

Dietary ω -3 Polyunsaturated Fatty Acids Inhibit Phosphoinositide Formation and Chemotaxis in Neutrophils

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Abstract

Earlier studies demonstrated that dietary ω -3 polyunsaturated fatty acid (PUFA) supplementation attenuates the chemotactic response of neutrophils and the generation of leukotriene (LT) B₄ by neutrophils stimulated with calcium ionophore; however, the mechanisms and relationship of these effects were not examined. Neutrophils and monocytes from eight healthy individuals were examined before and after 3 and 10 wk of dietary supplementation with 20 g SuperEPA daily, which provides 9.4 g eicosapentaenoic acid (EPA) and 5 g docosahexaenoic acid. The maximal neutrophil chemotactic response to LTB₄, assessed in Boyden microchambers, decreased by 69% after 3 wk and by 93% after 10 wk from prediet values. The formation of [³H]inositol tris-phosphate (IP₃) by [³H]inositol-labeled neutrophils stimulated by LTB₄ decreased by 71% after 3 wk (0.033±0.013% [³H] release, mean±SEM) and by 90% after 10 wk (0.011±0.011%) from prediet values (0.114±0.030%) as quantitated by β -scintillation counting after resolution on HPLC. LTB₄-stimulated neutrophil chemotaxis and IP₃ formation correlated significantly ($P < 0.0001$); each response correlated closely and negatively with the EPA content of the neutrophil phosphatidylinositol (PI) pool ($P = 0.0003$ and $P = 0.0005$, respectively). Neither the affinities and densities of the high and low affinity LTB₄ receptors on neutrophils nor LTB₄-mediated diglyceride formation changed appreciably during the study. Similar results were observed in neutrophils activated with platelet-activating factor (PAF). The summed formation of LTB₄ plus LTB₅ was selectively inhibited in calcium ionophore-stimulated neutrophils and was also inhibited in zymosan-stimulated neutrophils. The inhibition of the summed formation of LTB₄ plus LTB₅ in calcium ionophore-stimulated neutrophils and in zymosan-stimulated neutrophils did not correlate significantly with the EPA content of the PI pool. The data indicate that dietary ω -3 PUFA supplementation inhibits the autoamplification of the neutrophil inflammatory response by decreasing LTB₄ formation through the inactivation of the LTA epoxide hydrolase and independently by inhibiting LTB₄- (and PAF) stimulated chemotaxis by attenuating the formation of IP₃ by the PI-selective phospholipase C. This is the initial demonstration that dietary ω -3 PUFA

supplementation can suppress signal transduction at the level of the PI-specific phospholipase C in humans. (*J. Clin. Invest.* 1993. 91:651–660.) Key words: fish oil • inositol phosphates • monocytes • platelet-activating factor • phospholipids

Introduction

AA (20:4, ω -6), the major substrate for both the cyclooxygenase and 5-lipoxygenase pathways in humans on a typical Western diet, is derived from ω -6 polyunsaturated fatty acids (PUFA)¹ of land-based plants and animals. Eicosapentaenoic acid (EPA, 20:5, ω -3) and docosahexaenoic acid (DHA; 22:6, ω -3), found primarily in the form of esterified triglycerides, are each major fatty acids in marine organisms. Dietary marine fish oils rich in ω -3 PUFA have an antiinflammatory effect in murine models of lupus (1, 2) and RA (3). Several small studies of the effects of dietary ω -3 PUFA supplementation in patients with RA have each demonstrated modest clinical benefit (4–8). In healthy individuals dietary supplementation with ω -3 PUFA inhibits leukotriene (LT) B₄ formation by human neutrophils activated *ex vivo* with calcium ionophore A23187 (9). However, its effects on LTB₄ formation in receptor-stimulated neutrophils are unknown. Dietary supplementation with ω -3 PUFA in healthy volunteers inhibits neutrophil chemotaxis to a range of concentrations of LTB₄ (9) and suppresses the LTB₄-mediated enhancement of adherence to endothelial cell monolayers (9, 10). Both functional responses of the neutrophils returned nearly to baseline levels 6 wk after the dietary fish oil was discontinued, further supporting a causal relationship. These results were subsequently confirmed by others (11, 12). No changes in neutrophil membrane fluidity were detected by fluorescence polarization in cells doped with the fluorescent probe, 1,6-diphenyl-1,3,5-hexatriene (9). The mechanism(s) by which dietary ω -3 PUFA supplementation modulates transmembrane stimulation of neutrophils by chemotactic ligands were not addressed in previous studies. Early events in the signal transduction of the neutrophil chemotactic ligands LTB₄, platelet-activating factor (PAF), and FMLP are believed to involve the activation of a G-protein-associated, phosphatidylinositol (PI)-selective phospholipase C (13, 14). The activated phospholipase C hydrolyzes PI-4,5-bis-phosphate, resulting in the formation of inositol-1,4,5-tris-phos-

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1. Abbreviations used in this paper: alkylacylIPC, 1-O-alkyl-2-acyl-sn-glycero-3-phosphocholine; alkenacylIPE, 1-O-alk-1'-enyl-2-acyl-sn-glycero-3-phosphoethanolamine; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; 5-HEPE, 5S-hydroxy-eicosapentaenoic acid; 5-HETE, 5S-hydroxy-eicosatetraenoic acid; hpf, high power field; IP₁, inositol phosphate; IP₂, inositol bis-phosphate; IP₃, 1,4,5-inositol tris-phosphate; PI, phosphatidylinositol; PAF, platelet-activating factor; PUFA, polyunsaturated fatty acid(s); RP-HPLC, reverse-phase HPLC; THA, Tyrode's-Hepes-albumin buffer.

phate (IP₃) and diacylglycerols. IP₃ binds to specific receptors on the endoplasmic reticulum, resulting in the release of intracellular stores of calcium and activation of phospholipases A₂ and D (15). The diacylglycerols, in conjunction with the increased cytoplasmic calcium ion concentration, activate protein kinase C and lead to activation of other specific cellular pathways (16).

In this study, we demonstrate that dietary supplementation with ω -3 PUFA in healthy human volunteers leads to the inhibition of chemotaxin-mediated phosphoinositide formation in neutrophils in parallel with inhibition of chemotaxis without affecting appreciably neutrophil chemotaxin receptor density or affinity or diglyceride formation. The suppression of receptor-mediated stimulation of LTB₄ generation is attributed to the inactivation of the LTA epoxide hydrolase by the EPA-derived intermediate, LTA₅. Unlike chemotaxis, this suppression is not significantly correlated either with the EPA content of PI or with the suppression of the second messenger, IP₃. These dual effects of dietary supplementation with ω -3 PUFA could attenuate substantially the amplification of the neutrophil inflammatory response mediated by LTB₄. Furthermore, the profound inhibition of ligand-stimulated IP₃ formation and chemotaxis demonstrates a novel action of dietary ω -3 PUFA on PI pathway-mediated signal transduction.

