Effects of Perfluorinated Fatty Acids with Different Carbon Chain Length on Fatty Acid Profiles of Hepatic Lipids in Mice

Naomi KUDO,[a] Tohru YAMAZAKI,[a] Takeshi SAKAMOTO,[a] Katsuyoshi SUNAGA,[a] Tadashi TSUDA,[a] Atsushi MITSUMOTO,[b] and Yoichi KAWASHIMA*,[a]

[a] *Faculty of Pharmaceutical Sciences, Josai University; 1–1 Keyakidai, Sakado, Saitama 350–0295, Japan: and* [b] *Faculty of Pharmaceutical Sciences, Josai International University; Gumyo 1, Togane, Chiba 283–8555, Japan.*
Received December 21, 2010; accepted March 9, 2011; published online March 23, 2011

Alterations by perfluorinated fatty acids (PFCAs) with a chain length of 6—9 carbons in the fatty acid profile of hepatic lipids of mice were investigated. The characteristic changes caused by all the PFCAs examined were increases in the contents and proportions of oleic acid (18 : 1), palmitoleic acid (16 : 1) and 8,11,14-eicosatrienoic acid (20 : 3) in hepatic lipids. Hepatic contents of palmitic acid were also increased by the treatments with the PFCAs. These effects were almost dependent on the hepatic concentrations of PFCA molecules regardless of their carbon chain length. Perfluorooctanoic acid elevated the expressions of mRNA encoding acetyl-CoA carboxylase, fatty acid synthase, malic enzyme, stearoyl-CoA desaturase (SCD) (SCD1 and 2), chain elongase (ELOVL5), Δ6 desaturase (Fads2), 1-acylglycerophosphocholine acyltransferase (LPCAT) (LPCAT3). The four PFCAs examined induced microsomal SCD and LPCAT in hepatic concentration-dependent manners regardless of carbon chain length. One linear regression line was confirmed between LPCAT activity and hepatic concentration of PFCA at wide range of the concentration, whereas the induction of SCD was saturable at relatively low concentration of PFCAs. These results suggest (i) that PFCAs with a chain length of 6—9 carbons change the fatty acid profile of hepatic lipids by increasing contents and proportions of 16 : 1, 18 : 1 and 20 : 3, (ii) that these alterations in fatty acid profile are caused by up-regulation of SCD, *de novo* fatty acid synthesis, chain elongase and Δ6 desaturase and (iii) that the mechanism underlying SCD induction is, in part, mediated through peroxisome proliferator-activated receptor α.

Key words perfluorinated fatty acid; carbon chain length; fatty acid profile; liver lipid; stearoyl-CoA desaturase; mouse

A growing body of evidence supporting the key role of fatty acid species in maintaining systemic metabolic homeostasis has been accumulated in recent years. Many studies showed that free fatty acids and a particular molecular species of phosphatidylcholine (PC), palmitoyl-oleoyl-PC, extend molecular effects to cellular homeostasis as lipid signaling molecules through nuclear receptors.[1—3] Palmitic acid (16 : 0) and stearic acid (18 : 0) are known to cause endoplasmic reticulum stress, leading to inductions of insulin resistance, and apoptosis of liver cells and pancreatic β-cells.[4—8] Moreover, oleic acid (18 : 1) in a large amount was shown to induce hepatic endoplasmic reticulum stress, resulting in inhibition of very low-density-lipoprotein secretion.[9] 18 : 1 is a major component of triacylglycerol (TG), the accumulation of which causes fatty liver, obesity and muscle insulin resistance.[10—12] Recently, palmitoleic acid (16 : 1) was demonstrated to strongly stimulate muscle insulin action and suppress hepatosteatosis.[13] Thus, the nature of the fatty acid species is now considered crucial for maintaining homeostasis in organs. Accordingly, it is plausible that xenobiotics affect systemic metabolic homeostasis through changing the fatty acid profile of organs, especially the liver, because the changes that occur in the liver extend to the fatty acid composition of extrahepatic tissues.[14] In this context, it is clearly important to accumulate information with respect to the effects of xenobiotics on the fatty acid profile for hepatic lipids.

