Dietary Supplementation with Ethyl Ester Concentrates of Fish Oil (n-3) and Borage Oil (n-6) Polyunsaturated Fatty Acids Induces Epidermal Generation of Local Putative Anti-Inflammatory Metabolites

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Clinical reports have attributed the amelioration of chronic inflammatory skin disorders to the presence of certain polyunsaturated fatty acids (PUFA) in dietary oils. To test the hypothesis of a local modulatory effect of these PUFA in the epidermis, the basal diet of normal guinea pigs was supplemented with ethyl esters of either fish oil [rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] or borage oil [rich in gamma-linolenic acid (GLA)]. Our data demonstrated that dietary oils influence the distribution of PUFA in epidermal phospholipids and the epidermal levels of PUFA-derived hydroxy fatty acids. Specifically, animals supplemented with ethyl esters of fish oil markedly incorporated EPA and DHA into epidermal phospholipids, which paralleled the epidermal accumulation of 15-hydroxyeicosapentaenoic acid (15-HEPE) and 17-hydroxydocosahexaenoic acid (17-HDoHE). Similarly, animals supplemented with esters of borage oil preferentially incorporated dihomogammalinolenic acid (DGLA), the epidermal elongase product of GLA, into the epidermal phospholipids, which also was accompanied by epidermal accumulation of 15-hydroxyeicosatrienoic acid (15-HETrE). By factoring the epidermal levels of the 15-lipoxygenase products and their relative inhibitory potencies, we evolved a measure of the overall potential of dietary oils to exert local anti-inflammatory effect. For example, the leukotriene inhibition potentials (LIP) of both fish oil and borage oil were greatly enhanced when compared to controls. Thus, the altered profiles of epidermal 15-lipoxygenase products generated from particular dietary oils may be responsible, at least in part, for reported ameliorative effects of oils on chronic inflammatory skin disorders. *J Invest Dermatol 96:98–103, 1991*

Oxygenated metabolites of arachidonic acid (AA) (20:4n-6) are known to generate mediators (eicosanoids) of inflammatory reactions [1,2]. The possibility that eicosanoids formed from AA (particularly the 5-lipoxygenase products) may be involved in the pathogenesis of inflammatory skin disorders has been reviewed [3–7]. Interestingly, a weak inhibitor of leukotriene B_4 (LTB_4) synthesis has been described to alleviate the lesions of psoriasis [8,9], supporting the view that the inhibition of products of the lipoxygenase pathway (particularly LTB_4) may be critical in the management of at least the inflammatory aspects of psoriasis.

Excitement that other polyunsaturated fatty acids (PUFA), particularly those of the n-3 series (and their oxidative metabolites), can affect a variety of chronic and inflammatory diseases [10–13] has led to interest in the dietary intake of fish oil. The interest in psoriasis was accentuated because of an epidemiologic study of Eskimos in Greenland by Kromann and Green [14], which revealed a 20 times decrease in the estimated incidence in psoriasis when compared to a

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Abbreviations:

AA: arachidonic acid
DGLA: dihomogammalinolenic acid
DHA: docosahexaenoic acid
EPA: eicosapentaenoic acid
GLA: gammalinolenic acid
HOFA: hydroxyfatty acid
IC: inhibitory concentration
LA: linoleic acid
LT: leukotriene
PUFA: polyunsaturated fatty acid
13-HODE: 13-hydroxyoctadecadienoic acid
15-HETrE: 15-hydroxyeicosatrienoic acid
15-HETE: 15-hydroxyeicosatetraenoic acid
12-HETE: 12-hydroxyeicosatetraenoic acid
15-HEPE: 15-hydroxyeicosapentaenoic acid
17-HDoHE: 17-hydroxydocosahexaenoic acid
18:2n-6/20:5n-3/22:6n-3: designations of fatty acids. The first number represents the number of carbons in a straight chain and the number following the colon indicates the number of methylene-interrupted *cis* double bonds. The number after n indicates the number of carbon atoms from the methyl end of the acyl chain to the nearest double bond.

