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Δ^6-Desaturase activity in liver microsomes of rats fed diets enriched with cholesterol and/or ω3 fatty acids

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The effect of feeding semipurified diets enriched in linseed (rich in $C_{18:3,\omega3}$ fatty acid) or fish (rich in $C_{20:5,\omega3}$ and $C_{22:6,\omega3}$ fatty acid) oil with and without cholesterol supplementation on the desaturation of linoleic acid ($C_{18:2,\omega6}$) by rat liver microsomal fractions was investigated. Animals fed diets supplemented with beef tallow were used as equal-energy controls. Both linseed-oil and fish-oil diets, without added cholesterol, decrease conversion of $C_{18:2,\omega6}$ fatty acid to γ-linolenic acid ($C_{18:3,\omega6}$). Reduction in Δ^6-desaturation was significantly greater for animals fed the diet containing fish oil than with animals fed the linseed-oil diet. The major effect of cholesterol supplementation was to decrease the rate of desaturation of $C_{18:2,\omega6}$, when fed in combination with the beef-tallow diet, whereas Δ^6-desaturation was unaffected when cholesterol was fed along with diets high in ω3 fatty acids (linseed oil or fish oil). The activity of the Δ^6-desaturase *in vitro* is consistent with the fatty acid composition observed for the microsomal membranes on which this enzyme is localized. Dietary linseed oil and fish oil lowered the arachidonic ($C_{20:4,\omega6}$) acid content of rat liver microsomes, with an accompanying increase in membrane eicosapentaenoic ($C_{20:5,\omega3}$) and docosahexaenoic ($C_{22:6,\omega3}$) acid content, in comparison with the group fed beef tallow. Inclusion of cholesterol into the beef-tallow or linseed-oil diets resulted in decreased membrane $C_{20:4,\omega6}$-fatty-acid content, with concomitant increase in $C_{18:2,\omega6}$-fatty-acid content. However, addition of cholesterol to the fish-oil diet did not alter the microsomal membrane content of $C_{20:4,\omega6}$ fatty acid. Thus it is suggested that (1) the decrease in prostaglandin E_2, thromboxane and prostacyclin levels generally observed after fish-oil consumption may be at least partly due to inhibition of $C_{20:4,\omega6}$-fatty-acid synthesis from $C_{18:2,\omega6}$ fatty acid; and (2) consumption of fish oil prevents the further decrease in $C_{20:4,\omega6}$-fatty-acid levels by dietary cholesterol that is apparent when cholesterol is fed in combination with diets high in saturated fat or $C_{18:3,\omega3}$ fatty acid.

INTRODUCTION

There is considerable evidence to suggest that polyunsaturated fatty acids of marine origin have beneficial effects against cardiovascular disease, including atherosclerosis and thrombosis [1]. Epidemiological data also indicates a very low incidence of ischemic heart disease in Eskimoes and the Japanese, populations that consume a diet rich in seafoods [2,3] etc. Further it was found that these same populations have low blood levels of cholesterol and triacylglycerols [4]. This lipid-lowering effect has been attributed to the high eicosapentaenoic ($C_{20:5,\omega3}$) and docosahexaenoic ($C_{22:6,\omega3}$) acid content in foods of marine origin [5–7]. The other known effects of $C_{20:5,\omega3}$ and $C_{22:6,\omega3}$ fatty acids include reduction in the synthesis of prostaglandin E_2 (PGE_2), thromboxane A_2 (TXA_2), prostacyclin (PGI_2) and leukotrienes. This leads to decreased platelet aggregation and enhanced bleeding time [8–11]. The prostanoids (PGE_2, TXA_2, PGI_2) are derived from endoperoxide intermediates (PGH_2) generated from $C_{20:4,\omega6}$ fatty acid, which is synthesized in various tissues by alternative desaturation and chain elongation of linoleic acid ($C_{18:2,\omega6}$). Conversion of $C_{18:2,\omega6}$ into $C_{18:3,\omega6}$ fatty acid by microsomal Δ^6-desaturation is considered to be rate-limiting in the synthesis of $C_{20:4,\omega6}$ from $C_{18:2,\omega6}$ fatty acid [12,13].

