Effects of Perfluorooctanoic Acid on the Synthesis of Phospholipids in the Liver of Mice Fed a Dietary Soybean Oil, Perilla Oil or Fish Oil

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The effects of perfluorooctanoic acid (PFOA) on the synthesis of phospholipids were studied in combination with feeding of various dietary oils (soybean oil (SO), perilla oil (PO) or fish oil (FO)). Hepatic contents of phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn) and phosphatidylinositol (PtdIns) did not differ between the three dietary groups except for a high level of PtdEtn in FO-fed mice. PFOA treatment increased the hepatic content of PtdCho and PtdEtn by 1.5 fold but did not affect that of PtdIns. Fatty acid compositions of phospholipids were different between the three groups reflecting the fatty acid composition in their diet. The rate of incorporation of [^{3}H]glycerol into PtdEtn in the FO-fed group was significantly higher and that into TG was lower, compared to other dietary groups in PFOA-untreated mice. PFOA treatment significantly increased the incorporation of [^{3}H]glycerol into PtdEtn and PtdSer and decreased that into TG, whereas those into other lipid classes were not altered by PFOA treatment. These results suggest that acceleration of PtdEtn synthesis *de novo* is responsible for a marked increase in the hepatic content of PtdEtn in PFOA-treated mice, whereas an increase in hepatic content of PtdCho is thought not to be due to increased synthesis *de novo*.

Key words —— perfluorooctanoic acid, soy bean oil, perilla oil, fish oil, phosphatidylcholine, phosphatidylethanolamine

INTRODUCTION

Perfluorooctanoic acid (PFOA), a peroxisome proliferator, causes marked changes in lipid metabolism in rodents.[1–8] The amounts of various phospholipids as well as triacylglycerol (TG) in the liver were greatly increased by the treatment of rats with PFOA.[2,5,8] In addition, fatty acid profile was greatly altered; octadecenoic acid (18 : 1) was increased whereas stearic acid (18 : 0) and arachidonic acid were decreased in phospholipids in rat liver.[3,7,8] Such changes are, at least in part, due to changes in the activities of lipid metabolizing enzymes in rat liver.[6–8] Compared to rats, little information is available for mice about PFOA-induced changes in lipid metabolism. In the previous study, we demonstrated that PFOA caused a significant accumulation of TG in mice fed a diet containing soybean oil (SO) or perilla oil (PO) but not in mice fed a fish oil (FO)-containing diet.[9] This suggested that n-3 fatty acids, especially docosahexaenoic acid (22 : 6(n-3)), play a crucial role in the accumulation of TG in mouse liver upon the PFOA treatment. In fact, recent studies have demonstrated the hypolipidemic effects of fish oil, enriched in eicosapentaenoic acid (20 : 5(n-3)) and 22 : 6(n-3), in patients of hyperlipidemia[10,11] and in experimental animals.[12–17] It is of interest, therefore, to understand how PFOA influences individual phospholipid metabolism and how n-3 fatty acids modify the effects of PFOA. In the present study, the effects of PFOA on *de novo* synthesis of individual phospholipids were extensively compared between mice fed a diet containing SO, PO or FO.

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MATERIALS AND METHODS

Materials —— PFOA was purchased from Aldrich Japan (Tokyo, Japan). Fish oil concentrate (TG25) was obtained from Maruha Co. (Tokyo). [1(3)-^{3}H]Glycerol (500 Ci/ mol) was purchased from Amersham (Amersham, UK). All other chemicals and reagents were of analytical grade.

Animals —— Male ddY mice 7 weeks old were purchased from SLC Inc. (Hamamatsu, Japan). Mice

Table 1. Fatty Acid Composition of Experimental Diets

Fatty acid	Standard diet	SO-diet	PO-diet	FO-diet
	(%)			
16 : 0	21.0	11.1	6.7	6.4
16 : 1	1.1	0.3	0.2	9.3
18 : 0	7.5	4.2	2.1	2.7
18 : 1	25.7	24.6	19.4	10.3
18 : 2(n-6)	40.7	52.8	14.6	18.6
18 : 3(n-3)	4.0	7.0	56.9	2.9
20 : 3(n-6)			0.9	
20 : 4(n-6)			1.1	
20 : 5(n-3)			33.1	
22 : 4(n-3)			0.7	
22 : 5(n-3)			1.9	
22 : 6(n-3)			10.8	

