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The Effect of Eicosapentaenoic Acid on Leukotriene B Production by Human Neutrophils*

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Eicosapentaenoic acid, which is a major fatty acid in fish oil, previously has been shown to competitively inhibit the cyclooxygenase-catalyzed metabolism of arachidonic acid in platelets. In the present study the effect of eicosapentaenoic acid on the production of leukotriene B via the lipoxygenase pathway in human neutrophils was examined. Eicosapentaenoate was incorporated into complex lipids of neutrophils at the same rate as arachidonate; release of the two homologous fatty acids in response to calcium ionophore A23187 was equivalent and both fatty acids were metabolized to a leukotriene B. The products derived from eicosapentaenoic acid were identified as leukotriene B_5 and its stereoisomers. Eicosapentaenoate was a less favorable substrate for leukotriene B_5 synthesis (94 ng/10^7 cells/5 min at 20 μM exogenous fatty acid) than arachidonate was for leukotriene B_4 (401 ng under the same conditions). However, eicosapentaenoate or an oxygenated product inhibited arachidonate metabolism since at equimolar concentrations of eicosapentaenoate and arachidonate leukotriene B_4 production was decreased by 68%. The inhibitory effect occurred at the level of leukotriene A hydrolase. The biological activity of eicosapentaenoate-derived products was tested; leukotriene B_5 was found to have only approximately 10% of the potency of leukotriene B_4 in inducing the aggregation of neutrophils, and the stereoisomers of leukotriene B_5 were inactive. These data suggest that diets enriched in eicosapentaenoic acid affect neutrophils by decreasing the quantity of leukotriene B and by the production of a less potent leukotriene.

Eicosapentaenoic acid is known to compete with arachidonic acid in the cyclooxygenase pathway that leads to synthesis of prostaglandins, thromboxanes, and prostacyclins (1, 2). It is via this mechanism that eicosapentaenoate is thought to favorably influence the occurrence of cardiovascular disease in humans (3) and experimental animal models (4, 5), as well as to prevent the onset of an autoimmune disease in NZB/NZW mice (6). The metabolism of eicosapentaenoate via the lipoxygenase pathway to leukotrienes C_5 and B_5 has been demonstrated in mouse mastocytoma cells (7, 8).

A prominent product of arachidonate production in neutrophils (PMNs[1]) is leukotriene B (9–11), which is a potent chemotactic stimulus that has been implicated in the genesis of the inflammatory response in several diseases (12–14). PMNs are an important component of the acute unflammatory response and recently have been implicated in the vascular damage seen in a variety of diseases (15).

In the studies described here it was reasoned that eicosapentaenoic acid or its products might alter LTB production by PMNs and that such an alteration might explain some of the beneficial effects observed with an eicosapentaenoate-rich diet. The results of experiments testing the first hypothesis are reported here. It is shown that (*a*) eicosapentaenoate can be incorporated and released by PMNs; (*b*) eicosapentaenoate is metabolized to LTB_5, but not as efficiently as arachidonate is converted to LTB_4; (*c*) the presence of eicosapentaenoate results in inhibition of the production of LTB_4 from arachidonate; and (*d*) LTB_5 is a less potent stimulus for PMN activation than LTB_4.

EXPERIMENTAL PROCEDURES

Materials — [1-^{14}C]Eicosapentaenoic (55 mCi/mmol) and [5,6,8,9,11,12,14,15-^{3}H]arachidonic (91.2 Ci/mmol) acids were purchased from New England Nuclear. Eicosapentaenoic and arachidonic acids were from Sigma and repurified by thin layer chromatography prior to use. Neutral lipid standards were from Sigma and phospholipids from Avanti-Polar Lipids, Birmingham, AL. Solvents were reagent, or HPLC, grade and were purchased from Fisher or J. T. Baker Chemical Co. Dextran T-70 was from Pharmacia. All other reagents were from Sigma or Fisher.

Assay of LTB Production—Human PMNs were separated by the technique of Boyum (16). The preparations resulted in suspensions in which more than 99% of the leukocytes were PMNs and the platelet contamination was less than one platelet per PMN. PMNs were suspended in Tyrode's buffer (138 mM NaCl, 2.7 mM KCl, 1.8 mM $CaCl_2$, 0.5 mM $MgCl_2 \cdot 6H_2O$, 0.4 mM $NaH_2PO_4 \cdot H_2O$, 12 mM $NaHCO_3$, pH 7.4, 5.6 mM glucose) and stimulated with the calcium ionophore A23187 dissolved in Me_2SO. Control samples received an equal amount of Me_2SO. The final concentration of Me_2SO was never more than 0.2% and did not stimulate LTB production or release of eicosapentaenoate or arachidonate. The final concentration of A23187 was 10 μM. Following a 5-min incubation at 25 °C the suspensions were centrifuged at 800 × *g* for 5 min. The supernatant buffer was removed and extracted with 2.5 volumes of $CHCl_3$:CH_3OH:HCOOH (12:12:1.0). The lower phase was removed, the upper phase washed with 1 ml of $CHCl_3$, and the resultant lower phase combined with the previous one. The chloroform extract was evaporated under a stream of nitrogen and redissolved in methanol. For RP-HPLC analysis this solution was used directly. For straight phase

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[1] The abbreviations used are: PMN, polymorphonuclear leukocyte neutrophil; HPLC, high performance liquid chromatography; RP-, reversed phase; TLC, thin layer chromatography; BSA, bovine serum albumin; LTB, LTA, etc., leukotriene B, leukotriene A, etc.; diHETE, 5,12-dihydroxyeicosatetraenoic acid; diHEPE, 5*S*,12*S*-dihydroxyeicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; HEPE, hydroxyeicosapentaenoic acid; Me-, methyl ester; Me_2SO, dimethyl sulfoxide.

HPLC the methyl ester was produced by adding ethereal diazomethane at room temperature for 15 min. The product was dried with nitrogen and redissolved in the starting solvent for HPLC.

