Effect of Dietary n-9 Eicosatrienoic Acid on the Fatty Acid Composition of Plasma Lipid Fractions and Tissue Phospholipids

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ABSTRACT: n-9 Eicosatrienoic acid (ETrA), also known as Mead acid, is a minor fatty acid in essential fatty acid (EFA)-sufficient healthy subjects but is found at increased levels in EFA deficiency. This study examined the influence of dietary ETrA from a biological source on plasma and tissue ETrA. A synthetic fat-free diet was prepared to which was added Mut 48 oil which contains 19% ETrA (wt%) as well as other n-9 fatty acids. Blends of vegetable oils were used to achieve overall diets with 5% fat (wt%) and varying amounts of ETrA at two different dietary levels of linoleic acid (LA), approximately 4.4 and 19% of total fatty acids. These diets were fed to 5-week-old Dark Agouti rats for four weeks. Plasma lipid fractions and liver, spleen, and peritoneal exudate (PE) cells were analyzed for fatty acid composition. ETrA was present at up to 20% total fatty acids in plasma triglyceride, cholesterol ester, and phospholipid fractions. ETrA also accumulated to substantial levels in phospholipids of liver and spleen (up to 15% of total fatty acids) and PE cells (up to 11%). ETrA was found in plasma and tissue phospholipids in proportion to the amount of ETrA present in the diet. The incorporation was reduced in diets with higher LA content compared to diets containing similar amounts of ETrA but lower LA. All rats remained apparently healthy, and histological survey of major organs revealed no abnormality. While the long-term implications for health of ingestion of diets rich in ETrA remain to be established, rats appear to tolerate high levels of dietary ETrA without adverse effects. Dietary enrichment with ETrA warrants further investigation for possible beneficial effects in models of inflammation and autoimmunity, as well as in other conditions in which mediators derived from n-6 fatty acids can affect homeostasis adversely.
Lipids 31, 829–837 (1996).

Dietary n-6 polyunsaturated fatty acids (PUFA) are present in the Western diet at levels far in excess of essential requirements (1). As a result, arachidonic acid (AA; 20:4n-6) is the dominant substrate for eicosanoid-forming enzymes which are pivotal in platelet-vascular homeostasis and control of inflammation. Consequently, the balance of eicosanoids produced is prothrombotic and proinflammatory as evinced by the antithrombotic and antiinflammatory effects of agents such as aspirin, which inhibit prostaglandin H synthase. These effects of n-6 eicosanoids can be reduced by dietary fortification with the n-3 homologue of AA, eicosapentaenoic acid (EPA; 20:5n-3). This fatty acid competes for eicosanoid-forming enzymes, and is metabolized to products, which on balance, reduce risk for thrombotic events and modulate inflammation.

Dietary fortification with the n-9 homologue of AA and EPA, eicosatrienoic acid (ETrA; 20:3n-9), also can antagonize n-6 eicosanoid formation (2). However, the lack of a suitable biological source of ETrA has been a barrier to experimental feeding studies. The present study examines the effects of Mut 48 oil, an ETrA-rich oil derived from a strain of the fungus *Mortierella alpina*, on the fatty acid composition of plasma lipid fractions and cellular phospholipids in rats. Because accumulation of EPA in plasma and cell phospholipids is reduced by dietary linoleic acid (LA; 18:2n-6), ETrA accumulation was assessed at two levels of dietary LA (3). ETrA is a normal metabolite which is typically present at low levels in plasma and cells, except in the setting of n-6 essential fatty acid (EFA) deficiency when levels increase. Evidence for signs of the EFA deficiency syndrome (coat changes and growth retardation) were, therefore, sought in rats fed the ETrA-fortified diets.

METHODS

Materials. Mortierella alpina oil was obtained from Suntory Ltd. (Osaka, Japan). Sunflower oil and Sunola™ were obtained from Meadow Lea Foods Ltd. (Sydney, Australia). Sunola is an oil obtained from a sunflower strain which yields an oil with a low LA content (~4.4% total fatty acids) compared to regular sunflower oil which contained approximately 70% LA.

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Abbreviations: AA, arachidonic acid; EFA, essential fatty acid; EPA, eicosapentaenoic acid; ETrA, n-9 eicosatrienoic acid; LA, linoleic acid; PE cells, peritoneal exudate cells, PUFA, polyunsaturated fatty acids.

TABLE 1
Fatty Acid Composition of Diets[a]

	Dietary groups[b]								
Fatty acids	16:4	12:4	12:19	8:4	8:19	4:4	4:19	0:4	0:19
Total saturated	22.6	19.8	20.3	16.4	17.2	13.3	13.9	10.0	10.7
Totals *trans*	0.2	0.1	0.1	0.3	0.1	0.3	0.3	0.4	0.3
18:1n-9	34.4	47.1	31.5	59.6	43.6	71.8	56.3	84.0	68.6
Total monosaturated	38.4	50.4	34.9	62.2	46.3	73.6	58.2	85.1	69.7
18:2n-9	13.7	10.1	10.3	6.7	7.0	3.4	3.5	—	0.2
20:2n-9	2.5	1.9	1.9	1.3	1.2	0.6	0.6	—	—
20:3n-9	16.5	12.2	12.2	8.1	8.1	4.0	4.0	—	—
22:3n-9	0.2	0.1	0.2	0.1	0.1	—	—	—	—
Total n-9	68.4	72.3	56.9	76.3	60.6	80.1	64.8	84.0	68.8
18:2n-6	4.6	4.4	19.2	4.4	19.3	4.4	19.1	4.4	18.9
Total n-6	5.2	4.9	19.7	4.7	19.6	4.5	19.2	4.4	18.9
Total n-3	0.9	0.6	0.7	0.4	0.5	0.3	0.4	0.2	0.3

[a]Expressed as percentage of total fatty acids as determined by gas–liquid chromatography analysis.
[b]The test diets are designated according to the ratios of their approximate content of linoleic acid and n-9 eicosatrienoic acid.

Diets. A set of oil mixtures containing approximately 12, 8, or 4% (wt%) of ETrA in combination with LA at levels of 4.4 and 19% (wt%) were prepared by using varying amounts of sunflower, Sunola and *M. alpina* oils. In a further preparation, *M. alpina* oil was blended with a small amount of sunflower oil to yield an oil mix containing 16.5% (wt%) of ETrA and 4.4% (wt%) of LA. In addition, Sunola alone and a blend of Sunola with sunflower oil were used to achieve oils with undetectable ETrA and 4.4 or 19% (wt%) of LA, respectively. These preparations were mixed with a fat-free diet which was based on the AIN-76 diet, as published previously (4). The total fat content of the final diet was 5% (wt%). The salient details of the test diets in relation to fatty acids of interest are detailed in Table 1. The full fatty acid analysis is detailed elsewhere (5).

Rats. Female Dark Agouti rats were used with groups of four rats being fed the test diets from five weeks of age for four weeks. The rats were observed and given fresh food daily, and weighed weekly. Peritoneal exudates (PE) were induced with peptone 3% (g/100 mL) four hours prior to sacrifice as described previously (4).