Perfluorinated fatty acids (PFCAs) are straight-chain fatty acid analogues of which all aliphatic hydrogens are substituted with fluorine. Because of their surfactant properties and their chemical and thermal stability, PFCAs are primarily used as industrial materials.[15] Since perfluorooctanoic acid (PFOA) accumulates in the environment and in the serum of not only occupationally exposed workers, but also the general population,[16—19] most research has been focused on the study of the toxicological effects of PFOA, so toxicological information pertaining to PFCAs is limited largely to PFOA. However, PFCAs having shorter perfluoroalkyl chains are candidates of substitutes for PFOA, and PFCAs having longer perfluoroalkyl chains exist in the environment as pollutants. Therefore, more information on the toxicological aspects of PFCAs having shorter or longer perfluoroalkyl chains must be accumulated.

The primary target organ of PFCAs is considered to be the liver.[19,20] PFOA was shown to be accumulated in the liver of rats and mice and to cause hepatomegaly,[21] induction of enzymes including peroxisomal acyl-CoA oxidase (AOX) in particular[22—24] and accumulation of TG.[24] Our previous studies showed that, although a striking difference exists in the effects of PFCAs on the liver when they are estimated by the induction of AOX as a parameter, the induction of AOX by PFCAs depends on only the number of PFCA molecules, but not the difference in their perfluoroalkyl chain length with regard to the difference in the extent of the induction.[22—24] These findings suggest that PFCAs with a perfluoroalkyl chain of 6—10 carbons have almost the same potency as a ligand for peroxisome proliferator-activated receptor (PPAR)α in the liver of rats,[22,24] since AOX is considered to be induced by peroxisome proliferators through the activation of PPARα.[25] Our previous study demonstrated that the treatment of male rats with PFOA evidently changed the acyl composition of hepatic phospholipids and that the changes were, in part, elucidated by the induction of stearoyl-CoA desaturase (SCD) by PFOA.[26] To date, however, little informa-

* To whom correspondence should be addressed. e-mail: ykawash@josai.ac.jp

tion is available about the effect of PFCAs other than PFOA on the fatty acid composition of hepatic lipids.

In the present study, we aimed to investigate (i) whether there are perfluoroalkyl chain length-related differences in the response of fatty acid composition to PFCAs with a chain length of 6—9 carbons and (ii) the mechanism underlying the changes in fatty acid profile by the PFCAs.

MATERIALS AND METHODS

Materials Stearoyl-CoA, oleoyl-CoA, bovine serum albumin, perfluorohexanoic acid (PFHeA), perfluoroheptanoic acid (PFHA), and PFOA were purchased from Sigma Aldrich Japan (Tokyo, Japan). Perfluorononanoic acid (PFNA) was from Lancaster Synthesis (Lancashire, U.K.); nicotinamide adenine dinucleotide reduced (NADH) was from Oriental Yeast Co. (Tokyo, Japan); triheptadecanoin, methyl heptadecanoate, and nonadecanoic acid were from Nu-Chek-Prep Inc. (Elysian, MN, U.S.A.); 1-acylglycerophosphocline (LPC) was from Avanti Polar Lipid (Pelham, AL, U.S.A). 3-Bromoacetyl-7-methoxycoumarin was prepared as described previously.[27] All other chemicals used were of analytical grade.

Animals and Treatments Male ddY mice aged 7 weeks were purchased from SLC (Hamamatsu, Japan). After 1 week of acclimatization, the mice were intraperitoneally administered once a day for 5 d with PFCAs at the doses of 150 mg/kg of body weight for PFHeA, of 20 and 50 mg/kg of body weight for PFHA, and 0.5, 1, 2.5, 5 and 10 mg/kg of body weight for PFOA and PFNA. PFCAs were dissolved in propyleneglycol : water (1 : 1, v/v) after neutralization with 1 M NaOH. After mice were killed under diethyl ether anesthesia, livers were excised. One part of the liver was frozen in liquid nitrogen and then stored at −80 °C for determination of mRNA. The other part of the liver was perfused with ice-cold 0.9% NaCl. Liver was homogenized with 4 volumes of 0.25 M sucrose/1 mM ethylenediaminetetraacetic acid (EDTA)/10 mM Tris–HCl (pH 7.4) in a Potter glass-Teflon homogenizer. An aliquot of the homogenates was frozen in liquid nitrogen and stored at −80 °C for determination of PFCAs and for analyses of hepatic lipids. The remaining homogenates were centrifuged at 18000×***g*** for 20 min, and the supernatant was centrifuged under the same conditions. The resulting supernatant was centrifuged at 105000×***g*** for 60 min. The pellet obtained was resuspended in 0.25 M sucrose/0.1 mM EDTA/10 mM Tris–HCl (pH 7.4) and recentrifuged under the same conditions. The resulting pellet was resuspended in a small volume of 0.25 M sucrose/0.1 mM EDTA/10 mM Tris–HCl (pH 7.4) and used as microsomes. All operations were carried out at 0—4 °C. Protein concentrations were determined by the method of Lowry *et al.*[28] using bovine serum albumin as a standard. All animal studies complied with the regulations of the institutional board for animal studies, Josai University.

