Antithetic relationship of dietary arachidonic acid and eicosapentaenoic acid on eicosanoid production in vivo

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Abstract Eicosanoids are oxidative derivatives of arachidonic acid. When produced in excessive amounts, many are proinflammatory and/or prothrombotic agents. N-3 polyunsaturated fatty acids (PUFA) have been used to attenuate tissue arachidonic acid (AA, 20:4 n-6) levels and thus modulate eicosanoid production. However, there is growing evidence that dietary arachidonic acid may also be able to modulate eicosanoid formation by enriching tissue phospholipids with AA. Therefore, the effects of dietary AA and n-3 PUFA are in diametric opposition. This study investigates the antithetic relationship of dietary AA and eicosapentaenoic acid (EPA, 20:5 n-3) on fatty acid composition of hepatic phospholipids and eicosanoid production in vivo. Forty-nine CD-1 male mice were randomly divided into four dietary groups. Identical diets were supplemented with ethyl esters (1.5% w/w) of the following fatty acids: oleic acid (OA, 18:1 n-9), AA, EPA or AA+EPA. After 4 weeks on diet, peritoneal cells were stimulated in vivo with opsonized zymosan and the peritoneal exudates were analyzed for eicosanoid production (PGE_2, 6-keto-$PGF_{1\alpha}$, TXB_2, LTB_4, LTE_4, and LTE_5). Hepatic phospholipids were enriched with AA when AA was included in the diet, and EPA was enriched at the expense of AA when EPA was added to the diet. However, when AA was added to the diet containing equivalent amounts of EPA (AA+EPA), any effect EPA had on modulating hepatic phospholipid fatty acid composition was almost completely eliminated. Similar effects were observed with eicosanoid production. The pooled eicosanoid production in the AA group was 41% and 300% higher compared to the OA (control) and EPA groups, respectively. The pooled eicosanoid production in the EPA group was 47% that of the OA group. When equivalent amounts of AA and EPA (AA+EPA) were included in the diet, the pooled eicosanoid production was 29% and 274% higher compared to the OA and EPA groups, respectively. These data demonstrate that dietary AA *1)* enriches tissue phospholipids with AA even in the presence of equal amounts of EPA, *2)* enhances eicosanoid production under in vivo conditions, and *3)* abrogates virtually all of the effects observed with dietary EPA when both are included in the diet in equivalent amounts.—**Li, B., C. Birdwell, and J. Whelan.** Antithetic relationship of dietary arachidonic acid and eicosapentaenoic acid on eicosanoid production in vivo. *J. Lipid Res.* 1994. **35:** 1869-1877.

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Arachidonic acid (AA, 20:4 n-6) is arguably one of the most important polyunsaturated fatty acids associated with membrane phospholipids. When liberated, free AA can be oxidatively metabolized to a variety of eicosanoids, many of which, when chronically or excessively produced, can be proinflammatory, prothrombotic, and proatherogenic (1-4). The amounts synthesized are influenced by the levels of AA in membrane phospholipids.

We and others (5-12) have presented evidence suggesting that low to moderate levels of dietary AA can have a significant impact on tissue AA content and thus, augment eicosanoid production. Recent evidence also suggests that the effect of dietary AA transcends those effects observed with dietary linoleic acid (LA, 18:2 n-6), its metabolic precursor (9-13).

In opposition, dietary long chain n-3 PUFA (as derived from marine oils) are very effective in attenuating tissue AA levels and eicosanoid production in vitro and in vivo, and many of the beneficial physiological effects of n-3 PUFA have been linked to their abilities to inhibit the AA cascade (1-4). However, the antithetic effects of n-3 PUFA and AA have yet to be carefully investigated. Therefore, this study was designed to provide new information on the antithetic relationship of dietary AA and n-3 PUFA. Specifically, we studied the effects of dietary AA and eicosapentaenoic acid (EPA, 20:5 n-3), separately and in combination, on the fatty acid content of hepatic phospholipids and in vivo eicosanoid production in CD-1 male mice.

Abbreviations: AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; LT, leukotriene; OA, oleic acid; PG, prostaglandin; PUFA, polyunsaturated fatty acid; TBHQ, tertiary butylhydroxyquinone; TX, thromboxane.

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MATERIALS AND METHODS

Animals

Forty-nine CD-1 male mice (HSD, Indianapolis, IN) (21–24 g) were randomly assigned to four dietary groups upon arrival, 12 or 13 animals per group. All animals were maintained on chow diet for 1 week prior to being placed on experimental diets for 28 days.