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Danish group. This report was followed by tests of the possible efficacy of dietary fish oil in the management of psoriasis, with varying degrees of success [15–17]. Similarly, reports are emerging of the clinical improvement of patients with atopic eczema after oral administration of primrose oil [18–20], a vegetable oil that is rich in gammalinolenic (GLA) acid. Despite these emerging reports, the biochemical mechanisms of these reported therapeutic effects have remained unclear.

As a first step in elucidating the role of PUFA in cutaneous biology, we investigated whether a relationship exists between the dietary intake of fish-oil (an n-3 PUFA, containing predominantly eicosapentaenoic acid [EPA]) (20:5n-3) and docosahexaenoic acid (DHA) (22:6n-3), the intake of borage oil (an n-6 PUFA containing linoleic acid) (18:2n-6) and gammalinolenic acid (GLA) (18:3n-6) fatty acids, and the in vivo generation of oxidative anti-inflammatory metabolites of these PUFA. To attain these objectives, guinea pig diets were supplemented with ethyl ester concentrates of either fish oil (an n-3 PUFA) or borage oil (an n-6 PUFA). After 8 weeks, we determined 1) whether the PUFA constituents of each dietary oil are incorporated into distinct epidermal phospholipid pools; 2) whether the incorporation into phospholipids is accompanied by the generation and accumulation of in vivo epidermal 15-lipoxygenase products of the dietary PUFA; and 3) whether these metabolites possess the ability to inhibit generation of proinflammatory LTB_4 by rat basophilic leukemic (RBL-1) cell line (a model of LTB_4 generation).

MATERIALS AND METHODS

Dietary Treatment Male weanling Hartley guinea pigs (Simonsen, n = 15) were housed individually at 22–24°C with a 12-h diurnal light cycle; deionized water (0.05% ascorbic acid) and diet were provided ad libitum. After acclimating for 2 weeks on a standard chow diet (Purina), the animals were randomly assigned into three groups and fed purified diets as described previously [21,22]. These three groups (n = 5 per group) varied only in the type of oil used in the diet, such that each dietary group received 1% safflower oil (providing > 1.5 cal% 18:2n-6) and either 5% hydrogenated coconut oil (control), 5% ethyl ester concentrate of GLA-enriched borage oil (+GLA), or 5% ethyl ester concentrate of EPA- and DHA-enriched fish body oil (+EPA/DHA). The fatty acid composition of the diets is shown in Table I. The diets were mixed in a crude, powdered form and stored under N_2 at −20°C. As needed, the powdered form of the diet was mixed with 2% agar in water to produce a gel (stored under N_2 at 4°C, which was the form of the diet provided to the animals for consumption. Diets were periodically analyzed for auto-oxidation by checking fatty acid profiles. Fresh diet was provided daily after diets not consumed was removed. Body weights were recorded 3 times per week throughout the study. There were no significant differences in the growth curves of the three groups and no macroscopical differences in the appearances of the animals. Thus, there were no indications of essential fatty acid (EFA) deficiency in any of the dietary groups. After 8 weeks on the experimental diets, the animals were sacrificed for analyses.

Table I. Polyunsaturated Fatty Acid Compositions of Dietary Oils[a]

Fatty Acids	Control	+GLA	+EPA/DHA
LA (18:2n-6)	[15.64][b]	[47.62]	14.71
GLA (18:3n-6)		[39.48]	0.35
DGLA (20:3n-6)			0.27
AA (20:4-n-6)			1.40
EPA (20:5-n-3)			[26.42]
DHA (22:6n-3)			[20.68]

[a] mg/100 mg total PUFA fatty acids. Values are expressed as mg/100 mg of total PUFA. Control represents 5% hydrogenated coconut oil plus 1% safflower oil; +GLA represents 5% ethyl ester concentrate of borage oil plus 1% safflower oil and +EPA/DHA represents ethyl ester concentrate of fish oil plus 1% safflower oil.

[b] Brackets represent major polyunsaturated fatty acids.