Methods

Subjects and study design. Informed consent was obtained from eight healthy volunteers, three men and five women aged 43±11 y (mean±SD, range 25–59 y); the volunteers were chosen to be demographically representative of the population of patients with RA entering clinical trials at the Robert B. Brigham Arthritis Center of the Brigham and Women's Hospital. The subjects supplemented their usual diet with 20 g SuperEPA (Pharmacaps, Elizabeth, NJ) daily, providing 9.4 g EPA, 5 g DHA, and 180 kcal daily, for 10 wk, beginning 4 wk after they entered the study. During the study, seven of the volunteers experienced mild gastrointestinal symptoms, which included eructation, diarrhea, and occasional mild steatorrhea; none of the volunteers withdrew from the study because of these symptoms. Pill counts and analyses of the fatty acid composition of leukocyte membranes indicated compliance with the prescribed regimen. A complete blood count with platelet count was obtained on entry to the study and after 10 wk of dietary supplementation with ω -3 PUFA. One unit of venous blood was obtained at three time points during the course of the study for the analysis of selected neutrophil and monocyte cellular functions: on entry (4 wk before starting dietary supplementation with ω -3 PUFA) and after 3 wk and 10 wk of dietary supplementation was begun. Chemotaxis, receptor affinity and density, and inositol phosphate and diglyceride formation by LTB₄ and PAF-stimulated neutrophils, and the formation of 5-lipoxygenase pathway products by calcium ionophore- and zymosan-stimulated neutrophils were evaluated. Calcium ionophore and PAF-stimulated 5-lipoxygenase pathway product generation and PAF-stimulated inositol phosphate and diglyceride formation were investigated in monocytes.

Activation of neutrophils and monocytes and quantitation of 5-lipoxygenase pathway products. Neutrophils and monocytes were isolated from the venous blood by dextran sedimentation and Ficoll-Hypaque discontinuous gradient centrifugation (9, 17). Neutrophils were purified further from contaminating red cells by hypotonic lysis. Monocytes were isolated further by adhesion on silanized glass culture dishes, as previously described (18). Before investigation, the monocyte monolayers were cultured overnight in RPMI 1640 (Gibco Laboratories, Grand Island, NY) with 100 U/ml penicillin, 100 μ g/ml streptomycin, 2 mM L-glutamine, and 5% heat-inactivated autologous serum.

5-Lipoxygenase products were quantitated by reverse-phase HPLC (RP-HPLC) in parallel, duplicate portions of neutrophil suspensions

and monocyte monolayers that had been preincubated in Tyrode's buffer with 30 mM Hepes, pH 7.4, and 1 mg/ml bovine serum albumin (THA) for 5 min at 37°C before activation. Albumin in the incubation buffer minimized the underestimation of 5*S*-hydroxy-eicosatetraenoic acid (5-HETE) formation by decreasing the esterification into cellular phospholipids of the 5-HETE that was generated (19). Duplicate portions of 4 × 10⁶ neutrophils suspended in 400 μ l THA were activated by the addition of 400 μ l of buffer alone or containing calcium ionophore A23187 at a final concentration of 10 μ M or 8 × 10⁸ boiled zymosan particles (gift of Dr. Joyce Czop), and the samples were incubated at 37°C for 15 min. The reactions were terminated by rapid cooling to 0°C. After centrifugation of the samples at 10,000 g for 1 min at 0°C, the supernatants were harvested and the cell pellets were extracted with 800 μ l of methanol (9). Both fractions were stored under a nitrogen atmosphere at -70°C until analyzed. The monocyte monolayers were preincubated in THA at 37°C for 15 min, the supernatants were discarded, and duplicate monolayers were incubated for 15 min with either 1 ml of THA alone or containing 10 μ M calcium ionophore A23187 or 1 μ M PAF (Bachem Bioscience, Inc., Torrance, CA). At the end of the incubation period, the fluid over the monocyte monolayers was decanted and retained, the monocyte monolayers were extracted with 1 ml of methanol, and both fractions were stored as described above.

For analysis on RP-HPLC, equal portions of supernatants and cell extracts were combined and the methanol in the samples was diluted by the addition of 250 μ l of distilled, deionized water. Samples were injected onto an Ultrasphere ODS 5 μ m column (4.6 × 150 mm) (Beckman Instruments, Inc., Berkeley, CA), preequilibrated with 100% solvent A (0.1% [vol/vol] aqueous acetic acid/ammonium acetate, pH 5.6: methanol:acetonitrile, 70:15:15 [vol/vol]). The column was eluted at a flow rate of 1 ml/min beginning with 100% solvent A for 3 min, followed by a step gradient to 71.4% solvent A and 28.6% 85:15 (vol/vol) methanol:acetonitrile (solvent B), with a 15-min linear gradient to 50% solvent B, followed by a 15-min linear gradient to 68.4% solvent B, and then a 12-min linear gradient to 100% solvent B. The column was recycled by washing with 100% solvent B and then reequilibrated with 100% solvent A. Product elution was quantitated by integrated optical density, continuously monitored at 269 nm (for LTs) and 234 nm (for HETEs). The column was calibrated with known quantities of synthetic standards, and the quantities reported are corrected for recovery. Retention times were as follows (mean±SD): 20-carboxy-LTB₄, 10.81±1.00 min; 20-hydroxy-LTB₅, 14.75±0.84 min; 20-hydroxy-LTB₄, 17.91±0.78 min; 5*S*,12*R*-6-*trans*-LTB₅, 24.30±0.59 min; 5*S*,12*S*-6-*trans*-LTB₅, 24.96±0.61 min; LTB₅, 26.32±0.47 min; 5*S*,12*R*-6-*trans*-LTB₄, 28.00±0.44 min; 5*S*,12*S*-6-*trans*-LTB₄, 29.68±0.47 min; LTB₄, 31.15±0.80 min; 5*S*-hydroxy-eicosapentaenoic acid (5-HEPE), 45.04±0.81 min; and 5-HETE, 50.49±2.31 min. The recoveries (mean±SD) of [³H]LTB₄ and [³H]5-HETE from the HPLC column were 75.5±7.1 and 55.4±2.3%, respectively. Synthetic LTB₄ and 6-*trans*-LTB₄ diastereoisomers were prepared as described (20); synthetic 5-HETE, 20-hydroxy-LTB₄, and 20-carboxy-LTB₄ were obtained from Cayman Chemical Co. Inc. (Ann Arbor, MI). The generation of 5-lipoxygenase pathway products is reported in ng/10⁶ cells, as the mean ±SEM, corrected for recovery. The formation of LTB₄ and LTB₅ was confirmed by RIA of the fractional eluates (9) using synthetic standards, as only modest quantities of LTB₅ were formed. Quantitation of these products by integrated optical density and RIA yielded similar results.

Chemotaxis assay. The chemotactic responses of neutrophils and mononuclear cells to concentrations (10⁻⁹–10⁻⁶ M) of synthetic LTB₄ and PAF were assessed in Boyden microchambers by determining the number of cells migrating the same distance as the leading edge in control samples; a nitrocellulose membrane with a 5- μ m pore size was used (9, 21, 22). The experiments were performed in duplicate with interassay and intraassay coefficients of variation of < 35 and < 25%, respectively (9). Results are expressed as the average number of neutrophils per 5 high power fields (hpf) after correction for background migration; 10 hpf were counted per sample.

Measurement of LTB₄ and PAF receptor density and affinity. The density and affinity of the neutrophil LTB₄ and PAF receptors were determined by the displacement by unlabeled ligand of the binding of labeled ligand (23). Duplicate portions of 2.5×10^6 neutrophils were incubated with $\sim 1.5 \times 10^{-10}$ M [³H]LTB₄ (specific activity 174 Ci/mmol; NEN-Dupont, Boston, MA) or 7.2×10^{-10} M [³H]PAF (specific activity 80 Ci/mmol; NEN-Dupont) plus concentrations (0 – 10^{-6} M) of unlabeled ligand at 0°C. The neutrophils were separated from the unbound ligand by rapid filtration on glass fiber filters under vacuum; the bound counts, retained on the filter, were eluted in 10 ml of Aquasol and counted by β-scintillation. Receptor density and affinity were determined by computer modeling with the Ligand Program for the Macintosh Computer (National Institutes of Health, Bethesda, MD). This method minimized the number of cells needed to assay receptor density and affinity.

