Comparison of Cell Membrane Phospholipid Fatty Acids in Five Rat Strains Fed Four Test Diets

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The fatty acid composition of phospholipids in peritoneal exudate cells and spleen cells was assessed in five rat strains fed four test diets of differing fatty acid composition. Distinctive patterns of fatty acids were seen in the total phospholipid preparations in both cell types in response to the diets which contained either olive, sunflower, linseed or fish oil. In general, similar fatty acid profiles were seen in each of the rat strains fed the same diet with the only evidence of possible genetic (strain) variation being a relative deficiency of Δ4 desaturase in Dark Agouti rats.
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Following absorption, dietary fatty acids are transported in lipoproteins and taken up by hepatic and other cells, where they may be elongated and desaturated through multiple enzymatic steps (1). Twenty-carbon fatty acids derived from dietary precursors or provided in the diet can be transferred *via* plasma lipoproteins to cells throughout the body where they act as substrates for several oxygenase enzymes. Two of these enzymes (cyclooxygenase and 5-lipoxygenase) are pivotal in the multienzyme pathways which lead to the synthesis of the prostaglandins and leukotrienes (eicosanoids), which have important mediating functions in many homeostatic and pathological processes throughout the body. Thus, the effects of treatments which involve changing the proportions of fatty acids in the diet will depend upon multiple metabolic transformations, each of which is potentially subject to varying degrees of biological variation. Under these circumstances, possible genetically determined diversity in response becomes an important issue. A search for such diversity in rat strains is important as a basis for assessing the likely generality of findings obtained in a single rat strain and may also yield a model for metabolic diversity in human populations.

Important differences between rat strains have been observed in some relevant experimental settings. For example, rat strains may differ in their clinical responses to the anti-inflammatory effects of fish-oil supplemented diets on adjuvant induced polyarthritis (2). Rat strains have also been shown to differ in the rate at which they metabolize chylomicrons following fat ingestion (3). Accordingly, the present study was undertaken to assess the extent to which rat strains may vary in their responses to modification of dietary fatty acids in relation to the composition of phospholipid fatty acids in leucocyte membranes.

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Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; DGLA, dihomoylinolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GLC, gas-liquid chromatography; LA, linoleic acid; PBS, phosphate buffered saline; TLC, thin-layer chromatography.

METHODS

Rats. Five rat strains were studied—Dark Agouti, Ginger Hooded, Porton, Sprague Dawley and Hooded Wistar. Weanling rats were obtained from the Gilles Plains Animal Breeding Facility of the South Australian Department of Agriculture. Cohorts of 16 rats were divided into four groups providing four rats for each dietary treatment group. The rat strains were entered sequentially at 1-week intervals with all animals entering the dietary treatment period at six weeks of age. Studies with Dark Agouti and Hooded Wistar (control) rats were repeated to corroborate differences seen in the Dark Agouti rats in the initial study.

Diets. Diets were prepared from fat-free rat chow (Milne Feeds, Perth, Australia) to which the oil supplements were added to achieve a final fat content of 10% (w/w, dry weight). Four oil supplements were used—sunflower (Flora, Unilever Pty. Ltd., Adelaide, Australia), olive (F.H. Faulding & Co. Pty. Ltd., Adelaide, Australia), linseed (Diggers Trading Co., Adelaide, Australia) and fish oil (Maxepa, R.P. Scherer, Melbourne, Australia). Vitamin E was added to achieve a final concentration of 1.84 mg/g oil. The oils and other constituents were mixed with sufficient water to allow 3 × 3 × 1 cm biscuits to be prepared. These were air dried overnight at room temperature. The food was not examined for lipid oxidation, but all diets contained the same levels of vitamin E. The proportions of major fatty acids in the diets are shown in Table 1. The diets were stored at −20°C until used. The rats were fed test diets and water *ad libitum*, with food being changed each evening. The diets were continued for four weeks until induction of peritoneal exudates. All diets resulted in similar weight gains; e.g., the average weight gain over the four-week period for Porton rats were; olive, 182 g; sunflower, 167 g; linseed, 169 g; and fish, 176 g.

TABLE 1

Fatty Acid Profile of Diets[a]

	Diet			
	Olive	Sunflower	Linseed	Fish
Oleic 18:1n-9	74.0	26.6	22.1	10.0
Linoleic 18:2n-6	8.0	57.7	17.0	1.7
Arachidonic 20:4n-6	—	—	—	1.1
α-Linolenic 18:3n-3	1.0	1.6	46.8	0.8
Eicosapentaenoic 20:5n-3	—	—	—	16.5
Docosahexaenoic 22:6n-3	—	—	—	11.7

[a]Expressed as percentage of total fatty acids.

