Effect of dietary fat on lipids and enzyme activities of rat hepatic membranes

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The effect of consumption of ω-3 fatty acids on membrane lipid composition and specific activities of some membrane-bound enzymes was investigated in rats fed a semisynthetic diet containing 14% by weight of either menhaden oil (MO), safflower oil (SO), or beef fat (BF). These diets also contained 2% corn oil to assure an adequate supply of essential fatty acids. The diets were fed to the experimental animals ab libitum for 2 or 7 weeks. The data obtained on lipid analysis indicated a significant increase in phospholipid:cholesterol ratio in membranes of animals fed the MO diet than those fed the SO diet, but was lower than that of animals fed the BF diet. These changes were due to the significantly higher phospholipid content in membranes of animals fed the MO diet when compared with the other diets, and to the significantly lower cholesterol content of animals fed the BF diet. These findings in lipid composition were observed after both 2 and 7 weeks of feeding the experimental diets. Significant effects from dietary MO were observed in the contents of two major phospholipids: phosphatidyl choline and phosphatidyl ethanolamine. No significant effect from the diet was observed in membrane phosphatidyl inositol content. There were significant differences in the fatty acid composition that were reflected on the unsaturation index (UI) of the membranes. Membranes of animals fed the MO diet had a 36% increase in membrane UI as compared with those fed either SO or BF. Hepatic alkaline phosphatase was 33% higher in membranes of animals fed the MO diet as compared to the other diets. No significant effect of MO feeding on hepatic (Na^+, K^+)-ATPase was observed. There was no significant difference between membrane glucose 6-phosphatase activity of animals fed MO and BF diets, which were, in turn, significantly higher than that of animals fed the SO diet. All the above enzyme activities were found to be uninfluenced by the length of the feeding period. The observed alterations in the lipid composition and specific activities of membrane-bound enzymes in addition to the increase in liver weight in animals fed the MO diet indicate significant effects on the liver and therefore warrant further studies on the effect of MO feeding on liver function.

Keywords: liver; lipids; alkaline phosphatase; phospholipids; fish oil; membranes

Introduction

The consumption of ω-3 fatty acids has been steadily increasing since it was discovered that they lower blood lipids, especially triglycerides.[1,2] It has been shown by some investigators that this hypotriglyceridemic effect is due to the inhibition of triglyceride synthesis in the liver.[3] Moreover, ω-3 fatty acid consumption has been shown to inhibit the development of some forms of cancer including breast[4] and colon.[5] It has been shown that this protective role of ω-3 fatty acids could be mediated through their alteration of prostaglandin synthesis.[6] However, the consequence of the long-term consumption of ω-3 fatty acids on the structure and function of mammalian membranes has received little attention. Recently, Zuniga et al.[7] reported on the effect of the consumption of a diet containing 10% by weight of ω-3 fatty acid for 3 weeks on rat hepatic 5′-nucleotidase. These authors found an increase in the Vmax, but a decrease in the breakpoint temperature in the Arrhenius plot of the enzyme with

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feeding menhaden oil. The level of fat intake (20% of the calories) used in the latter studies is lower than the current level in the American diet, ie, 42% of calories, or as suggested by the American Heart Association, 30% of the calories. In the present studies we investigated this effect on membrane lipids and some membrane bound enzymes after feeding a diet containing 16% by weight (32% of the calories) for 2 or 7 weeks. These enzymes are alkaline phosphatase (EC 3.1.3.1), (Na^+, K^+)-ATPase (EC 3.6.1.37), Mg-ATPase (EC 3.6.1.3), and glucose 6-phosphatase (EC 3.1.3.9). In these studies we demonstrated that the dietary-induced alteration in membrane lipids resulted in different effects on these enzymes that could reflect their locations and/or orientations within the membranes.