Quantitation of phosphoinositide formation. The formation of phosphoinositides was quantitated in myo-[³H]inositol-labeled neutrophils and monocytes by an HPLC assay. Neutrophils and monocytes were labeled by incubation with myo-[³H]inositol (Amersham Corp., Arlington Heights, IL) by a modification of the technique of DiVirgilio et al. (24). Briefly, the labeling medium was inositol-free RPMI 1640 (Gibco Laboratories) with 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, 25 μCi/ml myo-[³H]inositol, and 5% heat-inactivated autologous serum. Neutrophils (75×10^6) were incubated in 5 ml of labeling medium at 37°C for 2 h. The labeled cells were washed three times in HBSS without divalent cations with 1 mg/ml BSA, resuspended in THA, and prewarmed at 37°C for 5 min before being activated. Samples of neutrophils (10^7 cells/150 μl) were activated in duplicate by the addition of LTB₄ or PAF (final concentration 1 μM) or of THA buffer alone for 15 s. The monocyte monolayers were cultured overnight in labeling medium, washed three times with HBSS without divalent cations with 1 mg/ml BSA, preincubated in THA for 15 min, and then activated in duplicate with 1 μM PAF or THA buffer alone for 15 s. The reactions of neutrophils and monocytes were terminated, and inositol phosphates were extracted by the method of Seiss (25). The extracts were evaporated to dryness under reduced pressure and redissolved in 200 μl of distilled water; 2×10^{-7} mol of AMP, ADP, and ATP were added to each sample as retention time standards. The samples were injected into a Partisil SAX10 column (250×4.6 mm) with a 10-μm pore size (Whatman Labsales, Hillsboro, OR) and eluted with increasing concentrations of ammonium formate/phosphoric acid, pH 3.7, to 2 M, using a modification of the program of Irvine et al. (26). The elution of the adenosine phosphate standards was monitored by on-line ultraviolet absorption at 257 nm, and the [³H]inositol phosphates eluted were quantitated by on-line HPLC β-scintillation counting using a scintillation counter (Radiomatic β-One; Packard Instruments Co., Inc., Canberra Industries, Meriden, CT). The retention times (mean±SD) in this system were as follows: AMP, 16.5±1.7 min; IP₁, 17.3±1.8 min; ADP, 22.6±2.5 min; IP₂, 25.0±2.7 min; ATP, 32.5±3.2 min; and IP₃, 38.2±3.6 min. The recovery of [³H] standards (Amersham Corp.) were as follows: IP₁, 76.8±1.3%; IP₂, 76.1±1.7%; and IP₃, 70.6±5.0%; the quantum efficiency of measurement of the tritiated products by the on-line β-scintillation counter was 22%.

Quantitation of 1,2-diacylglycerol formation. Diacylglycerols were quantitated by mass measurement by the method of Preiss et al. (27). Duplicate portions of 10^7 neutrophils were suspended in 400 μl THA containing 5 μg/ml cytochalasin B and were warmed at 37°C for 5 min. The duplicate portions were activated by the addition of LTB₄ or PAF at a final concentration of 1 μM in THA with 5 μg/ml cytochalasin B or by the addition of cytochalasin B and buffer alone for control cells; all portions were incubated at 37°C for 2 min. Duplicate monocyte monolayers were preincubated at 37°C with 1 ml THA containing 5 μg/ml cytochalasin B for 5 min. The overlying buffer was discarded, and duplicate monolayers were activated by the addition of 1 μM PAF in THA with 5 μg/ml cytochalasin B or by the addition of buffer containing 5 μg/ml cytochalasin B alone for control cells, and the samples were incubated at 37°C for 2 min. The reactions were terminated by

the addition of an equal volume of methanol and rapid cooling on a dry ice/acetone slurry. Lipids were extracted by the method of Bligh and Dyer as modified by Preiss et al. (27), and 1,2-diacylglycerols were quantitated as [³²P]phosphatidic acids after [³²P]_i-labeling with γ-[³²P]ATP catalyzed by diacylglycerol kinase as described (27). The incubation period and the concentration of cytochalasin B were chosen to maximize the formation of 1,2-diacylglycerols.

Assays of the fatty acid composition of neutrophil and monocyte membranes. The fatty acid composition of the major phospholipid subclasses of neutrophil and monocyte cellular lipids were determined for each individual subject before and after 3 and 10 wk of dietary supplementation with ω-3 PUFA to assess whether changes in the fatty acid composition of a particular phospholipid subclass(es) resulted in the observed functional changes. Cellular lipids were extracted by the Folch et al. procedure (28), and the phospholipids were separated into classes by straight-phase HPLC (29). To separate the ether-linked phospholipids, the phosphatidylethanolamine and phosphatidylcholine fractions were digested with phospholipase C from *Bacillus cereus*, and the resulting diradylglycerols were benzoylated to form the corresponding 3-benzoyl derivatives (30). The benzoyl-diradylglycerols were separated into the three major fractions, the alk-1-enyl and alkyl ethers and the diacyl fractions by straight-phase HPLC (30). The elution of the fractions was monitored and quantitated by benzoyl group absorbance at 230 nm. The fatty acid composition of each phospholipid class and subclass was determined after acid-catalyzed transmethylation of each class and subclass, resolution of the fatty acids as the methyl ester derivatives by capillary gas chromatography, and quantitation by flame ionization (31).

Data analysis and statistics. The data were analyzed for overall statistical significance by a repeated measures design analysis of variance, followed by the Student's protected least-significant difference *t* test to make individual pairwise comparisons on a Macintosh IIcx computer using the StatView 512+ statistics package (Abacus Concepts, Inc., Berkeley, CA). The formation of phosphoinositides and maximal chemotaxis were analyzed as above after a logarithmic transformation, and the dose-response data for chemotaxis were analyzed by a repeated-measures design multivariate analysis of variance with zero between and two within variables after logarithmic transformation, followed by means comparisons to make individual pairwise comparisons, on a Macintosh IIcx computer using the SuperANOVA statistics package (Abacus Concepts, Inc.). Correlations were made with the simple regression program in the StatView 512+ statistics package using the data from each time point for each individual. Data are presented as the mean±SEM unless otherwise stated.

Results

Neutrophil fatty acid composition. The fatty acid composition of the major neutrophil phospholipid classes and subclasses were modified considerably after 3 and 10 wk of dietary supplementation with ω-3 PUFA (Table I). The AA contents of the pools of 1,2-diacyl-*sn*-glycero-3-phosphocholine (diacylIPC), 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphocholine (alkylacylIPC), 1,2-diacyl-*sn*-glycero-3-phosphoethanolamine (diacylPE), and 1-*O*-alk-1'-enyl-2-acyl-*sn*-glycero-3-phosphoethanolamine (alkenylacylPE) decreased significantly after 3 and 10 wk of dietary supplementation with ω-3 PUFA, and there were no significant differences between the 3 and 10 wk time points. However, the AA content of the PI pool was not significantly affected. The EPA contents of each of these subclasses increased substantially after 3 and 10 wk of dietary supplementation with ω-3 PUFA above prediet values.

