Interactions of saturated, n-6 and n-3 polyunsaturated fatty acids to modulate arachidonic acid metabolism

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Abstract Anti-thrombotic effects of omega-3 (n-3) fatty acids are believed to be due to their ability to reduce arachidonic acid levels. Therefore, weanling rats were fed n-3 acids in the form of linseed oil (18:3n-3) or fish oil (containing 20:5n-3 and 22:6n-3) in diets containing high levels of either saturated fatty acids (hydrogenated beef tallow) or high levels of linoleic acid (safflower oil) for 4 weeks. The effect of diet on the rate-limiting enzyme of arachidonic acid biosynthesis (Δ^6-desaturase) and on the lipid composition of hepatic microsomal membrane was determined. Both linseed oil- or fish oil-containing diets inhibited conversion of linoleic acid to γ-linolenic acid. Inhibition was greater with fish oil than with linseed oil, only when fed with saturated fat. Δ^6-Desaturase activity was not affected when n-3 fatty acids were fed with high levels of n-6 fatty acids. Arachidonic acid content of serum lipids and hepatic microsomal phospholipids was lower when n-3 fatty acids were fed in combination with beef tallow but not when fed with safflower oil. Similarly, n-3 fatty acids (18:3n-3, 20:5n-3, 22:5n-3, and 22:6n-3) accumulated to a greater extent when n-3 fatty acids were fed with beef tallow than with safflower oil. These observations indicate that the efficacy of n-3 fatty acids in reducing arachidonic acid level is dependent on the linoleic acid to saturated fatty acid ratio of the diet consumed. —**Garg, M. L., A. B. R. Thomson, and M. T. Clandinin.** Interactions of saturated, n-6 and n-3 polyunsaturated fatty acids to modulate arachidonic acid metabolism. *J. Lipid Res.* 1990. **31:** 271-277.

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Anti-thrombotic effects of marine oils rich in eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) acid are mediated by alteration in the ratio of thromboxane A_2 to prostacyclin produced in platelets and vascular endothelium, respectively (1-5). In this regard, 20:5n-3 is apparently responsible for changes in prostanoid metabolism (6) by competitively inhibiting the formation of thromboxane A_2, prostacyclin, and leukotrienes from arachidonic acid (20:4n-6) at the level of the cyclooxygenase and lipoxygenase enzyme complex (7, 8). Some 20:5n-3 is also converted to eicosanoids of the series-3 type (thromboxane A_3, prostacyclin) (7-11) which have different biological activities than eicosanoids of series-2 type (12). Recent evidence has suggested that a limited amount of 20:5n-3 is utilized for eicosanoid formation and thus the biosynthetic pathway leading to prostaglandins of the 3-series may be of minor importance (13). Therefore, it is apparent that eicosanoids produced from 20:4n-6 constitute the major eicosanoid pool even under a high dietary load of n-3 fatty acids. Under these circumstances, availability of 20:4n-6 may be the most important factor for eicosanoid biosynthesis. Our recent research has shown that dietary enrichment with 18:3n-3 or 20:5n-3 and 22:6n-3 inhibits desaturase enzyme activities in rat hepatic microsomes and reduces 20:4n-6 levels in plasma and tissue phospholipids (14, 15). In the present study we present evidence that the ability of n-3 fatty acids to impair 20:4n-6 biosynthesis is dependent on the linoleic acid to saturated fatty acid ratio of the diet consumed.

MATERIALS AND METHODS

[1-^{14}C]Linoleic acid (18:2n-6; sp act 59.0 mCi/mmol) was purchased from New England Nuclear Corp. (Boston, MA). Fish oil concentrate (EPA-28, NISHHIN) was supplied by Nishho Iwai Corporation, Tokyo, Japan. Linseed oil was obtained from a local health food store, fully hydrogenated beef tallow from Canada Packers, and safflower oil from a supermarket. Unlabeled linoleic acid and other biochemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Rats were obtained from Bioscience

Abbreviations: HBT, hydrogenated beef tallow; LNA, linolenic acid; EPA, eicosapentaenoic acid; SFO, safflower oil.