Materials and methods

Animals and diets

A total of 35 Sprague-Dawley weanling male rats (Harlan-Sprague, Indianapolis, IN, USA) were fed a semi-purified diet (*Table 1*) containing 14% of a test fat or oil and 2% corn oil. The fats or oils used were beef fat (BF), safflower oil (SO), and menhaden oil (MO) supplemented with 0.02% of butylated hydroxytoluene as antioxidant. Diets were mixed in small batches and kept under N_2 and refrigerated until used. The fatty acid analysis of these diets was conducted using a gas chromatograph as described below. The fatty acid composition of these diets is given in *Table 2*. The diets were fed ad libitum and the rats had free access to tap water. They were housed individually in a temperature-controlled room (22° ± 1°C) that was equipped with 12 hr light: 12 hr dark cycle. Daily food intake and weekly body weights were recorded. After 2 weeks, six animals from each group were killed while the rest continued on their diets for an additional 5 weeks. Rats were killed by cervical decapitation and their livers were excised, weighed, and frozen in liquid nitrogen and kept at −70° C until analyzed.

Preparation of hepatic crude membranes

Liver samples were thawed on ice and homogenized in 20 volumes of buffer containing 0.25% M sucrose and 20 mM Tris, pH 7.6[8] using a Tissumizer (Tekmar Co., Cincinnati, OH, USA). The homogenization was done twice, each 10 seconds, at 70% of the maximum speed, while the tissue was kept on ice. Tissue homogenates were centrifuged at 105,000*g* for 1 hour at 4° C. Pellets were resuspended in the same buffer using a hand glass homogenizer and teflon pestle and used as crude membrane fractions.

Table 1 Composition of the experimental diets

Diet ingredient	% by weight
Corn starch	30.0
Casein	26.0
Sucrose	16.5
Fat or oil[a]	14.0
Celufil (fiber source)	6.0
Mineral mix[b]	4.0
Corn oil	2.0
Vitamin mix[b]	1.0
DL-methionine	0.4
Choline chloride (70%)	0.1
Caloric value (Kcal/gm)	4.3

[a] Beef fat, safflower oil, or menhaden oil.
[b] American Institute of Nutrition '76.

Table 2 Fatty acid composition of the experimental diets

Fatty acid[a]	Experimental diets		
	Beef fat	Safflower oil	Menhaden oil
		(%)	
14:0	3.0	10.4	8.6
14:1	1.4	tr[b]	0.5
16:0	20.7	—	15.6
16:1	5.2	—	12.3
17:0	—	—	1.8
18:0	11.8	3.2	4.4
18:1	44.8	22.5	13.9
18:2	11.4	62.9	9.0
18:3	0.5	—	1.3
20:3	—	—	1.4
20:4	—	—	1.0
20:5	—	—	13.6
22:5	—	—	2.2
22:6	—	—	8.3
Σ saturates	35.5	13.2	30.4
Σ monosaturates	51.4	22.5	26.7
Σ omega 6	11.9	62.9	12.7
Σ omega 3	—	—	24.1

[a] Number of carbon atoms: number of double bonds
[b] tr (trace) ≤ 0.5%. The difference between the sum of the percentages and 100 represents minor and unidentified fatty acids.

Lipid analysis

Lipids were extracted from membranes by the method of Folch et al.[9] Cholesterol,[10] phospholipids,[10] and free fatty acids[11] were measured in aliquots of the lipid extracts. Fatty acid analysis was conducted on aliquots of lipid extract after methylation using the method of Lepage and Roy.[12] The composition of the methylated fatty acids was examined using a gas liquid chromatograph equipped with a 6-ft glass column filled with 10% SP-2330 on 100/120 Chromosorb, WAW (Supelco, Bellefonte, PA, USA). The temperatures of the oven and injection port were maintained at 190° C and 250° C, respectively. Identification of the peaks was accomplished using the retention time of authentic standards obtained from NuChek, Prep, Inc., (Elysian, MN, USA). The areas under the peaks were measured using an integrator (Shimadzu, Columbia, MD, USA).

Thin-layer chromatography was used to isolate phospholipid species by the method of Emilsson and Sundler.[13] The silica gel spots containing the phospholipids were charred and their inorganic phosphorus contents were measured according to Pollet et al.[10] A factor of 23.7 was used to convert the inorganic phosphorus data to phospholipids. The factor represents the ratio of the atomic weight of phosphorus to the molecular weight of dipalmitoyl phosphatidyl choline.