Neutrophil receptors for LTB₄ and PAF. The binding of LTB₄ to neutrophils was fitted to a two-site model with the Ligand program. Neither the density nor the affinity of the high and low affinity receptors of neutrophils for LTB₄ was signifi-

Table I. Fatty Acid Composition of the Major Neutrophil Phospholipid Classes and Subclasses before and 3 and 10 wk after Dietary Supplementation with ω -3 PUFA

	Prediet	Week 3	Week 10
Phosphatidylinositol			
Arachidonic acid	22.8±1.7	25.9±1.0	19.9±1.4
Total ω -6 PUFA	37.7±2.2	38.9±0.6	32.4±1.6*
Eicosapentaenoic acid	0.2±0.1	1.7±0.2**	1.9±0.2**
Docosahexaenoic acid	1.1±0.1	0.2±0.0	1.4±0.1
Total ω -3 PUFA	2.2±0.5	3.7±0.3	5.2±1.0 [§]
diacyl-Phosphatidylcholine			
Arachidonic acid	2.7±0.3	1.5±0.1 [†]	1.6±0.1 [†]
Total ω -6 PUFA	16.7±1.1	13.9±1.1	14.9±0.9
Eicosapentaenoic acid	0.1±0.0	0.8±0.1**	0.9±0.2**
Docosahexaenoic acid	0.4±0.1	0.3±0.0	0.7±0.2
Total ω -3 PUFA	0.9±0.2	1.6±0.1 [‡]	2.2±0.4
alkylacyl-Phosphatidylcholine			
Arachidonic acid	13.1±0.7	9.2±0.5**	8.4±0.7**
Total ω -6 PUFA	41.3±1.6	35.6±1.1 [§]	33.9±1.5 [§]
Eicosapentaenoic acid	0.4±0.1	4.8±0.4**	8.4±0.7**
Docosahexaenoic acid	1.7±0.2	1.6±0.1	2.7±0.5
Total ω -3 PUFA	3.6±0.4	10.6±0.7**	12.3±0.8**
diacyl-Phosphatidylethanolamine			
Arachidonic acid	8.3±1.1	4.0±0.3 [§]	5.2±0.6 [§]
Total ω -6 PUFA	20.6±2.3	12.6±0.5 [§]	14.3±0.7 [‡]
Eicosapentaenoic acid	0.1±0.0	1.0±0.1**	1.5±0.1**
Docosahexaenoic acid	0.8±0.1	0.5±0.1	1.2±0.2
Total ω -3 PUFA	1.6±0.3	2.8±0.2 [§]	4.4±0.4**
alk-1-enylacyl-Phosphatidylethanolamine			
Arachidonic acid	30.2±1.6	18.4±0.9**	19.8±1.0**
Total ω -6 PUFA	54.0±1.9	37.0±0.7**	39.6±1.5**
Eicosapentaenoic acid	0.9±0.1	10.4±0.6**	9.6±0.8**
Docosahexaenoic acid	2.7±0.2	3.1±0.2	3.7±0.4*
Total ω -3 PUFA	9.3±1.3	24.5±1.1**	22.4±1.5**

* $P < 0.05$. [‡] $P < 0.01$. [§] $P < 0.005$. ^{||} $P < 0.001$. [†] $P < 0.0005$. ** $P < 0.0001$.

cantly affected by dietary supplementation with ω -3 PUFA. The binding of PAF to neutrophils was fitted by the Ligand program to a one-site model; the specific activity of [³H]PAF used in this study was insufficient to assess the high affinity receptor. The dietary supplementation did not significantly affect the density or the affinity of the PAF receptors on neutrophils.

Neutrophil phosphoinositide formation. In the prediet period, levels of IP₃ in LTB₄- and PAF-stimulated neutrophils were 3.2- and 5-fold higher than in the buffer-treated neutrophils ($P = 0.012$ and 0.0004 , respectively, $n = 8$). In Fig. 1, the suppression at 3 and 10 wk of dietary supplementation with ω -3 fatty acids of phosphoinositide formation in neutrophils stimulated with LTB₄ in a representative subject is shown in relation to the baseline. The net formation (buffer-control levels subtracted) of IP₃ in neutrophils stimulated by LTB₄ for 15 s decreased by 71% after 3 wk of dietary supplementation ($0.033 \pm 0.013\%$, $P = 0.025$) and by 90% after 10 wk of dietary supplementation ($0.011 \pm 0.011\%$, $P = 0.015$) from prediet values ($0.114 \pm 0.030\%$) (Fig. 2A). Similarly, net formation of IP₃ by PAF-stimulated neutrophils decreased by 62% after 3 wk ($0.074 \pm 0.029\%$, $P = 0.005$) and by 96% after 10 wk ($0.008 \pm 0.005\%$, $P = 0.0004$) from prediet values ($0.194 \pm 0.038\%$)

(Fig. 2B). Comparable, statistically significant decreases were observed in the net formation of IP₁ and IP₂ by LTB₄-stimulated neutrophils (IP₁: 76%, $P = 0.012$, week 3 and 100%, $P = 0.01$, week 10; IP₂: 65%, $P = 0.0046$, week 3 and 100%, $P = 0.0005$, week 10) and for PAF-stimulated neutrophils (IP₁: 71%, $P = 0.0052$, week 3 and 75%, $P = 0.0052$, week 10; IP₂: 82%, $P = 0.012$, week 3 and 95%, $P = 0.0098$, week 10). Although LTB₄- and PAF-stimulated net IP₃ formation correlated positively and significantly with the AA contents of the alkylacylPC and alkenylacylPE pools, generally, LTB₄- and PAF-stimulated IP₃ formation correlated negatively but much better with the EPA contents of each of the major phospholipid subclasses in the neutrophil ($P = 0.0005$ – 0.0076) as evaluated for each subject at each time point. Despite the minimal EPA contents of the PI pool (~ 2 mol%), LTB₄-stimulated net IP₃ formation correlated negatively and most strongly with the EPA content of the PI pool ($P = 0.0005$). PAF-stimulated IP₃ formation also correlated strongly and negatively with the EPA content of the PI pool ($P = 0.0012$).

Neutrophil diacylglycerol formation. In the prediet period, the stimulation of neutrophils with 1 μ M LTB₄ or 1 μ M PAF for 2 min in the presence of cytochalasin B resulted in significant increases in diglyceride formation above buffer control

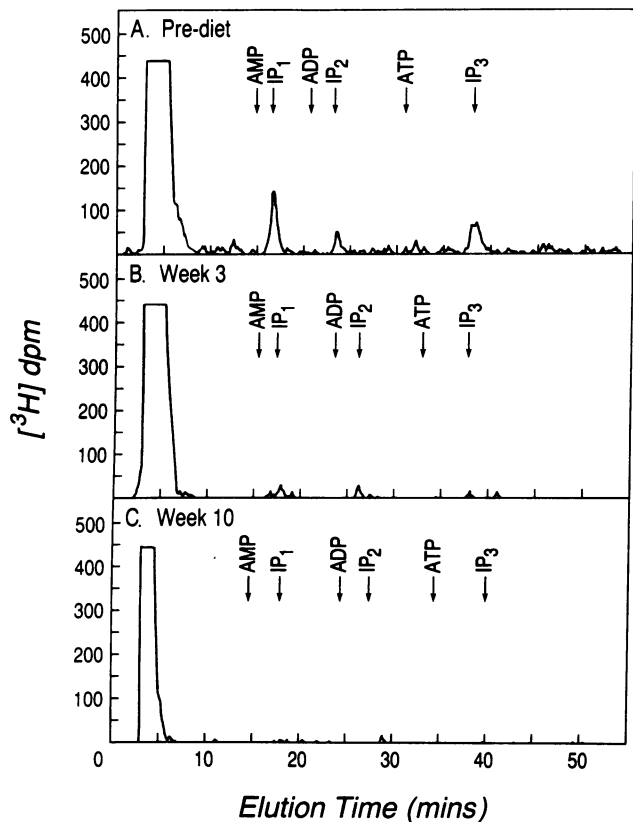


Figure 1. The inositol phosphates released by LTB₄-stimulated neutrophils. The chromatograms from one study subject in the prediet period (A) and after 3 wk (B) and 10 wk (C) of dietary supplementation with ω -3 PUFA. Neutrophils were labeled with [³H]myo-inositol and stimulated with 1 μ M LTB₄. Inositol monophosphate (IP₁), inositol bisphosphate (IP₂), and inositol trisphosphate (IP₃) were quantitated by on-line β -scintillation counting after resolution on anion-exchange HPLC. Data are expressed as dpm.

levels (data not shown). Dietary supplementation with ω -3 PUFA did not induce statistically significant changes in LTB₄- and PAF-stimulated net diglyceride formation (data not shown) under conditions where most of the diglyceride derives from the phospholipase D pathway (32, 33).