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Male Sprague-Dawley rats weighing 40–50 g at the beginning of the experiment were randomized into six groups of five rats in each group. Each group was fed a semi-synthetic nutritionally adequate diet (16) containing 20% (w/w) fat but differing in the fat composition: HBT diet, 90% hydrogenated beef tallow plus 10% safflower oil; HBT-LNA diet, 70% hydrogenated beef tallow, 5% safflower oil plus 25% linseed oil; HBT-EPA diet, 66% hydrogenated beef tallow, 9% safflower oil plus 25% fish oil concentrate; SFO diet, 100% safflower oil; SFO-LNA diet, 75% safflower oil plus 25% linseed oil; SFO-EPA diet, 75% safflower oil plus 25% fish oil concentrate. The fatty acid composition of the diets was similar to the data presented elsewhere (17). The diets contained by weight 20% fat, 20% starch, 20.7% glucose, 27% casein, 5% non-nutritive cellulose, 1% vitamin mix, 5% mineral mix, 0.25% L-methionine, 0.625% inositol, and 0.275% choline (Teklad Test Diets, Madison, WI). The diets were prepared on a weekly basis and stored at −20°C under nitrogen. Animals were housed in stainless-steel cages in a well-ventilated room maintained at 22 ± 2°C with a 12/12 h dark–light cycle. Food and water were available to the rats at all times. Fresh diet containing fish oil was mixed weekly, stored at −20°C, fed fresh on a daily basis; the left over food was discarded. Body weights of the animals were monitored weekly.

After 4 weeks of feeding the experimental diets, animals were killed between 0800 and 1000 h using a sharp guillotine. Trunk blood was collected and serum was obtained after centrifugation at 1800 rpm for 15 min (IEC Centra-7 centrifuge). Livers were excised immediately and immersed in ice-cold saline solution.

Preparation of hepatic microsomes

Livers were homogenized in a 0.25 M sucrose buffer (pH 7.2) containing 0.25 M sucrose, 0.1 M KH_2PO_4, 1 mM EDTA, and 1 mM dithiothreitol (pH 7.2) using Polytron at setting 8 for 30 sec, and microsomes were obtained and characterized for membrane purity as previously described (14). Briefly, the homogenate was spun at 1500 *g* for 10 min to get rid of unbroken cells and nuclei. The supernatant was centrifuged at 10,000 *g* for 15 min (Beckman, model JA-21) to sediment mitochondria. Microsomes were pelleted by centrifuging the supernatant at 105,000 *g* for 60 min in an ultracentrifuge (Beckman, model LC-5B). The microsomes were suspended in 0.25 M sucrose, 0.15 M potassium chloride (pH 7.2) buffer (10 mg microsomal protein/ml) for desaturase assay. 5'-Nucleotidase (18), succinic dehydrogenase (19), and glucose-6-phosphatase (20) activities were assayed as markers for plasma membrane, mitochondria, and microsomes, respectively. Microsomal preparations were found to be greater than 80% endoplasmic reticulum. Protein was estimated using the method of Lowry et al. (21) with bovine serum albumin as standard.