Enzyme Assays SCD activity was assayed spectrophotometrically by employing microsomes as an enzyme source according to the method of Oshino *et al.*[29] as the stearoyl-CoA-stimulated reoxidation of NADH-reduced cytochrome b_5 with some modifications as previously described.[30] The activity of SCD was represented as the rate constant for stearoyl-CoA-stimulated reoxidation of NADH-reduced cytochrome b_5.[30] Microsomal LPC acyltransferase (LPCAT) was assayed with oleoyl-CoA as substrate by the optical assays that are based on the reaction of liberated coenzyme A with 5,5′-dithiobis(2-nitrobenzoic acid) as described previously.[31]

Reverse Transcription and Real-Time Polymerase Quantitative Chain Reaction Total RNA was extracted from mouse liver using QIAzol reagent and RNeasy kit (QIAGEN, Hilden, Germany). cDNA was synthesized from 500 ng of total RNA with avian myeloblastosis virus reverse transcriptase (Takara, Shiga, Japan). Polymerase chain reaction (PCR) amplification was carried out using QuantiTect SYBR green PCR master mix (QIAGEN, Hilden, Germany). The amplification and detection were performed with an iCycler IQ real-time detection system (Bio-Rad, Hercules, CA, U.S.A.). The sequences of primers used in this study are listed in Table 1. The thermal cycling program was as follows: 15 min denaturaton step at 95 °C followed by 40 cycles of 15 s denaturation at 94 °C, 30 s annealing at 57 °C, and 30 s extension at 72 °C. Changes in gene expression were calculated by using the comparative threshold cycle (C_t) method. C_t values were first normalized by subtracting the C_t value obtained from β-actin (control).

Lipid Analyses After the addition of known amounts of nonadecanoic acid (for the determination of total fatty acids) and triheptadecanoin (for the determination of TG) as internal standards, total lipid was extracted from liver homogenates according to Bligh and Dyer.[32] An aliquot of the lipid extract was taken to dryness, and to the total lipid 1 ml of 10% (w/v) KOH/90% methanol was added. The mixture was heated at 80 °C for 60 min for saponification. After the addition of 2 ml of water, non-saponified materials were removed by the extraction three times with *n*-hexane. The aqueous phase was acidified by the addition of 6 M HCl and free fatty acids were extracted three times with *n*-hexane. Methyl esters of fatty acids were prepared from each extract with boron trifluoride in methanol. Phospholipid (PL) and TG in total lipid were separated by TLC on silica-gel G plates (Merck, Darmstadt, Germany), which were developed with *n*-hexane : diethyl ether : acetic acid (80 : 30 : 1, v/v). After visualizing by spraying 0.001% (w/v) primuline in acetone, individual spots corresponding to PL and TG were scraped off the plates and transferred to tubes. To the tubes containing PL was added a known amount of methyl heptadecanoate as an internal standard. The lipids were extracted from the silica as described previously.[33] Total lipid was extracted from liver microsomal suspension according to Bligh and Dyer.[32] PC, phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS) were separated by TLC on silica-gel G plates as described by Holub and Skeaff,[34] and were extracted from silica by the same method as mentioned above. Methyl esters of fatty acids were prepared from each extract using sodium methoxide in methanol. The amounts and composition of the methyl esters of fatty acids were determined by GLC as described previously.[33]

Determination of PFCAs After adding an internal standard, PFCAs were extracted from liver homogenates as an ion pair with tetrabutylammonium, derivatized with 3-bromoacetyl-7-methoxycoumarin and quantified as described previously.[27] PFHeA was used as an internal standard for the