Neutrophil chemotaxis. In the prediet period neutrophil chemotactic responsiveness both to LTB₄ and to PAF increased with increasing concentrations (10^{-9} – 10^{-6} M) of synthetic LTB₄ and PAF as assessed in Boyden microchambers (Fig. 3). Dietary supplementation with ω -3 PUFA significantly lowered the neutrophil chemotactic dose-response curve to LTB₄ and PAF; significant decreases were observed 3 wk after the dietary supplement ($P = 0.0016$ for LTB₄, $P = 0.019$ for PAF) as compared with the baseline chemotactic dose-response curves. Further lowering of the neutrophil chemotactic dose-response curves to LTB₄ and PAF was observed after 10 wk of dietary supplementation as compared with the week 3 ($P \leq 0.0001$ for LTB₄ and $P = 0.0018$ for PAF) and prediet periods ($P \leq 0.0001$ for both LTB₄ and PAF). The mean of the neutrophil chemotactic responses to LTB₄ at the most effective concentration over the entire dose-response curve for each subject (mean maximal response) decreased by 69% after 3 wk (113 ± 19 cells/5 hpf, $P = 0.0034$) and by 93% after 10 wk of dietary supplementation (26 ± 5 cells/5 hpf, $P \leq 0.0001$), as compared with the prediet period (361 ± 78 cells/5 hpf). Similarly, the mean maximal response to PAF was decreased by 64% after 3 wk (130 ± 33 cells/5 hpf, $P = 0.0033$) and by 92% after 10 wk of dietary supplementation (28 ± 5 cells/5 hpf, $P \leq 0.0001$), as compared with the prediet period (358 ± 89 cells/5 hpf). Neutrophil chemotaxis to both agonists correlated well with the AA contents of the alkylacylPC and alkenylacylPE pools, and it correlated highly and negatively with the EPA contents of each of the major phospholipid pools ($P = 0.0009$ – 0.0001 for LTB₄; $P = 0.0067$ – 0.0005 for PAF). Although the EPA content of the PI pool was affected minimally (<2 mol%) but significantly ($P \leq 0.0001$) by the dietary supplementation

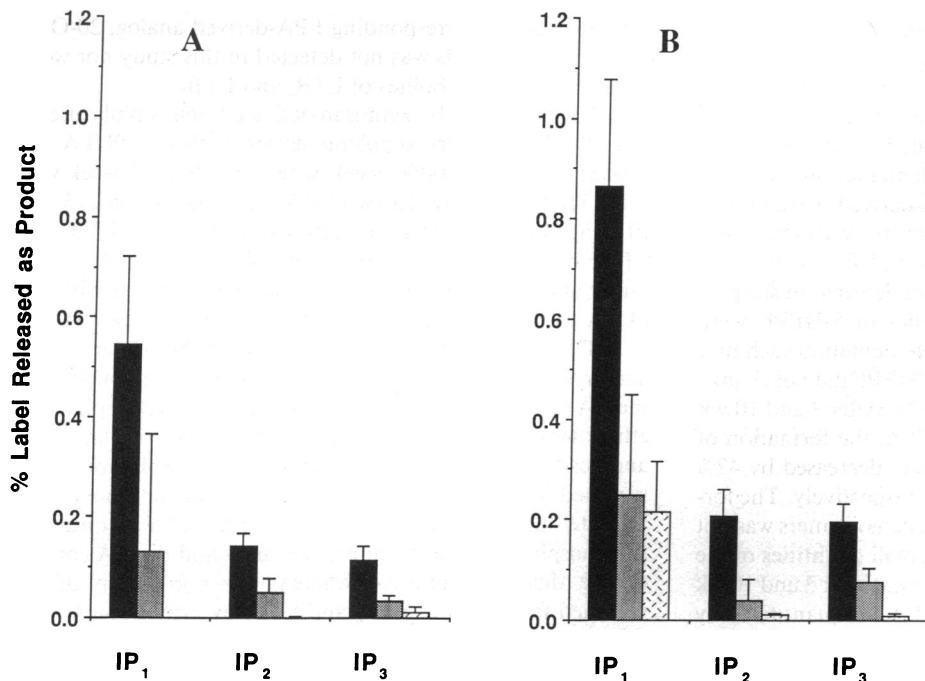


Figure 2. The effects of dietary ω -3 PUFA on the release of inositol phosphates from neutrophils. Net percent release of [³H] as IP₁, IP₂, and IP₃ in the prediet period (black bars) and after 3 (gray bars) and 10 wk (patterned bars) of dietary supplementation with ω -3 PUFA as quantitated by on-line β -scintillation counting after resolution on anion-exchange HPLC. (A) LTB₄-stimulated neutrophils. (B) PAF-stimulated neutrophils. Data are expressed as net percent release, mean \pm SEM, $n = 8$, minus buffer control levels.

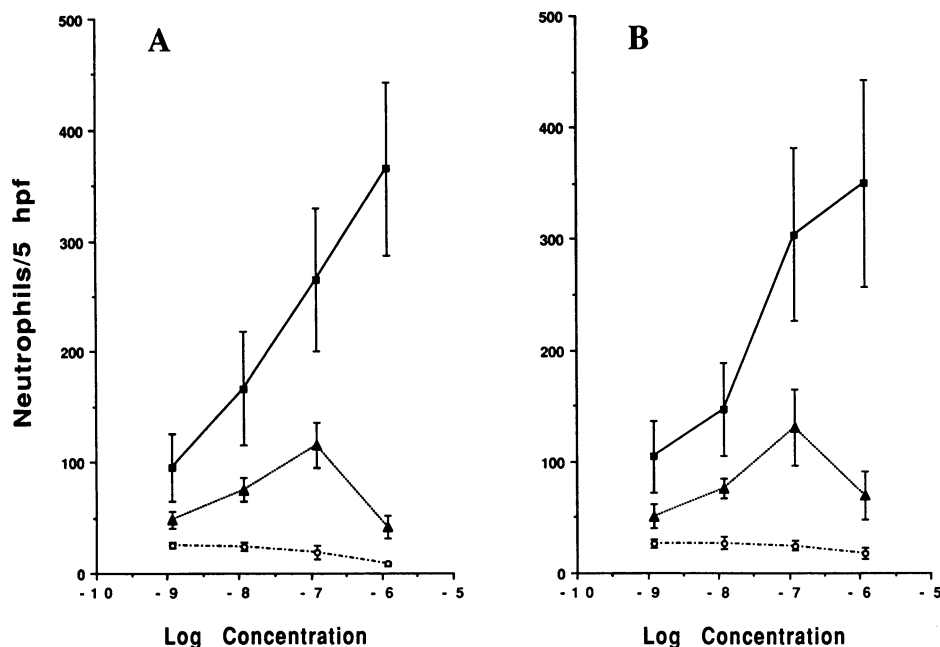


Figure 3. The effects of dietary ω -3 PUFA on the chemotaxis of human neutrophils in the prediet period (\blacksquare — \blacksquare) and after 3 (\blacktriangle — \blacktriangle) and 10 wk (\circ — \circ) of dietary supplementation with ω -3 PUFA as quantitated ex vivo in Boyden microchambers. (A) Chemotaxis of neutrophils to concentrations of LTB_4 . (B) Chemotaxis of neutrophils to concentrations of PAF. Data are expressed as neutrophils per 5 hpf, mean \pm SEM, $n = 8$.