The RP-HPLC assay was performed on a Beckman (Altex) model 342 gradient liquid chromatograph as described in the legend of Fig. 3 (10). The straight phase HPLC assays were performed as described in the legend of Fig. 4 (17). The quantity of the compounds was estimated by making photocopies of a strip chart recording and then cutting out and weighing individual peaks. These values were compared with a standard curve obtained by performing the same analysis on known amounts of LTB_4. The usual internal standard (prostaglandin B) could not be used because of coelution with eicosapentaenoate-derived products. However, the recovery of known LTB_4 in parallel extractions and separations was excellent (>90%) and reproducible (<5% variation).

Uptake and Release of Eicosapentaenoate and Arachidonate—PMNs (10^6) were suspended in 1.0 ml of Tyrode's buffer that contained 0.5 mg of fatty acid-free BSA. Radiolabeled fatty acids, of known specific activity, were added at different concentrations as a suspension in 20 μl of pH10 carbonate buffer (Beckman) (18). After varying times of incubation at 25 °C the cells were removed from the suspension by centrifugation at 6600 × *g* for 3 min. The supernatant was removed, and the cellular pellet resuspended in buffer with fatty acid-free BSA and centrifuged again. The second supernatant was removed, and the pellet resuspended and transferred to a vial for counting in a Beckman LS6800 scintillation spectrometer.

In parallel tubes the pellet was extracted by the modifier Bligh/Dyer technique (19) and the organic phase subjected to TLC. Samples were applied to glass plates coated with Silica Gel 60 (Merck-Darmstadt) that had been activated at 110 °C for 60 min. The solvent system was $CHCl_3$:MeOH:H_2O:HOAc (25:17:4:2) for phospholipid separation and ethyl ether:hexane:HOAc (70:30:1.0) for neutral lipids. The areas corresponding to standards (run in parallel lanes) were scraped into vials and counted in a scintillation spectrometer. Some samples had the neutral and polar lipids separated by activated silica, as previously described (20), prior to TLC. In other samples the esterified fatty acids were converted to their methyl esters with 14% boron trifluoride in methanol (21). The esters were run in the neutral lipid system for TLC which clearly separates Me-arachidonate and Me-eicosapentaenoate from methylated mono- and dihydroxyarachidonate.

PMNs were incubated with radiolabeled eicosapentaenoate or arachidonate (described above), washed in Tyrode's buffer containing 0.5 mg/ml of fatty acid-free BSA, and resuspended at a concentration of 0.5 × 10^7/ml. One ml of suspension was stimulated with A23187 (10 μM) or equivalent volume of Me_2SO and allowed to incubate at room temperature for the indicated period. The cells were rapidly removed by centrifugation at 6600 × *g* for 3 min. The supernatants and pellets were individually transferred to vials for scintillation spectrometry.

Purification of Leukotrienes Derived from Eicosapentaenoic Acid—Suspensions of PMNs were stimulated with 10 μM calcium ionophore A23187 in the presence of 50 μM eicosapentaenoate. After 5 min the cells were removed by centrifugation and the supernatant was extracted as described above. The products were redissolved in methanol and applied to a Ultrasphere ODS 5 μ column (10 × 250 mm) on the HPLC instrument described above. Elution was performed using MeOH:H_2O:HOAc (80:20:0.1) at 3.0 ml/min, and samples corresponding to peaks I–III (Fig. 1) were collected (22). The individual peaks were further purified by reapplication to, and elution from, the same column with acetonitrile:H_2O:HOAc (65:35:0.1) at 1.5 ml/min. This removes the 5*S*,12*S*-dihydroxyeicosapentaenoic acid that contaminates peak III just as described for the arachidonate homologues (22). Following these steps each peak was examined for purity in the analytical reversed phase and straight phase systems described above as judged by the A_{270}. Each compound gave a single peak under conditions in which as little as 2–4% contamination could have been detected.

Measurement of Degradation of LTB—PMNs were stimulated with A23187 in the presence of either radiolabeled arachidonate or eicosapentaenoate and the products purified as described above. The purified radiolabeled LTB from each precursor was incubated with PMNs for varying times. The cells were removed by centrifugation and the supernatant extracted as described above except that unlabeled carrier LTB_4 was added. The samples were then methylated and separated by HPLC (straight phase system). Fractions were collected and the amount of radioactivity in the LTB peaks was estimated by scintillation spectrometry.

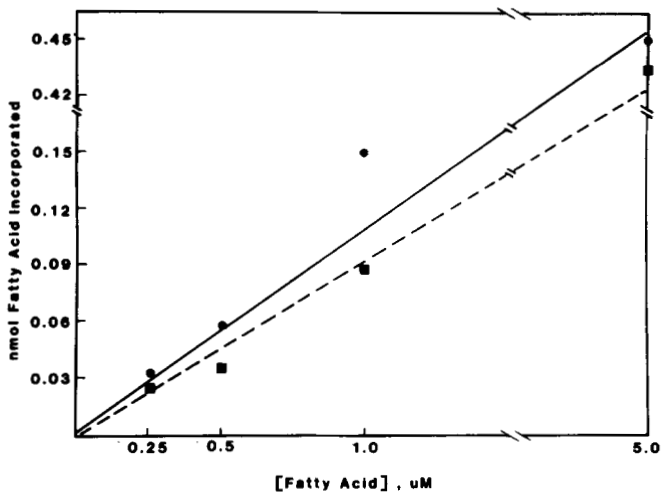

FIG. 1. **Incorporation of arachidonic and eicosapentaenoic acids by human neutrophils.** Isolated PMNs (10^6/ml) were incubated for 60 s with varying concentrations of fatty acid. The cells were removed from the reaction mixture, washed, and the amount of fatty acid incorporated was estimated by scintillation spectrometry. Control experiments demonstrated that all of the radioactivity associated with the cells under these conditions was fatty acid that was esterified in complex lipids. The results are representative of four experiments; in one experiment the uptake was shown to saturate between 5 and 10 μM for each exogenous fatty acid. ●, arachidonate; ■, eicosapentaenoate.