Collection of samples. Rats were sacrificed by cervical dislocation under halothane/nitrous oxide anesthesia. Blood samples were taken by cardiac puncture. PE cells were obtained by lavage as described (4). The liver, spleen, kidney, heart, and skin specimens were excised. The study was

TABLE 2
Plasma Phospholipid Fatty Acids[a]

	Dietary group[b]								
Fatty acids	16:4	12:4	12:19	8:4	8:19	4:4	4:19	0:4	0:19
Total saturated	45.4 ± 0.2	46.2 ± 0.4	48.1 ± 0.6	45.9 ± 0.2	47.8 ± 1.1	48.3 ± 0.9	48.3 ± 0.6	46.1 ± 1.0	47.8 ± 0.7
Total monosaturated	10.0 ± 0.4	10.1 ± 0.3	9.1 ± 0.4	12.8 ± 0.4	10.2 ± 1.1	13.2 ± 0.8	11.1 ± 1.1	17.2 ± 1.8	12.4 ± 0.3
18:2n-9	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
20:2n-9	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	n.d.[c]
20:3n-9	20.2 ± 1.3	17.2 ± 1.4	6.6 ± 0.5	14.4 ± 1.3	4.2 ± 0.2	8.5 ± 0.7	2.7 ± 0.4	3.0 ± 0.8	0.3 ± 0.0
22:3n-9	0.7 ± 0.0	0.7 ± 0.0	0.5 ± 0.1	0.5 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	n.d.
Total n-9	28.3 ± 1.3	25.5 ± 1.4	14.0 ± 0.9	24.5 ± 1.1	11.9 ± 1.2	19.0 ± 0.8	11.4 ± 1.2	16.7 ± 2.2	10.2 ± 0.2
18:2n-6	4.0 ± 0.1	4.0 ± 0.3	7.0 ± 0.9	4.5 ± 0.2	5.8 ± 0.4	4.7 ± 0.3	7.3 ± 0.6	6.1 ± 0.5	7.0 ± 0.5
20:3n-6	0.6 ± 0.0	0.7 ± 0.0	0.6 ± 0.1	0.8 ± 0.1	0.6 ± 0.0	1.0 ± 0.1	0.7 ± 0.1	1.3 ± 0.2	0.6 ± 0.1
20:4n-6	13.5 ± 0.1	15.2 ± 1.1	21.7 ± 1.3	15.6 ± 0.9	24.6 ± 0.7	18.6 ± 0.8	23.3 ± 1.2	20.1 ± 2.2	25.2 ± 0.5
22:4n-6	0.2 ± 0.0	0.2 ± 0.0	0.5 ± 0.0	0.2 ± 0.0	0.5 ± 0.0	0.3 ± 0.0	0.6 ± 0.0	0.4 ± 0.0	0.7 ± 0.0
22:5n-6	0.5 ± 0.0	0.7 ± 0.1	1.0 ± 0.2	0.7 ± 0.1	1.3 ± 0.2	1.0 ± 0.1	1.6 ± 0.3	1.3 ± 0.1	1.9 ± 0.3
Total n-6	18.9 ± 0.8	21.0 ± 1.0	31.0 ± 0.5	22.0 ± 0.7	33.0 ± 0.5	25.6 ± 0.6	33.7 ± 0.6	29.3 ± 1.3	35.6 ± 0.8
20:5n-3	0.2 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	n.d.
22:5n-3	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
22:6n-3	3.6 ± 0.3	3.6 ± 0.4	3.5 ± 0.2	3.1 ± 0.1	3.5 ± 0.2	3.1 ± 0.2	2.8 ± 0.4	3.0 ± 0.4	2.7 ± 0.2
Total n-3	4.4 ± 0.3	4.3 ± 0.2	4.1 ± 0.3	3.9 ± 0.2	3.9 ± 0.2	3.6 ± 0.2	3.2 ± 0.4	3.5 ± 0.4	3.0 ± 0.2

[a]Expressed as percentage of total fatty acids; mean ± SD, $n = 4$ rats in each group. [b]n-9 Eicosatrienoic acid/linoleic acid in diet expressed as percentage of total fatty acids. [c]n.d. = Not detected.

TABLE 3
Plasma Triglyceride Fatty Acids[a]

	Dietary group[b]								
Fatty acids	16:4	12:4	12:19	8:4	8:19	4:4	4:19	0:4	0:19
Total saturated	27.8 ± 2.1	28.7 ± 0.7	21.1 ± 1.0	22.5 ± 0.8	23.9 ± 1.8	26.3 ± 0.7	24.1 ± 1.1	25.4 ± 0.7	25.7 ± 0.6
Total monosaturated	39.1 ± 1.0	41.7 ± 0.7	33.4 ± 1.9	47.7 ± 3.4	36.7 ± 1.5	55.0 ± 2.0	45.2 ± 1.5	62.6 ± 3.2	51.1 ± 2.1
18:2n-9	2.6 ± 0.3	2.0 ± 0.3	2.5 ± 1.2	1.6 ± 0.2	1.2 ± 0.1	1.8 ± 0.4	1.5 ± 0.6	1.3 ± 0.4	2.4 ± 0.7
20:2n-9	1.4 ± 0.1	0.9 ± 0.2	1.0 ± 0.3	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.0	n.d.[c]	n.d.
20:3n-9	22.4 ± 2.5	20.2 ± 0.8	22.7 ± 2.3	19.3 ± 1.6	15.3 ± 1.8	8.5 ± 1.8	8.8 ± 1.8	2.4 ± 0.3	1.0 ± 0.0
22:3n-9	1.3 ± 0.2	0.9 ± 0.1	0.8 ± 0.2	0.8 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.2 ± 0.1	n.d.
Total n-9	58.5 ± 2.3	58.7 ± 1.0	54.5 ± 1.3	62.9 ± 1.6	48.5 ± 2.0	58.9 ± 1.1	49.8 ± 1.3	58.3 ± 2.5	48.3 ± 1.9
18:2n-6	4.1 ± 0.6	3.8 ± 0.2	11.7 ± 0.9	4.6 ± 0.5	11.6 ± 0.5	4.8 ± 0.3	11.3 ± 1.2	4.7 ± 0.9	11.0 ± 0.9
20:3n-6	0.1 ± 0.1	n.d.	0.2 ± 0.1	n.d.	0.2 ± 0.0	n.d.	0.2 ± 0.2	0.1 ± 0.1	0.2 ± 0.2
20:4n-6	1.6 ± 0.2	1.9 ± 0.4	5.2 ± 1.2	1.9 ± 0.7	8.0 ± 0.8	2.5 ± 0.2	6.2 ± 1.3	2.7 ± 1.3	6.7 ± 1.2
22:4n-6	0.1 ± 0.1	n.d.	0.4 ± 0.1	n.d.	0.5 ± 0.1	0.1 ± 0.2	0.7 ± 0.2	0.2 ± 0.2	0.9 ± 0.2
22:5n-6	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.2	n.d.	0.4 ± 0.0	0.1 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.6 ± 0.1
Total n-6	6.0 ± 0.7	6.0 ± 0.5	18.2 ± 0.6	6.6 ± 1.1	21.2 ± 1.2	7.6 ± 0.8	19.5 ± 0.5	8.0 ± 2.4	19.7 ± 1.9
20:5n-3	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	n.d.	0.3 ± 0.1	n.d.	0.1 ± 0.1	n.d.	n.d.
22:5n-3	0.1 ± 0.1	n.d.	n.d.	n.d.	0.1 ± 0.1	n.d.	n.d.	n.d.	n.d.
22:6n-3	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.1	0.2 ± 0.3
Total n-3	0.8 ± 0.2	0.5 ± 0.1	0.9 ± 0.1	0.5 ± 0.2	0.9 ± 0.2	0.4 ± 0.2	0.6 ± 0.1	0.3 ± 0.1	0.2 ± 0.3