Induction of peritoneal exudates. Exudates were induced 4 hr before sacrifice by injection into the peritoneal cavity of 3% bacteriological grade peptone (Oxoid Ltd., Hampshire, U.K.) in 10 mL of normal saline. At 4 hr, rats were anaesthetized (Halothane) and blood was collected by cardiac puncture. The anaesthetized animals were sacrificed by cervical dislocation. Peritoneal exudate cells were harvested by lavage with 20 mL phosphate buffered saline (PBS). Typically, 1×10^8 leucocytes were obtained. The cells comprised 80–90% neutrophils as assessed microscopically following nuclear staining with Gentian Violet. The cell preparations were washed twice in PBS, which included one red cell lysis treatment with hypotonic saline (30 seconds) and finally resuspended in normal saline. Membrane lipids were then extracted in chloroform/methanol (2:1, v/v) containing butylated hydroxy anisole (0.005% w/w) according to the method of Bligh and Dyer (4) and stored at −20°C prior to gas-liquid chromatographic (GLC) analyses.

Preparation of spleen cells. The spleen was removed and cells were dispersed by passage through a fine wire mesh. Cells were sedimented by centrifugation. Erythrocytes were lysed by 20-second exposure to distilled water. The wash and lysis procedure was repeated once, after which cells were washed and finally resuspended in normal saline. Membrane lipids were then extracted in chloroform/methanol as described above for peritoneal exudate cells.

Separation of tissue phospholipids. Phospholipids in cell membrane extracts were separated from the total extracted lipid by thin-layer chromatography (TLC) on silica gel H plates developed in petroleum ether/diethyl ether/acetic acid (90:15:1, v/v/v). The band containing the total phospholipids remaining at the origin was scraped into the methylating solution and methylated in 1% (v/v) H_2SO_4 in methanol by heating at 70°C for 3 hr. The resulting fatty acid methyl esters were extracted and analyzed by gas chromatography.

Tissue phospholipid fatty acid analysis. Analyses of the fatty acid methyl esters of total phospholipids were performed using capillary gas chromatography (Hewlett Packard HP 5880 gas chromatograph; Hewlett Packard, Palo Alto, CA). The column was a 50-meter glass column (0.56 mm, I.D.) coated with SP2340 (Supelco Inc.) prepared by Chromolytic Technology Ltd. (Boronia, Victoria). The esters were separated using a carrier gas (helium) flow of 25 cm/sec with a temperature gradient of 120°C to 200°C at 5°C per min. The injection/split temperature was 250°C and the flame ionization detector temperature was 300°C. Fatty acid methyl esters were identified against authentic lipid standards supplied by Nuchek Prep (Elysian, MN). The flame ionization detector was calibrated so that the response obtained for each of the fatty acids between C_{14}–C_{24} was identical regardless of the number of double bonds.

Statistical analysis. Newman-Keuls multiple comparisons analysis was used to identify dietary or strain differences (5).

RESULTS

Consistency and reproducibility of findings within groups. Distinctive patterns in the percentage of fatty acids in phospholipids of cell preparations were seen in response to each of the dietary treatments in all rat strains. The effects of the diets were consistent within strains with little variability between animals; e.g., the coefficients of variation for peritoneal exudate cell linoleic acid (LA, 18:2n-6) determinations for Porton rats on the different diets were: olive, 3%; sunflower, 6%; linseed, 11%; and fish, 9%. All determinations had similar values. With one exception (detailed below), no systematic difference was seen between strains given the same dietary treatments.

n-9 Fatty acids. The oleic acid-rich olive oil diet was associated with substantially higher proportions of oleic acid (18:1n-9) in phospholipids in cell membranes than found with the other diets (Table 2). Levels of 20:3n-9 were slightly increased in the cell membranes of olive oil-fed rats as compared with the other treatment groups. However, all levels were less than 2%.

n-6 Fatty acids. There appeared to be no simple relationship between dietary LA and n-6 fatty acids in membranes of peritoneal exudate cells or spleen cells. Although by far the highest proportion of dietary linoleic acid was present in the sunflower oil diet (Table 1), the highest proportions of linoleic acid in cell membrane phospholipids were found in samples from the linseed oil-fed and sunflower oil-fed rats (Table 3). Substantially lower levels were found in olive oil- and fish oil-fed rats. However, arachidonate (AA, 20:4n-6) levels in both cell types were highest in olive oil- and sunflower oil-fed rats and lowest in linseed oil- and fish oil-fed animals. AA was a smaller proportion of the phospholipid fatty acids of spleen cells than peritoneal exudate cells in all treatment groups. Proportions of dihomoγlinolenic acid levels (DGLA, 20:3n-6) were consistently less than 2% in both cell types (data not shown).