Table 1. Sequences of Primers Used for Real-Time PCR

	Primer (5′—3′)	Accession No.
SCD1	F: GAATGACGTGTACGAATGGGC	BC007474
	R: GAATTGTGAGGGTCGGCGT	
SCD2	F: CCAGCTGTCAACACTGTTGCA	AB032243
	R: TTGTTAGGCGAAATGCCCAG	
AOX	F: TTCGTGCAGCCAGATTGGTAG	NM_015729
	R: CGGCTTTGTCTTGAATCTTGG	
Fads1	F: TGGATGCTCAGCTTCTATGCC	BC063053
	R: ACAATGAAGAAAAGGCCCAGG	
Fads2	F: CTGGATGGCTGCAACATGACT	BC057189
	R: AGTTGGCTGAGGCACCCTTTA	
ELOVL1	F: GGGTCCCCCTTGCTAATAACA	BC006735
	R: GATTAGCCATGATTCGAGGCC	
ELOVL2	F: ACCACGAGATTCTCGAGTTCG	NM_019423
	R: GCCATATCGAGAGCAGGTACG	
ELOVL3	F: ATGAATTTCTCACGCGGGTT	NM_007703
	R: AGGGCCTTAAGTCCTGAAACG	
ELOVL5	F: TCCATGCGTCCCTACCTCTG	NM_134255
	R: GACCAGCTGCCCTTGAGTGA	
ELOVL6	F: CAGCCCCAATGAACATGTCAG	BC100576
	R: GCGGCTTCCGAAGTTCAAA	
FAS	F: AGATCCTGGAACGAGAACACGA	BC046513
	R: GACATTTCCTGAAGTTTCCGCA	
ACC	F: TTTGGCGCTTACATTGTGGA	BC056500
	R: ACAACCCAAGAACCACCCC	
ME	F: TGCATGGCCAGAGGATGTC	NM_008615
	R: CATTACAGCCAAGGTCTCCCA	
LPCAT1	F: CGTGAATATGTGGTCGCCTTG	NM_145376
	R: ATGCTGGCCATCCTCAGGAGAT	
LPCAT3	F: TTTCTGGTTCCGCTGCATGT	AB294194
	R: CCGACAGAATGCACACTCCTTC	
LPCAT4	F: TTCGGTTTCAGAGGATACGACAA	NM_001083341
	R: AATGTCTGGATTGTCGGACTGAA	
β-Actin	F: CGTGCGTGACATCAAAGAGAA	NM_007393
	R: AGGAAGAGGATGCGGCAGT	

determination of PFHA and PFOA; PFHA was for the determination of PFNA; PFOA was for the determination of PFHeA.

Statistical Analyses Homogeneity of variance was established using one-way analysis of variance. When a difference was significant ($p<0.05$), Dunnet multiple range test was used as a *post-hoc* test. Statistical significance between two means was estimated by either Student's *t*-test or Welch's test after *F*-test. Linear regression analysis was performed to evaluate the correlation between two parameters.

RESULTS

Effects of PFCAs on Fatty Acid Profiles of Hepatic Lipids Table 2 shows the effects of PFHeA, PFHA, PFOA and PFNA on the fatty acid profile of hepatic lipids. Upon the treatment of mice with PFOA, the proportions of 16 : 1, 18 : 1 and 8,11,14-eicosatrienoic acid (20 : 3) of total lipid were increased in dose-dependent manners. These elevations were compensated with the reductions in the proportions of 18 : 0, linoleic acid (18 : 2), arachidonic acid (20 : 4) and docosahexaenoic acid (22 : 6). The changes that were caused by the treatment of mice with PFNA were almost the same as those observed with the mice treated with PFOA. The administration of PFHA at doses of 20 and 50 mg/kg of body weight caused alterations similar to those produced by PFOA with respect to the contents and proportions of fatty acids, although the extent of the changes was not as evident as those induced by PFOA. The treatment of mice with PFHeA at a dose of 150 mg/kg of body weight caused a modest, but significant, increase in content and proportion of 18 : 1. The treatment of mice with PFOA markedly increased the hepatic content of TG in a dose-dependent manner; the proportions of 16 : 1 and 18 : 1 were elevated, and conversely that of 18 : 2 was decreased in TG. The changes caused by PFNA in acyl composition of TG were similar to those observed with PFOA, but PFNA increased the hepatic content of TG to a lesser extent. The proportions of 16 : 1 and 18 : 1 were increased and that of 18 : 2 was decreased by the treatment with PFHA and PFHeA, although the extents were not as prominent as those observed with PFOA and PFNA. The administration of PFOA increased the proportions of 16 : 1, 18 : 1 and 20 : 3 and decreased the proportions of 18 : 0, 18 : 2 and 20 : 4 in PL. The changes caused by PFNA were similar to those observed with PFOA, but were slightly smaller. The administration of PFHA and PFHeA slightly increased the proportions of 16 : 1 and 18 : 1, and did not substantially change the proportions of the other fatty acids in PL.