[a]Expressed as percentage of total fatty acids; mean ± SD, $n = 4$ rats in each group. [b]n-9 Eicosatrienoic acid/linoleic acid in diet expressed as percentage of total fatty acids. [c]n.d. = Not detected.

approved by the Animal Ethics Committee of the Institute of Medical and Veterinary Science (Adelaide, Australia) where the animals were housed.

Fatty acid analyses. Lipids were extracted from plasma or homogenized tissue samples with chloroform/methanol (7:3), and extracts were evaporated to dryness under nitrogen. The residues were dissolved in chloroform/methanol (9:1) and stored at −70°C prior to fatty acid analysis. Plasma triglycerides, cholesterol esters, and phospholipids and tissue phospholipid fractions were separated by thin-layer chromatography (6). The samples were then hydrolyzed and methylated in 1% H_2SO_4 and methanol at 70°C for three hours. The resulting methyl esters were separated and quantified by gas–liquid chromatography as described previously (7). All organic solvents contained butylated hydroxyanisole 0.005% (g/100 mL).

Table 4
Plasma Cholesterol Ester Fatty Acids[a]

	Dietary group[b]								
Fatty acids	16:4	12:4	12:19	8:4	8:19	4:4	4:19	0:4	0:19
Total saturated	9.3 ± 1.1	7.1 ± 1.1	6.2 ± 0.3	6.1 ± 0.1	5.4 ± 0.4	6.0 ± 0.2	5.5 ± 0.4	5.6 ± 0.2	5.4 ± 0.1
Total monounsaturated	11.9 ± 0.9	10.3 ± 0.4	8.1 ± 0.4	13.0 ± 0.1	8.1 ± 0.1	13.7 ± 0.6	9.3 ± 1.4	16.2 ± 2.0	10.1 ± 0.7
18:2n-9	2.4 ± 0.1	1.7 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.3 ± 0.0	0.5 ± 0.1
20:2n-9	0.2 ± 0.0	0.1 ± 0.1	0.1 ± 0.2	0.1 ± 0.0	n.d.	n.d.	n.d.	n.d.	n.d.
20:3n-9	24.9 ± 2.1	22.1 ± 1.6	7.0 ± 0.6	17.9 ± 1.7	4.5 ± 0.3	9.9 ± 0.5	2.8 ± 0.4	3.3 ± 1.1	0.3 ± 0.0
22:3n-9	0.1 ± 0.0	n.d.[c]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total n-9	36.4 ± 2.1	31.8 ± 2.1	14.6 ± 0.9	29.2 ± 1.6	11.4 ± 0.2	21.7 ± 1.0	10.5 ± 1.4	16.8 ± 2.5	9.1 ± 0.6
18:2n-6	4.7 ± 0.9	5.1 ± 0.4	8.1 ± 0.6	5.6 ± 0.4	7.2 ± 0.5	5.8 ± 0.3	8.8 ± 0.5	7.6 ± 0.9	8.8 ± 0.6
20:3n-6	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.6 ± 0.1	0.3 ± 0.0
20:4n-6	42.8 ± 2.4	49.8 ± 3.5	65.8 ± 1.3	52.4 ± 2.3	71.1 ± 1.1	60.8 ± 1.2	70.4 ± 2.5	63.7 ± 4.1	72.6 ± 1.0
22:4n-6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
22:5n-6	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.4 ± 0.1
Total n-6	48.4 ± 2.4	55.9 ± 3.2	75.2 ± 1.2	59.2 ± 1.9	79.5 ± 0.6	67.7 ± 1.0	80.5 ± 2.1	72.8 ± 3.1	82.6 ± 0.6
20:5n-3	1.1 ± 0.1	1.0 ± 0.1	0.3 ± 0.0	1.0 ± 0.1	0.3 ± 0.0	0.6 ± 0.1	0.2 ± 0.0	0.7 ± 0.1	0.2 ± 0.0
22:5n-3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
22:6n-3	1.4 ± 0.1	1.4 ± 0.1	1.3 ± 0.0	1.2 ± 0.0	1.2 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	1.0 ± 0.1
Total n-3	2.5 ± 0.1	2.4 ± 0.1	1.7 ± 0.2	2.2 ± 0.1	1.5 ± 0.1	1.8 ± 0.1	1.2 ± 0.1	1.9 ± 0.0	1.1 ± 0.1

[a]Expressed as percentage of total fatty acids; mean ± SD, $n = 4$ rats in each group. [b]n-9 Eicosatrienoic acid/linoleic acid in diet expressed as percentage of total fatty acids. [c]n.d. = Not detected.

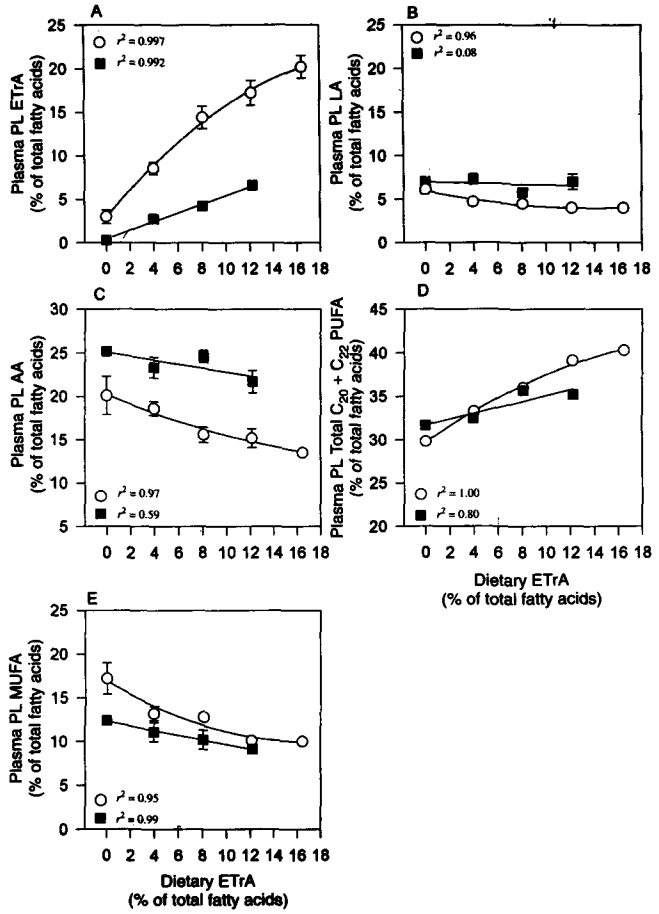

FIG. 1. Plasma phospholipids (PL): Levels of selected fatty acids in plasma lipid fractions from rats fed for four weeks diets containing linoleic acid (LA) at 4.4 % of total fatty acids (O----O) or LA at 19% of total fatty acids (■-----■) and increasing amounts of n-9 eicosatrienoic acid (ETrA). A, ETrA; B, LA; C, arachidonic acid (AA); D, total C_{20} + C_{22} polyunsaturated fatty acids (C_{20} +C_{22} PUFA); E, monounsaturated fatty acids (MUFA).