The diet were composed of 18.8% (w/w) soybean oil (SO), perilla oil (PO), or fish oil (FO), 26.69% corn starch, 1.0% α-starch, 16.0% sucrose, 5% cellulose, 24.5% casein, 1% vitamin mix (AIN76), 7% mineral mix (AIN76), and 0.01% of butylated hydroxytoluene. Fatty acids are designated by the number of carbon atoms and double bonds; 16 : 0, palmitic acid; 16 : 1, palmitoleic acid; 18 : 0, stearic acid; 18 : 1, octadecenoic acid; 18 : 2(n-6), linoleic acid; 18 : 3(n-3), α-linoleic acid; 20 : 4(n-6), arachidonic acid; 20 : 5(n-3), eicosapentaenoic acid; 22 : 4(n-3), docosatetraenoic acid; 22 : 5(n-3), docosapentaenoic acid; 22 : 6(n-3), docosahexaenoic acid.

fed a standard diet (CE-2 from Clea Japan, Tokyo) for 1 week, were switched to a semi-synthetic high-fat diet (40% of energy from fat), and fed the diet for 4 weeks. The semi-synthetic diet contained either SO, PO or FO. To the FO containing diet, 1.1% (w/w) safflower oil was added to supplement linoleic acid (18 : 2(n-6)). The fatty acid compositions of the three diets are shown in Table 1; all were prepared weekly and stored under nitrogen at –20°C. The left-over food was discarded daily. Some of the mice were intraperitoneally injected with PFOA (10 mg/kg body weight) or 0.9% (w/w) NaCl as a vehicle daily for 7 days before being killed. PFOA was dissolved in 0.9% (w/v) NaCl and the pH of the solution was adjusted to 8.0 with 1 M NaOH. Mice were killed 24 hr after the last injection of PFOA. Livers were isolated, perfused with 0.9% (w/v) NaCl, weighed, frozen in liquid nitrogen and stored at –80°C until use.

Lipid Analyses —— Total lipids were extracted from livers according to the method of Bligh and Dyer.[18] Phospholipids, triacylglycerol and diacylglycerol were separated by TLC on silica gel G plates (Merck, Darmstadt, Germany), which were developed with hexane: diethyl ether: acetic acid (80 : 30 : 1, v/v). Phospholipids were separated by TLC on silica gel G, developed with chloroform : methanol : acetic acid : water (50 : 37.5 : 5 : 3.5, v/v). Individual spots were visualized by spraying 0.005% (w/v) purimuline/90% acetone, scraped off from the plates and transferred to glass tubes. To the tubes was added a known amount of methyl pentadecanoate as an internal standard. Each lipid was extracted from the silica with 10 ml of chloroform: methanol: 0.1 M HCl (4 : 4 : 1, v/v). A phase of the extract was broken by adding 3 ml of 0.1 M HCl, and the chloroform layer was collected. Methylesters of fatty acids were prepared from each extract by the reaction with 14% (w/v) boron trifluoride/methanol solution (Wako Pure Chemical Ind., Osaka, Japan), and analyzed by gas-liquid chromatography (GLC) as described previously.[19] All solvents employed for lipid analyses contained 0.005% (w/v) butylated hydroxytoluene.

***In vivo* Synthesis of Glycerolipids in Mouse Liver** —— [1(3)-^{3}H]glycerol was dissolved in 0.9% (w/v) NaCl at a concentration of 50 μCi/0.15 ml for injection. Mice, which had been fed SO-, PO- or FO-diet in combination with PFOA treatment, were intraperitoneally injected with 50 μCi of [^{3}H]glycerol 15 min before being killed. Hepatic lipids were extracted and separated as described above. Each lipid extract was transferred to a glass scintillation vial and was dried under nitrogen stream. To the residue was added 1 ml of water and 9 ml of toluene/Triton X-100 (2 : 1, v/v)-based scintillation cocktail. The radioactivity was determined by a liquid scintillation counter.

Statistical Analysis —— Analysis of variance was used to test the significance of the difference between the three dietary groups. Where difference was significant, the statistical significance ($p < 0.05$) between any two means was determined using Shèffe's multiple range test. Statistical significance ($p < 0.05$) between control and PFOA-treated group was determined by Student's *t*-test or Welch's test after *F*-test for two means.