Enzyme activity

Alkaline phosphatase (AP) was assayed using the method of Chu et al.[14] Preliminary work in our laboratory indicated that the maximum activity of AP was attained by incubating the membranes in the presence of 0.033% sodium dodecyl sulfate (SDS) prior to the assay.

Glucose 6-phosphatase (G6P) was assayed according to Sigma Co. (St. Louis, MO, USA) using a modification of two published methods.[15,16] Both SDS and deoxycholic acid were found to inhibit the enzyme activity. Therefore, the enzyme activities were measured in the absence of any detergent.

The methods of Largensen and Skou[17] was used to measure total ATPase, Mg-ATPase, and (Na^+ + K^+) ATPase. Preliminary work indicated that the maximum activation for these enzymes was attained by incubating the membranes with SDS for 10 min at 37° C before measuring enzymatic activity. Protein was assayed by the method of Lees and Paxman.[18]

Statistical analysis

Data were analyzed by a two-way analysis of variance (ANOVA). Differences between the means were tested for significance by the Newman-Keuls method.[19] The computer program used was NCSS version 5.1 (Kayesville, UT, USA).

Results

Analysis of variance indicated that the average daily weight gain was not influenced by the diet and there was no significant diet × feeding period interaction. There was significant reduction in daily weight gain as the animals grew older (*Table 3*). The average daily weight gain was 3.8 ± 0.3 gm (mean ± SEM, n = 35) in the first 2 weeks as compared to 3.0 ± 1.0 gm (n = 17) for those killed after 7 weeks of feeding. ANOVA indicated that neither diet nor feeding period had a significant effect on average daily food consumption. Animals ate an average of 15.7 ± 0.6 gm/day at 2 weeks and 16.3 ± 0.5 gm/day at 7 weeks.

ANOVA indicated that both diet and feeding period affected the liver weight, but there was no interaction between diet and feeding period. Animals fed the MO diet, regardless of the period of feeding, had heavier livers whether expressed as an absolute weight or as a percentage of body weight, than those of the other two groups (*Table 4*). The absolute and the relative liver weights were 12%–18% and 9% higher, respectively, than those of animals fed the other diets. There was no difference between liver weights (absolute and relative) of animals fed BF and SO diets. Regardless of the diet, liver weight increased 13% between weeks 2 and 7. On the other hand, as the body weight increased, the relative liver weight decreased by 13% (*Table 3*).

Table 3 The effect of dietary fat and feeding period on rat liver weight

Factor	n	Liver weight*	
		gm	% of body weight
Dietary fat			
BF	12	11.6 ± 0.4[a]	3.5 ± 0.1[a]
SO	11	11.0 ± 0.3[a]	3.5 ± 0.1[a]
MO	12	13.0 ± 0.5[b]	3.8 ± 0.1[b]
Feeding period			
2 weeks	18	11.2 ± 0.3[a]	3.8 ± 0.1[a]
7 weeks	17	12.6 ± 0.3[b]	3.3 ± 0.1[b]

* Values (mean ± SEM) carrying the same superscript in one column within a factor are not significantly ($P \leq 0.05$) different. Analysis of variance indicated no dietary fat × feeding period interaction. Thus, values of the two feeding periods were pooled in examining dietary fat effect. Samples for different dietary fats were pooled when feeding period effect was examined.

Table 4 Effect of dietary fat on lipid content of hepatic membranes

Diet	n†	Membrane Lipids*			
		PL	C	P/C	FFA
Beef fat	12	181 ± 4[a]	54.5 ± 1.6[a]	3.3 ± 0.1[a]	231 ± 15[a]
Safflower oil	11	194 ± 5[a]	79.4 ± 2.1[b]	2.5 ± 0.1[b]	279 ± 13[a]
Menhaden oil	12	217 ± 7[b]	79.4 ± 1.9[b]	2.8 ± 0.1[c]	256 ± 12[a]

* PL, Phospholipid (μg/mg protein); C, cholesterol (μg/mg protein); P/C, phospholipid/cholesterol ratio (μg/μg); FFA, free fatty acids (nmol/mg protein).
† Samples were pooled from week 2 and 7 feeding periods because there was no significant effect for both diet and diet × feeding period interaction.
[a-c] Values (mean ± SEM) carrying the same superscript in a column are not significantly ($P \leq 0.05$) different.