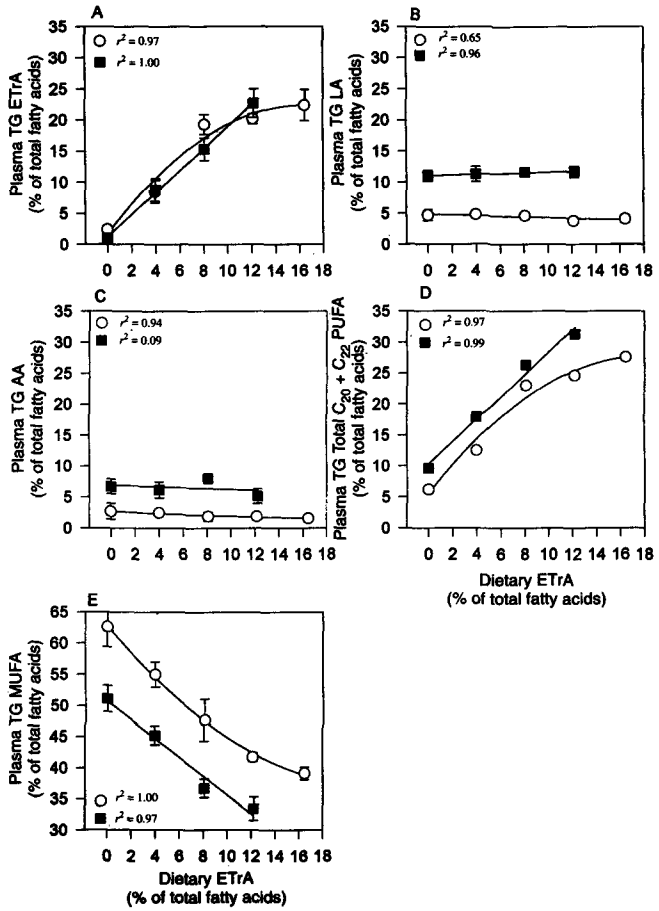

FIG. 2. Plasma triglycerides (TG): See Figure 1 caption for data.

Histological survey. Formalin fixed sections of liver, spleen, kidney, skin, and heart were stained with haematoxalin and eosin and examined by light microscopy.

RESULTS

Fatty acid composition of plasma lipid fractions (Tables 2–4 and Figs. 1–3). With the diets containing the lower level of LA (4.4% total fatty acids), ETrA attained levels of ~20% total fatty acids in all of the plasma lipid fractions, and incorporation was curvilinear in relation to dietary ETrA. Within the ambit of levels attained when the diets contained the higher level of LA (19% total fatty acids), incorporation of ETrA was essentially linear. In the phospholipid and cholesterol ester fractions, but not the triglycerides, ETrA accumulation was reduced substantially by dietary LA.

Accumulation of LA was greater with the higher LA diets than with the lower LA diets, although the differences were modest, except in the triglycerides. By contrast, AA accumulation was strongly influenced by dietary LA in the case of the plasma phospholipids and cholesterol esters. The levels of LA were modestly affected or unaffected by dietary ETrA. However, with increasing dietary ETrA, dose-dependent reductions of AA were seen in all fractions with the exception of the triglycerides where AA levels were low. With increasing dietary ETrA, total C_{20} and C_{22} PUFA increased by up to 10% in the phospholipids and by more than 20% in the triglycerides (with reciprocal displacement of monounsaturates) but were unchanged in the cholesterol esters.

Fatty acid composition of cellular phospholipids (Tables 5–7 and Figs. 4–6). The effects of dietary ETrA and LA on the levels of ETrA, LA, and AA in liver, spleen, and PE cell phospholipids resembled broadly the pattern seen in plasma phospholipids. Compared with liver and plasma phospholipids, spleen and PE cell phospholipids were distinctive in displaying significant accumulations of 22:3n-9, a metabolite of ETrA absent or present in trace amounts only in the diets. With increasing dietary ETrA, there was an overall increase in C_{20} and C_{22} PUFA as a proportion of total fatty acids, with an increment of ~10% of total fatty acids for spleen and PE cells and ~5% of total fatty acids for liver. In liver and spleen, but not PE cells, monounsaturates decreased with increasing dietary ETrA.

General observations and growth. All rats appeared normal in terms of general behavior and condition. Growth was

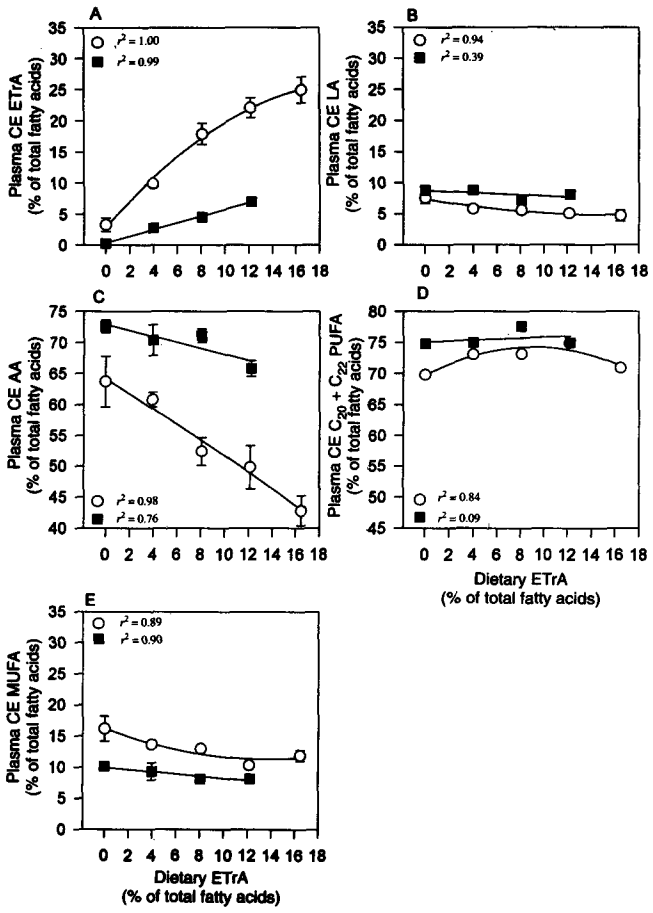

FIG. 3. Plasma cholesterol esters (CE): See Figure 1 caption for data.