RESULTS AND DISCUSSION

Effects of PFOA on Body and Liver Weight of Mice Fed a Diet Containing SO, PO or FO

Body weights of mice were not different between 6 experimental groups. Food consumption was not affected by the treatment with PFOA. PFOA treatment significantly increased liver weight in all dietary groups: 2.25 ± 0.36 g *vs*. 3.89 ± 0.64 g in SO-fed groups; 2.73 ± 0.25 g *vs*. 4.41 ± 0.38 g in PO-fed groups; 2.49 ± 0.13 g *vs*. 4.57 ± 0.15 g in FO-

Table 2. Fatty Acid Composition of PtdCho in Mouse Liver

	SO		PO		FO	
PFOA	−	+	−	+	−	+
	(%)					
16 : 0	31.8 ± 1.0[a)]	40.0 ± 1.9[a),*]	34.0 ± 2.5[a)]	37.9 ± 2.9[a)]	40.6 ± 1.4[b)]	44.9 ± 2.2[b),*]
18 : 0	18.3 ± 1.8	6.9 ± 1.1*	16.0 ± 0.9[a,b)]	6.5 ± 0.4*	13.9 ± 0.4[b)]	6.8 ± 0.8*
18 : 1(n-9)	4.4 ± 0.2[a)]	9.8 ± 0.7[a),*]	6.9 ± 1.4[b)]	14.9 ± 2.0[b),*]	5.5 ± 0.4[a,b)]	9.8 ± 2.1[a),*]
18 : 2(n-6)	26.4 ± 1.9[a)]	32.4 ± 2.4[a),*]	21.7 ± 1.7[b)]	27.8 ± 2.2[b),*]	5.4 ± 1.1[c)]	11.1 ± 2.0[c),*]
18 : 3(n-3)	0.2 ± 0.1[a)]	0.8 ± 0.2	2.9 ± 0.4[b)]	1.7 ± 1.5	0.1 ± 0.1[a)]	0.0 ± 0.0*
20 : 3(n-6)	1.1 ± 0.2[a)]	2.6 ± 0.4[a),*]	0.7 ± 0.5[a,b)]	1.3 ± 0.3[b)]	0.4 ± 0.0[b)]	0.8 ± 0.3[b),*]
20 : 4(n-6)	11.7 ± 0.5[a)]	2.8 ± 0.5[a),*]	2.4 ± 0.5[b)]	0.9 ± 0.1[b),*]	4.7 ± 0.2[c)]	2.2 ± 0.9[a,b),*]
20 : 5(n-3)	0.3 ± 0.2[a)]	0.2 ± 0.0[a)]	6.7 ± 0.7[b)]	2.0 ± 0.3[a),*]	9.1 ± 0.3[c)]	9.0 ± 2.0[b)]
22 : 5(n-3)	0.2 ± 0.0[a)]	0.3 ± 0.0[a),*]	0.7 ± 0.0[b)]	0.8 ± 0.1[b)]	1.2 ± 0.3[c)]	1.0 ± 0.1[c)]
22 : 6(n-3)	4.7 ± 0.6[a)]	2.9 ± 0.3[a),*]	6.2 ± 1.1[a)]	2.4 ± 0.4[a),*]	17.1 ± 1.1[b)]	11.7 ± 2.0[b),*]
	Total fatty acid (μmol/g liver)					
	25.01 ± 1.55	36.62 ± 2.89*	24.28 ± 4.75	33.68 ± 1.27*	23.21 ± 1.85	33.55 ± 4.12*

Mice fed a diet containing SO, PO or FO for 4 weeks were intraperitoneally injected with PFOA (10 mg/ kg body weight) daily for 1 week before being killed. Values represent means ± S.D. for 4 animals. *a*, *b*, *c*) Differences between SO-, PO- and FO-fed animals are statistically significant without a common superscript ($p < 0.05$). If no superscript appears, differences between three dietary groups are not statistically significant. *, Differences are statistically significant between PFOA-treated group and untreated group ($p < 0.05$).

fed groups.