The data obtained on the effect of dietary fat on total hepatic membrane lipids are given in *Table 4*. ANOVA indicated that there was no difference between the lipid composition of membranes of animals fed the diets for 2 or 7 weeks. Thus, the data of the two feeding periods were pooled. Animals fed the MO diet had a significantly higher proportion of membrane phospholipids as compared to those of BF- or SO-fed animals. Rats fed the BF diet had significantly lower cholesterol in their membranes as compared to the rats fed the other diets. These changes in phospholipid and cholesterol contents resulted in the highest phospholipid:cholesterol ratio in the membranes of BF animals followed by MO diet animals, and the least in membrane of animals fed the SO diet. There was no significant effect of the diet on membrane free fatty acids.

ANOVA for data obtained on the concentration of different phospholipid species in hepatic membranes revealed an effect for both diet and feeding duration. *Table 5* data indicate that MO feeding resulted in significantly lower phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) content than either BF or SO. These two phospholipids make up more than one-half of the membrane lipids. Membranes of rats fed MO had a 16% lower concentration of phosphatidyl serine (PS) concentration as compared to SO feeding. There was no significant difference between SO and BF or between BF and MO feeding in membrane PS concentration. There was no significant effect for either the diet or feeding period on phosphatidyl inositol concentration in these membranes. Increasing the feeding period from 2 to 7 weeks on these diets resulted in higher PC and lower PS in these membranes. Duration of feeding period was without an effect on the PE concentration in the membranes (*Table 5*).

Two minor phospholipids, phosphatidic acid (PA) and sphingomyelin (SP) (*Table 6*) had a significant in-

Table 5 Factors affecting some phospholipid species of hepatic membranes*

Factor	n	Phospholipids			
		PC	PE	PS	PI
		(μg/mg protein)			
Dietary fat					
BF	10	73.3 ± 3.1[a]	36.2 ± 1.5[a]	22.3 ± 0.5[ab]	16.2 ± 0.9[a]
SO	10	73.4 ± 2.9[a]	37.2 ± 2.3[a]	26.6 ± 1.7[a]	18.4 ± 1.1[a]
MO	10	93.1 ± 2.6[a]	45.5 ± 3.0[b]	22.4 ± 1.5[b]	19.1 ± 1.3[a]
Feeding period					
2 weeks	15	76.4 ± 3.5[a]	39.5 ± 2.3[a]	25.5 ± 1.2[a]	17.6 ± 1.1[a]
7 weeks	15	83.5 ± 2.9[a]	39.7 ± 2.0[a]	22.0 ± 1.0[b]	18.2 ± 0.7[a]

* Analysis of variance indicated no dietary fat × feeding period interaction. Thus, values of the two feeding periods were pooled in examining the dietary fat effect. Samples for different dietary fats were pooled when the feeding period effect was examined. n refers to the number of samples.
BF, beef fat; SP, safflower oil; MO, menhaden oil; PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PS, phosphatidyl serine; and PI, phosphatidyl inositol.
[a,b] Values (mean ± SEM) carrying the same superscript in one column within a factor are not significantly different ($P > 0.05$)

Table 6 Effect of dietary fat and feeding protein on sphingomyelin and phosphatidic acid content (μg/mg protein) of hepatic membanes*