The proportions of long chain metabolites, 22:4n-6 and 22:5n-6, were higher in phospholipids from olive oil- and sunflower oil-fed rats (Table 4) as compared with their counterparts from linseed oil- and fish oil-fed rats in which these fatty acids were all less than 1%.

In rats fed olive oil or sunflower oil diets, significantly less 22:5n-6 was found in spleen cell phospholipids of Dark Agouti rats as compared with the other rat strains (Newman-Keuls, $p < 0.05$) (Table 4). These differences were confirmed in a second experiment in which Dark Agouti and Hooded Wistar rats were given the same dietary treatments (data not shown). Less 22:5n-6 was also found in peritoneal exudate cells from Dark Agouti rats fed sunflower oil.

n-3 Fatty acids. Despite the high level of α-linolenic acid (ALA, 18:3n-3) in the diet of linseed oil-fed rats, only small amounts of ALA were found in the cell membrane phospholipids (Table 5). ALA was detected in very small amounts or was undetectable in the phospholipids from the other treatment groups.

Fish oil-fed rats had two to three times higher proportions of eicosapentaenoic acid (EPA, 20:5n-3) in cellular phospholipids compared with linseed oil-fed rats (Table 5). EPA levels were <1% or undetectable in phospholipids from olive oil- and sunflower oil-fed rats.

In both fish oil- and linseed oil-fed rats, peritoneal exudate cell phospholipids were found to have higher proportions of EPA compared with phospholipids from spleen cells. The proportion of 22:5n-3, one of the metabolites of EPA, in membrane phospholipids was greater in spleen cells than peritoneal exudate cells in all

TABLE 2

n-9 Fatty Acid Content[a]

Diet	Rat strain	Peritoneal exudate cells		Spleen cells	
		18:1	20:3	18:1	20:3
Olive oil	DA	25.1 ± 2.9	0.7 ± 0.1	20.5 ± 2.7	0.9 ± 0.1
	GH	18.9 ± 1.3	0.9 ± 0.1	16.7 ± 2.0	1.0 ± 0.0
	P	18.3 ± 0.7	1.3 ± 0.3	17.9 ± 0.8	1.3 ± 0.2
	SD	20.7 ± 0.6	1.5 ± 0.1	16.8 ± 0.1	1.7 ± 0.2
	HW	22.9 ± 1.6	0.7 ± 0.1	19.9 ± 0.7	1.0 ± 0.1
Sunflower oil	DA	13.1 ± 3.0	n.d.[b]	8.9 ± 0.4	n.d.
	GH	8.8 ± 0.4	n.d.	7.9 ± 0.5	n.d.
	P	8.2 ± 0.4	n.d.	7.8 ± 0.4	n.d.
	SD	11.1 ± 0.2	n.d.	8.0 ± 0.2	n.d.
	HW	13.2 ± 2.6	n.d.	9.7 ± 2.3	n.d.
Linseed oil	DA	12.5 ± 0.9	0.4 ± 0.1	11.0 ± 0.1	0.2 ± 0.0
	GH	11.8 ± 0.7	0.1 ± 0.1	10.5 ± 0.1	0.1 ± 0.0
	P	13.0 ± 0.9	n.d.	11.6 ± 0.5	n.d.
	SD	14.5 ± 0.8	0.1 ± 0.0	11.1 ± 0.3	0.1 ± 0.1
	HW	12.8 ± 0.3	0.3 ± 0.1	10.8 ± 0.2	0.2 ± 0.0
Fish oil	DA	12.2 ± 1.2	n.d.	10.0 ± 0.2	0.2 ± 0.0
	GH	12.4 ± 1.8	0.1 ± 0.1	9.6 ± 0.4	0.2 ± 0.0
	P	11.4 ± 1.1	0.1 ± 0.0	10.0 ± 0.2	0.2 ± 0.0
	SD	14.3 ± 1.1	0.5 ± 0.5	9.9 ± 0.6	0.2 ± 0.0
	HW	11.9 ± 0.5	n.d.	9.3 ± 0.3	0.2 ± 0.0

[a]Results are expressed as % of total phospholipid fatty acids and represent the means ± S.D. of data from four rats in each strain. DA, Dark Agouti; GH, Ginger Hooded; P; Porton; SD, Sprague Dawley; HW, Hooded Wistar.