Effects of PFOA on Hepatic Content of Phospholipids and Their Fatty Acid Composition

Fatty acid composition of SO-diet was close to that of standard laboratory chow (CE-2) as shown in Table 1. The fatty acid composition of hepatic phosphatidylcholine (PtdCho) in PO-fed and FO-fed mice was compared to that in SO-fed mice (Table 2). Dietary intake of PO decreased the proportion of arachidonic acid (20 : 4(n-6)) and increased those of α-linolenic acid (18 : 3(n-3)) and 20 : 5(n-3). Upon feeding FO, the proportion of 20 : 4(n-6) was decreased and those of 20 : 5(n-3) and 22 : 6(n-3) were more markedly increased. These changes partially reflected the difference in fatty acid composition between the diets. It is noteworthy that dietary 18 : 3(n-3) increased the proportion of 20 : 5(n-3) but not 22 : 6(n-3) in hepatic PtdCho. In all dietary groups, PFOA treatment significantly increased the proportions of 18 : 1 and 18 : 2(n-6), but it decreased the proportions of 18 : 0 and polyunsaturated fatty acids such as 20 : 4(n-6), 20 : 5(n-3) and 22 : 6(n-3) with the exception that 20 : 5(n-3) was not changed in FO-fed mice. PFOA treatment caused approximately 1.5-fold increase in hepatic content of PtdCho on the basis of g liver in all dietary groups.

Changes induced by dietary intake of PO and FO in fatty acid composition of phosphatidylethanolamine (PtdEtn) were essentially the same as those observed with PtdCho (Table 3). PFOA treatment significantly increased in the proportions of 18 : 1 and 18 : 2(n-6) and decreased in the proportion of 18 : 0 in all dietary groups. The proportions of 20 : 4(n-6), 20 : 5(n-3) and 22 : 6(n-3) were decreased by the treatment of SO- and PO-fed mice with PFOA, whereas these were not altered in FO-fed mice. Hepatic contents of PtdEtn in FO-fed mice were higher than that in the SO-fed group on the basis of g liver. PFOA treatment significantly increased the hepatic content of PtdEtn in all dietary groups.

The manipulation of dietary oil produced similar changes in fatty acid composition of phosphatidylinositol (PtdIns) as were observed in PtdCho and PtdEtn (Table 4). PFOA treatment caused an increase in 18 : 1 and a decrease in 18 : 0, however, the proportions of polyunsaturated fatty acids were not altered by PFOA treatment. Hepatic contents of PtdIns were not significantly different among the 6 experimental groups. The effects of PFOA on hepatic contents of PtdCho, PTdEtn and PtdIns observed in mice were different from the findings derived from the experiments using rats where hepatic contents of these phospholipids were increased by PFOA treatment from 2.2 fold to 2.4 fold.[8)]

The increase in 18 : 1 and the decrease in 18 : 0 observed in the three phospholipid classes were thought to be due to an induction of stearoyl-CoA desaturase that catalyzes the conversion of 18 : 0 to

Table 3. Fatty Acid Composition of PtdEtn in Mouse Liver

	SO		PO		FO	
PFOA	−	+	−	+	−	+
			(%)			
16 : 0	24.0 ± 1.6	23.8 ± 1.7[a)]	23.4 ± 2.2	22.8 ± 2.3[a)]	27.2 ± 3.9	28.1 ± 1.6[b)]
18 : 0	22.6 ± 1.5	16.8 ± 1.8*	28.1 ± 1.1[b)]	17.3 ± 2.1*	27.4 ± 2.0[b)]	19.2 ± 1.7*
18 : 1	8.7 ± 0.8[a)]	14.9 ± 1.9[a),*]	7.2 ± 1.4[a)]	16.9 ± 2.3[a),*]	3.9 ± 0.5[b)]	8.3 ± 1.2[b),*]
18 : 2(n-6)	12.3 ± 1.3[a)]	19.7 ± 1.9[a),*]	8.2 ± 0.8[b)]	19.2 ± 1.9[a),*]	2.0 ± 0.2[c)]	3.8 ± 1.0[b),*]
18 : 3(n-3)	0.0 ± 0.0[a)]	0.1 ± 0.1*	2.5 ± 0.4[b)]	1.6 ± 1.4	0.1 ± 0.2[a)]	0.0 ± 0.0
20 : 3(n-6)	0.5 ± 0.0[a)]	1.5 ± 0.2[a),*]	0.4 ± 0.1[b)]	1.0 ± 0.2[b),*]	0.1 ± 0.0[c)]	0.3 ± 0.1[c),*]
20 : 4(n-6)	17.6 ± 1.0[a)]	9.9 ± 1.3[a),*]	4.4 ± 0.7[b)]	3.3 ± 0.4[b),*]	3.8 ± 0.3[b)]	3.0 ± 0.6[b)]
20 : 5(n-3)	0.3 ± 0.1[a)]	0.3 ± 0.1[a)]	9.9 ± 0.9[b)]	4.5 ± 0.6[b),*]	7.1 ± 0.4[c)]	8.4 ± 0.7[c),*]
22 : 5(n-3)	0.1 ± 0.1[a)]	0.8 ± 0.1[a),*]	1.2 ± 0.1[b)]	2.0 ± 0.1[b),*]	1.3 ± 0.3[b)]	1.2 ± 0.1[c)]
22 : 6(n-3)	13.6 ± 0.8[a)]	11.2 ± 1.2[a),*]	13.3 ± 1.3[a)]	9.3 ± 1.2[a),*]	26.0 ± 2.4[b)]	26.3 ± 2.4[b)]
			Total fatty acid (μmol/g liver)			
	10.20 ± 0.34[a)]	20.04 ± 0.87*	13.84 ± 2.37[a,b)]	20.29 ± 3.65*	14.95 ± 2.62[b)]	22.19 ± 1.85*