Δ^6-Desaturase assay

Δ^6-Desaturase activity was determined by the rate of conversion of [1-^{14}C]18:2n-6 to 18:3n-6 by a previously published procedure (14). The assay mixture contained (in a final volume of 1.2 ml) 4 μmol ATP, 0.1 μmol CoA 1.25 μmol NADH, 0.5 μmol nicotinamide, 5 μmol $MgCl_2$, 62.5 μmol NaF, 1.5 μmol glutathione, 62.5 μmol KH_2PO_4 (pH 7.2), and 200 nmol of [1-^{14}C]linoleic acid (700 dpm/nmol). The reaction was started by addition of 200 μl of microsomal preparation containing approximately 2 mg protein. The reaction was continued for 20 min at 37°C in a shaking water bath and was terminated by adding 3 ml of chloroform–methanol 2:1 (v/v) containing butylated hydroxytoluene (0.005% w/v) as an antioxidant. Lipids were extracted and methylated using BF_3–methanol reagent (14% w/w) at 100°C for 60 min. Fatty acid methyl esters were spotted on silver nitrate-impregnated (10% w/w in silica gel G) thin-layer chromatography plates and developed in toluene–acetone 95:5 (v/v). Standard methyl esters of 18:2n-6 and 18:3n-6 were spotted along with the sample. The spots were visualized by spraying the plate with amino-naphthol-sulfonic acid (0.1% w/v in water). Areas corresponding to dienes and trienes were scraped off directly into a scintillation vial containing 5 ml of scintillation fluor (Aquasol, NEN) and counted with an efficiency of more than 90% (Beckman, LS-5801). Raw data were adjusted for recovery and the Δ^6-desaturase activity was expressed as pmol of 18:3n-6 formed per min per mg of microsomal protein.

Analytical methods

Lipids from serum and liver microsomes were extracted with chloroform–methanol 2:1 (v/v) containing 0.005% w/v butylated hydroxytoluene (22). Total and unesterified cholesterol content were determined using an enzymatic assay kit (Boehringer Mannheim, West Germany; 23, 24). The differences between total and unesterified cholesterol gave a measure of esterified cholesterol content. Phospholipid concentrations were estimated by a previously published method (25). Microsomal total phospholipid was isolated by thin-layer chromatography on silica gel G plates (20 cm × 20 cm × 0.25 mm) developed in hexane–diethyl ether–acetic acid 80:20:1 (v/v/v) (26). The area corresponding to the phospholipid fraction was scraped off the plate and methylated using BF_3–methanol (14% w/w) reagent (27). Fatty acid methyl esters were stored at −70°C under nitrogen until analyzed. Fatty acid analysis was performed by gas–liquid chromatography (Varian, model 6000) using a fused silica capillary column (25 m × 0.25 mm I.D.) (BP-20, SGE Pty. Ltd., Melbourne, Australia) as detailed elsewhere (28). Fatty

acid peaks were identified by comparison of retention time with authentic standards. Raw data were stored and normalized using a chromatography data system (Varian, model DS 654).

Statistical analysis

The data are presented as mean ± SD. Analysis of variance was used to test the significance of the difference between dietary groups. Where differences were significant, statistical significance of the difference between any two means was determined using a Duncan's Multiple Range Test (29).

RESULTS

The fatty acid composition of the experimental diets is presented elsewhere (17). The HBT diet was enriched with saturated fatty acids (85.3%) while the SFO diet contained high levels of 18:2n-6 (73.5%). Both linseed oil-containing diets (HBT-LNA and SFO-LNA) provided a similar amount of α-linolenic acid (18.3% and 17.9%, respectively) but differed in linoleic acid to saturated fatty acid ratio. Both fish oil-containing diets (HBT-EPA and SFO-EPA) supplied similar amounts of long chain polyunsaturated n-3 fatty acids (18:4n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3; 11.5% and 11.6%, respectively) but differed in linoleic acid to saturated fatty acid ratio.

Δ^6-Desaturase activity

The rate of conversion of 18:2n-6 to 18:3n-6 was reduced by inclusion of n-3 fatty acids in the high saturated fat diet (**Table 1**). The inhibition of Δ^6-desaturase activity was greater when the diet contained 20:5n-3 and 22:6n-3 (HBT-EPA) compared with the diet containing 18:3n-3 (HBT-LNA diet). Desaturase activity in rat liver microsomes was not affected by dietary n-3 fatty acids when these fatty acids were fed in association with safflower oil (SFO-LNA and SFO-EPA diets). However, the rate of desaturation of 18:2n-6 was slower in rats fed diet high in n-6 fatty acid compared with animals fed the high saturated fat diet (Table 1).