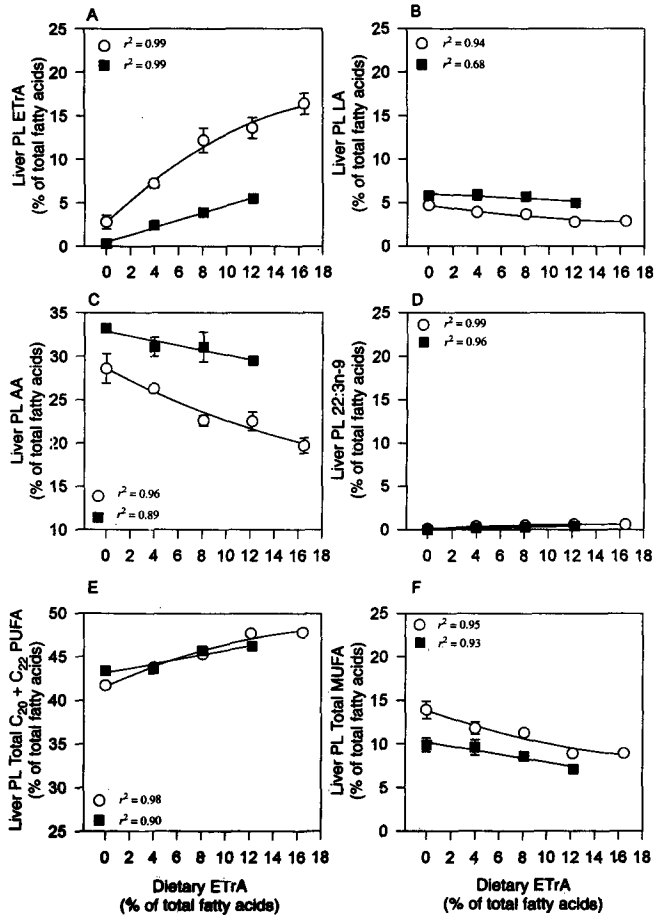

FIG. 4. Liver: Levels of selected fatty acids in tissue and cell phospholipid extracts from rats fed for four weeks diets containing LA at 4.4 % of total fatty acids (O----O) or LA at 19% of total fatty acids (■-----■) and increasing amounts of ETrA. A, ETrA; B, LA; C, AA; D, n-9 docosatrienoic acid (22:3n-9); E, total C_{20} + C_{22} PUFA; F, MUFA. See Figure 1 for abbreviations.

accelerated in rats receiving ETrA relative to rats receiving the diets with no ETrA, regardless of the LA content. There appeared to be a direct relationship between dietary ETrA and weight gain (Fig. 7).

Histological survey. All organs and tissues examined histologically (liver, spleen, kidney, heart, and skin) displayed normal appearances.

DISCUSSION

These investigations show that dietary ETrA appears prominently in plasma lipid fractions and that it is incorporated efficiently into cellular phospholipids in a dose-dependent manner. Levels of ETrA attained 20% of total fatty acids in each of the plasma fractions and 11–16% in PE cell, spleen, and liver. Our data suggest that both plasma fractions and cell membrane phospholipids are saturable in relation to ETrA. The facility with which ETrA accumulates suggests that it competes favorably with potential fatty acid competitors for enzymes responsible for acylation of fatty acids into cell membranes and plasma phospholipids.

Dietary LA had an important negative effect on incorporation of dietary ETrA into plasma lipid fractions and membrane phospholipids. For a given dietary intake of ETrA, levels of ETrA in phospholipids from rats fed the higher LA diets were only 25 to 30% of the values attained with the lower diets. We have reported similar effects of dietary LA on incorporation into plasma lipid fractions and cell membranes of EPA, the n-3 homologue of ETrA (3).

The major n-9 PUFA incorporated into all the plasma fractions and tissues examined was ETrA despite the fact that the diets contained a range of n-9 PUFA. In particular, 18:2n-9 (~14%) was only slightly less abundant in the Mut 48 oil than 20:3n-9 (~16%). That the level of 18:2n-9 only once exceeded 2% total fatty acids in any fraction tested suggests that metabolism of this species occurs readily. One possible route of metabolism of 20:2 n-9 is desaturation by δ5 desaturase to ETrA, which, in contrast to δ6 desaturase, does not appear to be rate-limiting in relation to desaturation of PUFA (8).

In rats, ETrA can be metabolized further to 22:3n-9 (2). This fatty acid was present in only trace amounts in the Mut 48 oil-containing diets and was found in only small amounts (<1% fatty acids) in the plasma fractions and liver phospholipids. By contrast, more substantial accumulation of 22:3n-9

TABLE 5
Liver Phospholipid Fatty Acids[a]

Fatty acids	Dietary group[b]								
	16:4	12:4	12:19	8:4	8:19	4:4	4:19	0:4	0:19
Total saturated	40.1 ± 0.4	40.4 ± 0.2	40.9 ± 0.2	39.1 ± 1.2	38.7 ± 1.8	39.7 ± 0.6	39.9 ± 0.5	38.6 ± 0.3	39.6 ± 0.7
Total monounsaturated	8.9 ± 0.0	8.9 ± 0.4	7.1 ± 0.3	11.3 ± 0.4	8.6 ± 0.5	11.8 ± 0.7	9.6 ± 0.9	13.9 ± 1.0	9.9 ± 0.8
18:2n-9	0.6 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.0	1.1 ± 0.3	0.6 ± 0.2	0.5 ± 0.2	0.7 ± 0.1	0.7 ± 0.2
20:2n-9	0.5 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	n.d.[c]	n.d.
20:3n-9	16.4 ± 1.2	13.6 ± 1.2	5.5 ± 0.4	12.2 ± 1.4	3.9 ± 0.2	7.2 ± 0.5	2.4 ± 0.4	2.8 ± 0.8	0.3 ± 0.0
22:3n-9	0.6 ± 0.0	0.6 ± 0.1	0.4 ± 0.1	0.5 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	n.d.
Total n-9	22.9 ± 1.2	20.1 ± 1.2	10.7 ± 0.4	20.3 ± 1.1	10.3 ± 0.4	15.7 ± 0.7	9.0 ± 1.2	12.7 ± 1.6	7.2 ± 0.6
18:2n-6	2.9 ± 0.1	2.8 ± 0.1	5.0 ± 0.1	3.7 ± 0.4	5.7 ± 0.3	3.9 ± 0.2	5.9 ± 0.6	4.7 ± 0.3	5.8 ± 0.4
20:3n-6	0.7 ± 0.0	0.8 ± 0.0	0.7 ± 0.1	1.0 ± 0.1	0.7 ± 0.0	1.1 ± 0.1	0.9 ± 0.1	1.4 ± 0.2	0.8 ± 0.1
20:4n-6	19.7 ± 0.9	22.5 ± 1.1	29.5 ± 0.3	22.6 ± 0.6	31.1 ± 1.7	26.3 ± 0.4	31.1 ± 1.1	28.6 ± 1.7	33.2 ± 0.2
22:4n-6	0.2 ± 0.0	0.2 ± 0.0	0.5 ± 0.0	0.2 ± 0.0	0.5 ± 0.0	0.3 ± 0.0	0.6 ± 0.0	0.4 ± 0.0	0.7 ± 0.1
22:5n-6	0.7 ± 0.0	1.1 ± 0.1	1.5 ± 0.2	1.0 ± 0.1	1.8 ± 0.3	1.4 ± 0.1	2.3 ± 0.3	1.8 ± 0.2	2.7 ± 0.5
Total n-6	24.2 ± 1.0	27.5 ± 1.0	37.5 ± 0.2	28.7 ± 0.6	40.1 ± 1.8	33.2 ± 0.2	41.1 ± 0.5	37.2 ± 1.0	43.6 ± 0.5
20:5n-3	0.5 ± 0.0	0.4 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.1 ± 0.0
22:5n-3	0.5 ± 0.1	0.5 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
22:6n-3	8.0 ± 0.4	7.7 ± 0.5	7.3 ± 0.3	6.8 ± 0.3	6.7 ± 0.6	6.5 ± 0.2	5.5 ± 0.5	6.0 ± 0.6	5.3 ± 0.5
Total n-3	9.0 ± 0.4	8.5 ± 0.5	7.9 ± 0.4	7.5 ± 0.3	7.2 ± 0.6	7.0 ± 0.2	6.0 ± 0.5	6.5 ± 0.6	5.7 ± 0.5