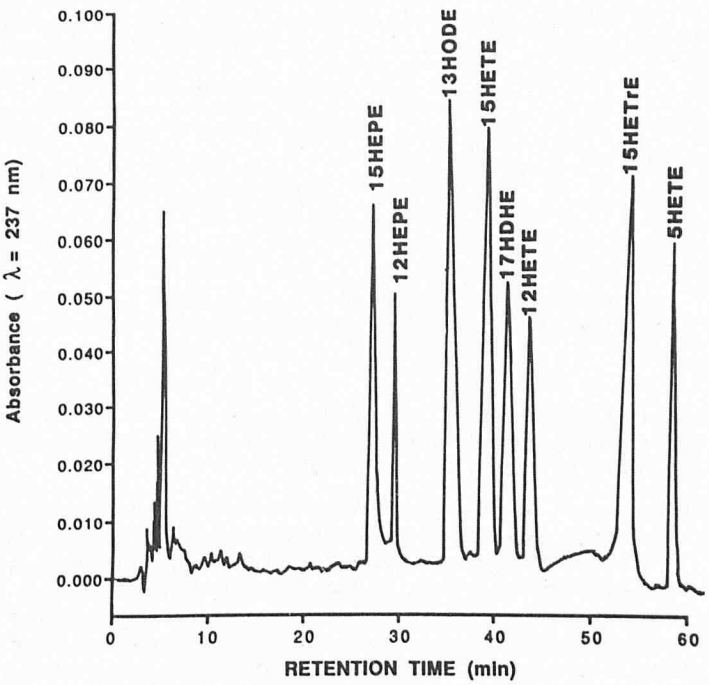

Figure 1. A typical chromatogram of hydroxy fatty acid standards separated using a system of MeOH (74%) and H_2O (acidified to pH 3.0 with acetic acid) run isocratically from 0–60 min at flow rate of 1.0 ml/min.

Lipid Analysis Epidermal strips were taken from the guinea pig and homogenized as reported previously [22]. A portion of the crude epidermal homogenate was used to quantitate protein concentration by a modified Lowry method [23]. Lipids from a portion of the crude epidermal homogenate were extracted with $CHCl_3$:MeOH (2:1, v/v) [24] and the individual phospholipids fractionated by thin-layer chromatography on 0.25-mm silica gel G plates (Merck, Darmstadt, FDR) using $CHCl_3$:MeOH:HOAc:H_2O (50:37.5:3.5:2, v/v/v/v) [25,26]. The individual phospholipids were visualized under UV light after spraying with 0.2% 2′,7′-dichlorofluorescein in ethanol. The respective bands were eluted from the silica gel and dried under N_2 gas after a known concentration of methylheptadecanoate was added as an internal standard to determine the relative contribution of fatty acids from each phospholipid class. The fatty acid profiles from the individual phospholipids were determined by gas chromatography after transmethylation in 6% methanolic HCl [22]. The gas chromatograph (Hewlett-Packard model 5730A) was equipped with a DB-225 fused silica capillary column (50% cyanopropylphenyl, 0.15-mm film thickness, 30 m × 0.25 mm i.d.; J&W Scientific, Rancho Cordova, CA). Hydrogen (36 cm/sec) was used as the carrier gas, the oven was run isothermically at 200°C, and detection was performed by flame ionization detector (FID).

Hydroxy fatty acids from the epidermal homogenate were extracted with ice-cold $CHCl_3$:MeOH (2:1, v/v) after acidification to pH 3.0. The profiles were determined by reverse phase-high performance liquid chromatography (RP-HPLC) using a Beckman 5 μm octadecylsilica (ODS) column (25 cm × 4.6 μm i.d., Beckman), which 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 [27]. The separated hydroxy fatty acids were monitored at 237 nm with a Beckman Model 165 Variable Wavelength Detector. Quantitation was determined with external standards of 13-hydroxyoctadecadienoic acid (13-HODE), 15-hydroxyeicosatrienoic acid (15-HETrE), 15-hydroxyeicosatetraenoic acid (15-HETE), 12-hydroxyeicosatetraenoic acid (12-HETE), 15-hydroxyeicosapentaenoic acid (15-HEPE), and 17-hydroxydocosahexaenoic acid