TABLE 1. Effect of dietary n-3 fatty acids on Δ^6-desaturase activity in rat liver microsomes

Diet	Δ^6-Desaturase Activity
	pmol/min/mg protein
HBT	617 ± 84[a]
HBT-LNA	218 ± 37[b]
HBT-EPA	141 ± 24[c]
SFO	329 ± 38[d]
SFO-LNA	240 ± 60
SFO-EPA	263 ± 45[d]

Values are the mean ± SD for five separate microsomal preparations (n = 5) from each diet group.
[a,b,c]Values are significantly different within the hydrogenated beef tallow-based diets, $P < 0.05$.
[d]Values are significantly different from the corresponding values in the hydrogenated beef tallow-fed animals ($P < 0.05$).

Lipid composition of hepatic microsomes

Cholesterol content of rat liver microsomal membrane was not affected by n-3 fatty acids when fed in combination with either beef tallow or safflower oil (**Table 2**). The cholesterol content in hepatic microsomes of rats fed the SFO diet was significantly higher compared with that of animals fed the beef tallow (HBT) diet. The increase in cholesterol content after feeding of the SFO diet was mainly associated with change in the esterified cholesterol content (7.5% in HBT vs 19.1% SFO groups). Microsomal membrane phospholipid content was not altered by diet. Therefore, increase in cholesterol content in rats fed the SFO diet increased the cholesterol to phospholipid ratio in the microsomal membranes (Table 2).

TABLE 2. Effect of dietary n-3 fatty acids on cholesterol and phospholipid content of rat liver microsomes

	HBT	HBT-LNA	HBT-EPA	SFO	SFO-LNA	SFO-EPA
			μmol/mg protein			
Cholesterol						
Total	47.3 ± 3.6	48.6 ± 3.9	53.0 ± 6.2	64.9 ± 6.2[a]	57.2 ± 4.7	61.0 ± 5.7
Unesterified	43.7 ± 2.6	44.0 ± 2.8	48.4 ± 6.5	52.5 ± 5.7	49.4 ± 3.6	51.7 ± 5.2
Esterified	3.6 ± 1.0	4.7 ± 1.0	4.7 ± 1.6	12.4 ± 0.8[a]	7.8 ± 1.3[b]	9.3 ± 1.8[a]
% Esterified	7.5 ± 1.9	9.7 ± 1.6	8.5 ± 2.5	19.1 ± 1.4[a]	13.4 ± 1.5[b]	15.6 ± 1.9[a]
Phospholipid	563 ± 20	571 ± 25	585 ± 33	619 ± 52	604 ± 36	585 ± 28
Unesterified cholesterol/phospholipid (mol/mol)	0.077 ± 0.002	0.077 ± 0.008	0.083 ± 0.012	0.086 ± 0.004[a]	0.082 ± 0.009	0.089 ± 0.010

Values are the mean ± SD for five separate microsomal preparations (n = 5) from each diet group.
[a]$P < 0.05$ versus corresponding values in the beef tallow-based diets.
[b]$P < 0.01$ versus SFO.

TABLE 3. Effect of dietary n-3 fatty acids on fatty acid composition (%) of rat serum total lipids