[b]Not detectable; minimum detectable level, 0.1.

TABLE 3

n-6 Fatty Acid Content[a]

Diet	Rat strain	Peritoneal exudate cells		Spleen cells	
		18:2	20:4	18:2	20:4
Olive oil	DA	5.5 ± 0.4	20.7 ± 2.2	6.5 ± 0.2	19.3 ± 2.0
	GH	4.8 ± 1.2	26.6 ± 2.4	5.2 ± 0.8	23.4 ± 2.0
	P	4.8 ± 0.1	24.7 ± 0.3	5.3 ± 0.2	22.1 ± 0.3
	SD	4.5 ± 0.2	23.3 ± 0.4	5.5 ± 0.2	21.4 ± 0.2
	HW	6.0 ± 0.1	24.6 ± 1.9	6.5 ± 0.4	21.4 ± 0.8
Sunflower oil	DA	11.9 ± 0.4	26.4 ± 3.2	12.8 ± 0.2	23.7 ± 0.4
	GH	11.7 ± 0.5	29.2 ± 1.1	11.9 ± 0.8	24.2 ± 0.5
	P	13.2 ± 0.8	29.0 ± 1.0	13.8 ± 1.0	24.6 ± 0.8
	SD	12.1 ± 0.6	24.5 ± 0.7	12.6 ± 0.7	22.1 ± 0.4
	HW	12.8 ± 0.1	27.7 ± 1.9	12.7 ± 0.3	23.9 ± 2.0
Linseed oil	DA	19.6 ± 2.6	14.0 ± 0.9	14.5 ± 0.2	12.4 ± 0.3
	GH	12.2 ± 0.2	20.9 ± 0.9	13.6 ± 0.1	16.3 ± 0.3
	P	12.1 ± 1.3	15.3 ± 1.1	13.4 ± 0.5	12.8 ± 0.7
	SD	11.6 ± 0.4	18.7 ± 1.0	13.5 ± 0.5	14.5 ± 0.6
	HW	14.9 ± 1.5	18.2 ± 0.5	14.9 ± 0.3	13.8 ± 0.3
Fish oil	DA	4.8 ± 0.8	14.1 ± 0.5	3.7 ± 0.2	9.3 ± 0.1
	GH	5.3 ± 1.0	13.2 ± 1.6	4.2 ± 0.5	9.8 ± 0.3
	P	3.3 ± 0.3	12.7 ± 0.7	3.1 ± 0.2	9.3 ± 0.1
	SD	4.1 ± 0.3	14.0 ± 0.2	3.4 ± 0.2	9.7 ± 0.3
	HW	4.6 ± 0.1	14.5 ± 0.8	4.0 ± 0.1	10.0 ± 0.1

[a]Results are expressed as % of total phospholipid fatty acids and represent the means ± S.D. of data from four rats in each strain. Abbreviations as in Table 2.

TABLE 4

22-Carbon n-6 Fatty Acid Content[a]

Diet	Rat Strain	Peritoneal exudate cells		Spleen cells	
		22:4	22:5	22:4	22:5
Olive oil	DA	1.9 ± 0.0	n.d.[b]	1.7 ± 0.2	0.2 ± 0.1[c]
	GH	2.4 ± 0.4	0.4 ± 0.2	2.5 ± 0.1	0.6 ± 0.3
	P	1.7 ± 0.1	0.4 ± 0.1	2.1 ± 0.2	0.5 ± 0.1
	SD	1.9 ± 0.1	0.4 ± 0.0	2.3 ± 0.0	0.7 ± 0.1
	HW	1.4 ± 0.4	0.1 ± 0.1	1.9 ± 0.1	0.3 ± 0.1
Sunflower oil	DA	3.7 ± 0.5	0.3 ± 0.1	3.5 ± 0.1	0.4 ± 0.0[c]
	GH	2.8 ± 0.2	1.0 ± 0.2	3.5 ± 0.2	1.5 ± 0.2
	P	2.3 ± 0.3	0.5 ± 0.1	3.9 ± 0.2	0.7 ± 0.2
	SD	2.5 ± 0.2	0.6 ± 0.1	3.7 ± 0.1	1.0 ± 0.2
	HW	2.3 ± 0.5	0.7 ± 0.1	3.8 ± 0.3	1.2 ± 0.2

[a]Results are expressed as % of total phospholipid fatty acids and represent the means ± S.D. of data from four rats in each strain. Abbreviations as in Table 2.

