLI	BOR	FBO
PC <sup>2</sup>	18:2(n-6)	8.04 ± 0.16 <sup>a</sup>	7.92 ± 0.19 <sup>a</sup>	4.55 ± 0.34 <sup>b</sup>	7.28 ± 0.17 <sup>a</sup>	7.80 ± 0.62 <sup>a</sup>	5.09 ± 0.17 <sup>b</sup>	6.56 ± 0.31 <sup>a</sup>	8.22 ± 0.35 <sup>b</sup>	5.23 ± 0.34 <sup>c</sup>
	20:3(n-6)	tr <sup>3</sup>	0.81 ± 0.03 <sup>b</sup>	tr <sup>3</sup>	tr <sup>3</sup>	1.03 ± 0.19 <sup>b</sup>	tr <sup>3</sup>	tr <sup>3</sup>	0.90 ± 0.15 <sup>b</sup>	tr <sup>3</sup>
	20:4(n-6)	1.70 ± 0.30 <sup>a</sup>	4.13 ± 0.41 <sup>b</sup>	2.68 ± 1.08 <sup>ab</sup>	2.07 ± 0.36 <sup>a</sup>	3.93 ± 0.34 <sup>b</sup>	2.26 ± 0.58 <sup>ab</sup>	2.58 ± 0.19 <sup>a</sup>	3.79 ± 0.26 <sup>b</sup>	2.59 ± 0.18 <sup>a</sup>
	20:5(n-3)	tr <sup>3</sup>	tr <sup>3</sup>	0.69 ± 0.14 <sup>b</sup>	tr <sup>3</sup>	tr <sup>3</sup>	0.52 ± 0.32 <sup>b</sup>	tr <sup>3</sup>	tr <sup>3</sup>	0.50 ± 0.07 <sup>b</sup>
PE <sup>2</sup>	22:6(n-3)	tr <sup>3</sup>	tr <sup>3</sup>	tr <sup>3</sup>	tr <sup>3</sup>	tr <sup>3</sup>	tr <sup>3</sup>	tr <sup>3</sup>	tr <sup>3</sup>	tr <sup>3</sup>
	18:2(n-6)	3.78 ± 0.12 <sup>a</sup>	3.10 ± 0.12 <sup>b</sup>	2.10 ± 0.05 <sup>c</sup>	3.44 ± 0.19 <sup>a</sup>	2.77 ± 0.23 <sup>ab</sup>	2.12 ± 0.02 <sup>b</sup>	3.26 ± 0.18 <sup>a</sup>	3.26 ± 0.06 <sup>a</sup>	2.15 ± 0.02 <sup>b</sup>
	20:3(n-6)	tr <sup>3</sup>	0.66 ± 0.03 <sup>b</sup>	tr <sup>3</sup>	tr <sup>3</sup>	0.71 ± 0.06 <sup>b</sup>	tr <sup>3</sup>	tr <sup>3</sup>	0.61 ± 0.05 <sup>b</sup>	tr <sup>3</sup>
	20:4(n-6)	4.73 ± 0.17 <sup>a</sup>	6.17 ± 0.22 <sup>b</sup>	4.21 ± 0.24 <sup>a</sup>	4.73 ± 1.36 <sup>a</sup>	6.01 ± 0.46 <sup>a</sup>	4.49 ± 3.91 <sup>a</sup>	5.06 ± 0.50 <sup>a</sup>	6.30 ± 0.08 <sup>a</sup>	5.15 ± 0.23 <sup>a</sup>