Diets

All diets contained 90% (w/w) fat-free mouse chow diet (Dyets Inc., Bethlehem, PA) and 10% (w/w) fat (**Table 1** and **Table 2**). The LA content of the diets, as provided primarily by safflower oil (75% LA) (Hollywood, St. Louis, MO). Each diet was supplemented with 3.0% (w/w) fatty acid ethyl esters (Nu-Chek Prep, Elysian, MN). The control diet (oleic acid diet, OA) contained 3.0% (w/w) oleic acid ethyl ester (Table 2). In the arachidonic acid diet (AA diet), 1.5% (w/w) of the oleic acid ethyl ester, as supplied in the OA group, was replaced by arachidonic acid ethyl ester. In the eicosapentaenoic acid diet (EPA diet), 1.5% (w/w) of the oleic acid ethyl ester as supplied in the OA group, was replaced by eicosapentaenoic acid ethyl ester (EPA group). (Eicosapentaenoic acid ethyl esters were kindly supplied by the National Oceanographic and Atmospheric Administration, Charleston, SC.) In the arachidonic acid plus eicosapentaenoic acid dietary group (AA + EPA diet), AA ethyl ester (1.5%, w/w) and EPA ethyl ester (1.5%, w/w) replaced OA ethyl ester (3.0%, w/w) (Table 2). Diets were prepared in bulk, prepackaged separately in individual Whirl-Pak bags (NASCO, Atkinson, WI) and stored under nitrogen at −80°C. α-Tocopherol (RRR) (60 mg/kg diet) (Eastman Chemical, Kingsport, TN) and tertiary butylhydroquinone (TBHQ) (6 mg/kg diet) (Eastman Chemical, Kingsport, TN) were supplemented as antioxidants to prevent oxidation of PUFA during storage. Water and food were provided ad libitum for 28 days. Fresh diets were provided daily and uneaten food was discarded to minimize oxidation prior to consumption. Fatty acid analysis of the diets is presented in Table 3.

TABLE 1. Composition of the diet[a]

Ingredient	Amount
	g/kg diet
Casein	200
DL-methionine	3
Cornstarch	150
Sucrose	450
Cellulose	50
Salt mix #200000[b]	35
Vitamin mix #300050[c]	10
Choline bitartrate	2
Fat[d]	100

[a]All diets contain identical amounts of carbohydrates, protein, lipids, vitamins, and minerals.
[b]AIN-76 salt mix contains (mg/kg diet): calcium (5200), phosphorus (4000), potassium (3600), sodium (1020), chloride (1560), sulfur (337), magnesium (507), iron (35), copper (6), manganese (54), zinc (30), chromium (2), iodine (0.2), selenium (0.1).
[c]AIN-76A vitamin mix contains (mg/kg diet): thiamine HCL (6), riboflavin (6), pyridoxine (7), niacin (30), calcium pantothenate (16), folic acid (2), biotin (0.2), cyanocobalamin (B-12, 0.1%) (10), menadione sodium bisulfite (0.8), vitamin A (4000 I.U.), vitamin E (50 I.U.), vitamin D_3 (1000 I.U.).
[d]See Tables 2 and 3 for details.

Stimulation of eicosanoid production with opsonized zymosan

Opsonized zymosan was prepared as described previously (14). Briefly, zymosan (10 mg) (Sigma, St. Louis, MO) was boiled in saline (0.9%, 1 ml) for 1 h, then incubated in rabbit serum (1 ml, Sigma, St. Louis, MO) for

TABLE 2. Lipid composition of the diets

	OA Group[a] (n = 13)	AA Group (n = 12)	EPA Group (n = 12)	AA + EPA Group (n = 12)
Fat free diet	900	900	900	900
Safflower oil	20	20	20	20
Olive oil	15	15	15	15
Tripalmitin	35	35	35	35
18:1n-9 Ethyl ester	30	15	15	0
20:4n-6 Ethyl ester	0	15	0	15
20:5n-3 Ethyl ester	0	0	15	15
Cholesterol	1	1	1	1
α-Tocopherol (RRR)[b]	60 mg	60 mg	60 mg	60 mg
TBHQ[c]	6 mg	6 mg	6 mg	6 mg

All data are expressed as g/kg diet except α-tocopherol (RRR) and TBHQ. Upon arrival 49 animals were randomly assigned to four dietary groups. Animals were put on chow diet for 1 week prior to being placed on the experimental diets for 28 days.
[a]OA group: oleic acid group; AA group: arachidonic acid group; EPA group: eicosapentaenoic acid group; AA + EPA group: arachidonic acid + eicosapentaenoic acid group.
[b]α-Tocopherol (RRR) was supplemented to the diets which contained 50 I.U. vitamin E.
[c]TBHQ, tertiary butylhydroquinone.

TABLE 3. Fatty acid composition of the diet

	OA Group	AA Group	EPA Group	AA + EPA Group
16:0	41.4	41.0	41.0	40.0
18:1n-9	40.2	28.1	27.0	15.9
18:2n-6	18.3	18.1	18.0	17.9
20:4n-6	ND	12.8	ND	12.3
20:5n-3	ND	ND	14.0	14.1

All data are expressed as g/kg diet. ND, not detectable.