It can be hypothesized that decrease in prostanoid levels after fish-oil supplementation is due to inhibition of arachidonic acid synthesis blocking the arachidonic acid cascade. Therefore, to obtain better understanding of the mechanism(s) of action of fish oil in decreasing prostanoid levels and inducing fatty acid compositional changes at a subcellular level, we have investigated the effects of dietary supplementation of ω3 fatty acids with or without added cholesterol, on liver microsomal Δ^6-desaturase activity and fatty acid composition of liver membrane lipids. To identify the specificity of fish oil in decreasing prostanoid levels, animals were also fed linseed-oil-supplemented diets. Animals fed on beef-tallow-supplemented diets were used as equal-energy controls.

EXPERIMENTAL

Materials

[1-^{14}C]Linoleic acid (59 mCi/mmol), with more than 98% radiochemical purity, was purchased from New England Nuclear Corp., Boston, MA, U.S.A., and was used without further purification. Unlabelled fatty acids, fatty acid methyl ester standards and other biochemical products were obtained from Sigma Chemical Co. (St.

Abbreviations used: TXA_2, thromboxane A_2; PGE_2, prostaglandin E_2; PGI_2, prostacyclin.

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Table 1. Composition of the fats added to the semipurified basal diet

The basal diet contained the following components (g/kg of diet): casein, 270; starch, 200; glucose, 207; non-nutritive cellulose, 50; vitamin mix, 10; mineral mix, 50; choline, 2.75; L-methionine, 6.25, as described previously [14].

		Content (g/kg of diet)					
	Diet ...	Beef tallow	Beef tallow and cholesterol	Linseed oil	Linseed oil and cholesterol	Fish oil	Fish oil and cholesterol
Fat source	Cholesterol added (g/kg of diet) ...	1.2	21.2	1.2	21.2	–	20.0
Beef tallow		180	180	40	40	–	–
Safflower oil		20	20	–	–	–	–
Linseed oil		–	–	160	160	–	–
Fish oil		–	–	–	–	200	200

Louis, MO, U.S.A.). Fish oil (S-28GA) was supplied by Nissho Iwai American Corp., New York, NY, U.S.A., and beef tallow by Canada Packers Edmonton, Alberta, Canada; linseed oil was obtained from a local health food store. The semipurified basal diet was described previously [14].

Animals and diets

Male weanling (Sprague–Dawley) rats weighing 50–60 g at the beginning of the experiment were systematically randomized into six groups containing six rats in each group, so that the mean body weight per group was the same. The animals were housed individually in stainless-steel cages, in a well-ventilated room maintained at 22±2 °C on a 12 h:12 h light/dark cycle. Experimental diets and tap water were provided *ad libitum*. All diets were prepared weekly and stored at 4 °C. Animals were fed semisynthetic diets supplemented with 20% (w/w) fat providing different fatty acid combinations (Table 1). These were named as beef tallow, beef tallow with added cholesterol, linseed oil, linseed oil with added cholesterol, fish oil and fish oil with added cholesterol. The oils added to the basal diet are indicated (Table 1) along with the fatty acid composition of each diet (Table 2). The cholesterol content of the beef-tallow and linseed-oil diets was adjusted by adding 1.2 g of cholesterol per kg of diet as the fish oil contained 0.6% (w/w) cholesterol. Therefore the low-cholesterol diets (beef tallow, linseed oil and fish oil) contained 1.2 g of cholesterol per kg of total diet and the high-cholesterol diets (beef tallow with added cholesterol, linseed oil with added cholesterol and fish oil with added cholesterol) contained 21.2 g of cholesterol per kg of diet.

Preparation of hepatic microsomes

After 28 days of feeding experimental diets, rats were killed between 08:00 and 10:00 h by decapitation; livers were quickly excised, rinsed with ice-cold physiological saline, blotted, weighed and minced with scissors. Livers were homogenized in a medium containing 0.25 M-sucrose, 0.1 M-potassium phosphate, 1 mM-EDTA, 1 mM-dithiothreitol, pH 7.2, by using five up-and-down strokes of a Potter–Elvehjem tissue homogenizer. Homogenates were centrifuged at 800 *g* for 10 min to remove cell debris, and the supernatant was centrifuged at 15000 *g* for 20 min. The microsomal pellet was

Table 2. Fatty acid composition of experimental diets

Addition of 2% cholesterol to the beef-tallow, linseed-oil or fish-oil diets had no effect on the fatty acid composition of diets.