Table II. Effects of Diets Enriched with Ethyl Ester Concentrates of n-6 and n-6 PUFA on Distribution of Fatty Acids in Phosphatidylcholine and Phosphatidylethanolamine[a]

	Phosphatidylcholine (PC)			Phosphatidylethanolamine (PE)		
PUFA	Control	+GLA	+EPA/DHA	Control	+GLA	+EPA/DHA
18:2n-6	11.60 + .80[a]	9.96 + .29[a,b]	8.54 + .54[b]	11.64 + .66[a]	9.88 + 1.03[a]	12.43 + 1.74[a]
20:3n-6	0.26 + .02[a]	1.27 + .09[b]	0.41 + .15[a]	0.90 + .21[a]	2.85 + .31[b]	1.46 + .31[a]
20:4n-6	2.45 + .17[a]	3.77 + .17[b]	2.45 + .10[a]	11.30 + 1.02[a]	14.26 + 1.23[a]	4.46 + 1.30[b]
20:5n-3	tr[a]	tr[a]	1.32 + .40[b]	0.11 + .09[a]	tr[a]	2.59 + .14[b]
20:6n-3	tr[a]	tr[a]	0.36 + .03[b]	tr[a]	tr[a]	2.49 + .23[b]

[a] mg/100 mg total phospholipid fatty acids. Values are expressed as mg/100 mg total phospholipid fatty acids and represent means ± SEM (n = 3). Values not sharing a common superscipt letter are significantly different ($p < 0.05$). *tr* indicates levels are not detectable. See footnote to Table I for key to diet abbreviations.

(17-HDoHE) (Cayman Chemicals, Philadelphia, PA). A representative chromatogram of the hydroxy fatty acid standards as separated by this HPLC system is depicted in Fig 1.

Preparation of 15-Lipoxygenase Products of Polyunsaturated Fatty Acids by Guinea Pig Epidermal Homogenates To obtain and evaluate the putative anti-inflammatory potentials of 15-lipoxygenase products that are generated from dietary polyunsaturated fatty acids (PUFA), the respective 15-lipoxygenase products of AA (20:4n-6), DGLA (20:3n-6), EPA (20:5n-3), and DHA (22:6n-3) were first synthesized by incubating each precursor fatty acid (NuChek Prep, Inc. Elysian, MN) with soybean lipoxidase (Sigma, St. Louis, MO) [22]. The crude products were purified by HPLC using a silica column (5 μm, μPorosil; Beckman) and the solvent system of hexane/ethanol/acetic acid (983/16/1; v/v/v) and quantitated via spectrophotometric analyses and by co-chromatography with authentic standard (Biomol; Philadelphia, PA). The identity of each 15-lipoxygenase product derived from the incubation of each respective PUFA and soybean lipoxygenase was confirmed by reverse-phase (RP)-HPLC and gas chromatography/mass spectrometry (GC/MS), as described previously.

Effects of Epidermal 15-Lipoxygenase Metabolites of Dietary PUFA on RBL-1 LTB_4 Generation We tested the effects of varying concentrations of each of the generated hydroxy fatty acids on LTB_4 generation by rat basophilic leukemia cells (RBL-1), as published previously [22]. Specifically, RBL-I cells were placed in buffer (50 mM phosphate, pH 7.4, 1 mM EDTA) at a concentration of 3×10^7 cells/ml and then homogenized by sonication. The crude homogenate was centrifuged at 10,000 g to obtain a supernatant containing the 5-lipoxygenase activity. Supernatant enzyme preparations were pre-incubated with 2 mM $CaCl_2$ and varying concentrations (0–50 μM) of either 15-HETrE, 15-HEPE, or 17-HDoHE at 37°C for 10 min. Reactions were initiated by the addition of [1-^{14}C] 20:4n-6 (DuPont, New England Nuclear Products Division, Boston, MA) at a final concentration of 20 μM and 0.2 μCi. Incubations continued for an additional 20 min and were then terminated by placement in ice, followed by acidification to pH 3.0. The incubation products were extracted with $CHCl_3$:MeOH (2:1, v/v) and the conversion of [1-^{14}C] 20:4n-6 into ^{14}C-LTB_4 and ^{14}C-5-HETE was ascertained by a gradient reverse-phase–HPLC system using a modified solvent system of methanol/water (0.08% HOAc, pH 6.2), according to Henke et al [28].