30 min at 37°C. The opsonized zymosan was then washed with saline once and resuspended in saline at 2 mg/ml for storage at 4°C. Thirty minutes prior to euthanization, animals were anesthetized by methoxyflurane (Pitman-Moore, Mendelian, IL) inhalation. Following anesthetization, opsonized zymosan (1 mg) (at 37°C) was injected intraperitoneally to stimulate the production and release of eicosanoids from resident peritoneal macrophages. Previous studies revealed that macrophages comprise >95% of the resident peritoneal cell population in this animal model (9). Due to experimental error, fatty acid analysis and accurate cell counts for the resident peritoneal cells are unavailable. However, we have previously demonstrated that population profiles and cell numbers are unaffected by dietary regimens similar to those used in these experiments (9, 10, 14). In addition, changes in phospholipid fatty acid composition of peritoneal macrophages closely parallel changes in the composition of hepatic phospholipid fatty acids (9, 10, 14–16).

Analysis of leukotrienes by high performance liquid chromatography (HPLC)

Thirty minutes after the zymosan injection, animals were killed by cervical dislocation and the peritoneal cavities were washed 3 times with a total of 10 ml cold saline containing 1 mM EDTA. The internal standard, PGB_1 (100 ng) (Cayman Chemical, Ann Arbor, MI), was included in the first wash. The peritoneal washes were centrifuged at 700 *g* for 4 min. Supernatants were removed and diluted with an equal volume of a 20% (v/v) methanol solution containing 3 mM formic acid. Ten percent of the supernatant-methanol mixture was removed for prostaglandin determination by radioimmunoassay (RIA) (see next section of methodology). The leukotrienes (LT) were separated via solid phase extraction using a C-18 cartridge (Burdick & Jackson, Muskegon, MI), washed once with distilled water, and eluted off the cartridge with approximately 2 ml methanol (15). (All leukotriene standards were purchased from Cayman Chemical, Ann Arbor, MI.) After being evaporated to dryness under nitrogen, the LT were dissolved in the high performance liquid chromatography (HPLC) solvent system of methanol–water 665:335 (v/v), pH 5.6, containing 5 mM ammonium acetate and 1 mM EDTA. The LT were analyzed by reverse phase HPLC using an HP 1090 diode array detector system (Hewlett-Packard, Atlanta, GA). The leukotrienes were separated using a C-18 column (10 cm × 4.6 mm) (Hewlett-Packard, Kennett Square, PA) with a flow rate of 0.8 ml/min, monitoring at 280 nm. Representative chromatograms are presented in **Fig. 1** demonstrating the ability to separate the cysteinyl leukotrienes (LTE_4, 11-*trans* LTE_4, LTE_5, 11-*trans* LTE_5) and LTB_4. Due to interfering peaks, LTC_4 and LTC_5 could not be quantitated (14, 15). Peaks were identified by their distinctive UV absorption spectra, and by comparing the retention times with that of known standards (14, 15). Leukotrienes were quantitated using the internal standard, PGB_1, and known extinction coefficients of the identified compounds. The

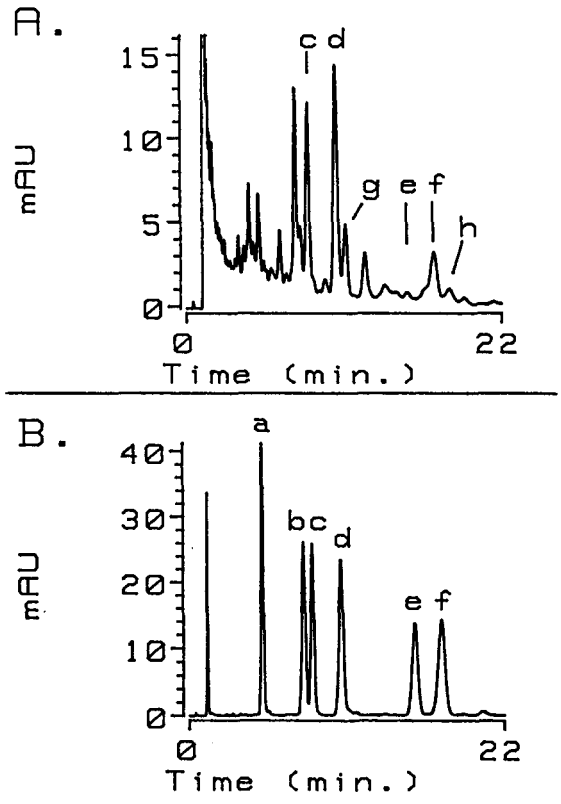

Fig. 1. Representative HPLC chromatogram of the separation of leukotrienes generated in vivo after intraperitoneal stimulation of resident peritoneal cells with opsonized zymosan. Chromatogram A was generated from an animal in the EPA dietary group. Chromatogram B contains standards. Other symbols: a, LTC_5; b, LTC_4; c, PGB_1 (internal standard); d, LTE_5; e, LTB_4; f, LTE_4; g, 11-*trans* LTE_5; h, 11-*trans* LTE_4.

recovery of PGB_1 (an average of 82%) from the peritoneal cavities was not different among dietary groups.