	Composition (%, w/w)		
Fatty acid / Diet ...	Beef tallow	Linseed oil	Fish oil
$C_{14:0}$	0.6	0.1	6.1
$C_{16:0}$	26.3	10.3	7.6
$C_{16:1}$	3.0	0.7	11.3
$C_{17:0}$	1.3	0.3	2.0
$C_{18:0}$	17.2	6.3	6.2
$C_{18:1,\omega9}$	31.9	23.5	7.8
$C_{18:1,\omega7}$	2.6	1.1	2.8
$C_{18:2,\omega6}$	10.3	16.2	1.6
$C_{18:3,\omega3}$	0.3	39.9	6.0
$C_{20:4,\omega6}$	–	0.1	1.2
$C_{20:5,\omega3}$	–	–	27.5
$C_{22:5,\omega3}$	–	0.1	2.2
$C_{22:6,\omega3}$	–	–	8.9
Total			
Saturated	45.4	17.0	25.9
Monounsaturated	37.5	25.3	21.9
$\omega6$	10.3	16.2	2.8
$\omega3$	0.3	40.1	44.6

obtained by centrifugation of the 15000 *g* supernatant at 105000 *g* for 60 min. The pinkish microsomal pellets were suspended in a cold solution containing 0.25 M-sucrose and 0.15 M-KCl, pH 7.2, for measurements of Δ^6-desaturase activity.

The purity of microsomes obtained was assessed by assaying for glucose-6-phosphatase [15], 5′-nucleotidase [16] and succinic dehydrogenase [17] activities to check for any plasma-membrane and mitochondrial contamination, respectively. Protein was assayed by the method of Lowry *et al.* [18], with bovine serum albumin as standard.

Δ^6-Desaturase assay

Desaturation of $C_{18:2,\omega6}$ fatty acid by liver microsomes was estimated by measuring the amount of γ-[1-^{14}C]-linolenic acid produced from [1-^{14}C]linoleic acid as described previously [19]. The reaction medium con-

Table 3. Food consumption, body weight and liver weight of rats fed various lipid-supplemented diets

Results are means ± S.D. for six animals in each group.

Diet	Cholesterol level (%)	Food consumption (g/day)	Body wt. (g)	Liver wt. (g)	$100 \times \frac{\text{Liver wt.}}{\text{Body wt.}}$
Beef tallow	0.12	18.9 ± 0.9	277 ± 11	11.9 ± 0.8	4.3 ± 0.2
	2.00	19.0 ± 1.0	271 ± 12	11.8 ± 1.0	4.4 ± 0.4
Linseed oil	0.12	19.1 ± 1.2	277 ± 12	11.8 ± 0.5	4.3 ± 0.3
	2.00	19.1 ± 1.3	277 ± 18	11.9 ± 0.9	4.3 ± 0.1
Fish oil	0.12	18.6 ± 1.0	242 ± 8*	10.0 ± 1.1	4.2 ± 0.4
	2.00	18.3 ± 1.1	245 ± 10*	10.4 ± 1.0	4.3 ± 0.3

* Significantly different from all other dietary groups ($P < 0.05$).