PS/PI <sup>2</sup>	20:5(n-3)	tr <sup>3</sup>	tr <sup>3</sup>	1.17 ± 0.27 <sup>b</sup>	tr <sup>3</sup>	tr <sup>3</sup>	1.06 ± 1.32 <sup>b</sup>	tr <sup>3</sup>	tr <sup>3</sup>	1.06 ± 0.01 <sup>b</sup>
	22:6(n-3)	tr <sup>3</sup>	tr <sup>3</sup>	1.19 ± 0.15 <sup>b</sup>	tr <sup>3</sup>	tr <sup>3</sup>	1.17 ± 1.34 <sup>b</sup>	tr <sup>3</sup>	tr <sup>3</sup>	1.39 ± 0.01 <sup>b</sup>
	18:2(n-6)	1.62 ± 0.10 <sup>a</sup>	1.33 ± 0.10 <sup>ab</sup>	0.94 ± 0.10 <sup>b</sup>	1.54 ± 0.07 <sup>a</sup>	1.35 ± 0.08 <sup>ab</sup>	1.03 ± 0.08 <sup>b</sup>	1.03 ± 1.19 <sup>a</sup>	1.39 ± 0.15 <sup>ab</sup>	0.91 ± 0.03 <sup>b</sup>
	20:3(n-6)	0.20 ± 0.01 <sup>a</sup>	0.65 ± 0.07 <sup>b</sup>	0.12 ± 0.03 <sup>a</sup>	0.19 ± 0.02 <sup>a</sup>	0.59 ± 0.02 <sup>b</sup>	0.08 ± 0.01 <sup>c</sup>	0.21 ± 0.05 <sup>a</sup>	0.60 ± 0.06 <sup>b</sup>	0.04 ± 0.01 <sup>c</sup>
PS/PI, combined	20:4(n-6)	1.88 ± 0.08 <sup>ab</sup>	1.94 ± 0.03 <sup>a</sup>	1.51 ± 0.14 <sup>b</sup>	1.89 ± 0.07 <sup>ab</sup>	1.90 ± 0.13 <sup>a</sup>	1.47 ± 0.06 <sup>b</sup>	1.79 ± 0.08 <sup>ab</sup>	2.01 ± 0.10 <sup>a</sup>	1.41 ± 0.11 <sup>b</sup>
	20:5(n-6)	tr <sup>3</sup>	tr <sup>3</sup>	0.11 ± 0.06 <sup>a</sup>	tr <sup>3</sup>	tr <sup>3</sup>	0.06 ± 0.03 <sup>a</sup>	tr <sup>3</sup>	tr <sup>3</sup>	0.14 ± 0.09 <sup>a</sup>
	22:6(n-3)	tr <sup>3</sup>	tr <sup>3</sup>	0.23 ± 0.14 <sup>a</sup>	tr <sup>3</sup>	tr <sup>3</sup>	0.16 ± 0.02 <sup>b</sup>	tr <sup>3</sup>	tr <sup>3</sup>	0.14 ± 0.02 <sup>b</sup>