Dietary fat	Sphingomyelin		Phosphatidic acid	
	2 weeks	7 weeks	2 weeks	7 weeks
BF	17.5 ± 1.0[ax]	20.1 ± 2.4[ax]	13.4 ± 0.3[ax]	13.8 ± 0.7[ax]
SO	21.6 ± 1.4[ax]	23.4 ± 1.8[ax]	11.9 ± 1.0[ax]	18.8 ± 1.3[ay]
MO	27.3 ± 1.7[bx]	19.2 ± 2.0[ay]	21.1 ± 3.4[bx]	15.6 ± 3.0[a]

* Values are (mean ± SEM) of 5 samples; BF, beef fat; SO, safflower oil; and MO, menhaden oil.
[a,b] Values carrying the same superscript in a column (within a feeding period) are not significantly ($P \leq 0.05$) different.
[x,y] Values carrying the same superscript in a row (within a diet group) are not significantly ($P \leq 0.05$) different.

teraction between diet and feeding period. After 2 weeks of feeding, membranes of animals fed the MO diet had a PA 57%–77% higher than BF- and SO-fed animals. This effect disappeared after 7 weeks of feeding. The concentration of phosphatidic acid was significantly higher after 7 weeks than after 2 weeks of feeding. Regarding the concentration of sphingomyelin in hepatic membranes, membranes of animals fed the MO diet had 40% more of this phospholipid than that of either BF- or SO-fed animals. This dietary effect disappeared after feeding the diet for 7 weeks.

The ANOVA for fatty acid composition data indicates significant effects for dietary fat but not feeding period. With the exception of a few fatty acids, there was no diet × feeding period interaction effect on the fatty acid composition of hepatic membranes. Feeding a diet rich in MO resulted in more of ω-3 fatty acids (20:5, 22:5, and 22:6) in hepatic membranes (*Table 7*). On the other hand, liver membranes of animals fed MO contained less ω-6 fatty acids than those of SO and BF animals. This was mainly due to the two major ω-6 fatty acids, 18:2 and 20:4. BF feeding, however, resulted in membranes having a significantly higher saturated fatty acid content than those of animals fed

Table 7 Effect of dietary fat on fatty acid composition of hepatic membranes

Fatty acid*	Diets		
	Beef fat	Safflower oil	Menhaden oil
14:0	1.2 ± 0.1	1.1 ± .01	1.4 ± 0.1
14:1	0.8 ± 0.1[a]	0.7 ± 0.1[a]	1.3 ± 0.1[b]
16:0	10.2 ± 0.3	9.7 ± 0.3	9.7 ± 0.6
16:1 (ω7)	3.0 ± 0.3[a]	1.4 ± 0.1[b]	3.6 ± 0.2[c]
17:0	1.3 ± 0.1[a]	0.4 ± 0.1[b]	1.0 ± 0.03[c]
18:0	13.7 ± 0.3[a]	12.1 ± 0.3[b]	10.6 ± 0.5[c]
18:1 (ω9)	15.3 ± 0.4[a]	9.8 ± 0.3[b]	8.6 ± 0.3[c]
18:2 (ω6)	11.5 ± 0.3[a]	20.5 ± 0.6[b]	9.5 ± 0.4[c]
18:3 (ω6)	0.8 ± 0.04	0.8 ± 0.1	0.7 ± 0.1
20:4 (ω6)	25.9 ± 0.7[a]	25.8 ± 0.4[a]	12.5 ± 0.4[b]
20:5 (ω3)	0.2 ± 0.03[a]	0.1 ± 0.1[a]	11.9 ± 0.5[b]
22:4(ω6)	1.3 ± 0.1	3.4 ± 0.3	0.8 ± 0.1
22:5 (ω3)	0.6 ± 0.1[a]	0.3 ± 0.04[a]	5.7 ± 0.4[b]
22:6(ω3)	8.0 ± 0.4[a]	3.5 ± 0.4[b]	17.4 ± 0.8[c]
Unknown†	5.1 ± 0.3[a]	9.5 ± 0.9[b]	3.7 ± 0.4[a]
ΣSAT	26.3 ± 0.4[a]	23.2 ± 0.5[a]	22.7 ± 1.1[b]
Σω6	39.5 ± 0.7[a]	50.4 ± 0.5[b]	23.5 ± 0.6[c]
Σω3	8.8 ± 0.4[a]	3.9 ± 0.4[b]	35.0 ± 1.5[c]
ω6/ω3	4.6 ± 0.3	14.3 ± 1.7	0.7 ± 0.04
UI	2.2 ± 0.03[a]	2.2 ± 0.03[a]	3.0 ± 0.1[b]