Relationship between Fatty Acid Profiles and PFCA Concentration in the Liver The hepatic concentrations of PFCAs with different perfluoroalkyl chain lengths were determined (Fig. 1). Dose-dependent accumulation was observed with PFOA and PFNA in the liver. The hepatic concentrations of PFHA were very low, compared with those of PFOA and PFNA. The concentration of PFHeA in the liver was lower than the detection limit (<3 nmol/g liver).

The relationships between fatty acid contents of hepatic lipids and hepatic concentrations of PFCA in terms of nmol/g liver are shown in Fig. 2. In total lipid, the contents of 16 : 0, 16 : 1, 18 : 1 and 20 : 3 were strikingly elevated with the increase in hepatic concentration of PFCAs regardless of their carbon chain length at the concentrations below 200 nmol/g liver. To examine the relationship between the contents of the fatty acids and hepatic concentrations of PFCAs, linear regression analyses were carried out for the data obtained from the PFCA concentrations below 200 nmol/g liver. The correlations between the contents of 16 : 0, 16 : 1, 18 : 1 and 20 : 3 in total lipid and the PFCA concentrations were significant, with $r=0.890$ ($p<0.001$) for 16 : 0, $r=0.948$ ($p<0.001$) for 16 : 1, $r=0.904$ ($p<0.001$) for 18 : 1 and $r=0.851$ ($p<0.01$) for 20 : 3 (solid lines in Fig. 2), respectively. Likewise, the contents of 16 : 0, 16 : 1 and18 : 1 in TG and 20 : 3 in PL prominently increased with the elevation in hepatic concentration of PFCAs regardless of their carbon chain length at the concentrations below 200 nmol/g liver. The correlations between the contents of 16 : 0, 16 : 1 in TG, 18 : 1 in TG and 20 : 3 in PL and the PFCA concentrations were significant, with $r=0.825$ ($p<0.01$) for 16 : 0 in TG, $r=0.894$ ($p<0.001$) for 16 : 1 in TG, $r=0.866$ ($p<0.001$) for 18 : 1 in TG and $r=0.820$ ($p<0.01$) for 20 : 3 in PL (solid lines in Fig. 2).

The effects of PFCAs on fatty acid profiles of PC, PE, PI and PS in hepatic microsomes were examined. Figure 3 shows the relationships between fatty acid proportions in the four microsomal phospholipids and the concentrations of PFCAs in terms of nmol/g liver. With the increase in hepatic concentrations of PFCAs regardless of their carbon chain length, the proportion of 16 : 1 was increased in PC; the pro-

Table 2. Effects of the Treatment of Mice with PFHeA, PFHA, PFOA and PFNA on Fatty Acid Profile of Hepatic Lipids