Fatty Acid	HBT	HBT-LNA	HBT-EPA	SFO	SFO-LNA	SFO-EPA
14:0	2.4 ± 0.3^{a}	1.0 ± 0.2^{b}	1.3 ± 0.6^{b}	0.2 ± 0.1^{c}	0.2 ± 0.1^{c}	0.5 ± 0.1^{d}
15:0	0.6 ± 0.1^{a}	0.3 ± 0.1^{b}	0.5 ± 0.1^{a}	0.2 ± 0.1^{b}	0.2 ± 0.0^{b}	0.3 ± 0.1^{b}
16:0	22.1 ± 0.8^{a}	18.1 ± 1.6^{b}	19.9 ± 1.4^{ab}	11.2 ± 0.5^{c}	12.0 ± 0.6^{c}	14.8 ± 0.8^{d}
16:1n-7	2.1 ± 0.5^{a}	0.9 ± 0.3^{b}	1.7 ± 0.2^{a}	0.4 ± 0.1^{c}	0.6 ± 0.2^{c}	1.5 ± 0.3^{a}
17:0	0.3 ± 0.1	0.1 ± 0.0	0.3 ± 0.2	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
18:0	18.9 ± 1.2^{a}	20.3 ± 1.2^{a}	19.0 ± 0.6^{a}	13.6 ± 1.1^{b}	12.0 ± 1.2^{b}	11.5 ± 1.4^{b}
18:1n-9	16.0 ± 0.5^{a}	11.7 ± 0.7^{b}	10.0 ± 1.4^{b}	10.0 ± 1.0^{b}	11.4 ± 0.8^{b}	9.7 ± 0.6^{b}
18:1n-7	–	–	0.3 ± 0.1	–	–	0.5 ± 0.1
18:2n-6	17.5 ± 0.8^{a}	22.7 ± 2.8^{b}	15.6 ± 1.5^{a}	41.2 ± 1.8^{c}	37.9 ± 2.4^{cd}	35.1 ± 2.3^{d}
18:3n-6	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
18:3n-3	0.1 ± 0.1^{a}	9.4 ± 2.2^{b}	0.4 ± 0.1^{c}	0.3 ± 0.1^{c}	5.3 ± 0.9^{d}	0.3 ± 0.2^{c}
20:1n-9	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.0	0.4 ± 0.1
20:2n-6	0.7 ± 0.1^{a}	0.3 ± 0.1^{b}	0.2 ± 0.1^{b}	0.7 ± 0.1^{a}	0.6 ± 0.1^{a}	0.6 ± 0.2^{a}
20:3n-9	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
20:3n-6	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.1
20:4n-6	14.9 ± 0.7^{a}	7.1 ± 2.3^{b}	5.7 ± 0.9^{b}	18.5 ± 1.4^{c}	15.8 ± 3.0^{cd}	13.4 ± 2.6^{d}
20:5n-3	0.3 ± 0.1^{a}	2.9 ± 0.6^{b}	16.4 ± 1.4^{c}	0.2 ± 0.1^{a}	0.4 ± 0.1^{a}	4.5 ± 0.7^{d}
22:4n-6	0.3 ± 0.1^{a}	0.1 ± 0.1^{a}	0.2 ± 0.1^{a}	0.6 ± 0.1^{b}	0.2 ± 0.1^{a}	–
22:5n-6	0.7 ± 0.2^{a}	–	0.1 ± 0.0^{b}	0.7 ± 0.3^{a}	0.1 ± 0.0^{b}	–
22:5n-3	0.2 ± 0.1^{a}	1.0 ± 0.2^{b}	1.9 ± 0.5^{c}	0.1 ± 0.1^{a}	0.4 ± 0.1^{a}	1.2 ± 0.3^{bc}
22:6n-3	1.6 ± 0.1^{a}	2.6 ± 0.9^{a}	5.0 ± 0.6^{b}	0.7 ± 0.1^{c}	1.8 ± 0.2^{a}	4.4 ± 0.5^{b}

Values are the mean ± SD of five rats (n = 5) in each diet group. Values within a line without a common superscript are significantly different, $P < 0.05$.