with ω -3 PUFA, the negative correlation of neutrophil chemotaxis to both LTB_4 and PAF with the EPA content of the PI pool in neutrophils was highly significant (LTB_4 , $P = 0.0003$; PAF, $P = 0.0013$). Furthermore, the mean maximal chemotactic response to LTB_4 after dietary supplementation with ω -3 PUFA correlated strongly with IP_3 formation by LTB_4 -stimulated neutrophils ($P \leq 0.0001$). Likewise, the mean maximal chemotactic response to PAF correlated strongly with IP_3 formation by PAF-stimulated neutrophils ($P = 0.0032$).

Monocyte phosphoinositide and diglyceride formation. The net formation of total phosphoinositides in PAF-stimulated monocytes decreased modestly but did not reach statistical significance ($P = 0.19$), reflecting the absence of significant decreases in the net formation of IP_1 , IP_2 , and IP_3 ($P > 0.2$ for each). Similarly, the net formation of diglyceride in PAF-stimulated monocytes was not significantly affected by dietary supplementation with ω -3 PUFA ($P > 0.2$).

Formation of 5-lipoxygenase pathway products by neutrophils. The formation of AA-derived 5-lipoxygenase pathway products by calcium ionophore-activated neutrophils decreased substantially after dietary supplementation with ω -3 PUFA (Fig. 4 A). The formation of AA-derived 5-HETE decreased by 44% after 3 wk and by 65% after 10 wk of dietary ω -3 fatty acid supplementation ($P = 0.0034$, and $P = 0.0004$, respectively). EPA-derived 5-HEPE was not detected in the prediet period, whereas substantial quantities of 5-HEPE were formed after 3 and 10 wk of dietary supplementation such that the summed quantities of 5-HETE and 5-HEPE did not change appreciably over this time period ($P > 0.2$). After 3 and 10 wk of dietary supplementation with ω -3 PUFA, the formation of AA-derived 6-*trans*- LTB_4 diastereoisomers decreased by 42% ($P = 0.0013$) and by 57% ($P = 0.0002$), respectively. The formation of EPA-derived 6-*trans*- LTB_5 diastereoisomers was not detected in the prediet period, whereas small quantities of the 6-*trans*- LTB_5 diastereoisomers were observed after 3 and 10 wk of dietary supplementation with ω -3 PUFA. As quantitated by RIA, the formation of AA-derived LTB_4 by calcium ionophore-activated neutrophils decreased by 71% after 3 wk (P

$= 0.0061$) of dietary supplementation and by 77% after 10 wk ($P = 0.0058$) compared with the prediet period. RIA revealed the generation of small quantities of EPA-derived LTB_5 in the prediet period; after dietary supplementation increased quantities of LTB_5 were detected by both integrated optical density and RIA (256%, $P = 0.0008$, week 3; 205%, $P = 0.0022$, week 10; quantitated by RIA). The summed formation of LTB_4 and LTB_5 decreased significantly and substantially after 3 and 10 wk of dietary supplementation (66%, $P = 0.0083$, week 3; 73%, $P = 0.0077$, week 10; quantitated by RIA), suggesting a selective action on the 5-lipoxygenase pathway at this step, catalyzed by the LTA epoxide hydrolase enzyme. The formation of 20-OH- LTB_4 , a metabolite of AA-derived LTB_4 , decreased by 46% after 3 and 10 wk of dietary supplementation with ω -3 PUFA ($P = 0.017$ for each) relative to the prediet period; the formation of the corresponding EPA-derived analog, 20-OH- LTB_5 , by neutrophils was not detected in this study nor were the 20-carboxy metabolites of LTB_4 and LTB_5 .

LTB_4 formation by zymosan-activated neutrophils after 3 and 10 wk of dietary supplementation with ω -3 PUFA decreased (61%, $P = 0.0006$, week 3; 58%, $P = 0.0007$, week 10), and LTB_5 formation increased (325%, $P = 0.004$, weeks 3 and 10) (Fig. 4 B). However, the summed formation of LTB_4 and LTB_5 by zymosan-activated neutrophils decreased significantly after 3 and 10 wk of dietary supplementation with ω -3 PUFA (30%, $P = 0.02$, week 3; 28%, $P = 0.02$, week 10).

LTB_4 formation in calcium ionophore- and zymosan-stimulated neutrophils correlated positively and most strongly with the AA contents of the phosphatidylcholine and phosphatidylethanolamine subclasses, and LTB_5 formation correlated best and positively with the EPA content of the phospholipids. The summed formation of LTB_4 and LTB_5 by calcium ionophore-activated neutrophils correlated best with the EPA content of the phosphatidylethanolamine subclasses and the AA content of the diacylPE subclass, whereas the correlation of the summed formation of LTB_4 and LTB_5 by zymosan-activated neutrophils with the fatty acid composition of the neutrophil phospholipid subclasses did not reach statistical significance.

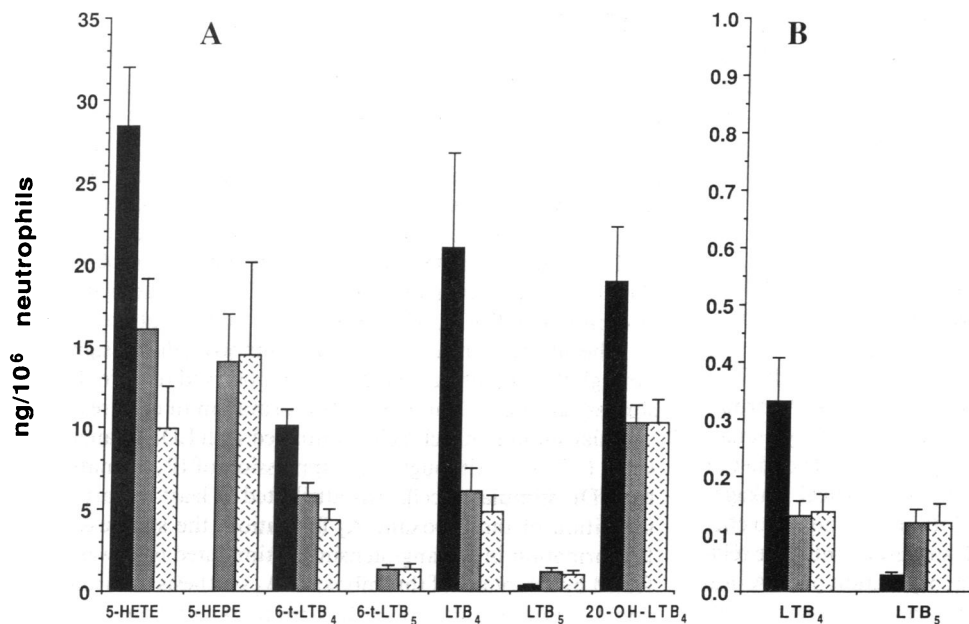


Figure 4. 5-Lipoxygenase pathway product generation by human neutrophils. LTB₄ and LTB₅ were quantitated by RIA after resolution on RP-HPLC, and the other 5-lipoxygenase products were quantitated by on-line integrated optical density after resolution on RP-HPLC, in the prediet period (black bars) and after 3 (gray bars) and 10 wk (patterned bars) of dietary supplementation with ω -3 PUFA. (A) Calcium ionophore A23187-activated neutrophils. (B) Zymosan-stimulated neutrophils. Data are expressed as ng/10⁶ neutrophils, mean \pm SEM, $n = 8$.