UV Absorption Spectroscopy and Mass Spectrometry—The purified compounds were dissolved in methanol and their UV absorption spectra measured in a Beckman DU8-B spectrophotometer. Their concentration was obtained by dividing the A_{270} by the molar extinction coefficient of LTB_4 (10). For determination of their mass spectrum the samples were converted to their methyl esters, by reaction with ethereal diazomethane, and subsequently to a trimethylsilyl derivative (10). The derivatized compounds were separated on a capillary column packed with OV-101 in a Hewlett-Packard 5985 gas chromatograph-mass spectrometer as described (23).

Neutrophil Aggregation—Aggregation of cytochalasin B-treated PMNs in response to various amounts of different agonists was performed as described (24, 32). The samples for bioassay were coded and the person performing the assay did not know the identity of the compounds.

RESULTS

Eicosapentaenoic Acid Is Incorporated into and Released from PMNs—Eicosapentaenoic acid was incorporated into PMNs with a concentration dependence similar to arachidonate (Fig. 1). The results shown are typical of four separate experiments. The rates of incorporation of the two fatty acids were similar over periods from 15 s to 60 min at concentrations of either 1 or 5 μM (*e.g.* at 1 μM substrate: eicosapentaenoate uptake = 1.2 nmol/10^7 PMNs in 1 min, and arachidonate = 1.4 nmol/10^7).

In agreement with the results of others (25) 61% of the arachidonate incorporated after 60 min (1 μM substrate) was esterified in triacylglycerols, with the remainder in phospholipid. Eicosapentaenoate (same conditions) also was found to be esterified in complex lipids but with a somewhat smaller percentage (37%) in triacylglycerols. In the phospholipid fraction arachidonate was found predominantly in phosphatidylcholine (57%) and phosphatidylinositol (31%), with much smaller amounts in phosphatidylethanolamine and phosphatidylserine. The phospholipid distribution of eicosapentaenoate was similar: phosphatidylcholine, 43%; phosphatidylinositol, 46%; phosphatidylethanolamine, 1%; phosphatidylserine, 7%. The patterns of distribution of both polyenoic

acids were also similar at an incubation time of 60 s and at a substrate concentration of 0.1 μM (not shown).

Based on the work of Stenson and Parker (25) the possibility that some of the radiolabel represented fatty acids that had been oxygenated prior to esterification was considered. That such was not the case was demonstrated by converting all of the fatty acids to their methyl esters and separating them by TLC. Over 90% of the radioactivity from either arachidonate or eicosapentaenoate incubations was found with the fatty acid methyl esters, and none of the radioactivity migrated with the methyl derivatives of 5-HETE, 12-HETE, or LTB_4 standards (data not shown).

The release of esterified arachidonate or eicosapentaenoate was measured after allowing the PMNs to incorporate the appropriate radiolabeled fatty acid. The cells were washed and then stimulated with A23187 (Fig. 2). At 5 min equivalent amounts of the arachidonate (3.2%) and eicosapentaenoate (3.0%) were released (specific release = total minus unstimulated control). These values are expressed as a percentage of the total radioactivity in the cell and likely were a mixture of free fatty acid and oxygenated metabolites.

Neutrophils Convert Eicosapentaenoic Acid to LTB_5—Human PMNs that were stimulated with the calcium ionophore A23187 in the presence of eicosapentaenoate produced three peaks (I–III) of absorption at 270 nm that preceded LTB_4 (VI) and its isomers (IV, V) in elution from RP-HPLC (Fig. 3, *lower*). In the absence of either A23187 or PMNs from the incubation no peaks were found (not shown). If eicosapentaenoic acid was omitted only peaks IV–VI were produced (Fig. 3, *upper*) (10). The products of parallel reaction mixtures were converted to methyl esters and analyzed by straight phase HPLC (Fig. 4). In this system an eicosapentaenoate-derived peak followed Me-LTB_4 as would be predicted by the insertion of an additional double bond (17). These results suggested that eicosapentaenoate is converted to LTB_5, the homologue of LTB_4, and to all-*trans*-isomers derived nonenzymatically from LTA_5.

Compounds I–VI were purified by serial RP-HPLC (see "Experimental Procedures"), and their UV absorption spectra were determined and compared to that of authentic LTB_4. Each of the compounds has a typical spectrum for conjugated trienes (10). Compounds III and VI have spectra identical to that of synthetic LTB_4 with λ_{max} = 270 nm and shoulders at 260 and 281 nm (10). Compounds I, II, IV, and V have λ_{max} = 269 with shoulders at 258 and 280. Peak VI is LTB_4, and peaks IV and V are its stereoisomers, based on their chromatographic bahavior and UV spectra. Analysis of compounds I–III by gas chromatography-mass spectrometry showed them to have C values of: I, 26.8; II, 26.8; III, 24.0. The methyl ester-trimethylsilyl ether derivatives of I–III had mass spectra characterized by ions *m/z* 492 (molecular ion), 477 (loss of —CH_3), 461 (loss of O—CH_3), 402 (loss of trimethylsilanol), and others as previously described for LTB_5 (8). Based on these results and by analogy with arachidonate products the following identifications have been made (although the stereochemistry was not determined): peaks I and II are 5,12-dihydroxy-6,8,10,14,17-eicosapentaenoic acid and are assumed to have an all-*trans* configuration in the conjugated triene portion of the molecule; peak III is 5*S*,12*R*-dihydroxy-6,8,10,14,17-eicosapentaenoic acid, *i.e.* LTB_5. In the RP-HPLC LTB_5 is contaminated with variable amounts of 5*S*,12*S*-dihydroxy-6,8,10,14,17-eicosapentaenoic acid, which was identified by its chromatographic mobility and UV spectrum (not shown) and presumably was a result of platelet contamination of the PMNs (17). Thus, these data support the conclusion that PMNs convert eicosapentaenoic acid to LTB_5 and its nonenzymatically generated isomers.