Fatty acid composition

The composition of major fatty acids present in serum lipids and microsomal membrane phospholipid is illustrated in **Table 3** and **Table 4**, respectively. Feeding of 18:3n-3 or 20:5n-3 in combination with saturated fat (HBT-LNA and HBT-EPA diets) lowered 20:4n-6 content and increased n-3 fatty acid (20:5n-3, 22:4n-3, 22:6n-3) content. The reduction in 20:4n-6 level in hepatic microsomes was greater for animals fed the HBT-EPA compared with HBT-LNA diet. Hepatic microsomes and serum prepared from rats fed the SFO diet were highly enriched in 18:2n-6 compared with that of animals fed the HBT diet. Feeding n-3 fatty acids with n-6 fatty acids (SFO-LNA or SFO-EPA diet) failed to alter 20:4n-6 content of serum lipids. Liver microsomes from rats fed the

TABLE 4. Effect of dietary n-3 fatty acids on fatty acid composition (%) of rat liver microsomal phospholipids

Fatty Acid	HBT	HBT-LNA	HBT-EPA	SFO	SFO-LNA	SFO-EPA
14:0	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.2 ± 0.1
16:0	14.9 ± 0.6^{a}	13.3 ± 0.9^{ad}	16.6 ± 0.4^{b}	9.5 ± 0.7^{cd}	11.7 ± 1.1^{d}	13.7 ± 0.6^{ad}
16:1n-7	0.8 ± 0.3^{a}	0.2 ± 0.1^{b}	1.7 ± 0.2^{c}	0.0 ± 0.0^{d}	0.2 ± 0.1^{b}	0.5 ± 0.1^{a}
17:0	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.0	0.5 ± 0.1	0.5 ± 0.1
18:0	30.3 ± 2.1^{a}	31.6 ± 0.6^{a}	29.2 ± 0.5^{a}	30.9 ± 0.7^{a}	29.1 ± 1.3^{a}	26.5 ± 1.0^{b}
18:1n-9	4.9 ± 0.3^{a}	3.3 ± 0.5^{b}	3.5 ± 0.8^{b}	2.3 ± 0.2^{c}	2.6 ± 0.3^{bc}	2.4 ± 0.2^{c}
18:1n-7	1.9 ± 0.6^{a}	1.0 ± 0.1^{b}	1.5 ± 0.1^{a}	1.7 ± 0.2^{a}	1.9 ± 0.2^{a}	2.6 ± 0.1^{c}
18:2n-6	9.1 ± 0.5^{a}	15.8 ± 1.3^{b}	11.2 ± 1.0^{c}	13.3 ± 1.2^{bc}	14.8 ± 0.5^{b}	13.3 ± 1.0^{bc}
18:3n-3	–	0.7 ± 0.2	0.3 ± 0.1	–	0.2 ± 0.1	0.2 ± 0.0
20:1n-9	–	–	0.1 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
20:2n-6	0.9 ± 0.2^{a}	0.2 ± 0.0^{b}	0.1 ± 0.1^{c}	2.0 ± 0.2^{d}	1.5 ± 0.2^{e}	1.1 ± 0.1^{a}
20:3n-9	0.5 ± 0.2^{a}	0.3 ± 0.1^{ac}	0.0 ± 0.0^{b}	0.2 ± 0.0^{c}	0.2 ± 0.0^{c}	0.0 ± 0.0^{b}
20:3n-6	1.0 ± 0.2^{a}	1.3 ± 0.1^{a}	0.8 ± 0.1^{b}	0.7 ± 0.1^{b}	1.0 ± 0.2^{a}	1.4 ± 0.1^{a}
20:4n-6	26.4 ± 0.4^{a}	16.0 ± 1.0^{b}	10.0 ± 0.7^{c}	32.0 ± 0.8^{d}	28.2 ± 0.7^{e}	22.5 ± 1.3^{f}
20:5n-3	–^{a}	4.8 ± 0.9^{b}	10.7 ± 0.6^{c}	–^{a}	0.4 ± 0.1^{d}	1.3 ± 0.2^{e}
22:4n-6	0.4 ± 0.1^{a}	0.1 ± 0.1^{b}	0.0 ± 0.0^{b}	0.8 ± 0.1^{c}	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}
22:5n-6	1.9 ± 0.4^{a}	1.2 ± 0.1^{b}	0.1 ± 0.0^{c}	3.0 ± 0.6^{d}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}
22:5n-3	0.3 ± 0.1^{a}	2.0 ± 0.3^{b}	3.1 ± 0.5^{c}	0.2 ± 0.1^{a}	0.7 ± 0.1^{d}	1.2 ± 0.2^{e}
22:6n-3	5.6 ± 0.2^{a}	8.4 ± 1.4^{b}	10.1 ± 1.8^{b}	2.7 ± 1.4^{c}	6.3 ± 0.2^{d}	8.0 ± 0.3^{b}
U.I.	183.9	198.2	202.8	201.0	198.7	190.6
$\Sigma\omega6/\Sigma\omega3$	6.73	2.18	0.92	17.86	6.35	3.6