Statistical Analysis Data were subjected to one-way analysis of variance by Student t test using the Bonferroni transformation for comparisons between three means. The upper level of significance chosen was $p < 0.05$.

RESULTS

Incorporation of Dietary PUFA into Individual Epidermal Phospholipids The fatty acid profiles of the phospholipids (Tables II, III) reflected the fatty acid contents of the oils ingested (Table I) by the three groups. The epidermal phospholipids of the animals fed the fish oil (EPA/DHA)-enriched diet had higher levels of the n-3 fatty acids than those animals fed safflower (LA)-enriched (control) or the borage oil (GLA)-enriched diets. The levels of EPA (20:5n-3) in the epidermal phospholipids of the fish oil (EPA/DHA)-supplemented animals were significantly high ($p < 0.05$) in the phosphatidylcholine (PC) and phosphatidylethanolamine (PE) fractions (Table II). In contrast, the incorporation of EPA/DHA was negligible in the phosphatidylserine (PS) and phosphatidylinositol (PI) fractions (Table III). Interestingly, the levels of EPA/DHA in fish oil-fed animals were higher in PE than in PC (Table II), indicating a special role of this phospholipid in epidermal PUFA homeostasis.

Similarly, the animals fed the GLA-enriched borage oil revealed high incorporation of DGLA (20:3n-6), the elongation product of GLA, into the epidermal phospholipids, when compared with the epidermal phospholipids of the animals fed either safflower oil (LA-enriched) or fish oil (EPA/DHA-enriched) diets. The level of DGLA (like EPA/DHA) was higher in the PE than in the PC fraction (Table II), further indicating the importance of PE for incorporating dietary DGLA and EPA/DHA. Additionally, unlike the n-3 PUFA (EPA/DHA), the DGLA, an n-6 PUFA, was notably high in PI and PS. This is interesting, as these latter two phospholipids are linked to cutaneous signal transduction mechanisms. The role of DGLA and/or its metabolites in cutaneous signal transduction mechanisms remains to be determined.

Table III. Effects of Diets Enriched with Ethyl Ester Concentrates of n-6 and n-3 PUFA on Distribution of Fatty Acids in Epidermal Phosphatidylserine and Phosphatidylinositol[a]

	Phosphatidylserine (PS)			Phosphatidylinositol (PI)		
PUFA	Control	+GLA	+EPA/DHA	Control	+GLA	+EPA/DHA
18:2n-6	4.45 + .57[a]	4.20 + .32[a]	3.79 + .65[a]	4.50 + .60[a]	4.52 + .37[a]	5.16 + .87[a]
20:3n-6	0.71 + .20[a]	2.50 + .22[b]	0.98 + .19[a]	0.64 + .04[b]	2.70 + .26[b]	1.22 + .11[a]
20:4n-6	1.91 + .31[a]	1.92 + .29[a]	1.36 + .32[a]	6.16 + .79[a]	7.30 + 1.30[a]	6.30 + .88[a]
20:5n-3	0.22 + .14[a]	tr[a]	0.25 + .10[b]	tr[a]	tr[a]	0.64 + .34[a]
20:6n-3	tr[a]	tr[a]	0.72 + .19[b]	tr[a]	tr[a]	0.84 + .33[a]

[a] Values are expressed as mg/100 mg total phospholipid fatty acids and represent means ± SEM (n = 3). Values not sharing a common superscipt letter are significantly different ($p < 0.05$). *tr* indicates levels are not detectable. See footnote to Table I for key to diet abbreviations.