[b]Not detectable, minimum detectable level, 0.1.

[c]$p < 0.05$, compared with all other strains, Newman-Keuls Analysis.

TABLE 5

n-3 Fatty Acid Content[a]

Diet	Rat strain	Peritoneal exudate cells		Spleen cells	
		18:3	20:5	18:3	20:5
Linseed oil	DA	1.6 ± 0.3	5.2 ± 0.6	1.8 ± 0.1	4.5 ± 0.2
	GH	1.1 ± 0.3	5.2 ± 0.3	1.9 ± 0.1	3.2 ± 0.2
	P	1.7 ± 0.2	5.9 ± 1.4	2.2 ± 0.4	4.1 ± 0.2
	SD	1.7 ± 0.2	4.3 ± 0.4	2.1 ± 0.1	3.6 ± 0.2
	HW	1.2 ± 0.2	5.2 ± 0.5	1.9 ± 0.1	3.8 ± 0.2
Fish oil	DA	0.5 ± 0.3	13.6 ± 0.9	n.d.[b]	10.7 ± 0.1
	GH	0.1 ± 0.1	13.5 ± 1.3	0.1 ± 0.1	9.9 ± 0.4
	P	0.5 ± 0.1	15.6 ± 0.7	n.d.	11.3 ± 0.2
	SD	n.d.	11.8 ± 1.1	n.d.	10.2 ± 0.3
	HW	0.5 ± 0.4	13.4 ± 0.6	0.1 ± 0.1	10.4 ± 0.3

[a]Results are expressed as % of total phospholipid fatty acids and represent the means ± S.D. of data from four rats in each strain. Abbreviations as in Table 2.

treatment groups (Table 6). Only trace amounts of 22:5n-3 were found in phospholipids from the olive oil- and sunflower oil-fed rats.

By far the highest proportions of docosahexaenoic acid (DHA) 22:6n-3 were found in the fish oil-fed animals. DHA constituted a higher proportion of phospholipids of spleen cells than peritoneal exudate cells in all dietary groups except the sunflower oil-fed group. In linseed oil-fed rats, DHA levels were significantly reduced in spleen cell phospholipids from Dark Agouti rats as compared with spleen cell preparations from all other strains (Newman-Keuls, $p < 0.05$) (Table 6). This was confirmed in a second experiment in which Dark Agouti and Hooded Wistar rats were given the same dietary treatments (data not shown). The DHA level in peritoneal exudate cells from linseed oil-fed Dark Agouti rats was lower than those in the other strains, but this difference was not statistically significant (Newman-Keuls, $p = 0.05$).

DISCUSSION

In the present investigations, the cell membrane fatty acid composition of the five rat strains responded in a similar manner to the different dietary treatments. A consistent exception was seen in Dark Agouti rats which, by comparison with the other strains fed similar diets, had reduced levels of long-chain fatty acids (22:5n-6 and 22:6n-3) in cellular phospholipids. DHA was reduced in Dark Agouti rats on the linseed oil diet which is rich in precursor α-linolenic acid (ALA). This reduction was not evident with the sunflower or olive oil diets, possibly due to the greatly reduced levels of dietary α-linolenic acid which may be at saturating levels for the metabolism of ALA to DHA on the linseed oil diet. The long-chain n-6 fatty acid, 22:5n-6, was reduced in Dark Agouti rats on the sunflower and olive oil diets with low levels (<1%) being present in all strains on the other diets. These

TABLE 6

22-Carbon n-3 Fatty Acid Content[a]