Treatment (mg/kg)		16 : 0	16 : 1	18 : 0	18 : 1	18 : 2	20 : 3	20 : 4	20 : 5	22 : 6	Total (μmol/g liver)
		(mol%)									
Total lipid											
Control		33.6±2.3	2.5±0.2	14.0±0.4	14.7±0.4	20.1±0.4	1.1±0.2	7.8±0.9	0.7±0.1	5.2±1.1	67.3±6.4
PFHeA	150	30.4±1.2	3.0±0.3	12.9±0.9	16.5±0.8*	19.0±0.4	1.8±0.2	8.5±0.7	0.8±0.1	6.5±0.5	63.9±2.8
PFHA	20	32.3±1.7	4.4±0.6*	10.1±0.7*	23.0±0.9*	15.7±1.1*	2.0±0.1*	6.6±0.4*	0.8±0.1	4.5±0.8*	85.9±4.2*
	50	30.1±1.8	2.9±0.2	10.7±1.2*	22.2±0.8*	18.6±0.5	2.7±0.3*	7.2±0.6	0.6±0.1	4.3±0.3*	88.4±5.9*
PFOA	0.5	31.2±1.1	4.2±0.8	10.5±1.4*	22.2±2.8*	16.0±1.6*	1.6±0.1	6.5±0.8	1.2±0.2*	5.4±0.2	97.7±13.1*
	1	30.8±1.7	5.5±0.5*	7.5±0.6*	27.1±0.4*	14.5±0.3*	2.0±0.2*	5.9±0.7	0.9±0.1	4.6±0.9	101.8±5.6*
	2.5	32.2±1.9	6.6±0.5*	6.6±0.3*	28.4±1.8*	13.3±1.8*	2.2±0.3*	5.1±0.7*	0.9±0.1	3.8±0.8	121.5±8.5*
	5	32.7±1.2	6.4±1.1*	6.5±1.2*	29.6±3.6*	12.8±3.0*	2.4±0.3*	4.6±0.8*	0.9±0.1	3.3±0.4*	110.8±14.1*
	10	31.4±1.8	5.1±0.7*	6.5±0.7*	25.2±1.7*	18.3±0.5	2.7±0.5*	4.8±0.9*	0.7±0.3	4.3±0.9	100.3±9.5*
PFNA	0.5	30.7±1.1	3.0±0.2	11.8±0.8*	16.7±0.6	19.5±0.1	1.8±0.1	7.8±0.2	1.1±0.1*	7.0±0.7*	82.4±2.9
	1	31.7±1.1	5.4±0.2*	8.3±0.4*	23.9±1.4*	15.8±1.2*	1.8±0.2	5.9±0.2*	1.0±0.2	5.7±0.6	115.9±15.2*
	2.5	31.2±0.6	6.4±0.3*	6.4±0.5*	25.7±1.1*	16.1±0.7*	2.6±0.2*	5.4±0.3*	1.2±0.2*	4.6±0.2	112.0±4.6*
	5	31.7±1.3	4.9±0.3*	6.5±0.3*	26.1±1.5*	17.0±0.6*	3.0±0.4*	5.4±0.4*	0.7±0.1	4.2±0.1	114.9±2.0*
	10	30.0±1.2*	5.8±0.7*	5.9±0.3*	26.1±1.5*	18.8±1.4	2.5±0.6*	4.5±0.9*	1.0±0.2	4.8±0.5	105.6±4.3*
TG											
Control		29.6±0.4	4.6±0.2	1.7±0.1	34.0±0.3	24.9±1.1	0.4±0.0	0.9±0.0	0.6±0.1	2.9±0.1	17.2±3.0
PFHeA	150	25.8±0.8*	5.7±0.2*	1.7±0.2	39.8±1.2*	22.0±1.0*	0.6±0.0	1.1±0.2	0.6±0.0	2.3±0.4*	11.0±0.9*
PFHA	20	28.5±0.9	7.4±0.5*	1.3±0.2	46.7±1.2*	13.1±0.8*	0.4±0.2	0.6±0.2*	0.4±0.1*	1.1±0.1*	26.6±1.1*
	50	25.0±0.4*	7.6±0.3*	1.2±0.3	51.4±0.7*	12.2±0.1*	0.5±0.2	0.6±0.3*	0.3±0.1*	1.0±0.1*	25.3±2.5*
PFOA	0.5	30.9±0.9	7.8±1.0*	1.2±0.1*	42.1±1.9*	14.8±2.4*	0.3±0.0	0.4±0.1*	0.5±0.1	1.6±0.3*	34.3±10.7*
	1	28.9±0.7	9.4±0.6*	0.8±0.1*	45.7±0.6*	13.3±0.7*	0.3±0.1	0.3±0.1*	0.3±0.1*	0.7±0.1*	42.6±2.4*