Prostaglandin and thromboxane analysis via radioimmunoassay (RIA)

Prostanoids (6-keto $PGE_{1\alpha}$, PGE_2, and TXB_2) were extracted with ethyl acetate (×3), evaporated to dryness under nitrogen, and resuspended in phosphate-buffered saline (0.9%) containing 0.1% gelatin. RIA were performed according to the manufacturer's instructions (Amersham, Arlington Heights, IL). Antibodies were purchased from Advanced Magnetics (Cambridge, MA) and ^3H-labeled antigens were purchased from New England Nuclear (Boston, MA). The standards (6-keto $PGF_{1\alpha}$, PGE_2, and TXB_2) were purchased from Cayman Chemical (Ann Arbor, MI). Cross-reactivities at half maximum binding of various prostanoids with PGE_2 antiserum are as follows: PGE_2 (100%), PGE_3 (26%) (16), 6-keto-$PGF_{1\alpha}$ (<1%), $PGF_{1\alpha}$ (1%), TXB_2 (<1%) and $PGF_{2\alpha}$ (1%). Cross reactivities of various prostanoids with 6-keto-$PGF_{1\alpha}$ antiserum are as follows: 6-keto-$PGF_{1\alpha}$ (100%), Δ-17, 6-keto-$PGF_{1\alpha}$ (14%) (personal communication, K. S. Broughton), PGE_2 (<1%), $PGF_{2\alpha}$ (2%), $PGF_{1\alpha}$ (8%), and TXB_2 (<1%). Cross reactivities of various prostanoids with TXB_2 antiserum are as follows: TXB_2 (100%), $PGF_{1\alpha}$ (<1%), $PGF_{2\alpha}$ (<1%), PGE_2 (<1%) and 6-keto-$PGF_{1\alpha}$ (<1%).

Analysis of fatty acid composition in liver and diet

After the wash of the peritoneum, livers of mice were perfused with cold saline (0.9%) via the portal vein after the hepatic artery and vein were severed. The livers were then removed, quick frozen in liquid nitrogen, and stored at −80°C for later analysis of fatty acids. Fatty acid compositions of extracted liver phospholipids were analyzed as described previously (15). Livers (100 mg) were homogenized in 0.8 ml cold saline (0.9%). Liver homogenates were resuspended in 3 ml methanol-chloroform 2:1 (v/v), extracted once with chloroform-saline 1:1 (v/v), and twice with chloroform. The pooled chloroform fractions were evaporated to dryness under nitrogen and resuspended in chloroform for separation of phospholipids. Phospholipids were separated by thin-layer chromatography (TLC) using HPTLC plates (10 cm × 10 cm) precoated with silica gel 60 (EM Science, Gibbstown, NJ) and chloroform-methanol 8:1 (v/v) as the developing solvent. The phospholipid fractions were scraped from the TLC plates, resuspended in toluene (0.25 ml), and saponified with 0.5 N methanolic KOH at 86°C for 8 min followed by acidification with 0.7 N methanolic HCl. An internal standard, pentadecanoic acid (15:0) methyl ester (100 ng), was added to each sample prior to the saponification step. After extraction with hexane (3 × 2 ml), fatty acids were methylated with ethereal diazomathane and resuspended in hexane for gas chromatographic (GC) analysis using a Hewlett-Packard 5890 series II gas chromatograph with a DB23 capillary column (0.25 mm × 30 m) (J and W Chromatography, Folsom, CA). Hydrogen was used as the carrier gas. Fatty acids were identified by comparing peaks with known standards (Nu-Chek Prep, Elysian, MN), and quantified using the internal standard and the protein contents of each sample.

Protein determination

Livers were homogenized as described above. An aliquot of the liver homogenate was removed for protein analysis. Protein was determined using a modified Lowry assay (17). A standard curve was generated using bovine serum albumin (Sigma, St. Louis, MO).

Statistical analysis

All data were tested for normality. ANOVA or Kruskal-Wallis ANOVA (when data were not normally distributed) were used for analysis of variance. Data with unequal variance were transformed and Fisher's least significant difference multiple comparison method was used to determine the differences among the dietary groups. Significant levels of linear regression were determined using simple linear regression test. True Epistat (Epistat Σ Services, Richardson, Texas) and SPSS statistic package (SPSS Inc., Chicago, IL) were used to test the normality and determine the differences. For statistical analysis of the pooled eicosanoids, the data were transformed to control for the differences in magnitude among the various eicosanoids. The transformed data were pooled and ANOVA was used to determine differences among the different dietary groups using SAS (SAS Institute, Inc., Cary, NC).

RESULTS

Weight gains of the animals were not significantly different among the dietary groups.

Fatty acid composition in liver phospholipids

Tissue AA level in the AA group was significantly higher compared to the OA group (control), while tissue linoleic acid (18:2 n-6, LA) levels were significantly lower compared to the OA group (**Table 4**). N-3 PUFA content in the AA group was not significantly different compared to the OA group. Including EPA in the diet resulted in characteristic changes in tissue phospholipid fatty acid composition compared to the OA group. The hepatic phospholipids became enriched in n-3 PUFA with a concomitant decline in tissue AA levels.