Mice fed a diet containing SO, PO or FO for 4 weeks were intraperitoneally injected with PFOA (10 mg/kg body weight) daily for 1 week before being killed. Values represent means ± S.D. for 4 animals. *a*, *b*, *c*) Differences between SO-, PO- and FO-fed animals are statistically significant without a common superscript ($p < 0.05$). If no superscript appears, differences between three dietary groups are not statistically significant. *, Differences are statistically significant between PFOA-treated group and untreated group ($p < 0.05$).

Table 4. Fatty Acid Composition of PtdIns in Mouse Liver

	SO		PO		FO	
PFOA	−	+	−	+	−	+
			(%)			
16 : 0	11.7 ± 1.5	5.7 ± 0.6[a),*]	11.4 ± 2.1	7.0 ± 0.0[a,b),*]	14.0 ± 1.3	11.2 ± 3.3[b)]
18 : 0	47.4 ± 1.0	44.1 ± 1.6[a),*]	50.5 ± 1.0	37.9 ± 0.7[b),*]	47.3 ± 2.4	43.0 ± 2.2[a)]
18 : 1	2.2 ± 0.1	5.8 ± 1.1[a),*]	2.3 ± 0.5	12.8 ± 1.3[b),*]	1.7 ± 0.5	4.0 ± 1.0[a),*]
18 : 2(n-6)	3.3 ± 0.4[a)]	4.5 ± 0.3[a),*]	3.2 ± 1.0[a)]	7.7 ± 1.5[b),*]	1.0 ± 0.1[b)]	2.0 ± 0.4[c),*]
18 : 3(n-3)	0.2 ± 0.1[a)]	0.0 ± 0.0[a),*]	0.8 ± 0.3[b)]	0.9 ± 0.3[b)]	0.1 ± 0.1[a)]	0.1 ± 0.2[a)]
20 : 3(n-6)	1.5 ± 0.3[a)]	5.9 ± 0.8[a),*]	3.2 ± 0.6[b)]	7.6 ± 2.0[a),*]	0.8 ± 0.1[a)]	2.3 ± 0.8[b),*]
20 : 4(n-6)	32.3 ± 2.0[a)]	32.6 ± 1.0[a)]	15.7 ± 2.2[b)]	15.4 ± 0.8[b)]	20.7 ± 2.3[b)]	19.3 ± 1.9[c)]
20 : 5(n-3)	0.0 ± 0.0[a)]	0.1 ± 0.1[a)]	5.8 ± 0.8[b)]	4.2 ± 1.1[b)]	4.5 ± 0.5[b)]	4.7 ± 1.0[b)]
22 : 5(n-3)	0.2 ± 0.0[a)]	0.5 ± 0.0[a),*]	2.8 ± 0.3[b)]	1.9 ± 0.3[b),*]	2.7 ± 0.6[b)]	3.1 ± 0.2[c)]
22 : 6(n-3)	0.6 ± 0.2[a)]	1.1 ± 0.3[a)]	2.3 ± 0.8[a)]	1.6 ± 0.1[a)]	6.3 ± 1.2[b)]	8.8 ± 1.2[b),*]
			Total fatty acid (μmol/g liver)			
	7.80 ± 0.62[a)]	7.16 ± 0.49	7.36 ± 1.71[a,b)]	6.60 ± 0.71	5.82 ± 0.12[b)]	7.41 ± 0.71*

Mice fed a diet containing SO, PO or FO for 4 weeks were intraperitoneally injected with PFOA (10 mg/kg body weight) daily for 1 week before being killed. Values represent means ± S.D. for 4 animals. *a*, *b*, *c*) Differences between SO-, PO- and FO-fed animals are statistically significant without a common superscript ($p < 0.05$). If no superscript appears, differences between three dietary groups are not statistically significant. *, Differences are statistically significant between PFOA-treated group and untreated group ($p < 0.05$).