tained, in a total volume of 1.2 ml: 4 μmol of ATP, 0.1 μmol of CoA, 1.25 μmol of NADH, 0.5 μmol of nicotinamide, 5 μmol of $MgCl_2$, 62.5 μmol of NaF, 1.5 μmol of glutathione, 62.5 μmol of potassium phosphate buffer, pH 7.0, and 200 nmol of [1-^{14}C]linoleic acid (750 d.p.m./nmol). Incubations were carried out with 3–5 mg of microsomal protein in a shaking water bath at 37 °C for 20 min. Under these assay conditions the rate of desaturation of $C_{18:2,\omega6}$ fatty acid was linear with respect to the microsomal protein, substrate concentration and the incubation time. The reaction was stopped by adding 2 ml of 10% (w/v) KOH in methanol. Lipids were saponified by heating for 2 h at 85 °C, acidified with 1 ml of 8 M-HCl, the fatty acids extracted with hexane and methylated by heating at 100 °C for 1 h with 14% (w/w) BF_3/methanol reagent. Fatty acid methyl esters were separated on t.l.c. plates [silica-gel G impregnated with 10% (w/w) $AgNO_3$]. Carrier methyl esters of $C_{18:2}$ and γ-$C_{18:3}$ fatty acid were spotted along with the labelled acids. Plates were developed in hexane/diethyl ether (17:3, v/v) for the separation of dienes (mainly $C_{18:2,\omega6}$) from trienes (mainly $C_{18:3,\omega6}$). The spots were made visible under u.v. light by spraying with 2′,7′-dichlorofluoroscein [0.2% (w/v) in ethanol], scraped off directly into the scintillation vials and counted for radioactivity with 5 ml of scintillation fluor (Aquasol) by using a liquid-scintillation counter (Beckman, model LS-5801). Enzyme activity was expressed as pmol of γ-$C_{18:3}$ fatty acid formed from $C_{18:2,\omega6}$ fatty acid/min per mg of microsomal protein.

Table 4. Influence of dietary cholesterol and/or ω3 fatty acids on Δ^6-desaturase activity of rat liver microsomes

Values are means ± S.D. for six separate microsomal preparations from six different rats. Values without a common superscript are significantly different ($P < 0.05$).

Cholesterol	Diet ...	Δ^6-Desaturase activity (pmol/min per mg of protein) Beef tallow	Linseed oil	Fish oil
−		338 ± 41.8^a	233 ± 32.1^b	132 ± 26.2^c
+		239 ± 33.1^b	201 ± 20.1^b	124 ± 35.4^c

Lipid analysis

Lipids from liver microsomal preparations were extracted with chloroform/methanol (2:1, v/v) [20]. Total phospholipid was assayed in portions of lipid extracts from liver microsomes [21]. For the determination of phospholipid composition, individual phospholipids were first separated by t.l.c., followed by densitometric quantification [22]. Fatty acid composition was determined by automated g.l.c. (Varian Model 6000) of methyl esters using a fused silica capillary column (BP20, bonded phase) as reported previously [23]. The cholesterol content of diets was determined by the method of Sale *et al.* [24].

Statistical analysis

The results shown are means ± S.D. The effect of dietary treatments was examined by analysis-of-variance procedures. Comparison between individual diets was made by using the Neuman–Keuls multiple-range test [25].

RESULTS

Animal characteristics

All animals appeared healthy after the 28-day experimental period. Animals fed diets containing fish oil exhibited significantly lower body weights as compared with beef-tallow- or linseed-oil-fed groups (Table 3). All groups of animals consumed the same amount of food irrespective of any fat supplementation and/or cholesterol inclusion. Liver weights and liver-weight-to-body-weight ratios were also unaffected by dietary lipid supplementation (Table 3).

Both linseed-oil and fish-oil diets contained the same amount of total ω3 fatty acids (approx. 40%), but qualitatively the linseed-oil diet was higher in $C_{18:3,\omega3}$ fatty acid, whereas the fish-oil diet was enriched with $C_{20:5,\omega3}$ and $C_{22:6,\omega3}$ fatty acid (Table 2).

Δ^6-Desaturase activity

Both the linseed-oil and fish-oil diets without cholesterol supplement inhibited microsomal Δ^6-desaturase activity in liver when compared with the isocaloric control group fed beef tallow (Table 4). The inhibition of Δ^6-desaturase activity is greater for animals fed fish oil (61%) than for animals fed linseed oil (31%). Addition of cholesterol to beef tallow decreased desaturation of

Table 5. Phospholipid content and composition of liver microsomes from rats fed various lipid-supplemented diets

Values are means ± S.D. (six animals). None of these values were significantly different ($P > 0.05$).