[a]Expressed as percentage of total fatty acids; mean ± SD, $n = 4$ rats in each group. [b]n-9 Eicosatrienoic acid/linoleic acid in diet expressed as percentage of total fatty acids. [c]n.d. = Not detected.

was observed in spleen and PE cell. This accumulation displayed a highly significant ($r^2 > 0.99$) second-order correlation with dietary ETrA with apparent saturation at levels of 4% for spleen and ~7% for PE cells. Reciprocal depression of the n-6 homologue 22:4n-6 was seen. Metabolism of dietary ETrA differs from EPA (and to a lesser extent AA) with regard to the lack of evident accumulation of the n-9 homologue of n-3 docosahexaenoic acid (DHA) in PE cells (2).

The accomodation of ETrA within plasma phospholipids and cell membranes was achieved by combinations of displacement of AA and monounsaturates and expansion of the sum contribution of C_{20} and C_{22} fatty acids. The differences in the patterns of accumulation of ETrA and other fatty acids between different plasma lipid fractions highlight the shortcomings of analyses confined to total plasma lipids. For example, in the cholesterol esters, an essentially reciprocal relationship between ETrA and AA levels was seen without the increase of $C_{20} + C_{22}$ fatty acids seen with the plasma phospholipids and triglycerides. As discussed above, with spleen and PE cell phospholipids, the ETrA metabolite C22:3n-9

TABLE 6
Spleen Phospholipid Fatty Acids[a]

Fatty acids	Dietary group[b]								
	16:4	12:4	12:19	8:4	8:19	4:4	4:19	0:4	0:19
Total saturated	37.6 ± 0.2	37.7 ± 0.2	38.7 ± 0.3	39.8 ± 0.0	40.7 ± 0.5	39.6 ± 0.2	40.3 ± 0.7	38.7 ± 0.5	39.9 ± 0.1
Total monounsaturated	20.3 ± 0.3	21.8 ± 0.4	18.3 ± 0.2	22.7 ± 0.4	18.4 ± 0.4	23.9 ± 0.4	19.3 ± 0.1	25.3 ± 0.4	20.4 ± 0.3
18:2n-9	1.8 ± 0.6	1.2 ± 0.2	1.2 ± 0.4	1.2 ± 0.2	1.5 ± 0.6	1.2 ± 0.1	1.7 ± 0.9	2.0 ± 1.0	1.2 ± 0.1
20:2n-9	1.8 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.0 ± 0.1	0.9 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.2 ± 0.0	n.d.[c]
20:3n-9	15.2 ± 1.0	13.1 ± 0.5	7.6 ± 0.2	10.1 ± 0.5	5.1 ± 0.2	6.0 ± 0.1	3.1 ± 0.0	1.5 ± 0.2	0.3 ± 0.2
22:3n-9	4.1 ± 0.1	3.9 ± 0.1	3.2 ± 0.1	3.2 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	1.6 ± 0.0	0.6 ± 0.1	0.2 ± 0.0
Total n-9	34.3 ± 0.8	32.5 ± 0.5	23.5 ± 0.5	29.8 ± 0.8	21.0 ± 0.7	25.9 ± 0.4	19.7 ± 0.9	22.7 ± 1.5	16.5 ± 0.3
18:2n-6	3.4 ± 0.1	3.3 ± 0.1	6.4 ± 0.1	3.2 ± 0.1	5.9 ± 0.1	3.3 ± 0.1	6.1 ± 0.2	3.4 ± 0.1	6.0 ± 0.0
20:3n-6	0.7 ± 0.0	0.7 ± 0.0	1.1 ± 0.0	0.8 ± 0.0	1.0 ± 0.0	0.9 ± 0.0	1.1 ± 0.0	1.0 ± 0.0	1.1 ± 0.0
20:4n-6	16.4 ± 0.7	17.8 ± 0.4	21.5 ± 0.3	18.2 ± 0.5	22.3 ± 0.3	21.0 ± 0.3	23.1 ± 0.3	23.2 ± 1.0	25.2 ± 0.2
22:4n-6	0.9 ± 0.1	1.1 ± 0.0	1.8 ± 0.0	1.3 ± 0.1	2.1 ± 0.1	1.8 ± 0.1	2.5 ± 0.1	2.5 ± 0.1	3.4 ± 0.1
22:5n-6	0.2 ± 0.0	0.3 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.5 ± 0.1	0.5 ± 0.0	0.7 ± 0.1	0.7 ± 0.0	0.9 ± 0.1
Total n-6	21.8 ± 0.8	23.4 ± 0.5	31.7 ± 0.5	24.1 ± 0.5	32.4 ± 0.3	27.6 ± 0.3	34.0 ± 0.3	31.0 ± 1.0	37.2 ± 0.2
20:5n-3	0.2 ± 0.0	0.1 ± 0.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
22:5n-3	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.2	0.3 ± 0.0
22:6n-3	1.0 ± 0.0	0.9 ± 0.0	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.0	0.7 ± 0.1	1.0 ± 0.1	0.8 ± 0.1
Total n-3	1.6 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	1.5 ± 0.3	1.2 ± 0.1

[a]Expressed as percentage of total fatty acids; mean ± SD, $n = 4$ rats in each group. [b]n-9 Eicosatrienoic acid/linoleic acid in diet expressed as percentage of total fatty acids. cn.d. = Not detected.