18 : 1.[3,7,8)] It is of interest that the PFOA-induced decrease in polyunsaturated fatty acids was not significant in FO-fed mice compared to other dietary groups. This may be due to the fact that polyunsaturated fatty acids of n-3 series are preferentially utilized to form phospholipids, especially PtdEtn.[20,21)] FO diet contains a high proportion of polyunsaturated fatty acids of n-3 series; therefore, the changes in fatty acid composition in FO-fed mice were less than in other dietary groups. The proportions of polyunsaturated fatty acids of PtdIns were not altered by PFOA treatment at all. These results suggest that PFOA influenced independently the processes responsible for the regulation of fatty acid composi-

Table 5. Incorporation of [1(3)-^{3}H]glycerol Among Various Lipids in Mouse Liver

	SO		PO		FO	
PFOA	−	+	−	+	−	+
	(%)					
TG	58.3 ± 4.1$^{a)}$	47.2 ± 1.2$^{a),*}$	61.9 ± 2.1$^{a)}$	45.2 ± 4.9$^{a),*}$	46.3 ± 6.8$^{b)}$	27.0 ± 6.0$^{b),*}$
DG	9.3 ± 5.8	6.2 ± 1.6	6.4 ± 1.7	4.3 ± 0.9	3.9 ± 1.2	6.4 ± 1.0*
PtdCho	19.2 ± 3.7$^{a,b)}$	16.4 ± 2.1	18.4 ± 1.6$^{a)}$	20.8 ± 5.5	26.2 ± 5.2$^{b)}$	24.3 ± 6.4
PtdEtn	8.0 ± 1.6$^{a)}$	20.9 ± 2.6$^{a),*}$	8.1 ± 1.6$^{a)}$	20.1 ± 1.8$^{a),*}$	14.6 ± 2.0$^{b)}$	29.2 ± 5.3$^{b),*}$
PtdIns	1.6 ± 0.4$^{a)}$	2.3 ± 0.9	1.5 ± 0.1$^{a)}$	3.3 ± 0.6*	2.8 ± 0.7$^{b)}$	3.4 ± 1.6
PtdSer	0.7 ± 0.6$^{a)}$	2.3 ± 0.6*	0.5 ± 0.2$^{a)}$	1.5 ± 0.3*	1.8 ± 0.6$^{b)}$	2.8 ± 1.1
Others	2.9 ± 1.2$^{a)}$	4.7 ± 1.5	3.2 ± 1.1$^{a,b)}$	4.9 ± 2.2	5.4 ± 1.1$^{b)}$	6.8 ± 2.0
	Radioactivity incorporated in liver lipids ($\times 10^5$ dpm/g liver)					
	9.69 ± 1.66	8.50 ± 0.99	10.51 ± 2.03	7.28 ± 0.36	8.14 ± 0.68	7.96 ± 0.20

Mice fed a diet containing SO, PO or FO and treated with PFOA were intraperitoneally injected with 50 μCi of [^{3}H]glycerol 15 min before being killed. Values represent means ± S.D. for 4 animals. a, b, c) Differences between SO-, PO- and FO-fed animals are statistically significant without a common superscript ($p < 0.05$). If no superscript appears, differences between three dietary groups are not statistically significant. *, Differences are statistically significant between PFOA-treated group and untreated group ($p < 0.05$).