Phospholipid	Diet ...	Composition (% of total phospholipid)					
		Beef tallow	Beef tallow with cholesterol	Linseed oil	Linseed oil with cholesterol	Fish oil	Fish oil with cholesterol
	Total phospholipid (g/mg of protein) ...	429±18	469±18	450±50	415±20	426±29	461±36
Phosphatidylcholine		60.4±2.0	59.8±5.4	58.1±8.0	52.9±3.4	54.0±3.4	54.1±4.4
Phosphatidylethanolamine		24.8±2.6	27.2±5.3	27.8±5.2	30.1±1.3	33.3±4.3	32.0±4.6
Phosphatidylinositol		8.8±1.4	8.7±3.4	7.7±3.0	9.5±2.2	7.0±2.8	7.3±3.1
Sphingomyelin		4.3±0.8	5.1±1.2	5.1±1.0	4.8±1.3	3.9±1.1	4.5±1.5
Phosphatidylserine		1.6±0.9	1.4±0.5	2.0±0.7	2.4±1.5	1.9±0.5	2.1±0.5

Table 6. Fatty acid composition of rat liver microsomal total lipids after dietary lipid treatments

Values are means ± S.D. for six replicates. Values without a common superscript are significantly different ($P < 0.05$).

Fatty acid	Diet ...	Composition (%, w/w)					
		Beef tallow	Beef tallow with cholesterol	Linseed oil	Linseed oil with cholesterol	Fish oil	Fish oil with cholesterol
$C_{16:0}$		15.3±0.6[a2]	15.4±0.4[a]	14.8±0.8[a]	13.0±0.6[a]	21.5±0.3[b]	21.9±0.7[b]
$C_{16:1}$		1.4±0.1[a]	1.8±0.2[b]	0.4±0.1[c]	0.2±0.1[c]	3.8±0.3[d]	5.0±0.2[e]
$C_{17:0}$		0.8±0.1	0.9±0.1	0.7±0.1	0.7±0.1	0.7±0.1	0.7±0.1
$C_{18:0}$		25.2±0.7[a]	23.2±0.8[a]	25.4±0.4[a]	24.5±0.7[a]	17.3±0.5[b]	14.4±0.5[c]
$C_{18:1,\omega9}$		14.1±0.7[a]	13.8±1.2[a]	9.1±0.9[b]	9.2±0.5[b]	6.6±0.4[c]	8.2±0.7[b]
$C_{18:1,\omega7}$		3.3±0.3[a]	4.4±0.4[b]	2.0±0.1[c]	2.2±0.3[c]	6.7±0.2[d]	7.2±0.3[d]
$C_{18:2,\omega6}$		9.9±1.0[a]	12.9±0.3[b]	16.9±2.1[c]	20.3±1.6[d]	2.6±0.1[e]	2.7±0.1[e]
$C_{18:3,\omega6}$		0.2±0.1	0.3±0.1	0.3±0.1	0.3±0.1	0.2±0.1	0.2±0.1
$C_{18:3,\omega3}$		–	–	3.2±0.2	4.0±0.9	0.4±0.1	0.4±0.1
$C_{18:4,\omega3}$		–	–	–	–	0.5±0.2	0.5±0.2
$C_{20:2,\omega6}$		0.5±0.2	0.3±0.2	0.3±0.1	0.4±0.1	0.1±0.1	0.1±0.1
$C_{20:3,\omega9}$		0.6±0.1	0.7±0.1	0.4±0.1	0.5±0.2	–	–
$C_{20:3,\omega6}$		1.3±0.1	1.6±0.1	1.3±0.1	1.6±0.1	0.9±0.1	0.9±0.1
$C_{20:4,\omega6}$		22.2±0.5[a]	20.5±0.6[b]	13.2±0.9[c]	9.5±1.0[d]	9.4±0.3[d]	9.2±0.3[d]
$C_{20:5,\omega3}$		0.2±0.1[a]	0.2±0.1[a]	4.9±0.9[b]	7.2±0.5[c]	11.5±0.8[d]	13.3±1.0[e]
$C_{22:4,\omega6}$		0.2±0.1	0.2±0.1	–	–	–	–
$C_{22:5,\omega6}$		2.4±0.6	1.5±0.2	–	–	–	–
$C_{22:5,\omega3}$		0.2±0.1[a]	0.2±0.1[a]	1.8±0.3[b]	2.4±0.1[c]	3.3±0.2[d]	2.7±0.1[e]
$C_{22:6,\omega3}$		2.7±0.2[a]	2.3±0.1[a]	5.5±0.6[b]	4.1±1.1[c]	14.6±0.7[d]	12.7±0.5[e]
Total							
Saturated		41.3	39.5	40.9	38.2	39.5	37.0
Monounsaturated		18.6	20.0	11.5	11.6	17.1	20.4
$\omega6$		36.7	37.3	32.0	32.1	13.2	13.1
$\omega3$		3.1	2.6	15.4	17.7	30.3	29.6
$C_{20:4,\omega6}/C_{20:5,\omega3}$ ...		111	102.5	2.7	1.3	0.8	0.7