Diet	Rat strain	Peritoneal exudate cells		Spleen cells	
		22:5	22:6	22:5	22:6
Olive oil	DA	0.3 ± 0.1	0.8 ± 0.4	0.9 ± 0.1	1.4 ± 0.3
	GH	0.2 ± 0.2	1.1 ± 0.5	0.4 ± 0.1	1.6 ± 0.2
	P	0.1 ± 0.0	0.9 ± 0.7	0.4 ± 0.0	1.3 ± 0.1
	SD	0.1 ± 0.1	0.5 ± 0.0	0.5 ± 0.1	1.3 ± 0.1
	HW	0.1 ± 0.2	0.8 ± 0.3	0.5 ± 0.0	1.7 ± 0.1
Sunflower oil	DA	0.2 ± 0.2	0.7 ± 0.6	0.2 ± 0.0	0.3 ± 0.1
	GH	n.d.[b]	1.4 ± 0.1	0.2 ± 0.0	1.1 ± 0.1
	P	n.d.	0.2 ± 0.1	0.2 ± 0.0	0.4 ± 0.1
	SD	n.d.	1.0 ± 0.1	0.2 ± 0.1	0.8 ± 0.1
	HW	0.1 ± 0.3	0.6 ± 0.4	0.2 ± 0.1	0.8 ± 0.5
Linseed oil	DA	2.2 ± 0.3	0.2 ± 0.0	4.2 ± 0.1	0.4 ± 0.0[c]
	GH	1.7 ± 0.1	0.9 ± 0.4	3.7 ± 0.3	1.8 ± 0.2
	P	1.7 ± 0.1	0.6 ± 0.1	3.6 ± 0.1	1.3 ± 0.2
	SD	1.6 ± 0.1	1.1 ± 0.1	3.7 ± 0.2	1.3 ± 0.1
	HW	1.3 ± 0.2	0.4 ± 0.1	4.1 ± 0.3	1.2 ± 0.2
Fish oil	DA	2.4 ± 0.1	2.4 ± 0.2	4.9 ± 0.2	4.8 ± 0.2
	GH	3.2 ± 1.3	3.2 ± 0.6	4.8 ± 0.2	5.4 ± 0.3
	P	2.7 ± 0.6	3.2 ± 0.5	4.7 ± 0.4	4.8 ± 0.4
	SD	2.0 ± 0.2	2.5 ± 0.5	4.9 ± 0.1	5.0 ± 0.3
	HW	2.1 ± 0.6	2.4 ± 0.3	4.9 ± 0.2	5.8 ± 0.2

[a]Results are expressed as % of total phospholipid fatty acids and represent the means ± S.D. of data from four rats in each strain. Abbreviations as in Table 2.

[b]Not detectable, minimum detectable level, 0.1.

[c]$p < 0.05$, compared with all other strains, Newman-Keuls Analysis.

findings, which were confirmed in a subsequent experiment, suggest the possibility of a relative deficiency in Dark Agouti rats of Δ4 desaturase activity, the enzyme putatively responsible for the synthesis of these fatty acids.

Each of the four test diets was associated with distinct effects common to all of the rat strains studied. However, the unique effects of each diet on phospholipid fatty acid profiles cannot be entirely predicted from the dietary fatty acid profiles. For example, olive oil and sunflower oil have similar ALA levels, but olive oil was associated with higher proportions of the 22-carbon n-3 fatty acids compared with the sunflower oil diet, possibly due to the greater LA/ALA ratio in sunflower oil (36:1) as compared with olive oil (8:1). This latter finding may be relevant to the association of olive oil rich diets with low risk of occlusive vascular disease in humans (6), an association also seen with fish oil diets which are rich in 20- and 22-carbon n-3 fatty acids (7).

The linseed oil diet and the fish oil diet contain substantial amounts of oleic acid (linseed 22% and fish 9%) and yielded similar proportions of n-9 fatty acids in the phospholipids. Both diets were associated with substantially lower levels of arachidonic acid than were found with the olive oil and sunflower oil diets. The fish oil diet was associated with low linoleic acid levels in the phospholipid fractions as expected from the low linoleic acid content of fish oil. However, the linseed oil diet was associated with high levels of linoleic acid in the phospholipids exceeding, or equal to, those found with the sunflower oil diet despite the fact that the content of linoleic acid in the linseed oil diet was only one third of that found in the sunflower oil diet. Similar results have been reported by other workers (8,9). These findings suggest that the effect of the linseed oil diet upon AA levels may result, at least in part, from inhibition of elongase and desaturase enzymes which convert LA to AA and the 22-carbon n-6 fatty acids. Our findings in these *in vivo* studies are thus concordant with *in vitro* biochemical studies in which it has been shown that ALA competitively inhibits the metabolism of LA and its products along this pathway (10). Fish oil fatty acids have been found to be even more potent inhibitors of the Δ6 desaturase than those in the linseed oil (11). In addition, the fish oil diet may depress the AA content of phospholipids through the competitive incorporation of C_{20} and C_{22} n-3 fatty acids found in the diet at the expense of their n-6 fatty acid counterparts.

The limited strain comparisons detailed here suggest that diet may in general be a more important determinant of membrane fatty acid composition than genetic (strain) factors. Nevertheless, there is suggestive evidence in favor of a relative Δ4 desaturase deficiency in one strain, indicating that variable expression of lipid remodeling enzymes can occur.

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