* Number of carbon atoms: number of double bonds (omega notation).
† These fatty acids have either feeding period effects or diet × feeding interaction.
[a-c] Values (mean ± SEM) carrying the same superscript in a row are not significantly ($P \leq 0.05$) different. Samples were pooled from weeks 2 and 7 for a total n = 10 for each fatty acid.
ΣSAT, sum of saturated fatty acids; Σω6, sum of omega 6 fatty acids; Σω3, sum of omega 3 fatty acids; ω6/ω3, ratio of ω6 to ω3 fatty acids; UI, unsaturation index (fatty acid × number of double bonds/total fatty acids).

the other two diets. There was no significant difference between the total saturated fatty acid concentration in membranes of animals fed the SO and MO diets. The calculated unsaturated indices for these membranes revealed a 40% higher value for MO fed animals when compared with either BF- or SO-fed animals.

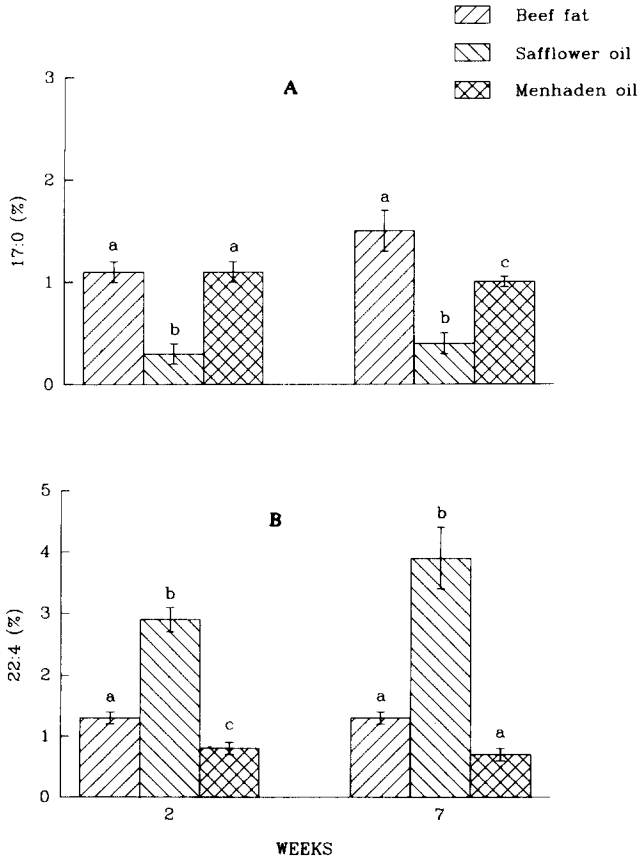

Figure 1 Effect of dietary fat and feeding period on percentage of 17:0 (*A*) and 22:4 (*B*) in hepatic membranes. a,b,c: values carrying the same superscript within a feeding period are not significantly different ($P > 0.05$). Values are mean ± SEM of 5 samples chosen at random from each group.

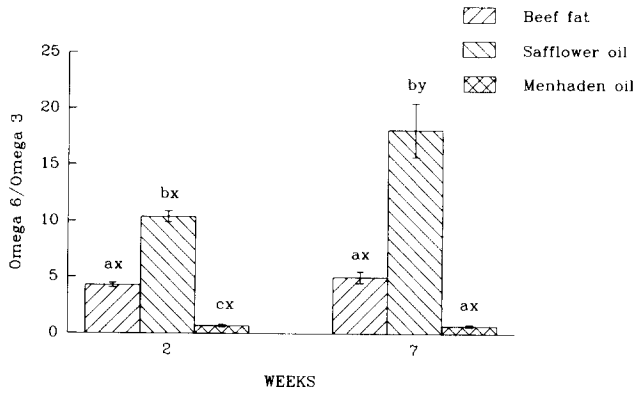

Figure 2 Effect of dietary fat and feeding period on the ω6/ω3 ratio of hepatic membranes. a,b,c: values carrying the same superscript within a feeding period are not significantly different ($P > 0.05$). x,y: values carrying the same superscript within one diet are not significantly different ($P > 0.05$). Values are the mean ± SEM of 5 samples chosen at random from each group.