Table IV. Effect of Diets Enriched with Ethyl Ester Concentrates of n-6 and n-3 Fatty Acids on In Vivo Generation of Epidermal Hydroxy Fatty Acids[a]

Precursor PUFA	Hydroxy Fatty Acids	(ng/mg protein) Control	+GLA	+EPA/DHA
LA (18:2n-6)	13-HODE	2.73 + .08[a]	2.12 + .33[a]	1.41 + .14[b]
DGLA (20:3n-6)	15-HETrE	0.07 + .01[a]	0.27 + .01[b]	0.07 + .01[a]
AA (20:4n-6)	15-HETE	0.67 + .02[a]	.75 + .08[b]	0.58 + .10[a]
AA (20:4n-6)	12-HETE	0.14 + .02[a]	0.19 + .03[b]	0.51 + .07[a]
EPA (20:5n-3)	15-HEPE	tr[a]	tr[a]	0.46 + .06[b]
DHA (22:6n-3)	17-HDoHE	tr[a]	tr[b]	0.08 + .01[a]

[a] Values are means ± SEM (n = 3). Values not sharing a common superscipt letter are significantly different ($p < 0.05$). *tr* indicates levels are not detectable. See footnote to Table I for key to diet abbreviations.

The two predominant PUFA in epidermal phospholipids, linoleic acid (LA) (18:2n-6) and arachidonic acid (AA) (20:4n-6) are also preferentially incorporated into PE. A notable effect of guinea pig diet supplementation with fish oil (EPA/DHA) is the increased incorporation of DGLA into the epidermal phospholipids when compared to the safflower oil-fed (control) animals (Table II). Although the mechanism for this n-3 PUFA–induced increase of DGLA is not immediately clear, the finding nonetheless suggests a possible cooperative effect of n-3 and n-6 PUFA. One possibility is the inhibition of Δ^5 desaturase activity (the enzyme that catalyzes the transformation of DGLA into AA) by increased ingestion of EPA/DHA (n-3 PUFA)-containing oils, which thereby will elevate epidermal DGLA.

Epidermal Hydroxy Fatty Acids Analyses of endogenous epidermal hydroxy fatty acids revealed that they reflect the increased incorporation of the respective PUFA from the dietary oils into epidermal phospholipids (Table IV). For instance, 13-HODE and 15-HETE, the most abundant hydroxy fatty acids, are 15-lipoxygenase products of LA and AA, respectively. The differences in the levels of 13-HODE and 15-HETE between the dietary groups are consistent with the differences in the levels of LA and AA in the epidermal phospholipids of the groups.

The most significant changes in epidermal hydroxy fatty acid levels were those of 15-HETrE, 15-HEPE, and 17-HDoHE, all 15-lipoxygenase products of DGLA, EPA, and DHA, respectively. Interestingly, the EPA/DHA-supplemented animals has significantly elevated levels of 15-HEPE and 17-HDoHE, whereas the GLA-supplemented animals had significantly elevated levels of 15-HETrE when compared to safflower-fed (control) animals. As with 13-HODE and 15-HETE, the levels of 15-HEPE and 15-HETrE correlate with the levels of incorporated substrate fatty acids in the epidermal phospholipids.

Additionally, analyses of the epidermal extracts revealed the presence of 12-HETE, a 12-lipoxygenase product of AA. In contrast to the 15-lipoxygenase products, the level of 12-HETE (S/R) did not correlate with the levels of its substrate (AA) in any of the individual epidermal phospholipid classes. For instance, the level of total 12-HETE (S/R) in the epidermis of the fish oil (EPA/DHA)-supplemented group was significantly elevated when compared with the level of 12-HETE (S/R) in the GLA- and LA-enriched dietary groups, which incorporated higher levels of AA into the phospholipids (Tables II, III).