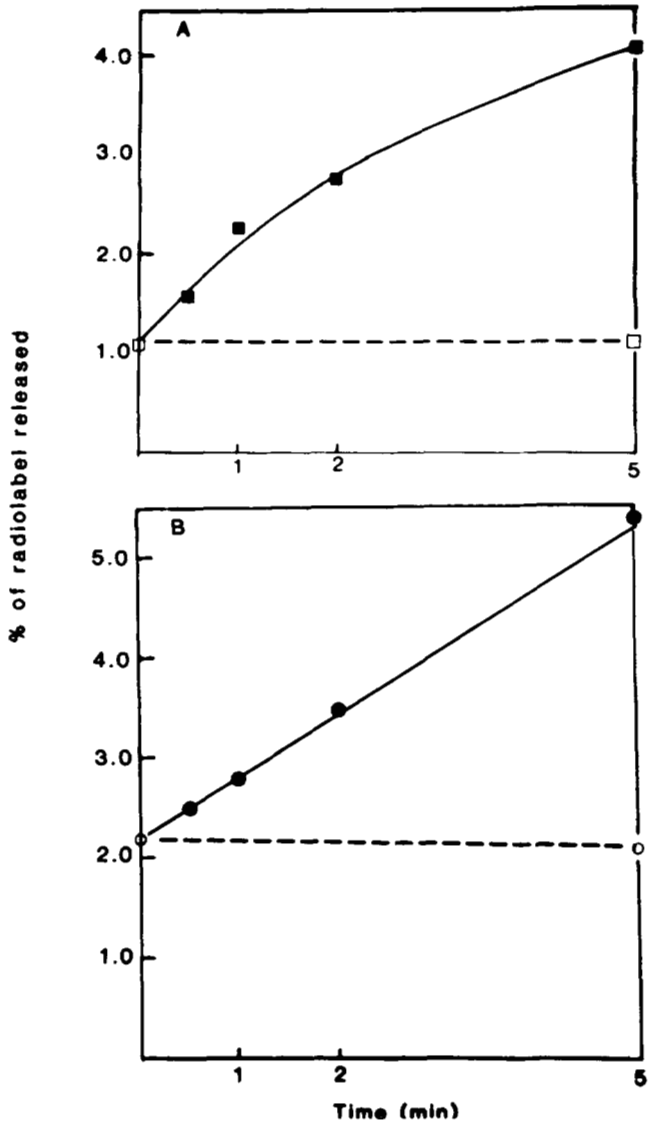

FIG. 2. **Release of arachidonic and eicosapentaenoic acids by stimulated neutrophils.** Neutrophils that had previously been allowed to incorporate radiolabeled arachidonic or eicosapentaenoic acid were stimulated with A23187 (10 μM) in buffer that contained 0.5 mg/ml of fatty acid-free BSA. At the indicated times the cells were removed by centrifugation, and a portion of the supernatant was taken for scintillation spectrometry. The amounts released are expressed as a percentage of the total cellular radioactivity. A second experiment gave the same results for stimulated release, varying only in the level of unstimulated release. In both panels the Me_2SO-only controls are shown in *open symbols with dashed lines*. *A*, release of [^{14}C]eicosapentaenoate (■); *B*, release of [^{3}H]arachidonate (●).

Eicosapentaenoic Acid Is a Poor Substrate for, but Good Inhibitor of, Leukotriene B Production—The production of LTB_5 is response to increasing concentrations of eicosapentaenoate was measured and compared to LTB_4 synthesis from equimolar amounts of exogenous arachidonate (Fig. 5). It is apparent that eicosapentaenoate was converted to LTB less efficiently than arachidonate. This observation suggested that the presence of eicosapentaenoate might lead to a decreased total LTB production. An alternative explanation, *i.e.* that the observed differences in LTB levels were due to a more rapid rate of degradation of LTB_5 than LTB_4, was excluded. Incubation of the radiolabeled LTBs with PMNs resulted in very similar rates of metabolism (*e.g.* at 5 min LTB_4 = 56.8%