$C_{18:2,\omega6}$ fatty acid by 29%, whereas inclusion of cholesterol into the fish-oil diet did not have any significant effect (Table 4). Cholesterol supplementation to the linseed-oil diet tended to decrease Δ^6-desaturase activity, but the difference did not reach a statistically significant level ($P > 0.05$).

Phospholipid content and composition of liver microsomes

The total phospholipid content of rat liver microsomal membranes remained unchanged by dietary fat and/or cholesterol treatment (Table 5). Phospholipid composition (i.e., the relative percentages of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine and sphingomyelin) was not altered by any of the lipid-supplemented diets. In each group of animals, phosphatidylcholine and phosphatidylethanolamine accounted for more than 80% of total phospholipids (Table 5).

Fatty acid composition

Marked differences in the fatty acid composition of

total liver microsomal lipids were observed for rats fed diets containing linseed oil or fish oil (Table 6). When compared with the control beef-tallow diet group, the fish-oil treatment resulted in increased microsomal $C_{16:0}$-fatty-acid content with an accompanied decrease in $C_{18:0}$ fatty acid. However, total saturated fatty acid content (approx. 40%) remained unchanged by dietary fat and/or cholesterol treatments (Table 6). Both linseed- and fish-oil diets, without added cholesterol, decreased the $C_{20:4,\omega6}$-fatty-acid content of microsomal membranes as compared with those of the beef-tallow-fed group, the decrease being greater for animals fed fish oil (58%) than for those fed linseed oil (41%). Microsomal membranes from animals fed on linseed oil were enriched with $C_{18:3,\omega3}$, $C_{20:5,\omega3}$, $C_{22:5,\omega3}$ and $C_{22:6,\omega3}$ fatty acids. Feeding fish oil enriched the microsomal membranes with $C_{20:5,\omega3}$, $C_{22:5,\omega3}$ and $C_{22:6,\omega3}$ fatty acids to a greater degree than was observed for linseed-oil-fed animals (Table 6).

The major effect of addition of 2% (w/w) cholesterol to beef-tallow or linseed-oil diets was to significantly decrease the $C_{20:4,\omega6}$-fatty-acid content of liver microsomes with a concomitant increase in $C_{18:2,\omega6}$ fatty acid. However, feeding 2% (w/w) cholesterol in combination with fish oil did not show significant effects on $C_{18:2,\omega6}$- or $C_{20:4,\omega6}$-fatty-acid content (Table 6). Dietary cholesterol treatment also altered metabolism of ω3 fatty acids in the rat liver microsomes. Addition of cholesterol to linseed-oil or fish-oil diets led to increase in $C_{20:5,\omega3}$ fatty acid accompanied by decrease in the $C_{22:6,\omega3}$-fatty-acid content in microsomal lipids when compared with the respective low-cholesterol diets (Table 6).