Inhibition of LTB_4 Synthesis by 15-Lipoxygenase Products Results in Fig 2 demonstrate that epidermal 15-lipoxygenase products exert varying dose-dependent inhibitory activities on Ca^{++}-ionophore induced-RBL-1 cell-5-lipoxygenase activity. The IC_{50} for 15-HETrE (a metabolite of DGLA) was 18 μM and is the most potent of the four tested monohydroxy fatty acids. The IC_{50} of 15-HEPE and 17-HDoHE (both 15-lipoxygenase metabolites of EPA and DHA) were intermediate (25 μM and 28 μM, respectively). In contrast, the IC_{50} for 15-HETE (a metabolite of AA) was 37 μM, indicating that it is apparently the least potent of the hydroxy fatty acids tested. Overall, the ability of the 15-lipoxygenase products to inhibit RBL-1-5-lipoxygenase activity follows the order 15-HETrE > 17-HDoHE > 15-HEPE > 15-HETE.

DISCUSSION

Our data indicate a strong sequential relationship between the PUFA constituents of the dietary oils, the distribution of constituent PUFA in epidermal phospholipids, the levels of 15-lipoxygenase products in the epidermis, and the potential of these epidermal products to inhibit the local production of inflammatory leukotrienes. Specifically, results from Tables II and III demonstrate the effects of dietary PUFA composition (Table I) on the PUFA profiles of individual epidermal phospholipids. For example, dietary supplementation with fish oil resulted in the incorporation of EPA/DHA into guinea pig epidermal phospholipids, whereas dietary supplementation with borage oil (enriched with GLA) resulted in the incorporation of higher levels of DGLA (the elongase produce of GLA). These results are in agreement with a past study by our laboratory showing a similar link between dietary PUFA and the distribution of PUFA in epidermal lipids [29].

The data in Table IV indicated that the levels of 15-lipoxygenase products in epidermal extracts reflect the levels of their corresponding PUFA precursors in the epidermal phospholipids. For example, in the fish oil-supplemented animals, the 15-lipoxygenase product of EPA (15-HEPE, 0.46 ng/mg protein) increased from non-detectable levels to levels that are similar in magnitude to the 15-lipoxygenase product of AA (15-HETE, 0.58 ng/mg protein), whereas the 15-lipoxygenase product of DHA (17-HDoHE, 0.08 ng/mg protein) increased from non-detectable levels to detectable levels.

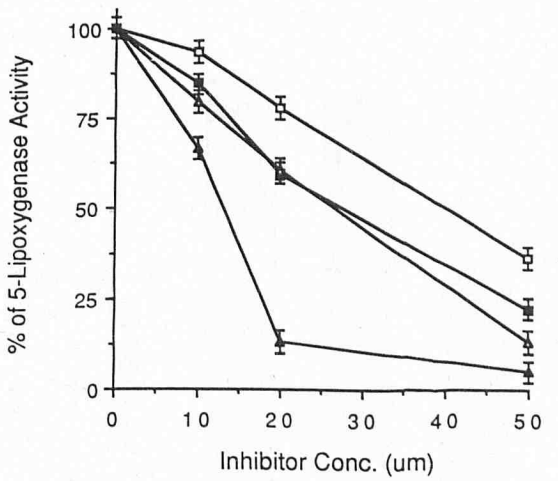

Figure 2. Inhibitory effects of 15-HETE *(open squares)*, 15-HEPE *(solid squares)*, 17-HoDHE *(open triangles)*, and 15-HETrE *(solid triangles)* on the activity of 5-lipoxygenase from RBL-1 cell homogenates. Each *point* and *error bar* represents the mean ± SEM of three experiments. Approximate IC_{50} values in μM for 15-HETE, 15-HEPE, 17-HoDHE, and 15-HETrE are 37, 28, 25, and 18 μM, respectively.

Similarly, in the borage oil-supplemented animals, the level of 15-HETrE (the 15-lipoxygenase product of DGLA) was significantly elevated (0.27 ng/mg protein) from non-detectable levels.