Values are the mean ± SD of five rats (n = 5) in each diet group. Values within a line without a common superscript are significantly different, $P < 0.05$. U.I.: Unsaturation Index Σ[(a) (b)], where a is the percentage of each unsaturated fatty acid and b is the number of double bonds for that particular fatty acid.

SFO-EPA diet exhibited a reduction in 20:4n-6 content. Enrichment of serum and liver microsomes with n-3 fatty acids was less prominent when n-3 fatty acids were fed in association with n-6 acids (SFO) than when fed in a diet high in saturated fat (HBT diet; Tables 3 and 4).

DISCUSSION

Previous studies have suggested that dietary n-3 polyunsaturated fatty acids have hypolipidemic and antithrombotic effects in animals as well as in humans (1-5). Inhibition of platelet aggregation by fish oils rich in 20:5n-3 and/or 22:6n-3 is apparently mediated via changes in the ratio of thromboxane A_2 to prostacyclin produced from 20:4n-6 by platelets and endothelium, respectively. Since endothelial cells or platelets are not able to synthesize 20:4n-6 from 18:2n-6 via desaturation and chain elongation. Δ^6-Desaturase in liver is located on microsomes and is a rate-limiting enzyme in 20:4n-6 biosynthesis (30-32). The present study suggests that both 18:3n-3 and 20:5n-3 inhibit conversion of 18:2n-6 to 18:3n-3 thus reducing 20:4n-6 content in rat serum and liver microsomal phospholipids. Inhibition of the rate of conversion of 18:2n-6 to 18:3n-6 was evident only when n-3 fatty acids were fed with low levels of 18:2n-6 (HBT-LNA and HBT-EPA diets). This indicates that maximum 20:4n-6 lowering and thus anti-thrombotic effects from a dose of n-3 fatty acids would be achieved when n-3 fatty acids are consumed in a diet high in saturated fat. The results of the present study also indicate that the dose of 18:3n-3 or 20:5n-3 required to have an effect on 20:4n-6 content may be lower for individuals who eat diets of low P/S type compared to those whose diets are rich in vegetable oils containing high levels of 18:2n-6. There is controversy in the literature regarding the anti-clotting effects of n-3 fatty acids. Depending on the aggregating-stimulant (such as 20:4n-6, ADP, collagen, and thrombin) used, n-3 fatty acids either have no effect or reduce platelet aggregation (8, 33-37). The results presented here indicate that these controversies may be due to different dietary 18:2n-6 intakes for subjects and these studies may potentially be resolved by carefully assessing the type of fat consumed during the n-3 fatty acid supplementation period (38).