	2.5	29.6±1.2	11.2±1.2*	0.7±0.1*	46.5±1.7*	10.7±2.3*	0.2±0.1	0.2±0.1*	0.2±0.0*	0.4±0.1*	50.4±6.7*
	5	29.9±0.7	10.6±0.7*	0.8±0.1*	48.6±0.7*	9.1±1.1*	0.2±0.1	0.2±0.1*	0.2±0.1*	0.4±0.1*	44.9±4.7*
	10	24.9±1.1*	8.0±0.6*	0.9±0.2*	43.8±1.0*	20.1±0.5	0.5±0.1*	0.5±0.2*	0.2±0.1*	0.7±0.3*	43.1±4.7*
PFNA	0.5	29.8±0.2	6.7±0.7*	1.1±0.0*	36.7±1.7	21.3±1.9	0.4±0.1	0.6±0.1*	0.7±0.1	2.2±0.2*	25.2±4.1*
	1	28.9±0.4	8.1±1.4*	0.8±0.0*	39.1±0.8*	20.4±0.8*	0.3±0.0	0.4±0.0*	0.4±0.0*	1.1±0.1*	46.3±6.3*
	2.5	27.4±1.4*	9.1±0.8*	0.8±0.0*	45.0±0.3*	16.0±1.4*	0.4±0.1	0.3±0.1*	0.3±0.0*	0.6±0.1*	39.0±5.2*
	5	28.0±0.4*	8.6±0.6*	0.7±0.0*	46.0±1.3*	15.0±1.6*	0.4±0.0	0.3±0.0*	0.2±0.0*	0.6±0.1*	38.9±4.7*
	10	24.5±0.5*	7.8±0.5*	0.9±0.1*	43.6±1.4*	20.8±1.2*	0.6±0.0*	0.5±0.0*	0.3±0.0*	0.8±0.0*	30.8±5.5*
PL											
Control		26.5±1.7	0.9±0.1	19.2±0.4	8.1±0.8	18.5±0.5	1.8±0.2	13.0±0.5	0.9±0.2	10.6±1.9	48.1±4.4
PFHeA	150	30.9±4.3	1.9±0.5*	15.3±1.0*	11.4±0.3*	17.2±0.7	2.2±0.3	11.3±1.5	0.9±0.2	8.1±1.6	56.5±3.8*
PFHA	20	29.4±1.2	1.7±0.2*	15.6±0.4*	11.4±0.4*	17.3±0.6	2.2±0.3	12.1±0.3	0.9±0.1	8.8±0.3	55.2±2.5*
	50	29.3±0.4	2.0±0.0*	13.2±0.7*	11.4±0.4*	17.6±0.5	3.6±0.3*	11.9±0.3	0.8±0.0	9.3±0.1	65.8±1.2*
PFOA	0.5	29.1±2.3	1.5±0.3*	15.0±0.6*	11.0±0.9*	15.9±0.8*	2.5±0.1	10.8±1.1*	1.8±0.3*	11.4±1.1	65.3±6.3*
	1	29.7±0.5	2.0±0.2*	12.2±0.6*	13.3±0.7*	14.8±0.6*	3.5±0.3*	11.0±0.3*	1.5±0.1*	10.7±0.4	62.0±1.7*
	2.5	30.5±0.8	2.6±0.2*	11.1±0.8*	15.1±1.2*	14.5±1.5*	3.9±0.2*	9.6±0.7*	1.5±0.1*	9.7±0.3	70.1±5.4*
	5	34.7±2.0*	3.4±0.2*	9.5±0.6*	17.0±0.9*	14.8±0.6*	3.6±0.3*	8.0±0.6*	1.4±0.2	6.6±0.5*	59.1±3.3*
	10	33.6±1.7*	3.0±0.3*	9.6±0.9*	16.7±1.0*	16.5±1.4	4.3±0.5*	7.4±0.3*	0.9±0.2	7.1±0.8*	61.2±0.9*
PFNA	0.5	27.6±2.3	1.3±0.1	15.7±0.8*	9.4±0.1	16.9±0.2	2.5±0.2	12.3±0.7	1.5±0.1	12.0±1.9	58.8±5.6*
	1	30.7±1.0	1.4±0.3	14.9±1.1*	10.1±1.3*	16.4±1.2	3.0±0.2*	11.0±0.8	1.2±0.4	10.4±1.1	63.0±3.9*
	2.5	34.0±2.9*	2.2±0.3*	11.2±0.8*	13.7±1.3*	15.3±0.5*	4.3±0.5*	8.9±0.8*	1.2±0.3	8.2±1.7	56.6±0.3*
	5	32.1±1.6*	1.8±0.3	11.2±0.7*	14.6±0.8*	16.1±1.0*	4.7±0.4*	9.3±1.0*	0.9±0.1	8.2±0.7	59.8±3.7*
	10	32.9±2.1*	2.7±0.8*	9.7±1.0*	17.0±1.1*	18.7±1.0*	3.5±0.7*	6.6±0.9*	1.2±0.1	7.0±1.0*	61.9±4.4*