Because linking the levels of epidermal 15-lipoxygenase products to their potential to inhibit local leukotriene synthesis is not easily measurable from in vivo parameters, we derived an arbitrary factor named "leukotriene inhibition potential" (LIP). This factor determines the potential of epidermal 15-lipoxygenase products from specific PUFA to inhibit the local generation of leukotrienes. As demonstrated from our studies (Fig 2) the validity of LIP determination depended on the ability of the 15-lipoxygenase products to inhibit proinflammatory LTB_4 generation by 5-lipoxygenase enzyme from RBL-homogenate. By dividing the epidermal level of each individual 15-lipoxygenase by its IC_{50}, a contribution for each individual 15-lipoxygenase product can be derived. By summing up the individual contributions, one can establish an overall LIP for each dietary oil. For example, both the fish oil- and borage oil-supplemented animals (as shown in Fig 3) revealed significant LIP increase when compared with control animals fed safflower oil-supplemented diet. Furthermore, the LIP of the fish oil-supplemented animals (0.038) is approximately 73% higher than the LIP of the control animals (0.022). This increase was largely due to contributions of EPA-derived 15-HEPE and DHA-derived 17-HDoHE. Similarly, the LIP of the borage oil-supplemented animals (0.037) is approximately 68% higher than the LIP of the control animals, largely due to the contribution of DGLA-derived 15-HETrE. These estimations imply that the level of 15-lipoxygenase products in the epidermis, particularly those derived from certain dietary PUFA, can greatly influence locally-derived, cutaneous anti-inflammatory products. The level in the tissue can in turn suppress the generation of leukotrienes in the skin. It is therefore reasonable to speculate that by suppressing local leukotriene synthesis, one can diminish further recruitment of leukocytes to an area of cutaneous inflammation.

Results from this study extend our previous observations [29], as well as those of other investigators, that the constituent PUFA in the diet can have profound effects on the levels and spectrum of tissue eicosanoids. A significant aspect of our findings is that constituent PUFA in dietary oils can be transformed enzymatically by epidermis into "putative" anti-inflammatory products. Thus, it is reasonable to speculate that dietary PUFA, on the one hand, can alter the epidermal environment such that the infiltration of inflammatory leukocytes to the tissue is markedly reduced and, on the other hand, can serve pharmacologically during an inflammatory condition to suppress the symptoms of chronic inflammatory skin disorders [15–20]. As data from these studies have established a link between dietary oils, the generation of anti-inflammatory local products, and their ability to suppress the generation of pro-inflammatory leukotrienes, future studies should focus on evolving dietary formulations to maximize epidermal levels of PUFA. Implications of these findings reach beyond the scope of cutaneous disorders to a variety of chronic inflammatory diseases in which eicosanoids may play an important role.

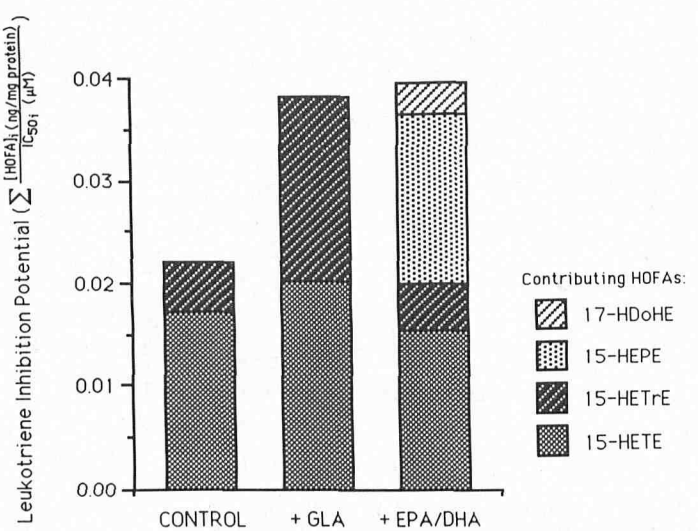

Figure 3. Leukotriene inhibition potential (LIP) of hydroxy fatty acids generated from dietary PUFA. Each *vertical box,* control, +GLA, or +EPA/DHA, represents inhibitory potentials of diets containing AA, GLA, or EPA/DHA, respectively.

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