Table 6. Fatty Acid Composition of DG in Mouse Liver

	SO		PO		FO	
PFOA	−	+	−	+	−	+
	(%)					
16 : 0	26.0 ± 4.0	27.4 ± 2.3$^{a)}$	26.6 ± 0.8	26.8 ± 1.3$^{a)}$	30.6 ± 3.7	35.1 ± 3.9$^{b)}$
18 : 0	9.5 ± 1.6	5.4 ± 0.4$^{a),*}$	9.0 ± 1.4	8.7 ± 0.4$^{b)}$	10.4 ± 0.9	10.3 ± 0.8$^{c)}$
18 : 1(n-9)	12.4 ± 2.7	29.3 ± 3.5$^{a),*}$	16.6 ± 2.6	25.1 ± 2.6$^{a),*}$	13.6 ± 1.9	19.3 ± 1.5$^{b),*}$
18 : 2(n-6)	45.2 ± 2.7$^{a)}$	30.6 ± 2.8$^{a),*}$	24.4 ± 1.9$^{b)}$	22.0 ± 2.6$^{b)}$	26.0 ± 5.3$^{b)}$	18.9 ± 1.8$^{b),*}$
18 : 3(n-3)	0.5 ± 0.6$^{a)}$	0.0 ± 0.0$^{a)}$	15.7 ± 0.4$^{b)}$	10.1 ± 0.7$^{b),*}$	0.0 ± 0.0$^{a)}$	0.0 ± 0.0$^{a)}$
20 : 4(n-6)	4.4 ± 1.3$^{a)}$	1.3 ± 0.6$^{a),*}$	1.5 ± 0.4$^{b)}$	1.2 ± 0.3$^{a)}$	2.6 ± 0.3$^{b)}$	2.3 ± 0.2$^{b)}$
20 : 5(n-3)	0.0 ± 0.0$^{a)}$	0.0 ± 0.0$^{a)}$	1.8 ± 0.2$^{b)}$	0.6 ± 0.1$^{a),*}$	3.3 ± 1.4$^{b)}$	2.5 ± 0.5$^{b)}$
22 : 5(n-3)	0.1 ± 0.1$^{a)}$	0.0 ± 0.0$^{a)}$	0.8 ± 0.3$^{a,b)}$	0.4 ± 0.4$^{a)}$	1.4 ± 0.7$^{b)}$	1.6 ± 0.3$^{b)}$
22 : 6(n-3)	1.7 ± 0.4$^{a)}$	0.4 ± 0.1$^{a),*}$	1.7 ± 0.5$^{a)}$	0.4 ± 0.2$^{a),*}$	6.4 ± 2.1$^{b)}$	4.8 ± 1.1$^{b)}$
	Total fatty acid (μmol/g liver)					
	3.27 ± 1.00	5.70 ± 0.52$^{a),*}$	3.30 ± 0.11	2.87 ± 0.34$^{b)}$	2.15 ± 0.59	2.14 ± 0.14$^{b)}$

Mice fed a diet containing SO, PO or FO for 4 weeks were intraperitoneally injected with PFOA (10 mg/ kg body weight) daily for 1 week before being killed. Values represent means ± S.D. for 4 animals. a, b, c) Differences between SO-, PO- and FO-fed animals are statistically significant without a common superscript ($p < 0.05$). If no superscript appears, differences between three dietary groups are not statistically significant. *, Differences are statistically significant between PFOA-treated group and untreated group ($p < 0.05$).

tion in each phospholipid.

Effects of PFOA on the Synthesis of Phospholipids

Table 5 shows the incorporation of [^{3}H]glycerol into various glycerolipids in mouse liver. The distribution of the radioactivity among glycerolipids in the liver of PO-fed mice was not different from that in SO-fed mice, whereas the proportions of labeled glycerol incorporated into PtdCho and PtdEtn were higher, and the distribution of radioactivity to TG was lower in FO-fed mice compared with the other two dietary groups. The higher rate of the sysnthesis of PtdEtn relative to TG in FO-fed mice may be explained by the facts that FO feeding increased diacylglycerol (DG) containing 22 : 6(n-3) (Table 6), and that the molecular species of DG containing 22 : 6(n-3) is a preferential substrate for CDP ethanolamine: DG ethanolaminephosphotransferase.[21)]

PFOA treatment markedly decreased the proportion of [^{3}H]glycerol incorporated into TG and increased that into PtdEtn in the three dietary groups.