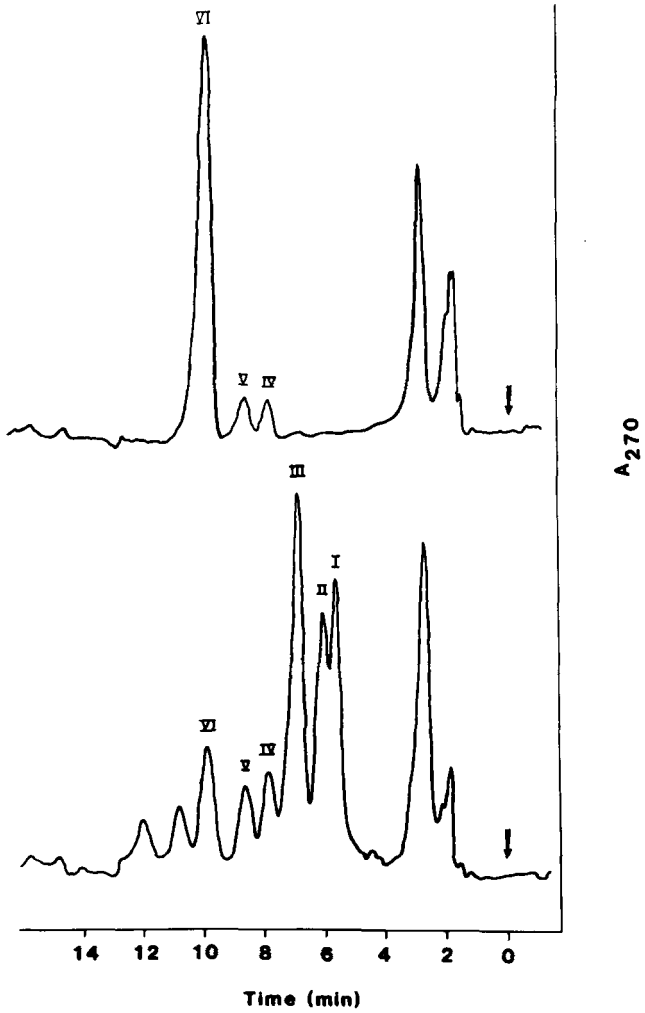

FIG. 3. **Leukotriene production by stimulated PMNs in the absence and presence of eicosapentaenoic acid.** Isolated human PMNs (10^7 cells in 1.0 ml) were stimulated with 10 μM calcium ionophore A23187 in the absence (*upper trace*) or presence (*lower trace*) of exogenous eicosapentaenoate (20 μM). The cells were removed after 5 min and the supernatant buffer extracted and then analyzed by RP-HPLC (4.6 × 250 mm C_{18} 5 μ column; MeOH:H_2O:HOAc, 75:25:0.01 at 1.5 ml/min). Peak VI is LTB_4 and peaks IV and V are its stereoisomers. The identity of peaks I–III is discussed in the text. The scale is the same for both traces but only one-third of the extract was analyzed in the assay shown in the *upper trace* while the entire sample was used for the *lower*.

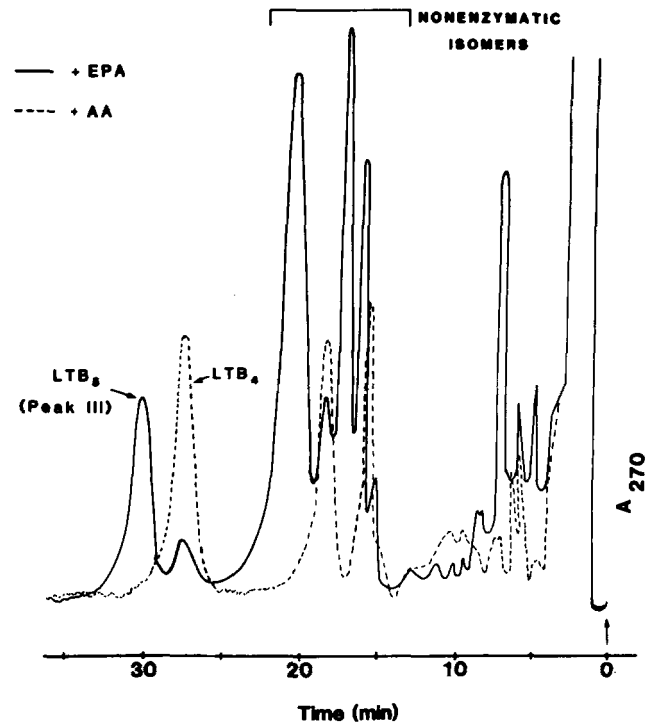

FIG. 4. **Straight phase HPLC analysis of leukotrienes produced by PMNs in the absence and presence of eicosapentaenoate.** PMNs were incubated as described in the legend of Fig. 3. The extracted products were converted to methyl esters (see "Experimental Procedures") and analyzed by HPLC on a Si-5 Micropak column (4.6 × 300 mm) with a mobile phase of hexane:2-propanol:HOAc, 96:4 0.1, at 2 ml/min. As in Fig. 3 the entire sample of cells exposed to eicosapentaenoate was analyzed and is compared here with one-third of the extract from cells stimulated in the presence of arachidonate. ——, PMNs + 20 μM eicosapentaenoate (*EPA*); – – –, PMNs + 20 μM arachidonate (*AA*).

and LTB_5 = 52.9% of control). The ability of eicosapentaenoate to inhibit the metabolism of arachidonate to LTB_4 was examined; PMNs were stimulated in the presence of a fixed amount of arachidonate and varying concentrations of eicosapentaenoate. As shown in Fig. 6, eicosapentaenoate was an effective inhibitor of LTB_4 production from arachidonate; at equimolar ratios of the two fatty acids there was only 32% as much LTB_4 synthesized as in the absence of eicosapentaenoate. In the experiment shown in Fig. 5 total LTB production (LTB_4 + LTB_5) was inhibited by 72% when 20 μM eicosapentaenoate was present (94 ng of LTB_5 + 19 ng of LTB_4 = 113 ng of total LTB) as compared to 20 μM arachidonate (401 ng of LTB_4 + 0 ng of LTB_5). This demonstrated that although it is not as good a substrate for LTB production as arachidonate is that eicosapentaenoate, or a product, is a good inhibitor. This further excluded the possibility that compartmentalization of substrate with the appropriate enzymes could account

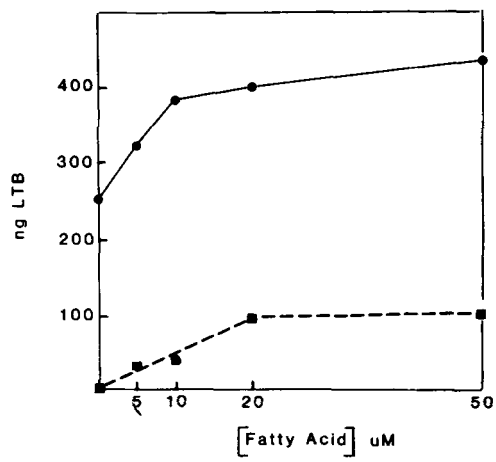

FIG. 5. **Comparison of eicosapentaenoic and arachidonic acids as substrates for LTB synthesis.** PMNs were stimulated with 10 μM A23187 in the presence of various concentrations of exogenous eicosapentaenoate or arachidonate. The supernatant buffer was extracted, derivatized, and analyzed as described in the legend of Fig. 4. The straight phase HPLC system was used for this analysis to eliminate any contribution of 5*S*,12*S*-diHETE or 5*S*,12*S*-diHEPE to the peaks. The results are the average of duplicate determinations from a single experiment that was representative of three separate experiments. ●, LTB_4 production from arachidonate; ■, LTB_5 production from eicosapentaenoate.