<sup>1</sup>Results are expressed in mg/100 mg total glycerophospholipid (PL) fatty acids and represent means ± SEM (n = 3). Values within a row for wk 4, 8 or 12 not sharing a common superscript letter are significantly different (P < 0.05). tr indicates levels are not detectable.

<sup>2</sup>OLI, 6% olive oil diet; BOR, 3% olive oil + 3% borage oil diet; and FBO, 6% olive oil + 3% fish body oil diet; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS/PI, combined (phosphatidyl serine and phosphatidylinositol).

TABLE 4

Effects of diets riched in (n-3) and (n-6) fatty acids on in vivo epidermal accumulation of lipoxygenase products in guinea pigs<sup>1</sup>

Hydroxy fatty acids	Week 4				Week 8				Week 12			
	OLI	BOR	FBO	OLI	BOR	FBO	OLI	BOR	FBO	OLI	BOR	FBO
	pg/mg protein											
13-HODE	2814 ± 17 <sup>a</sup>	2819 ± 525 <sup>ab</sup>	1243 ± 73 <sup>b</sup>	2415 ± 201 <sup>ab</sup>	3047 ± 373 <sup>a</sup>	1224 ± 316 <sup>b</sup>	2646 ± 112 <sup>a</sup>	3396 ± 322 <sup>a</sup>	1663 ± 162 <sup>b</sup>			
15-HETE	780 ± 206 <sup>a</sup>	1045 ± 144 <sup>a</sup>	644 ± 137 <sup>a</sup>	1154 ± 236 <sup>ab</sup>	1660 ± 137 <sup>a</sup>	873 ± 177 <sup>b</sup>	1002 ± 312 <sup>a</sup>	1513 ± 373 <sup>a</sup>	729 ± 16 <sup>a</sup>			
12-HETE	143 ± 37 <sup>a</sup>	131 ± 17 <sup>a</sup>	222 ± 40 <sup>a</sup>	123 ± 5 <sup>a</sup>	137 ± 8 <sup>a</sup>	301 ± 97 <sup>a</sup>	160 ± 44 <sup>a</sup>	210 ± 38 <sup>a</sup>	430 ± 67 <sup>b</sup>			
15-HETrE	44 ± 15 <sup>a</sup>	273 ± 48 <sup>b</sup>	40 ± 14 <sup>a</sup>	65 ± 25 <sup>a</sup>	335 ± 25 <sup>b</sup>	65 ± 22 <sup>a</sup>	66 ± 27 <sup>a</sup>	300 ± 63 <sup>b</sup>	62 ± 8 <sup>a</sup>			
15-HEPE	tr <sup>a</sup>	tr <sup>a</sup>	162 ± 20 <sup>b</sup>	tr <sup>a</sup>	tr <sup>a</sup>	191 ± 36 <sup>b</sup>	tr <sup>a</sup>	tr <sup>a</sup>	158 ± 32 <sup>b</sup>			
17-HDHE	tr <sup>a</sup>	tr <sup>a</sup>	tr <sup>a</sup>	tr <sup>a</sup>	tr <sup>a</sup>	tr <sup>a</sup>	tr <sup>a</sup>	tr <sup>a</sup>	tr <sup>a</sup>			

<sup>1</sup>Values are means ± SEM (n = 3). Values within a row for wk 4, 8 or 12 not sharing a common superscript letter are significantly different (P < 0.05). tr indicates levels are not detectable. See footnote to Table 3 for key to diet abbreviations. 13-HODE, 13-hydroxyoctadecadienoic acid; 12- or 15-HETE, 12- or 15-hydroxyeicosatetraenoic acid; 15-HETrE, 15-hydroxyeicosatrienoic acid; 15-HEPE, 15-hydroxyeicosapentaenoic acid; and 17-HDHE, 17-hydroxydocosahexaenoic acid.

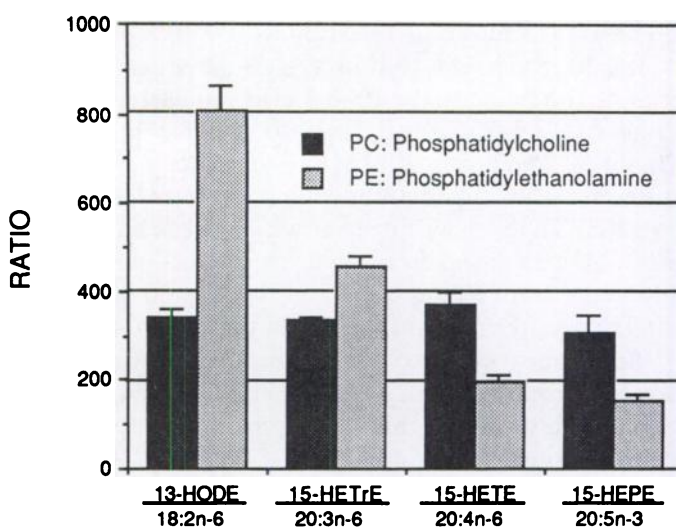


FIGURE 4 Ratios of epidermal hydroxy fatty acids (pg/mg protein) to total fatty acids (mg/100 mg glycerophospholipid fatty acids) in epidermal PC and PE. Data represent means ± SEM from guinea pigs (n = 27) of all three dietary groups combined (OLI, FBO and BOR).

epidermis reflects the fatty acid composition of the epidermal phospholipids (Table 4). The most abundant epidermal hydroxy fatty acids, 13-HODE and 15-HETE, are derived from the most abundant polyunsaturated fatty acids, 18:2(n-6) and 20:4(n-6), respectively. More interesting, though, were the significant diet-induced increases (wk 4, 8 and 12) in the epidermal levels of 15-HEPE [the 15-lipoxygenase product of 20:5(n-3)] and 15-HETrE [the 15-lipoxygenase product of 20:5(n-6)] in the animals fed FBO and BOR, respectively. Both 15-HETE (in the animals fed FBO) and 15-HETrE (in the animals fed BOR) contributed a significant proportion of total 15-lipoxygenase products, each representing 20–25% of the levels of 15-HETE. Notably absent in the epidermis of the animals fed FBO was any detectable amount of 17-HDHE, the 15-lipoxygenase product of 22:6(n-3).