When AA and EPA were simultaneously included in the diet (AA+EPA group), tissue AA levels were more than 4-fold higher compared to the EPA group. Tissue

TABLE 4. Fatty acid composition of liver phospholipids

	OA Group	AA Group	EPA Group	AA + EPA Group
16:0	24.29 ± 0.29[a]	25.61 ± 0.34[b]	26.67 ± 0.46[c]	26.85 ± 0.23[c]
	(80.33 ± 4.25)[a]	(86.52 ± 5.40)[a]	(98.31 ± 3.73)[b]	(97.67 ± 4.52)[b]
16:1	1.05 ± 0.14[a]	0.55 ± 0.17[b]	0.94 ± 0.17[a,b]	0.34 ± 0.13[b,c]
	(3.53 ± 0.50)[a]	(1.79 ± 0.55)[b]	(3.50 ± 0.68)[a]	(1.32 ± 0.50)[b]
18:0	14.85 ± 0.22[a]	16.86 ± 0.49[b]	15.05 ± 0.15[a]	16.53 ± 0.19[b]
	(54.69 ± 3.28)[a]	(63.50 ± 5.05)[a,b]	(62.16 ± 2.80)[a,b]	(67.09 ± 3.48)[b]
18:1n-9	13.66 ± 0.25[a]	8.76 ± 0.39[b]	10.06 ± 0.21[c]	6.66 ± 0.16[d]
	(49.93 ± 2.94)[a]	(32.54 ± 2.31)[b,c]	(38.50 ± 3.16)[c]	(26.62 ± 1.23)[b]
18:2n-6	15.13 ± 0.38[a]	12.68 ± 0.48[b]	19.15 ± 0.62[c]	14.08 ± 0.25[a]
	(55.15 ± 3.63)[a]	(47.38 ± 3.78)[a]	(79.48 ± 3.69)[b]	(56.42 ± 3.33)[a]
20:3n-6	1.86 ± 0.12[a]	ND	0.98 ± 0.20[b]	ND
	(7.34 ± 0.52)[a]	ND	(4.38 ± 0.94)[b]	ND
20:4n-6	21.29 ± 0.32[a]	26.33 ± 0.43[b]	5.19 ± 0.19[c]	22.01 ± 0.23[a]
	(84.24 ± 5.32)[a]	(106.03 ± 7.49)[b]	(22.80 ± 1.37)[c]	(95.23 ± 4.69)[a,b]
20:5n-3	ND	ND	6.53 ± 0.27[a]	0.41 ± 0.13[b]
	ND	ND	(28.67 ± 2.02)[a]	(1.80 ± 0.57)[b]
22:4n-6	ND	0.55 ± 0.14	ND	ND
	ND	(2.53 ± 0.71)	ND	ND
22:5n-6	1.03 ± 0.19[a]	1.96 ± 0.28[b]	ND	ND
	(4.48 ± 0.82)[a]	(8.57 ± 1.31)[b]	ND	ND
22:5n-3	ND	ND	2.37 ± 0.29[a]	2.38 ± 0.13[a]
	ND	ND	(11.49 ± 1.53)[a]	(11.36 ± 0.99)[b]
22:6n-3	6.84 ± 0.24[a]	6.70 ± 0.15[a]	12.49 ± 0.34[b]	10.72 ± 0.27[c]
	(29.19 ± 1.99)[a]	(28.99 ± 1.94)[a]	(59.00 ± 2.53)[b]	(50.15 ± 2.81)[c]
Total (n-3) FA	6.84 ± 0.24[a]	6.70 ± 0.15[a]	21.39 ± 0.47[b]	13.52 ± 0.20[c]
	(29.19 ± 1.91)[a]	(29.00 ± 1.94)[a]	(99.16 ± 5.03)[b]	(63.31 ± 3.56)[c]
Total (n-6) FA	39.32 ± 0.33[a]	41.51 ± 0.29[b]	25.82 ± 0.59[c]	36.09 ± 0.26[a]
	(151.21 ± 9.01)[a]	(164.52 ± 11.77)[a]	(106.65 ± 5.18)[b]	(151.65 ± 7.87)[a]
(n-3)/(n-6) ratio	0.17 ± 0.01[a]	0.16 ± 0.01[a]	0.84 ± 0.04[b]	0.38 ± 0.01[c]
	(0.19 ± 0.01)[a]	(0.18 ± 0.01)[a]	(0.94 ± 0.04)[b]	(0.42 ± 0.01)[c]

Data are mole% and are expressed as mean ± SEM. Data in parentheses are mg/100 mg protein and are expressed as mean ± SEM. ND, not detectable. Values with different letters in the same row are significantly different at $P < 0.05$.

EPA levels were lower (94%) in the AA+EPA group compared to the EPA group; however, 22:6 n-3 content was only 14% lower.