DISCUSSION

The present study was designed to determine the effect of dietary cholesterol supplementation and/or ω3 fatty acids on the conversion of $C_{18:2,\omega6}$ into $C_{18:3,\omega6}$ fatty acid, a rate-limiting reaction in the conversion of $C_{18:2,\omega6}$ into $C_{20:4,\omega6}$ fatty acids. Animals fed on the diet containing fish oil grew less than those fed diets containing beef tallow or linseed oil (Table 3). The amount of food eaten per day was similar in all dietary groups. Therefore lower body weights after fish-oil consumption cannot be due to difference in energy intake and may relate to changes in feed efficiency or body composition. Polyunsaturated fatty acids ($C_{20:5,\omega3}$ and $C_{20:6,\omega3}$) of fish oil are highly susceptible to oxidative changes and have previously been shown to increase lipid peroxides in rat liver microsomes [26], which may, in turn, decrease growth rate [27]. However, none of the lipid-supplemented diets altered liver weight or liver-weight-to-body-weight ratios in the present study.

Recent studies indicate that dietary ω3 fatty acids, particularly $C_{20:5,\omega3}$, have anti-aggregatory effects on the manifestation of thrombosis [28,29,30]. Mechanisms underlying decreased platelet aggregation for animals fed marine oils are not clearly understood. It has been proposed that the increased $C_{20:5,\omega3}/C_{20:4,\omega6}$ ratio in platelet membrane phospholipids, which occurs after fish-oil consumption, may be responsible for decreased platelet aggregation [2,31]. Others have shown that $C_{20:5,\omega3}$ decreases release of $C_{20:4,\omega6}$ from phospholipids of stimulated platelets [32]. $C_{20:5,\omega3}$ fatty acid also competes with $C_{20:4,\omega6}$ fatty acid at the level of cyclo-oxygenase to inhibit formation of TXA_2, a pro-aggregatory agent [33].

Results of the present study demonstrate that dietary ω3 fatty acids inhibit Δ^6-desaturase activity of liver microsomes (Table 4). Δ^6-Desaturase converts $C_{18:2,\omega6}$ into $C_{18:3,\omega6}$ fatty acid and is a rate-limiting step in synthesis of $C_{20:4,\omega6}$ from $C_{18:2,\omega6}$ fatty acid [12,13]. Thus a decrease in Δ^6-desaturase activity would indicate inhibition of $C_{20:4,\omega6}$-fatty-acid formation by liver microsomes. For tissues either dependent upon liver for synthesis of $C_{20:4,\omega6}$ fatty acid or exhibiting similar metabollic control for desaturation of $C_{18:2,\omega6}$ fatty acid, it would be reasonable to expect that this limitation in synthesis of $C_{20:4,\omega6}$ fatty acid would in turn block the arachidonic acid cascade. Although both the linseed oil (rich in $C_{18:3,\omega}$ fatty acid) and fish oil (rich in $C_{20:5,\omega3}$ and $C_{22:6,\omega3}$ fatty acid) decrease Δ^6-desaturation, the decrease was significantly greater with fish oil. It has previously been shown that $C_{18:2,\omega6}$ and $C_{18:3,\omega3}$ fatty acids compete for Δ^6-desaturation, with $C_{18:3,\omega3}$ fatty acid being the preferred substrate [34]. Therefore decreased Δ^6-desaturase activity in animals fed a linseed-oil-supplemented diet appears to be due to competitive inhibition of $C_{18:2,\omega6}$-fatty-acid desaturation, as $C_{18:3,\omega3}$ fatty acid is readily available for desaturation. The mechanism for inhibition of Δ^6-desaturase activity by fish oil may be different from that apparent for the linseed-oil diet. In this regard, $C_{20:5,\omega3}$ and $C_{22:6,\omega3}$ fatty acids from fish oil are preferentially incorporated into membrane and tissue phospholipids when compared with incorporation rates for $C_{20:4,\omega6}$ fatty acid. Thus $C_{20:5,\omega3}$ and $C_{22:6,\omega3}$ fatty acids may conceivably act as analogues of $C_{20:4,\omega6}$ fatty acid to inhibit Δ^6-desaturation by a feedback mechanism [35].