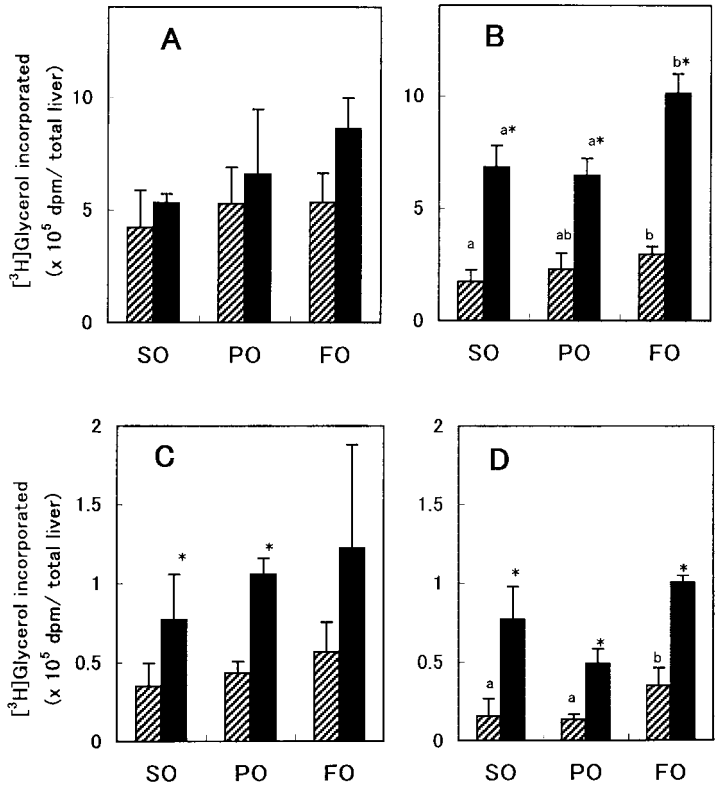

Fig. 1. Effects of PFOA on the Incorporation of [^{3}H]glycerol Among Individual Glycerolipids in the Liver of Mice Fed SO-, PO- or FO-Diet

Mice fed a diet containing SO, PO or FO with (closed bar) or without (hatched bar) PFOA treatment, were intraperitoneally injected with 50 μCi of [^{3}H]glycerol 15 min before being killed. PtdCho (A), PtdEtn (B), PtdIns (C) and phosphatidylserine (PtdSer, D) were separated by TLC and the radioactivity was determined. Values represent mean ± S.D. for 4 animals. Differences between SO-, PO- and FO-fed groups are statistically significant without a common superscript ($p < 0.05$). If no superscript appears, differences between three dietary groups are not statistically significant. *, Differences are statistically significant ($p < 0.05$) between PFOA-treated mice and their respective control.

The incorporation of radioactivity into total lipids did not differ among all experimental groups on the basis of g liver. These results suggest that PFOA lowered TG synthesis and accelerated PtdEtn synthesis. Since PFOA induces marked hepatomegaly, the radioactivity incorporated into PtdEtn was increased 3 times with PFOA treatment (Fig. 1B) on the basis of whole liver. An increase in radioactivity in PtdCho by PFOA, however, was not statistically significant (Fig. 1A). These results were inconsistent with the facts of the concomitant increase in hepatic content of both PtdCho and PtdEtn, as observed in Tables 2 and 3. This discrepancy can be explained by the facts that PFOA treatment inhibited VLDL secretion (Kudo *et al.*, unpublished data) and that hepatic accumulation of PtdCho was accompanied by a decrease in PtdCho in serum in PFOA-treated rats.[8,9] The accumulation of PtdCho, therefore, seems to be due to inhibition of VLDL secretion but not necessarily to an acceleration of synthesis *de novo*. The possibility cannot be ruled out that another synthetic pathway of PtdCho, such as *N*-methylation of PtdEtn, is accelerated by PFOA treatment although the activity was suppressed by PFOA treatment in rat liver.[8] In contrast, the present study demonstrated that PFOA accelerated the synthesis of PtdEtn *de novo*, resulting in marked increase in PtdEtn in mouse liver.

In conclusion, the effects of PFOA on the composition of polyunsaturated fatty acids were different between individual phospholipids and greatly influenced by dietary oil. In FO-fed mice, the synthetic rate of PtdEtn was significantly higher than other dietary groups. This may be due to the high proportion of 22 : 6(n-3) in DG, which is preferentially utilized for PtdEtn synthesis. PFOA markedly increased the hepatic contents of PtdCho and PtdEtn. The increase in the content of PtdCho is thought to be due to the inhibition of secretion but not to the increase in the synthesis *de novo*, whereas an increase in PtdEtn is mainly due to acceleration of synthesis *de novo* in PFOA-treated mice. Fish oil

feeding provided resistance against the PFOA-induced decrease in polyunsaturated fatty acids, which may be useful to protect against PFOA toxicity in the liver of mice.

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