Eicosanoid production from stimulated peritoneal macrophages in vivo

Leukotriene (LT) B_4 (LTB_4) production in vivo was 2-fold higher in the AA group compared to the OA group (control) (**Table 5**). While EPA-fed animals produced only trace amounts of LTB_4, including AA in the diet containing EPA (AA+EPA group) resulted in LTB_4 levels that were comparable to that observed in the AA group. LTE_4 production in the AA group was not significantly different from the OA group. LTE_4 production in the EPA group was significantly lower compared to all other dietary groups. However, the combination of AA+EPA feeding significantly increased LTE_4 production to a level intermediate to that of the AA ($P < 0.08$) and EPA ($P < 0.05$) groups, respectively (Table 5). LTE_5 was only detected in the dietary groups which included EPA. LTE_5 production was 82% lower in the AA+EPA group compared to the EPA group (Table 5).

TABLE 5. Effect of dietary unsaturated fatty acids on eicosanoid production in peritoneal macrophages in vivo stimulated with opsonized zymosan

	OA Group	AA Group	EPA Group	AA + EPA Group
LTB_4	3.56 ± 1.14[a]	6.64 ± 0.73[b]	trace	6.14 ± 0.65[b]
LTE_4[d]	83.65 ± 11.24[a]	101.39 ± 11.30[a]	38.31 ± 5.63[b]	75.56 ± 7.64[a]
6-keto $PGF_{1\alpha}$	4.79 ± 0.85[a]	8.27 ± 0.88[b]	0.63 ± 0.11[c]	9.09 ± 1.03[b]
PGE_2	0.73 ± 0.09[a,b]	1.03 ± 0.12[a]	0.55 ± 0.07[b]	0.90 ± 0.14[a]
TXB_2	0.18 ± 0.02[a,b]	0.31 ± 0.07[a]	0.15 ± 0.04[b]	0.27 ± 0.04[a]
LTE_5[d]	ND	ND	47.44 ± 9.64[a]	8.39 ± 1.85[b]

Eicosanoid production in vivo was stimulated by injecting opsonized zymosan intraperitoneally. The peritoneal washes were collected and analyzed for LT and PG. All data are ng/mouse and are expressed as mean ± SEM. ND, not detectable. Values with different letters in the same row are significantly different at $P < 0.05$.

[d]Data includes 11-trans LTE_4 and 11-trans LTE_5.

Similarly, prostaglandin (PG) E_2 (PGE_2) and thromboxane (TX) B_2 (TXB_2) levels were highest in the AA group compared to the OA group, and these differences approached statistical significance ($P < 0.08$ and $P < 0.06$, respectively) (Table 5). PGE_2 and TXB_2 production were significantly higher in the AA and AA+EPA groups compared to the EPA group (Table 5). 6-Keto $PGF_{1\alpha}$ production (the stable product of PGI_2) was significantly higher in the AA group and lower in the EPA group compared to the OA group (Table 5). Including AA in the diet containing EPA (AA+EPA group) resulted in a 6-keto $PGF_{1\alpha}$ level that was equivalent to that of the AA group.

Pooled eicosanoid data

The levels of eicosanoids formed from each animal were standardized to the percent of the mean of the control group (OA) for each corresponding eicosanoid. The standardized data were pooled to generate means ± SEM for each dietary group (**Fig. 2**). The standardized and pooled eicosanoid production was significantly higher in those groups containing AA compared to the OA and EPA groups. The EPA group had the lowest production compared to all other groups.

Correlation of eicosanoid production and phospholipid AA content

All eicosanoids produced in vivo were significantly correlated with hepatic phospholipid AA content (**Table 6**).

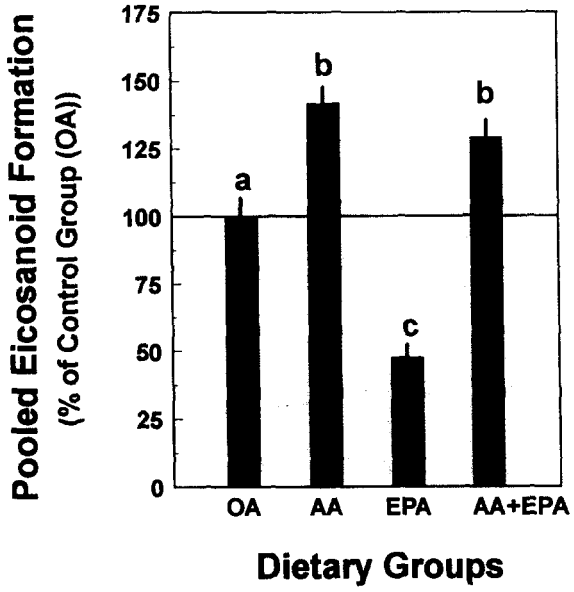

Fig. 2. The effects of diet on pooled eicosanoid formation. All of the data (each eicosanoid formed from each animal) were standardized to a percent of the mean of the control group (OA) for each corresponding eicosanoid. The standardized data were pooled to generate means ± SEM for each dietary group. Values with different letters are significantly different at $P < 0.05$.

TABLE 6. Relationship of eicosanoid production and hepatic AA content

	Correlation Coefficient (r)	Level of Significance (P)
LTB_4	0.22	<0.001
LTE_4	0.74	<0.001
6-keto $PGF_{1\alpha}$	0.76	<0.001
PGE_2	0.50	<0.003
TXB_2	0.41	<0.015
LTE_5	−0.68	<0.001

Eicosanoid levels of each animal were correlated with the AA content in liver phospholipids. Significance level of the correlation was determined using simple linear regression test.