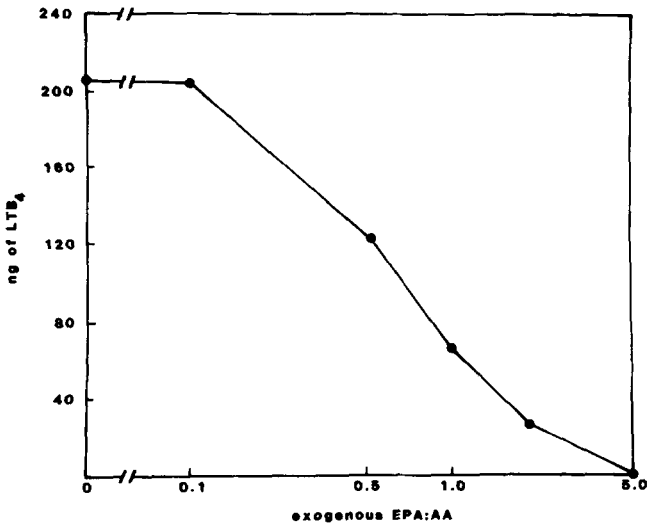

FIG. 6. **Eicosapentaenoate acid inhibits the production of LTB_4 from arachidonic acid.** Experiments were performed as described in the legend of Fig. 5 except that all of the tubes contained 10 μM arachidonic acid (*AA*), and the concentration of eicosapentaenoate (*EPA*) was varied from 0–50 μM. The production of LTB_4 is plotted against the ratio of the two exogenous fatty acids. At equimolar concentrations of the two polyenoic fatty acids the production of LTB_4 is inhibited by 68%. The results shown are the average of two separate experiments.

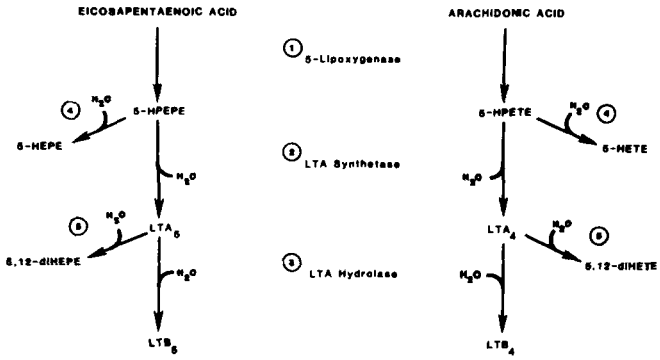

FIG. 7. **Pathway for leukotriene B synthesis.** Reactions 1–3 are catalyzed by the enzymes shown and lead to the production of LTB. Eicosapentaenoate acid is a good substrate, compared to arachidonate, for reactions 1 and 2 but not for reaction 3 as shown by the accumulation of large amounts of the products of the nonenzymatic reaction 5 (Table I).

for the decreased LTB_5 synthesis as compared to LTB_4 (Fig. 5).

The point in the pathway at which the inhibition occurred was determined by examining which metabolites accumulated, and thus would be proximal to the inhibited step, and which products were depleted, *i.e.* those beyond the inhibition. This issue was approached by measuring the production of the nonenzymatic isomers of both LTB_4 and LTB_5, as well as the LTBs, produced at different concentrations of exogenous substrate. The rationale was that inhibition at or prior to LTA synthetase (Fig. 7) would show a decrease in all LTA-derived products while inhibition of LTA hydrolase would show only a decrease in LTB. As shown in Table I the total LTA-derived nonenzymatic products were higher in the cells exposed to eicosapentaenoate than those incubated with arachidonate. In conjunction with the results in Fig. 5 this demonstrates that more of the intermediate LTA_5 was formed than LTA_4 in the presence of equivalent amounts of the

TABLE I

Production of leukotrienes B_4 and B_5 and their stereoisomers from arachidonate and eicosapentaenoate

Incubations were performed and analyzed as described in Fig. 3. All suspensions received 10 μM A23187. The results are expressed as nanograms per 10^7 PMNs.

Additions	5,12-Dihydroxy metabolites of eicosapentaenoic acid			5,12-Dihydroxy metabolites of arachidonic acid		
	Nonenzymatic (peaks I + II)	LTB_5[a]	Total	Nonenzymatic (peaks IV + V)	LTB_4[a]	Total
None	0	0	0	44	236	280
50 μM AA[b]	0	0	0	128	451	579
50 μM EPA	948	255	1203	0	0	0
20 μM each AA + EPA	359	183	542	205	57	262

[a] The LTB peaks (III and VI) were contaminated with the dioxygenation products; 5*S*,12*S*-diHEPE and 5*S*,12*S*-diHETE, respectively. The extract of cells stimulated in the presence of both fatty acids (20 μM each) was methylated and examined by straight phase HPLC as well (Fig. 4). This procedure separated the LTBs from the dioxygenation products, which accounted for 40% of peak III and 20% of peak VI. Thus, the values shown here for the LTBs are overestimates by approximately these percentages.

[b] AA, arachidonic acid; EPA, Δ5,8,11,14,17-eicosapentaenoic acid.

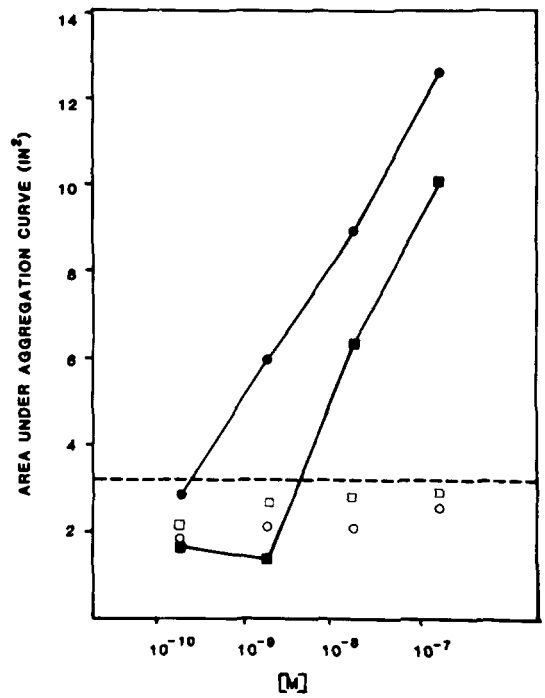

FIG. 8. **The effect of eicosapentaenoate-derived compounds on neutrophil aggregation.** Suspensions of cytochalasin B-treated PMNs were stirred in a Payton aggregometer, and aggregation was measured in response to the addition of LTB_5, its stereoisomers, and synthetic LTB_4. The final concentration of each agonist is plotted against the area of the aggregation curve. – – –, values obtained by the addition of an equivalent volume of buffer ("threshold"). The results are representative of two experiments, with duplicates at each point, using PMNs from different blood donors. ●, LTB_4; ■, LTB_5 (peak III); ○, peak I; □, peak II.