Because 22:6(n-3) was not incorporated into PC but was incorporated into PE [at levels equal to 20:5(n-3)], there may be selective phospholipid pools serving to provide substrate fatty acids for the epidermal 15-lipoxygenase. To explore this possibility further, we examined whether a relationship existed between levels of epidermal hydroxy fatty acids and profiles of individual epidermal phospholipid fatty acids. Figure 4 depicts the ratios of hydroxy fatty acids to precursor fatty acids (wt %) for all 15-lipoxygenase products that were detectable in the epidermal extracts. These ratios indicate that the levels of individual 15-lipoxygenase products correlate more closely with the levels of fatty acids in PC than with the levels of fatty acids in PE. In fact an analysis of correlation between the levels of epidermal 15-lipoxygenase products and the levels of epidermal phospholipid fatty acids yielded  $r = 0.958$  for PC ( $P < 0.00005$ )

and  $r = 0.413$  for PE ( $P < 0.0125$ ).

In addition to the 15-lipoxygenase products, an analysis of the epidermal extracts also indicated the presence of 12-HETE, the 12-lipoxygenase product of 20:4(n-6). The levels of 12-HETE were not significantly different at wk 4 and 8; the only significant differences in 12-HETE levels occurred at wk 12 in the animals fed FBO. There were no detectable levels of 12-lipoxygenase products from 20:5(n-3) (12-HEPE) or 20:3(n-6) (12-HETrE) in the epidermis of any of the animals.

**Epidermal cyclooxygenase products.** Analysis of the epidermal levels of prostaglandins at wk 4, 8 and 12 did not show any significant differences between the dietary groups; mean levels of PGE<sub>2</sub> were at 2600 ng/mg protein, followed by PGF<sub>2α</sub> and PGD<sub>2</sub> at 2100 ng/mg protein and 1600 ng/mg protein, respectively. Prostaglandin E<sub>1</sub>, a cyclooxygenase metabolite of 20:3(n-6), was present in the epidermis of the animals fed BOR, although the amounts present were relatively small (~200 pg/mg protein) and variable.

## DISCUSSION

Although the ability of dietary oils to alter the fatty acids present in cellular phospholipids has been established in several tissues, the exploration of this manipulation in the epidermis has not been well examined. Furthermore, the effects of these diet-induced changes in fatty acid content on epidermal levels of eicosanoids, the products of lipoxygenase and cyclooxygenase enzymes, were even less clear. **The present study indicates that the feeding of diets rich in eicosapentaenoic acid [20:5(n-3)] or γ-linolenic acid [18:3(n-6)] can profoundly affect epidermal phospholipid fatty acid composition and the local tissue content of eicosanoids. These findings suggest that it may be possible, via dietary manipulation, to alleviate cutaneous disorders associated with fatty acid or eicosanoid imbalance.**

Specifically, our data show that feeding guinea pigs a diet supplemented with fish oil (FBO), which is rich in (n-3) fatty acids, or a diet supplemented with borage oil (BOR), which is rich in 18:3(n-6), resulted in significant incorporation of (n-3) fatty acids [20:5(n-3)] and [22:6(n-3)] or an 18:3(n-6)-derived fatty acid [20:3(n-6)] into epidermal phospholipids, respectively. These findings agree with earlier reports in which dietary oils were shown to exert a profound effect on the fatty acid composition of epidermal total phospholipids (16, 17). The current study extends these previous studies by demonstrating the relationship between dietary intake of fish oils rich in (n-3) or vegetable oils rich in 18:3(n-6) on the incorporation of specific fatty acids into individual phospholipid classes.