LTB_4, LTE_4, 6-keto $PGF_{1\alpha}$, PGE_2, and TXB_2 were positively correlated with AA, while LTE_5 was negatively correlated with hepatic AA. It should be noted that while fatty acid composition data for the resident peritoneal cells was unable to be obtained, previous studies that were performed under similar experimental conditions have demonstrated that changes in the fatty acid composition in hepatic phospholipids strongly reflect fatty acid changes in other tissues (using dietary AA), including macrophages (9, 10, 15).

DISCUSSION

Previously, we demonstrated that dietary n-3 PUFA, as derived from marine oils, are very effective in augmenting tissue n-3 PUFA content and attenuating tissue AA levels and eicosanoid production in vivo and in vitro (14, 15). We also reported that the effects of dietary AA on these same parameters were diametrically opposed to that of n-3 PUFA (9, 10). This study was designed to provide new information on the antithetic relationship of dietary AA and n-3 PUFA, in particular, EPA.

Dietary AA and EPA have been shown to be potent modulators of tissue phospholipid fatty acid composition (10, 11, 15). This present study confirms that hepatic phospholipid AA content is significantly higher when AA is included in the diet and significantly lower when EPA is included compared to the OA control group (5, 9, 10). Upon close examination of the fatty acid composition data (Table 4), it appears that the potency of dietary AA to alter tissue AA content (AA group versus OA group) is matched by dietary EPA's ability to drive down the phospholipid content of AA and enrich the phospholipids with EPA (EPA group versus OA group). However, when AA and EPA were supplied in the diet concurrently (AA+EPA), the EPA content of the hepatic phospholipids virtually disappeared and AA content rose 4-fold (compared to the EPA group). While the direction of these changes was not all that surprising, the magnitude of the changes was. These data suggest that while

dietary n-3 PUFA are potent modulators of hepatic phospholipid fatty acid composition, the effect of dietary EPA appears to be much weaker compared to dietary AA. In other words, EPA appeared to be effective only when AA was not included in the diet (at least under these experimental conditions).

These results are not without support. Nilsson, Hjelte, and Strandvik (7) reported that dietary [^{14}C]AA is preferentially incorporated into tissue phospholipids compared to [^{3}H]EPA in various rat tissues when the radiolabeled markers were included in a dietary fish oil emulsion. These data are particularly important because *1)* the effects of AA were observed within 4 h of administration and *2)* the fish oil emulsion contained 34% n-3 PUFA (16.5% EPA) and only 0.8% AA. Evidently, AA appears to be effectively transported to extra hepatic tissues within 4 h and is a preferential substrate for the acyltransferases in phospholipid biosynthesis (18). Similarly, in a study by O'Dea and Sinclair (6), subjects were maintained on a fish-only diet for 2 weeks. Analysis of plasma fatty acid composition surprisingly exhibited a dramatic increase in AA content in animals following the dietary regimen compared to pre-study values. Analysis of the fatty acid content of the species of fish being consumed in the study revealed that the fish contained significant quantities of AA, thus abrogating the effects of the n-3 PUFA found in the fish.

It is also interesting to note that while EPA content of the phospholipids in the AA+EPA group virtually disappeared compared to the EPA levels in the EPA group, the differences in the DHA content of the hepatic phospholipids among the dietary groups were not as great as those observed with EPA. It is not known, for example, whether DHA effectively competes with AA for incorporation into the phospholipid pool or whether the decline in the EPA content (EPA group versus AA+EPA group) is due to an increase in the conversion of EPA to DHA. This effect on DHA appears to be species dependent (9, 10).

It had been previously established that including AA in the diet results in enhanced eicosanoid production (8, 10-12) and this effect is in opposition to the effects observed with dietary n-3 PUFA (14, 15). However, this antithetic relationship between dietary AA and EPA had not been previously investigated. This study is the first to evaluate this relationship. With every eicosanoid analyzed, the levels of eicosanoids formed in the AA+EPA group were significantly higher compared to the EPA group. The underlying mechanism for AA's effectiveness in altering eicosanoid formation, even in the presence of dietary EPA, is not certain. However, we suggest that it is, in part, related to the ability of dietary AA to potently modulate tissue phospholipid fatty acid composition. The production of each of the eicosanoids analyzed was significantly correlated with hepatic phospholipid AA content. Even LTE_5 production was significantly, but inversely, correlated.