respective precursor fatty acids. This indicated that either eicosapentaenoate or 5-HEPE, or both, are favored substrates at reactions 1 and 2 (Fig. 7), respectively. The accumulation of LTA_5, which is a less favorable substrate for, but a good inhibitor of, LTA hydrolase would then result in decreased total LTB production from both fatty acid precursors.

LTB_5 Is Not As Potent As LTB_4 in Inducing Neutrophil Aggregation—The purified compounds I–III were tested for their ability to induce aggregation in cytochalasin B-treated PMNs (Fig. 8). LTB_5 (compound III) induced the aggregation of PMNs but with only one-tenth the potency of LTB_4 over a broad range of concentrations. The purified stereoisomers (compounds I and II) did not promote aggregation of PMNs at the concentrations tested. These results support the conclusion that compound III is LTB_5 and that I and II are its inactive stereoisomers. The finding that LTB_5 has decreased biological activity suggests that replacement of arachidonate with eicosapentaenoate in PMN membranes might impair the function of PMNs even though a homologous LTB is formed.

DISCUSSION

This examination of competition between arachidonic and eicosapentaenoic acids for the enzymes of the leukotriene biosynthetic pathway in human neutrophils has shown that: (*a*) eicosapentaenoate can be converted to LTB_5 and its stereoisomers; (*b*) eicosapentaenoate is equivalent to arachidonate as a substrate through the synthesis of LTA_5 but at that point it, or a metabolite, inhibits the pathway; (*c*) the net effect of the addition of eicosapentaenoate is to decrease total LTB production; and (*d*) LTB_5 is a less potent stimulus for the aggregation of PMNs than is LTB_4. Additionally it has been demonstrated that neutrophils incorporate eicosapentaenoate into complex lipids and release it in response to a stimulus. This suggests that the changes observed *in vitro* with exogenous substrate might also be present *in vivo*.

The metabolism of Δ5,8,11,14,17-eicosapentaenoic acid via the cyclooxygenase pathway has been thoroughly examined in platelets and blood vessels by Needleman and co-workers (1, 2). They have shown that it can be converted to the 3-series endoperoxide and subsequently to thromboxane A_3 in platelets or to prostaglandin I_3 by microsomes from bovine aorta (1). They also have shown that eicosapentaenoate is a poor substrate for, but good inhibitor of, platelet cyclooxygenase resulting in a net inhibition of endoperoxide production. Whitaker *et al.* further showed that the cyclooxygenase-derived product prostaglandin H_3 is 10-fold less potent in activating platelets than is the arachidonate homolog (2). Our examination of the metabolism of eicosapentaenoate via the lipoxygenase pathway to leukotriene B has yielded analogous results although in this case the inhibition occurs not at the first step but at the final one in the pathway.

The metabolism of eicosapentaenoate via the lipoxygenase pathway has been examined by several groups. Hammarstrom identified leukotrienes C_5 and D_5 from mastocytoma cells that were stimulated in the presence of eicosapentaenoate (7). Interestingly, he found these products to be less active than LTC_4 and LTD_4 in provoking contraction of guinea pig ileum. Murphy *et al.* stimulated mastocytoma cells that had been harvested from mice raised on a fish oil-supplemented diet (8) and found both LTC and LTB derived from eicosapentaenoate. They concluded from the ratio of products that LTC synthesis was inhibited more than LTB, although LTB production was markedly decreased. The experiments reported here confirm that finding (in human PMNs) and extend it in that these studies have shown that eicosapentaenoate can account for the decreased LTB_4 production without invoking some other effect of the diet, excluded enhanced degradation of LTB_5 as an alternate explanation for the lower levels of LTB_5, demonstrated that the effect on LTB production is at the level of LTA hydrolase, and shown that purified LTB_5 has less biological activity than LTB_4.

Jakschik *et al.* (26) and Ochi *et al.* (27) have shown, with a partially purified enzyme, that eicosapentaenoate is a slightly better substrate for 5-lipoxygenase than is arachidonate. Jakschik *et al.* further showed that eicosapentaenoate is metabolized effectively to a diHEPE and, in the presence of reduced glutathione, to slow reacting substance (26). Their finding of more diHEPE than diHETE is consistent with our result of more total LTA_5-derived products than LTA_4-derived, which is an appropriate comparison since their TLC system would not have separated LTB from its stereoisomers.

The results reported here are consistent with the hypothesis that some of the beneficial effects of an eicosapentaenoate-rich diet on various animal models of disease may be mediated by an effect on neutrophils. This contrasts with the report of Lammi-Keefe and co-workers (28) who could not demonstrate an effect of such a diet on PMNs as measured by several functional assays. However, their subjects consumed only 1 g of eicosapentaenoate per day for 3 weeks, and they did not measure the fatty acid composition of the PMNs following the dietary supplement. Their use of inhibition of platelet thromboxane production as evidence for eicosapentaenoate incorporation may have been misleading since Corey *et al.* have shown that docosahexaenoic acid, which is an even larger component of most fish oils, is a potent inhibitor of cyclooxygenase but does not affect leukotriene synthesis (29). In a preliminary study of human volunteers consuming 8–10 g of eicosapentaenoate per day it was incorporated into PMNs, and LTB_5 was produced from the endogenous precursor.[2] An investigation of the functional effects of such changes is in progress. In addition, experiments utilizing PMNs from rats reared on a diet deficient in essential fatty acids have shown that the accumulation of another homolog of arachidonate, *i.e.* 5,8,11-eicosatrienoic acid, has similar effects on LTB production.[3] In conjunction with the results presented here this suggests that the methyl terminus of LTA homologs is an important determinant of suitability as a substrate for LTA hydrolase. Samuelsson has suggested that inhibitors at this point in leukotriene biosynthesis are particularly attractive, as are compounds that inhibit both the cyclooxygenase and leukotriene pathways (11). The results presented here suggest that a diet that has a high content of eicosapentaenoic acid may meet both criteria and that inhibition of leukotriene B synthesis may play a role in the favorable effect of such diets on a variety of diseases.