Formation of 5-lipoxygenase pathway products by monocytes. The formation of AA-derived 5-lipoxygenase pathway products of monocytes decreased substantially after dietary supplementation with ω -3 PUFA (Fig. 5 A). The formation of 5-HETE by calcium ionophore-activated monocytes decreased by 65% after 3 wk and by 47% after 10 wk of dietary supplementation ($P = 0.026$ and $P = 0.048$, respectively). EPA-derived 5-HEPE was not detected in the prediet period, but substantial quantities of 5-HEPE were formed after 3 and 10 wk of dietary supplementation such that the summed quantities of 5-HETE and 5-HEPE did not change appreciably over this time period ($P > 0.2$). After 3 and 10 wk of dietary supplementation with ω -3 PUFA, apparent decreases in the formation of AA-derived 6-*trans*-LTB₄ diastereoisomers were not statistically significant ($P = 0.17$). EPA-derived 6-*trans*-LTB₅ diaste-

reoisomers were not detected. The formation of LTB₄ by calcium ionophore-activated monocytes decreased significantly after 3 and 10 wk of dietary supplementation as compared with the prediet period (57%, $P = 0.0021$, week 3; 70%, $P = 0.0009$, week 10; quantitated by RIA). The generation of immunoreactive LTB₅ increased after dietary supplementation with ω -3 PUFA from the small quantities observed in the prediet period (432%, $P = 0.0007$, week 3; 295%, $P = 0.005$, week 10). Nevertheless, the summed formation of LTB₄ and LTB₅ by calcium ionophore-activated monocytes decreased significantly and substantially after 3 and 10 wk of dietary ω -3 PUFA supplementation (42%, $P = 0.025$, week 3; 58%, $P = 0.0093$, week 10; quantitated by RIA).

LTB₄ formation by PAF-activated monocytes decreased after 3 and 10 wk of dietary supplementation (57%, $P = 0.013$,

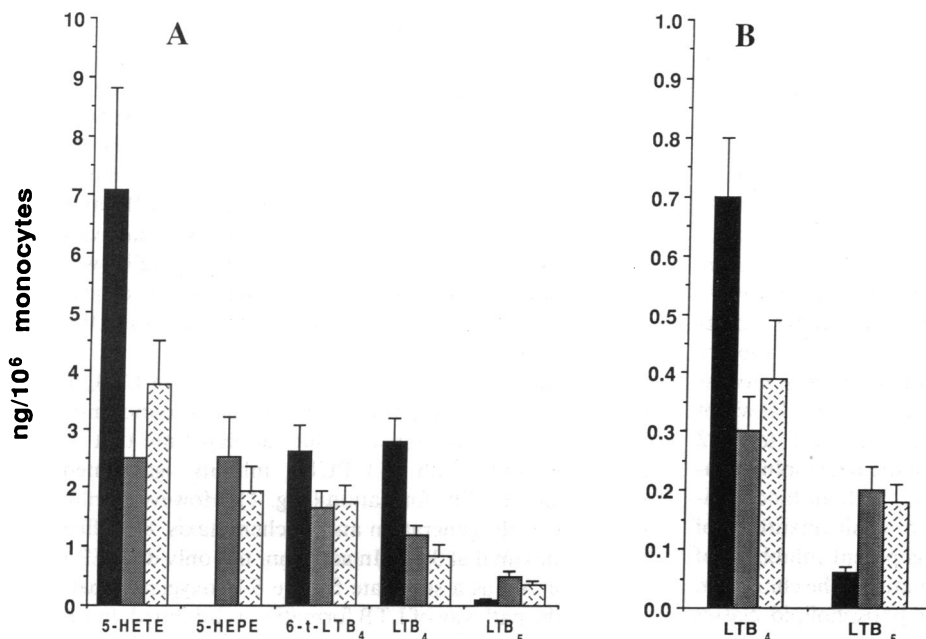


Figure 5. 5-Lipoxygenase pathway product generation by human monocyte monolayers. LTB₄ and LTB₅ were quantitated by RIA after resolution on RP-HPLC and the other 5-lipoxygenase products were quantitated by on-line integrated optical density after resolution on RP-HPLC, in the prediet period (black bars) and after 3 (gray bars) and 10 wk (patterned bars) of dietary supplementation with ω -3 PUFA. (A) Calcium ionophore A23187-activated monocytes. (B) PAF-stimulated monocytes. Data are expressed as ng/10⁶ monocytes, mean \pm SEM, $n = 8$.

week 3; 45%, $P = 0.022$, week 10) and LTB_5 formation increased (265%, $P = 0.006$, week 3; 235%, $P = 0.0072$, week 10) (Fig. 5 B). The decreases in the summed formation of LTB_4 and LTB_5 by PAF-activated monocytes after 3 and 10 wk of dietary supplementation with ω -3 PUFA (33%, week 3; 24%, week 10) approached but did not reach statistical significance ($P = 0.20$).

Discussion

This study of the effects of dietary enrichment with ω -3 PUFA differs from previous studies in the parallel assessment of ligand-initiated signal transduction (inositol phosphates and diglycerides) and function (chemotaxis) in neutrophils. The profound suppression of LTB_4 - and PAF-stimulated IP_3 formation and chemotaxis in human neutrophils induced by dietary ω -3 PUFA demonstrates a potentially significant role of diet in the modulation of cell signals. The tight statistical linkages among EPA incorporation into PI and IP_3 production and chemotaxis in response to LTB_4 and PAF suggest an intimate relationship in neutrophils between the incorporation of EPA into PI and the suppression of the biochemical and functional responses to the transmembrane signals. The absence of a statistically significant relationship between EPA incorporation into PI and the summed formation of LTB_4 and LTB_5 in response to either a particulate activator (zymosan) or the calcium ionophore indicates an independent, parallel action, through the formation of the EPA-derived intermediate LTA_5 , which inactivates the LTA epoxide hydrolase (34). Together, the inhibition of LTB_4 generation, the production of the functionally attenuated LTB_5 , and the suppression of the transduction of ligand-receptor signals negate the system of LTB_4 -mediated amplification of the neutrophil inflammatory response.