As stated before, the ANOVA indicated significant interaction between diet and feeding period in some fatty acids ie, 17:0, 22:4 (*Figure 1 a,b*). Rats fed animal fats (BF and MO) in their diets had significantly higher 17:0 in their lipids when compared with the SO feeding. Rats fed the MO diet for 7 weeks had a lower percentage of this fatty acid in their membranes than BF but it was still significantly higher than SO fed rats (*Figure 1A*). Membranes of animals fed the SO diet had a significantly higher percentage of 22:4 (ω-6) when compared with those of either BF or MO feeding at the two feeding periods (*Figure 1B*). In this regard, only at 2 weeks did BF feeding result in a higher 22:4 (ω-6) percentage in membranes than that of MO feeding.

In addition to the observed changes due to dietary fat, the feeding period was found to influence the percentages of 22:6 (ω-3) and unknown fatty acids in membranes. As the feeding period extended from 2 to 7 weeks, there was a lower percentage of 22:6 and a higher percentage of unknown fatty acid. The calculated ω-6:ω-3 fatty acid ratios were found to be influenced by the diet and had a diet × feeding period interaction (*Figure 2*). The values of the two feeding periods were not significantly different with the exception of SO feeding, where this ratio was increased from 10.4 ± 0.5 in 2 weeks to 18.1 ± 2.4 in 7 weeks. This suggests that there is a preference to accumulate ω-6 fatty acids more than ω-3 fatty acids in hepatic membranes with longer feeding periods.

The specific activities of four membrane-bound enzymes are given in *Table 8*. ANOVA of AP activity indicated that only diet had a significant effect on the specific activity of this enzyme (*Table 8*). Rats fed MO had a 33% higher activity than animals fed either BF or SO diet. As in the case of AP, only the diet was found to influence the specific activity of Mg-ATPase. Membranes of animals fed the MO diet had 36% and 43% lower activity of Mg-ATPase as compared to BF and SO diets, respectively. Similar activities of Mg-ATPase were found in membranes of animals fed

Table 8 Effect of dietary fat on the specific activities of membrane-bound enzymes

Enzyme	Diet	Specific activity*
Alkaline phosphatase	Beef fat	1.2 ± 0.05[a]
	Safflower oil	1.2 ± 0.02[a]
	Fish oil	1.6 ± 0.02[b]
(Na^+, K^+)-ATPase	Beef fat	0.67 ± 0.1[a]
	Safflower oil	0.73 ± 0.05[a]
	Fish oil	0.73 ± 0.05[a]
Mg^+-ATPase	Beef fat	9.4 ± 0.2[a]
	Safflower oil	8.3 ± 0.3[a]
	Fish oil	5.1 ± 0.4[b]
Glucose 6-phosphatase	Beef fat	2.7 ± 0.1[a]
	Safflower oil	0.8 ± 0.1[b]
	Fish oil	2.4 ± 0.1[a]

* Values are mean ± SEM of 11–12 samples. The samples of animals fed 2 weeks and 7 weeks were pooled because there was no effect for the length of feeding period on enzyme activities. Specific activities are expressed as nmol substrate/hr/mg protein for alkaline phosphatase and as μg phosphorus/hr/mg protein for other enzyme.

[a-c] Values for one enzyme carrying different superscripts are significantly ($P \leq 0.05$) different.