The data presented in Table 3 demonstrate that the constituent fatty acids of dietary oils are readily incorporated into individual epidermal phospholipids, as ev-

idenced by the analysis of fatty acid profiles in epidermal PC, PE and PS/PI phospholipid fractions. In the animals fed FBO, dietary 20:5(n-3) was incorporated into epidermal PC, PE and PS/PI; 22:6(n-3) was incorporated preferentially into epidermal PE, with lesser quantities incorporated into PS/PI and none detected in PC. This agrees with studies of fatty acid uptake in human platelets that have shown that labeled 22:6(n-3) is preferentially incorporated into PE compared with labeled 20:5(n-3), which is preferentially incorporated into PC (26). In the animals fed BOR, dietary 18:3(n-6) results first in the in vivo elongation of 18:3(n-6) to 20:3(n-6) followed by increased incorporation of 20:3(n-6) into epidermal PE, PS/PI and, particularly, PC. This agrees with similar studies with mice fed an 18:3(n-6) enriched diet that resulted in elevated absolute 20:3(n-6) in all macrophage phospholipid classes, with PC representing the largest gains (29).

**The importance of the fatty acid composition of epidermal phospholipids is evidenced by the relationship between epidermal phospholipid fatty acids and the epidermal levels of 15-lipoxygenase products.** Insignificant changes in 13-HODE and 15-HETE, the most abundant epidermal lipoxygenase products, reflect the insignificant changes in the levels of the precursor fatty acids 18:2(n-6) and 20:4(n-6). However, coinciding with the changes in the fatty acid profiles of the epidermal phospholipids described above, the epidermis of the animals fed the FBO diet had elevated levels of 15-HEPE [the 15-lipoxygenase product of 20:5(n-3)], whereas the epidermis of animals fed the BOR diet had elevated levels of 15-HETrE (the 15-lipoxygenase product of 20:3(n-6)) (Table 4). Notably absent in the epidermis of animals fed the FBO diet was any detectable amount of 17-HDHE, the 15-lipoxygenase product of 22:6(n-3). These data suggest that PE, into which 22:6(n-3) was preferentially incorporated, may resist hydrolysis by phospholipase A<sub>2</sub>, thus making 22:6(n-3) unavailable for lipoxygenation into 17-HDHE.

The possibility that the epidermal 15-lipoxygenase utilizes specific fatty acid pools (i.e., PC) remains to be confirmed. However, a comparison of the ratios of the levels of epidermal hydroxy fatty acids to the levels of precursor fatty acids in epidermal PC and PE (Fig. 4) suggests a specific relationship between the fatty acids in PC and their utilization by the epidermal 15-lipoxygenase. Analysis of correlation further implies that the levels of epidermal hydroxy fatty acids are related to the levels of fatty acids in epidermal PC ( $r = 0.958$ ;  $P < 0.00005$ ) but not to those in epidermal PE ( $r = 0.413$ ;  $P < 0.0125$ ). Although the exact relationship between epidermal PC and the epidermal 15-lipoxygenase remains to be fully elucidated, it has been demonstrated that PC is probably the major source of substrate fatty acid for human platelet 12-lipoxygenase (23) and for porcine aortic endothelial 5- and 15-lipoxygenases (21). It has also been reported that PC itself can be directly

oxygenated at carbon-15 of the PUFA moiety by 15-lipoxygenases in rabbit reticulocytes (30) and human polymorphonuclear leukocytes (31).

The importance of the generation of 15-lipoxygenase products in the epidermis *in vivo* relates to findings that the 15-lipoxygenase products inhibit the formation of pro-inflammatory 5-lipoxygenase products *in vitro*. Our results indicate that the feeding of diets rich in 20:5(n-3) or 18:3(n-6) can lead to increased levels of the 15-lipoxygenase products 15-HEPE and 15-HETrE, respectively. Both 15-HEPE (32) and 15-HETrE (20) are more potent inhibitors of 5-lipoxygenase *in vitro* than either 15-HETE or 13-HODE; thus, the increases in the levels of 15-HEPE and 15-HETrE observed in the epidermis of the guinea pigs that were fed diets enriched with 20:5(n-3) or 18:3(n-6) may lead to a substantial tissue increase of endogenous epidermal 5-lipoxygenase inhibitors. Such an elevation could conceivably suppress the *in vivo* activity of 5-lipoxygenase. **Because chronic inflammatory skin disorders such as psoriasis and atopic eczema are associated with increased levels of the 5-lipoxygenase product LTB<sub>4</sub> (5, 6, 15), the possibility that diets rich in 20:5(n-3) or 18:3(n-6) could enhance epidermal levels of potential 5-lipoxygenase inhibitors *in vivo* may explain partially how fish oil, rich in 20:5(n-3), was reported to improve the inflammatory aspects of psoriasis (1-3) and how evening primrose oil, rich in 18:3(n-6), was reported to be beneficial in the treatment of atopic eczema (13, 14).** Dietary supplementation with oils rich in specific PUFA may be an alternative therapy or may serve as an adjunct to conventional therapies used in the treatment of inflammatory disorders.