After 3 wk of high dose dietary supplementation with ω -3 PUFA, neutrophil chemotaxis to LTB_4 and PAF was dramatically inhibited (Fig. 3); mean maximal neutrophil chemotaxis to LTB_4 and PAF was inhibited by 69 and 64%, respectively; it was further suppressed after 10 wk of dietary supplementation. Perhaps because of the higher EPA and total ω -3 PUFA dose used in this study, the magnitude of the inhibition of neutrophil chemotaxis was greater and appeared earlier than in previous studies (9, 11, 12). The formation of IP_3 in LTB_4 - or PAF-stimulated neutrophils was nearly totally suppressed after dietary supplementation; the net formation of IP_1 and IP_2 was inhibited similarly (Figs. 1 and 2). The neutrophil chemotactic response to both LTB_4 and PAF correlated strongly with the net IP_3 formation by LTB_4 - and PAF-stimulated neutrophils, suggesting a causal relationship between the suppression of IP_3 formation and chemotaxis. The absence of a significant effect of the dietary ω -3 PUFA supplementation on the densities or affinities of the respective receptors and the previous study (9) showing no change in membrane fluidity demonstrates a post-receptor inhibition of the LTB_4 and PAF signals by the dietary supplementation with ω -3 PUFA. Despite only very modest alterations in the PI pool in neutrophils after 3 and 10 wk of dietary supplementation (EPA content increased to ~ 2 mol%), the EPA content of the PI pool of the neutrophils (Table 1) correlated significantly and negatively with the biochemical and functional responses. Thus even small quantities of EPA-containing species of PI may act as potent inhibitors of phospholipase C, thus inhibiting IP_3 formation. The changes in the fatty acid composition of the other phospholipid classes and subclasses may also contribute to the modulation of signal

transduction and function in neutrophils. Dietary ω -3 fatty acid supplementation did not substantially alter LTB_4 - or PAF-induced net increments in neutrophil diglyceride levels. We interpret our data that dietary PUFA supplementation inhibited IP_3 formation but did not appreciably affect diacylglycerol formation as consistent with the inhibition of a PI-selective phospholipase C and an absence of an effect on phospholipase D by dietary supplementation with ω -3 PUFA. The recent evidence that the major portion of the 1,2-diacylglycerol generated by stimulation of cytochalasin B-treated neutrophils with chemotactic agents is derived from PC by the activation of phospholipase D (32, 33) supports this interpretation.

The alteration by ω -3 PUFA of receptor-mediated signaling through the PI pathway has also been observed in vitro. In two studies, ω -3 PUFA suppressed IP_3 formation in cultures of rat vascular smooth muscle cells stimulated with LDL or angiotensin II (35, 36). Although this suppression of IP_3 formation in the LDL-stimulated cells was attributed at least in part to the inhibition of thromboxane A_2 formation, the suppression of IP_3 formation in the angiotensin II-stimulated cells was unaffected by inhibitors of thromboxane A_2 synthesis or thromboxane A_2 receptor antagonists. In vitro preincubation of aspirin-treated rabbit platelets with EPA inhibits inositol phosphate formation (including IP_3 formation) stimulated by the thromboxane A_2 receptor agonist, U46619 (37). Medini et al. (38) recently reported that dietary ω -3 PUFA also inhibits thrombin-stimulated inositol phosphate formation by rabbit platelets without measurably affecting thromboxane formation. Weber et al. (39) have suggested that in vitro preincubation with DHA blunts the rise in intracellular calcium ion concentration in PAF- and LTD_4 -stimulated, retinoic acid-differentiated U937 cells through a noncyclooxygenase-mediated oxidation product of DHA. In the present study, such an effect of a DHA metabolite appears unlikely because it would need to occur early in the 15-s activation period to inhibit IP formation and because the only significant change in the content of DHA in neutrophil phospholipids was a minimal increase in the DHA content of alkenylacylPE only at 10 wk. As neutrophils are short lived *ex vivo*, only studies of dietary enrichment allow the achievement of a steady state necessary for the optimal interpretation of the neutrophil fatty acid composition data. Furthermore, in platelets, in which an in vitro steady state modulation of the fatty acid composition is possible, in vivo and in vitro studies yield different results (40), demonstrating the importance of the in vivo dietary studies. The current approach of dietary supplementation with ω -3 PUFA has demonstrated for the first time a tight negative correlation between EPA incorporation into PI and agonist-induced formation of IP_3 and chemotaxis. In the only other published study (37) that evaluated the fatty acid composition of cellular PI in parallel with the quantitation of inositol phosphate formation, the exposure to ω -3 PUFA was in vitro, and no significant changes in the fatty acid composition of PI were observed.

The significant decreases in the formation of LTB_4 and in the summed formation of LTB_4 and LTB_5 by zymosan-stimulated neutrophils demonstrate for the first time that dietary supplementation with ω -3 PUFA inhibits ligand-mediated stimulation of LTB_4 formation (Fig. 4). However, unlike the decreases in IP_3 generation and in chemotaxis, this effect was already maximal at 3 wk. In addition, not only does EPA substitute for AA as a substrate for the 5-lipoxygenase pathway, but also the pathway of LTB_4 formation is inhibited. In calcium ionophore-activated neutrophils, a selective inhibition of the

summed formation of LTB₄ plus LTB₅, in parallel with a decrease in the ω -oxidation products, was observed; the summed formation of 5-HETE plus 5-HEPE remained unchanged by dietary ω -3 PUFA supplementation, suggesting inhibition of the LTA epoxide hydrolase. These results are in agreement with the findings of Terano et al. (11) and Hawthorne et al. (41) that dietary ω -3 fatty acid supplementation causes selective inhibition of calcium ionophore-stimulated neutrophil LTB formation, whereas Lee et al. (9) found that dietary fish oil supplementation inhibited formation of all 5-lipoxygenase pathway products in ionophore-stimulated neutrophils. Nathaniel et al. (34) demonstrated that LTA₅, derived from EPA, covalently binds to the LTA epoxide hydrolase and irreversibly inhibits the enzyme. The decreases in the formation of LTB₄ and the summed formation of LTB₄ and LTB₅ in zymosan-stimulated neutrophils were also significant.

In monocytes neither the decreases in PAF-stimulated levels of total phosphoinositides nor the changes in diglyceride formation after ω -3 PUFA supplementation were significant. In the studies of Schmidt et al. (12) the inhibition by dietary ω -3 PUFA supplementation of the chemotaxis in monocytes was less than that observed in neutrophils. In calcium ionophore-activated monocytes, a selective inhibition of LTB₄ plus LTB₅ formation was observed; the formation of 5-HETE plus 5-HEPE was unchanged by dietary supplementation with ω -3 PUFA, again indicating inhibition of the epoxide hydrolase (Fig. 5). LTB formation by PAF-stimulated monocytes was also inhibited, but the degree of inhibition was less than that in calcium ionophore-activated monocytes.

The significant, tight correlation of the inhibition of chemotaxis and inositol phosphate formation with the EPA content of the neutrophil PI pool in the absence of a significant effect on neutrophil LTB₄ and PAF receptor density or affinity and on diglyceride formation indicates that the inhibition of neutrophil chemotaxis to LTB₄ and PAF by dietary ω -3 PUFA supplementation is due to a selective postreceptor inhibition of the ligand-initiated signals via the PI-selective phospholipase C. The selective inhibition of the formation both of LTB and its metabolites in calcium ionophore-activated neutrophils and of LTB generation in zymosan-activated neutrophils plateaued at 3 wk and did not correlate with the incorporation of EPA into the PI pool. In contrast, the ligand-stimulated generation of IP₃ and chemotaxis correlated negatively with the incorporation of EPA into the PI pool and was suppressed further after 10 wk of dietary supplementation. These differences are most consistent with an independent but parallel inhibition of the LTA epoxide hydrolase enzyme. Together, these data on ligand stimulation of human neutrophils reveal that incorporation of dietary EPA attenuates proinflammatory responses mediated by LTB₄ by reducing the formation of this mediator, by substituting a functionally attenuated mediator (LTB₅) (42), and by inhibiting LTB₄ receptor-mediated signal transduction as exemplified by the decreases in the formation of the inositol phosphates and in chemotaxis. The effects on cell signal transduction in the neutrophil were dramatic and reveal a novel and reversible (9) dietary-based approach to the modulation of ligand-initiated signaling via the phosphatidyl inositol pathway.

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