BF and SO diets. On the other hand, (Na^+, K^+)-ATPase, which exists in the plasma membranes, was found to be resistant to dietary manipulation. The analysis of variance of glucose 6-phosphatase data indicated a significant dietary effect (*Table 8*). Animals fed the SO diet had 66%–70% lower specific activity in this enzyme as compared with those fed either BF or MO diets.

Discussion

The most significant effect of ω-3 fatty acids (MO) consumption observed in these studies was the significant alterations in hepatic membrane lipids and the higher specific activity of alkaline phosphatase as compared with BF and SO feeding. These effects were found at 2 weeks and 7 weeks. Alkaline phosphatase is a plasma membrane-bound enzyme[20,21] and recent evidence suggests that it is anchored to the membrane through phosphatidyl inositol.[22] Treating cells with IP-specific phospholipase C resulted in inhibition of the enzyme activity, suggesting that alteration in the lipid content of phosphatidyl inositol and/or its composition may influence the activity of the enzyme. Our present studies indicate that feeding fish oil (MO) resulted in significant alterations in the concentration of two major phospholipids, PC and PE, without influencing PI concentration.

The present studies indicate that feeding fish oil results in a significantly higher content of ω-3 fatty acids and lower ω-6 fatty acids in these membranes. This is in agreement with other reports[23,24] and suggests that the location of the double bond in the fatty acid moiety of membrane phospholipid may be an important factor in affecting the activity of alkaline phosphatase. The enrichment of these membranes with ω-3 fatty acid resulted in a higher unsaturation index, which could be another factor in affecting the fluidity of the microenvironment of the enzyme. The specific activity of another plasma membrane enzyme, (Na^+, K^+)-ATPase, was measured and was not found to be influenced by dietary modification of membranes. This is in agreement with other reports.[25] Previous work demonstrated that this enzyme spans the membrane bilayer[26] and thus may be less influenced by the observed alterations. However, there are some reports in the literature to indicate that membrane lipid alterations influence the activity of (Na^+, K^+)-ATPase.[26] In the case of Mg-ATPase, membranes of animals fed the MO diet exhibited a significantly lower specific activity of the enzyme when compared with those of animals fed either the BF or SO diets. On the other hand, membranes of animals fed ω-6 fatty acid rich diet had a three-fold lower activity of glucose 6-phosphatase than that of rats raised on ω-3 fatty acid (MO) or saturated fatty acid (BF) rich diets. These results suggest that these microsomal enzymes may require different degrees of fluidity in the adjacent lipids for their function. There is no method available to examine the lipid changes in the immediate vicinity of these proteins.

Rats fed the ω-3 fatty acid rich diet (MO) had more membrane phospholipids when compared with membranes of other rats. Recent work by Yeo and Holub[27] suggested that there was a shift from triglyceride to phospholipid synthesis in rat liver with feeding fish oil. Their studies also indicated that this increase in phospholipid associated with feeding fish oil was reflected in PC, PE, PS, and PI. Our data agree with these findings with the exception that we found a decrease in PS and no increase in PI with feeding ω-3 fatty acids, but these are minor components. The differences between the two findings may reflect the difference between the two types of studies. The studies by Yeo and Holub[27] were metabolic studies designed to examine the synthesis of triglycerides and phospholipids from precursors that could be modified in the liver by homeostatic mechanisms.

The present studies indicate that there was an increased liver weight, whether expressed as absolute weight or relative to body weight, with feeding fish oil as compared to the other two diets. This could be partially due to the increase in the phospholipid content of this organ in animals fed fish oil. The enlargement of the liver and the higher alkaline phosphatase activity may suggest malfunction of this organ. Alkaline phosphatase activity in the plasma, which is originated from organs such as the liver,[28] is usually used clinically as an indication for the presence of inflammation.[29] Accordingly, the ingestion of fish oil may mask the clinical symptoms of some diseases.

In conclusion, the fish oil diet resulted in significant alterations in the lipid composition of liver membranes. These changes induced alterations in the specific activities of some plasma and microsomal membrane-bound enzymes. The significantly higher liver alkaline phosphatase and the enlargement of the liver warrant further studies in the function of this organ in subjects ingesting fish oil.

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