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Addendum—During the preparation and review of this manuscript two papers describing the biological effects of LTB_5 have appeared (30, 31). Both describe a decreased potency of LTB_5, as compared to LTB_4, of approximately the magnitude reported here (Fig. 8).

REFERENCES

1. Needleman, P., Raz, A., Minkes, M. S., Ferrendelli, J. A., and Sprecher, H. (1979) *Proc. Natl. Acad. Sci. U. S.A.* **76,** 944–948
2. Whitaker, M. O., Wyche, A., Fitzpatrick, F., Sprecher, H., and Needleman, P. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76,** 5919–5923
3. Dyerberg, J., Bang, H. O., Stoffersen, E., Moncada, S., and Vane, J. R. (1978) *Lancet* **ii,** 117–119

[2] S. M. Prescott and G. A. Zimmerman, unpublished observations.
[3] W. F. Stenson, S. M. Prescott, and H. Sprecher, submitted for publication.

4. Culp, B. R., Lands, W. E. M., Lucchesi, B. R., Pitt, B., and Romson, J. (1980) *Prostaglandins* **20,** 1021–1031
5. Black, K. L., Culp, B., Madison, D., Randall, O. S., and Lands, W. E. M. (1979) *Prostaglandins* **3,** 257–268
6. Prickett, J. D., Robinson, D. R., and Steinberg, A. D. (1981) *J. Clin. Invest.* **68,** 556–559
7. Hammarström, S. (1980) *J. Biol. Chem.* **255,** 7093–7094
8. Murphy, R. C., Pickett, W. C., Culp, B. R., and Lands, W. E. M. (1981) *Prostaglandins* **22,** 613–622
9. Borgeat, P., and Samuelsson, B. (1979) *J. Biol. Chem.* **254,** 2643–2646
10. Borgeat, P., and Samuelsson, B. (1979) *J. Biol. Chem.* **254,** 7865–7869
11. Samuelsson, B. (1983) *Science* **220,** 568–575
12. Ford-Hutchinson, A. W., Bray, M. A., Doig, M. V., Shipley, M. E., and Smith, M. J. H. (1980) *Nature (Lond.)* **286,** 264–265
13. Palmblad, J., Malmsten, C. L., Uden, A-M., Radmark, O., Engstedt, L., and Samuelsson, B. (1981) Blood **58,** 658–661
14. Klickstein, L. B., Shapleigh, C., and Goetzl, E. J. (1980) *J. Clin. Invest.* **66,** 1166–1170
15. Sacks, T., Moldow, C. F., Craddock, P. R., Bowers, T. K., and Jacob, H. S. (1978) *J. Clin. Invest.* **61,** 1161–1167
16. Boyum, A. (1978) *Scand. J. Clin. Lab. Invest.* **21,** Suppl. 97, 77–89
17. Borgeat, P., Picard S., and Vallerand, P. (1981) *Prostaglandin Med.* **6,** 557–570
18. Neufeld, E. J., Wilson, D. B., Sprecher, H., and Majerus, P. W. (1983) *J. Clin. Invest.* **72,** 214–220
19. Cohen, P., Broekman, M. J., Verkley, A., Lisman, J. W. W., and Derksen, A. (1971) *J. Clin. Invest.* **50,** 762–772
20. Prescott, S. M., and Majerus, P. W. (1983) *J. Biol. Chem.* **258,** 764–769
21. Morrison, W. R., and Smith, L. M. (1964) *J. Lipid Res.* **5,** 600–608
22. Lin, A. H., Morton, D. R., and Gorman, R. R. (1982) *J. Clin. Invest.* **70,** 1058–1065
23. Jakschik, B. A., Morrison, A. R., and Sprecher, H. (1983) *J. Biol. Chem.* **258,** 12797–12800
24. Craddock, P. R., Hammerschmidt, D., White, J. G., Dalmasso, A. P., and Jacob, H. S. (1977) *J. Clin. Invest.* **60,** 260–264
25. Stenson, W. F., and Parker, C. W. (1979) *J. Clin. Invest.* **64,** 1457–1465
26. Jakschik, B. A., Sams, A. R., Sprecher, H., and Needleman, P. (1980) *Prostaglandins* **20,** 401–409
27. Ochi, K., Yoshimoto, T., Yamamoto, S., Taniguchi, K., and Miyamoto, T. (1983) *J. Biol. Chem.* **258,** 5754–5758
28. Lammi-Keefe, C. J., Hammerschmidt, D. E., Weisdorf, D. J., and Jacob, H. S. (1982) *Inflammation* **6,** 227–234
29. Corey, E. J., Shih, C., and Cashman, J. R. (1983) *Proc. Natl. Acad. Sci. U. S. A.* **80,** 3581–3584
30. Goldman, D. W., Pickett, W. C., and Goetzl, E. J. (1983) *Biochem. Biophys. Res. Commun.* **117,** 282–288
31. Lee, T. K., Mencia-Huerta, J. M., Shih, C., Corey, E. J., Lewis, R. A., and Austen, K. F. (1984) *J. Biol. Chem.* **259,** 2383–2389
32. Prescott, S. M., Zimmerman, G. A., and McIntyre, T. M. (1984) *Proc. Natl. Acad. Sci. U. S. A.*, in press