TABLE 7
Peritoneal Exudate Cell Phospholipid Fatty Acids[a]

	Dietary group[b]								
Fatty acids	16:4	12:4	12:19	8:4	8:19	4:4	4:19	0:4	0:19
Total saturated	36.4 ± 0.3	35.9 ± 0.8	36.6 ± 0.2	41.2 ± 0.9	41.7 ± 1.1	39.7 ± 1.5	40.9 ± 0.3	39.2 ± 0.5	40.5 ± 1.4
18:1n-9	10.5 ± 0.4	11.5 ± 0.6	9.8 ± 0.4	11.8 ± 0.8	10.0 ± 0.4	13.6 ± 0.6	11.2 ± 1.3	14.6 ± 1.3	12.4 ± 0.3
Total monounsaturated	23.2 ± 0.7	24.0 ± 0.9	18.9 ± 0.2	22.6 ± 0.6	18.1 ± 0.4	23.8 ± 1.8	19.8 ± 0.7	22.6 ± 0.9	18.9 ± 1.1
18:2n-9	3.7 ± 1.1	3.9 ± 1.1	3.9 ± 0.7	5.3 ± 1.9	6.1 ± 2.9	5.2 ± 4.9	5.7 ± 2.2	6.2 ± 1.0	5.8 ± 3.9
20:2n-9	2.2 ± 0.1	1.9 ± 0.1	1.5 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	0.5 ± 0.3	n.d.	0.1 ± 0.3	n.d.
20:3n-9	11.3 ± 0.6	9.3 ± 0.4	5.3 ± 0.0	6.7 ± 0.7	3.5 ± 0.2	4.0 ± 0.2	1.9 ± 0.1	1.0 ± 0.2	n.d.
22:3n-9	6.7 ± 0.3	6.6 ± 0.4	4.2 ± 0.2	4.9 ± 0.2	3.1 ± 0.3	2.8 ± 0.8	1.8 ± 0.1	0.7 ± 0.5	n.d.
Total n-9	36.5 ± 1.2	35.3 ± 0.8	26.4 ± 0.8	32.0 ± 1.1	25.6 ± 2.9	29.0 ± 4.2	23.6 ± 1.5	25.9 ± 1.3	20.9 ± 3.9
18:2n-6	2.8 ± 0.1	2.7 ± 0.2	5.1 ± 0.3	2.2 ± 0.1	4.4 ± 0.1	2.4 ± 0.2	4.4 ± 0.7	2.6 ± 0.3	4.6 ± 0.3
20:3n-6	0.7 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.1	0.8 ± 0.0	0.6 ± 0.4	0.9 ± 0.1	1.0 ± 0.1	0.7 ± 0.5
20:4n-6	17.0 ± 1.2	17.7 ± 1.0	22.1 ± 0.5	16.6 ± 0.4	19.6 ± 1.6	19.4 ± 0.7	20.5 ± 2.9	20.6 ± 1.5	21.6 ± 0.5
22:4n-6	2.5 ± 0.2	3.2 ± 0.3	4.2 ± 0.2	3.4 ± 0.5	4.5 ± 0.3	3.8 ± 0.8	5.0 ± 1.3	5.3 ± 1.1	7.0 ± 0.4
22:5n-6	n.d.	0.1 ± 0.2	0.4 ± 0.3	n.d.	0.2 ± 0.3	0.4 ± 0.3	0.8 ± 0.2	0.5 ± 0.4	0.7 ± 0.5
Total n-6	22.9 ± 1.4	24.5 ± 1	32.6 ± 0.9	23.0 ± 0.4	29.6 ± 2.0	26.7 ± 1.2	31.6 ± 2.5	30.1 ± 1.1	34.7 ± 1.7
20:5n-3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
22:5n-3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
22:6n-3	0.4 ± 0.4	0.5 ± 0.3	0.3 ± 0.3	0.1 ± 0.2	0.0 ± 0.0	0.2 ± 0.3	0.2 ± 0.3	0.1 ± 0.2	0.1 ± 0.2
Total n-3	0.4 ± 0.4	0.5 ± 0.3	0.3 ± 0.3	0.1 ± 0.2	0.0 ± 0.0	0.2 ± 0.3	0.2 ± 0.3	0.1 ± 0.2	0.1 ± 0.2

[a]Expressed as percentage of total fatty acids; mean ± SD, $n = 4$ rats in each group. [b]n-9 Eicosatrienoic acid/linoleic acid in diet expressed as percentage of total fatty acids. [c]n.d. = Not detected.

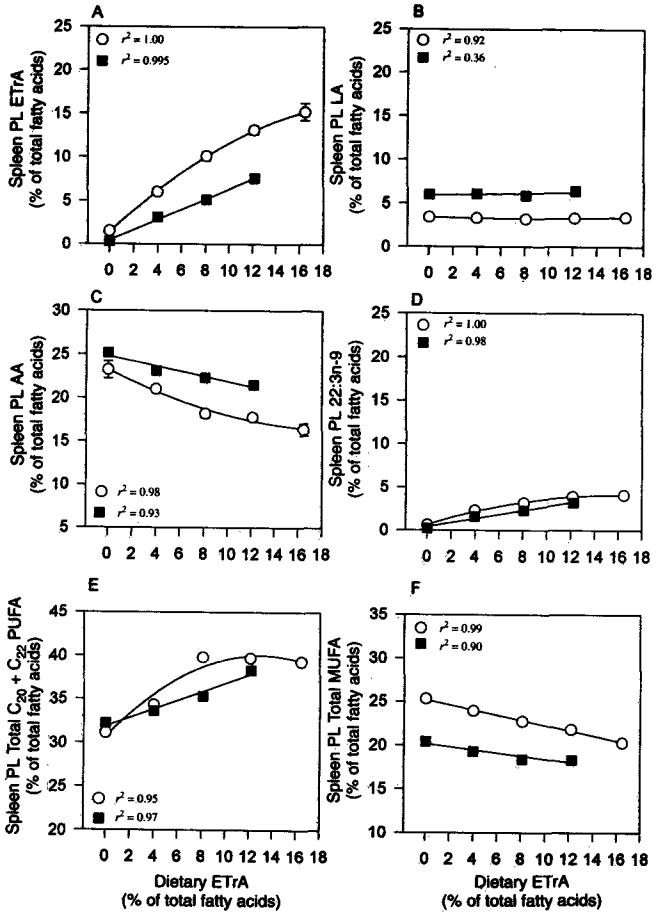

FIG. 5. Spleen: See Figure 4 caption for data.