The observation that fish oil prevents changes in Δ^6-desaturase activity induced by 2% (w/w) dietary cholesterol merits further investigation. Dietary cholesterol has previously been shown to decrease the Δ^6- and Δ^5-desaturase activity of rat liver microsomes, when fed in combination with saturated or unsaturated ω6 fatty acids [36,37]. The present study also indicates decreased Δ^6-desaturation when 2% (w/w) cholesterol is added to beef tallow. The combination of linseed oil and cholesterol in the diet also tended to lower Δ^6-desaturase activity. It is possible that the decrease in $C_{20:4,\omega6}$ fatty acid after feeding the diet supplemented with 2% cholesterol is due to an increased demand in utilization of $C_{20:4,\omega6}$ fatty acid for cholesterol ester formation and secretion into very-low-density lipoprotein. However, it seems unlikely, as after 2% cholesterol supplementation, the decrease in $C_{20:4,\omega6}$ fatty acid was accompanied by an increase in microsomal $C_{18:2,\omega6}$-fatty-acid content. Therefore $C_{18:2,\omega6}$ fatty acid was readily available for the synthesis of $C_{20:4,\omega6}$ fatty acid to fulfill any increased demand in utilization of $C_{20:4,\omega6}$ fatty acid. These results further support our observations that dietary cholesterol decreases $C_{20:4,\omega6}$-fatty-acid content by inhibiting Δ^6-desaturase enzyme activity. Inclusion of 2% cholesterol in the fish-oil diet, however, had no effect on Δ^6-desaturase activity or the $C_{20:4,\omega6}$-fatty-acid content of liver microsomes. It has been recently demonstrated that the rate of cholesterol ester formation by liver microsomal acyl-CoA:cholesterol acyltransferase is accelerated by supplementing the rat diet with fish oil (rich in $C_{20:5}$ and $C_{22:6,\omega3}$ fatty acids) [38,39]. This would mean that $C_{20:5,\omega3}$ and/or $C_{22:6,\omega3}$ fatty acids, instead of $C_{20:4,\omega6}$ fatty acid, are utilized for cholesterol esterification in animals fed on a fish-oil-supplemented diet and that $C_{20:5,\omega3}$ and $C_{22:6,\omega3}$

fatty acids are readily available for increased demand in utilization caused by 2% cholesterol. Therefore, the animals fed on a fish-oil-supplemented diet do not need to use $C_{20:4,\omega6}$ fatty acid for clearance of excessive cholesterol in the form of cholesterol esters, and thus enzyme activity remained unaffected.

Neither the phospholipid content nor the percentages of major phospholipids were altered by dietary lipid supplementation (Table 5). Changes in fatty acid composition presented reflect changes in microsomal phospholipid (Table 6), as phospholipids constitute more than 90% of the microsomal membrane lipids [40]. Changes observed in the fatty acid composition of microsomal lipids are consistent with diet-induced alterations of Δ^6-desaturase activity. For example, feeding diets containing linseed oil and fish oil decreased the $C_{20:4,\omega6}$-fatty-acid content of microsomal membrane lipids, the decrease being greater with fish oil. Dietary cholesterol supplementation decreased the $C_{20:4,\omega6}$-fatty-acid content only when animals were fed with beef tallow or linseed oil, whereas 2% cholesterol feeding with fish oil has no significant effect on the $C_{20:4,\omega6}$-fatty-acid level. The increase observed in $C_{20:5,\omega3}$ fatty acid with accompanied decrease in $C_{22:6,\omega3}$ fatty acid also suggests that dietary cholesterol may inhibit Δ^4-desaturase activity in liver microsomal fractions, diminishing the conversion of $C_{22:5,\omega3}$ into $C_{22:6,\omega3}$ fatty acid.

In summary, the results of the present study suggest that (1) decrease in prostanoid (PGE_2, PGI_2, TXA_2) levels generally observed after fish-oil feeding may be at least partly due to the inhibition of the pathway leading to formation of their common precursor, $C_{20:4,\omega6}$ fatty acid, and that (2) feeding fish oil can prevent further decrease in Δ^6-desaturation and $C_{20:4,\omega6}$-fatty-acid levels caused by addition of 2% (w/w) cholesterol to diets high in saturated fatty acids or high in $C_{18:3,\omega3}$ fatty acid.

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