## ACKNOWLEDGMENT

The authors wish to thank Georgia Brown for typing this manuscript.

## LITERATURE CITED

- ZIBOH, V. A., COHEN, K. A., ELLIS, C. N., MILLER, C., HAMILTON, T. A., KRAGBALLE, K., HYDRICK, C. R. & VOORHEES, J. J. (1986) Effects of dietary supplementation of fish oil neutrophil and epidermal fatty acids. *Arch. Dermatol.* 122: 1277-1282.
- MAURICE, P. D. L., ALLEN, B. R., BARKLEY, A. S. J., COCKBILL, S. R., STAMMERS, J. & BATHER, P. C. (1987) The effects of dietary supplementation with fish oil in patients with psoriasis. *Br. J. Dermatol.* 117: 599-606.
- BITTNER, S. B., CARTWRIGHT, I., TUCKER, W. F. G. & BLEEHEEN, S. S. (198) A double-blind, randomised, placebo-controlled trial of fish oil in psoriasis. *Lancet* 9: 378-380.
- CHOWANIEC, O., JABLONSKA, S., BEUTNER, E. H., PRONIEWSKA, M., JARZABEK-CHORZELSKA, M. & RZESA, G. (1981) Earliest clinical and histological changes in psoriasis. *Dermatologica.* 63: 42-51.
- GRABBE, J., CZARNETZKI, B. M., ROSENBAACH, T. & MARDIN, M. (1984) Identification of chemotactic lipoxygenase products of arachidonate metabolism in psoriatic skin. *J. Invest. Dermatol.* 82: 477-479.
- BRAIN, S., CAMP, R., DOWD, P., KOBZA-BLACK, A. & GREAVES, M.