Important to this discussion is the dietary relevance of AA. AA is a minor component of the average daily intake of PUFA consumed in the Western diet. Its dietary intake of 170-220 mg/day is approximately 100 times lower than the average intake of its metabolic precursor, LA (4, 9, 19-22). Dietary AA is almost exclusively associated with animal products. The data presented in **Table 7** represent the AA content of selected cooked foods (21, 22). While these data demonstrate that these foods possess only low to moderate levels of AA, it is clear that the average daily

TABLE 7. Arachidonic acid content of common foods[a]

Item	Preparation	Arachidonic Acid Content (mg/100 g cooked edible portion)
Beef		
Regular ground beef	pan fried	80
Liver	pan fried	350
Tenderloin	broiled	50
Dairy		
Egg (whole)	poached	90
Egg (yolk)	raw (fresh)	280
Milk (cow)	whole (fresh)	trace
Poultry		
Drumstick (chicken)	roasted	120
Wing (chicken)	roasted	170
Turkey (all classes)	roasted	210
Turkey (skin only)	roasted	110
Other		
Lamb (leg)	broiled	60
Veal (trimmed cuts)	broiled	110
Deer	roasted	130
Fish	broiled	varies

[a]See references 21 and 22.

intake of AA can be increased 3- to 4-fold without radically altering a Western type of diet.

Intakes of dietary AA, however, at relatively low levels can potentially be physiologically significant even though these intakes may appear to be quantitatively insignificant, especially when compared to LA (13). It has already been demonstrated that consuming dietary AA (6 g/day) can be physiologically significant (8). AA, at this intake, is enriched in platelet phospholipids and platelets are more sensitive to agonists, and urinary excretion of eicosanoids is increased (8). It has also been demonstrated that consuming foods rich in AA can have a significant physiological impact, even at intakes below 500 mg/day (23). Furthermore, n-3 PUFA may not be able to alter some of these effects if AA is consumed concurrently (6).

Our results clearly demonstrate that dietary AA can abrogate these effects observed with dietary EPA in stimulated peritoneal cells and that AA appears to be a more potent antithetic agent towards EPA than EPA is towards AA. The production of AA-derived eicosanoids can be restored when AA is included in the diet even in the presence of n-3 PUFA (AA+EPA group versus EPA group) and the production of EPA-derived eicosanoids can be effectively depressed (under the present experimental conditions). However, our data do not suggest that EPA cannot be a good antagonist if the dietary EPA/AA ratio is greater than one. But examination of the pooled eicosanoid data underscores the potency of dietary AA on elevating eicosanoid production, as the AA+EPA group acted as if EPA was not included in this diet.

In summary, we have previously proposed that the origin of tissue AA is LA when LA is the only n-6 PUFA provided in the diet (10). We have also suggested that as AA increases in the diet it becomes an increasingly important contributor to tissue AA, and the contribution of dietary LA, as a precursor for tissue AA, diminishes (13, 24). Based on the data provided in the present manuscript, a logical extension of our hypothesis is proposed in the following metabolic scheme (**Fig. 3**). When equivalent levels of AA and EPA are included in the diet (concomitantly), AA will have the greater of the two influences. That is, tissue AA content will increase, potentially increasing the production of AA-derived eicosanoids and diminishing the effect of EPA to alter tissue n-3 PUFA and AA composition, compromising the biochemical/physiological effects that may be associated with the consumption of n-3 PUFA.

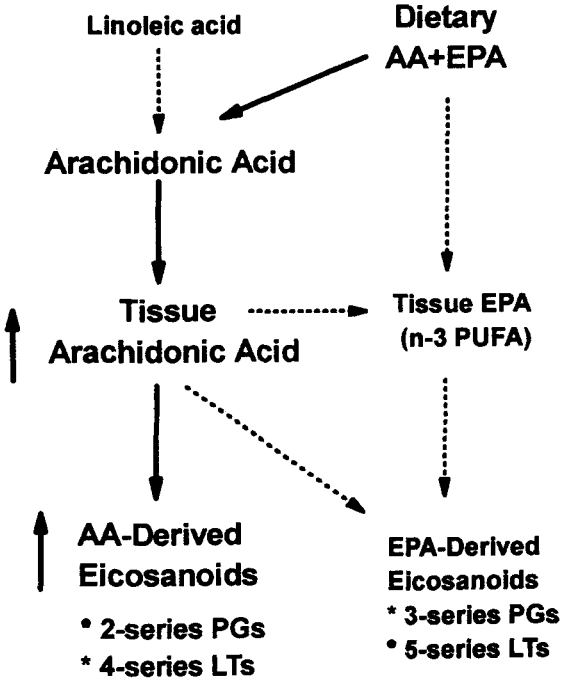

Fig. 3. The effects of dietary arachidonic acid (AA), in the presence of equal amounts of eicosapentaenoic acid (EPA), on tissue n-6 and n-3 PUFA content and eicosanoid production. When AA is added to a diet containing EPA (n-3 PUFA) it will increase tissue AA content at the expense of linoleic acid and EPA (n-3 PUFA) compared to diets containing only n-3 PUFA. This, in turn, results in an increased production of AA-derived eicosanoids with a concomitant decrease in eicosanoids derived from EPA. Bold solid line: metabolic pathway enhanced or positive effect; dotted line: pathway inhibited or negative effect. AA, arachidonic acid; EPA, eicosapentaenoic acid; PGs, prostaglandins; LTs, leukotrienes.

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