Δ^6-Desaturase activity was not affected by the presence of 18:3n-3 or 20:5n-3 when presented with high levels of 18:2n-6. However, the diets containing high levels of 18:2n-6 exhibited significantly lower Δ^6-desaturase activity compared with animals fed the SFA diet without supplementary n-3 fatty acid. Previous studies have shown either no change (39) or an increase (40) in Δ^6-desaturase activity in hepatic microsomes after feeding of diets containing high amounts of 18:2n-6. One of the problems encountered in desaturase asssay procedures, but generally ignored, is the participation of endogenous pools of substrate. As is evident from fatty acid composition data (Tables 3 and 4), 18:2n-6 levels in serum or liver microsomes were altered despite feeding diets containing similar amounts of 18:2n-6 (e.g., in HBT, HBT-LNA, and HBT-EPA diets, approximately 10%). It is not known what pools of 18:2n-6 (phospholipid, triacylglycerol, free fatty acids, or cholesteryl esters) participate in the Δ^6-desaturation reaction, or in what proportion. It is conceivable that the decrease in Δ^6-desaturase specific activity after feeding of the SFO diet may be attributable to dilution of the specific activity of [1-^{14}C]18:2n-6 by endogenous 18:2n-6 pools. However, the increase observed in 20:4n-6 content of serum and microsomal lipids, after feeding of the SFO diet indicates that the actual rate of desaturation and chain elongation of 18:2n-6 was higher.

Major determinants of membrane fluidity (physicochemical status) are phospholipid, cholesterol, fatty acyl chain length, and degree of unsaturation (41, 42). Phospholipid content of microsomal membranes was not affected by feeding n-3 fatty acids. Alteration in cholesterol content after feeding of the SFO diet changed cholesterol/phospholipid ratios of these membranes. Cholesterol has a condensing effect on molecular ordering of phospoholipid (43) and previously it has been shown that cholesterol enrichment of microsomal membranes inhibits Δ^6-desaturase activity (14, 44, 45). Therefore, it is possible that accumulation of cholesterol in microsomes induced by n-6 fatty acids present in the SFO diet may be responsible for inhibition of the observed conversion of 18:2n-6 to 18:3n-6.

Consistent with the alterations in Δ^6-desaturase activity, the 20:4n-6 content of rat serum was only lowered when n-3 fatty acids were fed in association with low levels of linoleic acid. However, the 20:4n-6 level in hepatic microsomal phospholipids decreased when 20:5n-3 (SFO-EPA), but not 18:3n-3 (SFO-LNA), was included in the SFO diet. The mechanism for inhibition of Δ^6-desaturase activity is different when 20:5n-3 or 18:3n-3 is fed in the diet. EPA (20:5n-3) appears to inhibit desaturation and chain elongation of 18:2n-6 by acting as an analogue of 20:4n-6, while inhibition by 18:3n-6 is competitive at the Δ^6-desaturase level (14). Therefore, inhibition of Δ^6-desaturase by 18:3n-3 would depend on relative availability of 18:2n-6 to 18:3n-3 ratio which is low in HBT- and high in SFO-based diets. Regardless of substrate availability, changes in microsomal phospholipid fatty acid composition evoked by dietary n-3 fatty acids may also potentially regulate the response of hepatic Δ^6-desaturase via changes in the physicochemical properties of the microsomal membranes (46).

Δ^6-Desaturase activity in rats fed the beef tallow diet was considerably higher compared to our previous study (14). A possible explanation for this difference could be that the beef tallow used in this study was fully hydrogenated while the tallow used in the previous study was not

hydrogenated and contained approximately 31% monounsaturated fatty acids. Monounsaturated fatty acids have been subject of interest recently due to their possible hypocholesterolemic and anti-thrombotic effects (47); therefore, for further studies, the effect of saturated and monounsaturated fatty acids should be examined separately.

It is evident from the present study that dietary 18:3n-3 is almost as effective as 20:5n-3 in lowering 20:4n-6 content in rat serum and microsomal membranes, provided the 18:2n-6 content of the diets is low, as in case of HBT-LNA diet. Extrapolation of the rat data to humans would suggest that the efficacy of n-3 fatty acids of linseed, canola, or fish oil in modulating eicosanoid precursor levels and having anti-aggregatory effects may depend upon the dietary fat composition.

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