- (1984) The release of leukotriene B<sub>4</sub>-like material in biologically active amounts from the lesional skin of patients with psoriasis. *J. Invest. Dermatol.* 83: 70-73.
- SOTER, N. A., LEWIS, R. A., COREY, E. J. & AUSTEN, K. F. (1983) Local effects of synthetic leukotrienes (LTC<sub>4</sub>, LTD<sub>4</sub>, LTE<sub>4</sub>, and LTB<sub>4</sub>) in human skin. *J. Invest. Dermatol.* 80: 115-119.
- CAMP, R., JONES, R. R., BRAIN, S., WOOLLARD, P. & GREAVES, M. (1984) Production of intraepidermal microabscesses by topical application of leukotriene B<sub>4</sub>. *J. Invest. Dermatol.* 82: 202-204.
- BAUER, P. M., VAN DE KERKHOFF, P. C. M. & MAASSEN-DE GROOT, R. M. (1986) Epidermal hyperproliferation following the induction of microabscesses by leukotriene B<sub>4</sub>. *Br. J. Dermatol.* 114: 409-412.
- STANSBY, M. E. (1986) Fatty acids in fish. In: *Health Effects of Polyunsaturated Fatty Acids in Seafoods.* (Simopoulos, A. P., Kifer, R. R. & Martin, R. E., eds.), pp. 389-401, Academic Press, New York.
- PRESCOTT, S. M. (1984) The effect of eicosapentaenoic acid on leukotriene B production by human neutrophils. *J. Biol. Chem.* 259: 7615-7621.
- 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.
- LOVELL, C. R., BURTON, J. L. & HORROBIN, D. F. (1981) Treatment of atopic eczema with evening primrose oil. *Lancet* i: 278.
- WRIGHT, S. & BURTON, J. L. (1982) Oral evening-primrose-seed oil improves atopic eczema. *Lancet* 99: 1120-1121.
- RUZICKA, T., SIMMET, T., PESKAR, B. A. & RING, J. (1986) Skin levels of arachidonic acid-derived inflammatory mediators and histamine in atopic dermatitis and psoriasis. *J. Invest. Dermatol.* 86: 105-108.
- CHAPKIN, R. S., ZIBOH, V. A. & MCCULLOUGH, J. L. (1987) Dietary influences of evening primrose and fish oil on the skin of essential fatty acid-deficient guinea pigs. *J. Nutr.* 117: 1360-1370.
- MILLER, C. C. & ZIBOH, V. A. (1988) Gammalinolenic acid enriched diet alters cutaneous eicosanoids. *Biochem. Biophys. Res. Commun.* 154: 945-954.
- CHAPKIN, R. S., ZIBOH, V. A., MARCELO, C. L. & VOORHEES, J. J. (1986) Metabolism of essentially fatty acids by human epidermal enzyme preparations: evidence of chain elongation. *J. Lipid Res.* 27: 945-954.
- VANDERHOEK, J. Y., BRYANT, R. W. & BAILEY, J. M. (1982) Regulation of leukocyte and platelet lipoxygenases by hydroxyeicosanoids. *Biochem. Pharmacol.* 31: 3463-3467.
- MILLER, C. C., MCCREEDY, C. A., JONES, A. D. & ZIBOH, V. A. (1988) Oxidative metabolism of dihomogammalinolenic acid by guinea pig epidermis: evidence of generation of anti-inflammatory products. *Prostaglandins* 35: 917-938.
- BROWN, M. L., JAKUBOWSKI, J. A., LEVENTIS, L. L. & DEYKIN, D. (1987) Ionophore-induced metabolism of phospholipids and eicosanoid production in porcine aortic endothelial cells: selective release of arachidonic acid from diacyl and ether phospholipids. *Biochim. Biophys. Acta* 921: 159-166.
- MAHADEVAPPA, V. G. & HOLUB, B. J. (1987) Quantitative loss of individual eicosapentaenoyl-relative to arachidonoyl-containing phospholipids in thrombin-stimulated human platelets. *J. Lipid Res.* 28: 1275-1280.
- FISCHER, S., SCHACKY, C. V., SEISS, W., STRASSER, T. & WEBER, P. C. (1984) Uptake, release and metabolism of docosahexaenoic (DHA, C22:6w3) in human platelets and neutrophils. *Biochem. Biophys. Res. Commun.* 120: 907-918.
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265-275.
- FOLCH, J., LEES, M. & SLOANE-STANLEY, G. H. (1957) A simple method for the isolation and purification of total lipids from

- animal tissues. *J. Biol. Chem.* 226: 497-509.
26. HOLUB, B. J. & SKEAFF, C. M. (1977) Nutritional regulation of cellular phosphatidylinositol. In: *Methods in Enzymology* (Conn, P. M. & Means, A. R., eds.), Vol. 141, pp. 234-244, Academic Press, New York.
27. CROSET, M. & LAGARDE, M. (1983) Stereospecific inhibition of PGH<sub>2</sub>-induced platelet aggregation by lipoxygenase products of icosanoic acids. *Biochem. Biophys. Res. Commun.* 112: 878-883.
28. SOKAL, R. R. & ROHLF, F. J. (1981) *Biometry, The Principles and Practice of Statistics in Biological Research*, pp. 228-262. Freeman, New York.
29. CHAPKIN, R. S. (1988) Dietary manipulation of macrophage phospholipid classes: selective increase of dihomogammalinolenic acid. *Lipids* 23: 766-770.
30. MURRAY, J. J. & BRASH, A. R. (1988) Rabbit reticulocyte lipoxygenase catalyzes specific 12(S) and 15(S) oxygenation of arachidonoyl-phosphatidylcholine. *Arch. Biochem. Biophys.* 265: 514-523.
31. JUNG, G., YANG, D-C. & NAKAO, A. (1985) Oxygenation of phosphatidylcholine by human polymorphonuclear leukocyte 15-lipoxygenase. *Biochem. Biophys. Res. Commun.* 130: 559-566.
32. MILLER, C. C. & ZIBOH, V. A. (1988) Human epidermal trans-forms eicosapentaenoic acid to 15-hydroxy-5,8,11,13,17-eicosapentaenoic acid: a potent inhibitor of 5-lipoxygenase. *J. Am. Oil Chem. Soc.* 65: 474 (abs.).