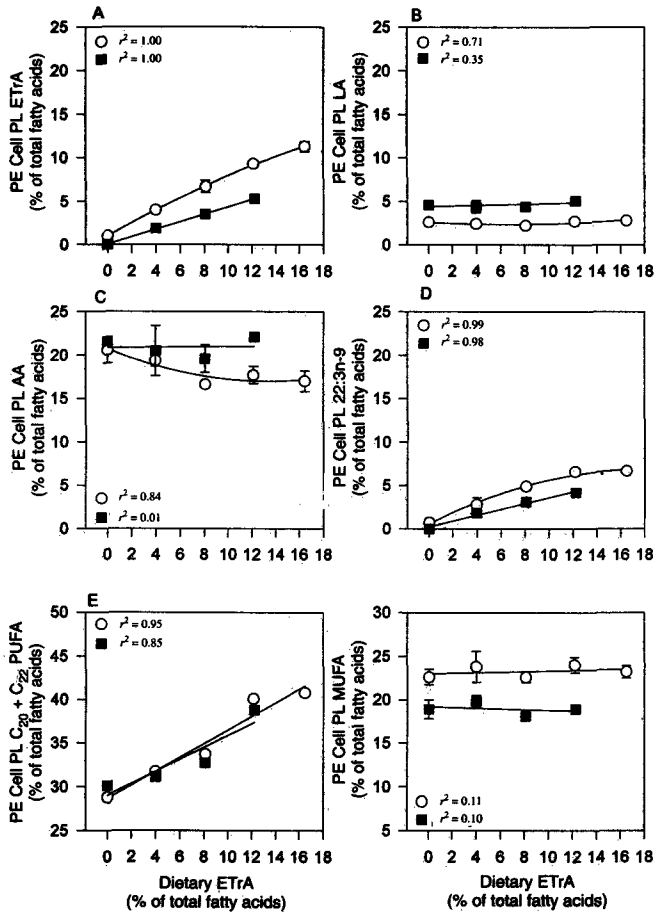

FIG. 6. Peritoneal exudate cells (PE cell): See Figure 4 caption for data.

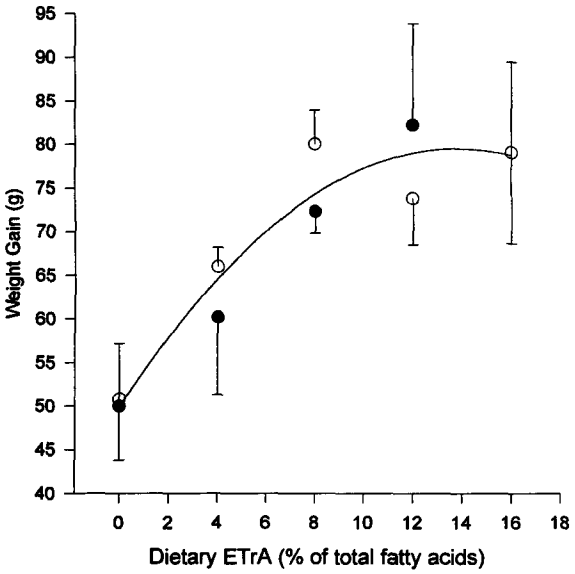

FIG. 7. Mean weight gain (g) observed in groups of rats ($n = 4$) during a four-week period of feeding with diets containing LA at 4.4% of total fatty acids (O----O) or LA at 19% of total fatty acids (■-----■) and increasing amounts of ETrA. See Figure 1 for abbreviations.

also accumulated. The accumulations of ETrA and 22:3n-9 in cells appeared to be potentially saturable as evinced by the observed hyperbolic relationships with dietary ETrA.

With the exception of the plasma cholesterol ester fraction, plasma and cellular AA were only partially displaced by dietary ETrA (as discussed above). This is similar to changes seen in EFA deficiency where AA levels are relatively conserved in the presence of LA depletion and ETrA accumulation (9). The ETrA-rich diets were associated with a modest reduction in LA in some, but not all, fractions or tissues. With the exception of the plasma triglycerides, dietary LA had little effect on LA accumulation in the various fractions, thus establishing the EFA-sufficiency of the lower LA diets. The salient biochemical point of distinction between the effects of ETrA-rich diets and EFA deficiency is the profound LA depletion in plasma and cells seen only in the latter. This is an important difference since the loss of cutaneous barrier function seen in EFA deficiency has been attributed directly to LA depletion (10).

The positive effect on growth of the ETrA-rich diets suggests that ETrA, or some other factor within Mut 48 oil, possesses general metabolic effects. However, only group data with large variation are available from this experiment, and further investigation on weight gain in individual rats is needed to confirm this apparent finding. Nevertheless, the present studies at least establish that dietary ETrA has no negative effects on growth *per se*, in contrast to the reported effects of EFA deficiency.

The very substantial incorporation of ETrA from the diet into cells observed raises questions as to the effects of this incorporation on cell metabolism. As a C_{20} PUFA, ETrA is a potential substrate competitor with AA for eicosanoid-forming enzymes. However, ETrA cannot be converted to a cyclic eicosanoid, such as a thromboxane or prostaglandin, because it lacks the n-6/δ14 double bond. Nevertheless, ETrA could influence eicosanoid synthesis through displacement of AA at the *sn*-2 position of phospholipids, resulting in reduced release of AA by phospholipases. This effect would be analogous to that seen with long-chain n-3 fatty acids where the antiinflammatory effects of purified EPA and DHA ethyl esters in a model sensitive to cyclooxygenase inhibition (carrageenan-induced paw oedema in the rat) correlated with the extent of reduction of AA as a proportion of total C_{20} and C_{22} PUFA in leucocyte phospholipids (11).

In a separate component of the present study, we analyzed the effects of the test diets on leukotriene B_4 (LTB_4) production by rat PE cells stimulated *in vitro*. Dietary ETrA inhibited LTB_4 production in a dose-dependent manner, and the effect was attributable to dose-dependent inhibition of LTA hydrolase (5). These observations confirm and extend our earlier studies into the effects of dietary supplementation with synthetic ETrA on LTB_4 production by leucocytes in rats (2), and are also consistent with *in vitro* studies into the effects of exogenous ETrA on LTB_4 production (9,12). The substantial incorporation of ETrA seen in spleen cells raises the possibility that dietary fortification of ETrA may have other important immunomodulatory effects. It is also possible that 22:3n-9, which accumulates in spleen and PE cell phospholipids, could also have immunomodulatory actions.

A clue to possible beneficial effects of ETrA may be found in the homology between ETrA and EPA, a constituent of fish oils which has been shown to have antiinflammatory effects (13,14). Conceivably, future investigations could be undertaken to assess the effects of ETrA in animal models of human diseases and, in particular, diseases in which there is an immunological pathogenesis or an important inflammatory component. The expected greater stability of ETrA-rich oils relative to n-3 fatty acid-rich oils, which have immunomodulatory effects, makes dietary ETrA fortification potentially a more acceptable and perhaps more effective strategy. If ETrA proves to be nontoxic and useful in reducing the incidence and/or severity of inflammatory diseases in susceptible animals, dietary studies could be undertaken in humans, involving, in the first instance, those with immunoinflammatory disorders or those identified as being at increased risk.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Health and Medical Research Council of Australia. The expert technical assistance of Cindy Hall is gratefully acknowledged.

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[Received February 12, 1996; Revision accepted June 3, 1996]