Mice were intraperitoneally injected PFCAs once a day for 5 d. Each value represents the mean±S.D. for four mice. * Statistically different from the control at $p<0.05$. The fatty acids are designated by the number of carbon atoms and double bonds: palmitic acid, 16 : 0; palmitoleic acid, 16 : 1; stearic acid, 18 : 0; oleic acid, 18 : 1; linoleic acid, 18 : 2; 8,11,14-eicosatrienoic acid, 20 : 3; arachidonic acid, 20 : 4; 5,8,11,14,17-eicosapentaenoic acid, 20 : 5; 4,7,10,13,16,19-docosahexaenoic acid, 22 : 6.

portion of 18 : 1 was elevated in PC and PI. The proportion of 20 : 3 was increased in the four phospholipids, and the extent of the elevation was the most striking in PI. The changes in the proportions of 16 : 1, 18 : 1 and 20 : 3 evidently occurred in PFCA concentration-dependent manners at the concentrations below 200 nmol/g liver. The proportions of 20 : 4 were decreased in the four phospholipids. The proportions of 22 : 6 were suppressed in PC and PE. The proportions of 18 : 2 were decreased in PC and were increased in PE.

Effects of PFOA on the Levels of mRNA Encoding Enzymes Involved in Fatty Acid Synthesis and LPCAT As shown in Fig. 4, the levels of mRNA encoding acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS) and malic enzyme (ME) were significantly increased by the treatments of mice with PFOA at the doses of 5 mg/kg of body weight. The mRNA levels of SCD1, SCD2 and ELOVL5 were also elevated by the treatments. The administrations of PFOA significantly increased the mRNA level of Fads2, whereas the treatments did not change that of Fads1. The treatments of mice with PFOA decreased the mRNA level of ELOVL3, but did not affected those of either ELOVL1, ELOVL2 or ELOVL6. The effects of PFOA on the levels of mRNAs encoding LPCAT and AOX were also examined. mRNA levels of LPCAT 3 and AOX were significantly increased in the liver by the treatments of mice with PFOA, whereas those of both LPCAT 1 and LPCAT 4 were not affected.

Effects of PFCAs on Induction of SCD The effects of

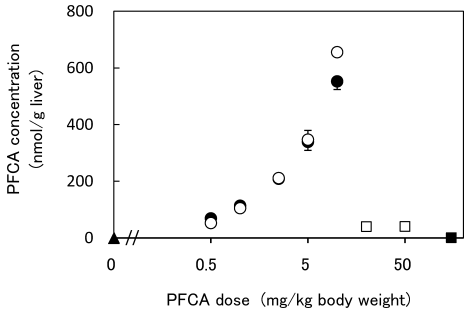

Fig. 1. Concentrations of PFCAs in the Liver of Mice after Repeated Injections

Mice were intraperitoneally injected with PFHeA, PFHA, PFOA or PFNA once a day for 5 d. The doses of the treatments were the same as indicated in Table 2. ▲, control; ■, PFHeA; □, PFHA; ●, PFOA; ○, PFNA. Values are mean±S.D. for four mice.

PFCAs on microsomal SCD were examined (Fig. 5). The SCD was induced by the treatments of mice with PFOA and PFNA in dose-dependent manners (Fig. 5A). The highest activity was observed with the mice treated with PFOA at a dose of 5 mg/kg of body weight and was 3.3 times that of the control. When comparing the doses of 1 and 2.5 mg/kg of body weight, PFOA induced SCD more effectively than did PFNA. PFHA and PFHeA also induced SCD, but to a lesser extent than PFOA and PFNA. The potencies of PFHeA at the dose of 150 mg/kg of body weight and PFHA at the dose of 20 mg/kg of body weight were approximately comparable to those of PFOA at the doses of 0.75 and 0.85 mg/kg of body weight, respectively. To estimate the contribution of SCD to the formation of monounsaturated fatty acids in the liver, linear regression analysis was performed between the activity of SCD and the content of 16 : 1+18 : 1 in total lipid, TG and PL. There were significant correlations between the two parameters (Fig. 6), with $r=0.771$ ($p<0.05$) for total lipid, $r=0.748$ ($p<0.01$) for TG, and $r=0.853$ ($p<0.001$) for PL.

Figure 5B shows the relationship between the hepatic concentration of PFCAs and the activity of SCD. At the PFCA concentrations below approximately 350 nmol/g liver, PFCAs induced SCD almost in a dose-dependent manner. When comparing the potency to induce SCD between PFOA and PFNA at the concentrations of 100—350 nmol/g liver, PFOA was found to be more effective than PFNA. At the concentrations over 400 nmol/g liver, the induction of SCD by PFOA and PFNA was saturable. When all the data in Fig. 5B were employed for the estimation of the correlation between hepatic concentrations of PFCAs and SCD activities, no linear regression line was found. Moreover, there was no significant correlation between the two parameters, even when comparing them at the concentrations of PFCAs below 200 nmol/g liver, with $r=0.626$ ($p>0.05$, solid line in Fig. 5B). When the data of mice treated with PFHeA and PFHA were excluded from the analysis, however, the correlation became significant, with $r=0.825$ ($p<0.01$, dotted line in Fig. 5B).

Effects of PFCAs on the Induction of LPCAT Microsomal LPCAT was induced by the treatments of mice with PFHeA, PFHA, PFOA and PFNA in dose-dependent manners, as was observed with SCD (Fig. 7A). The activity of LPCAT was elevated with the increase in hepatic concentration of PFCAs regardless of their carbon chain length at the concentrations below approximately 550 nmol/g liver (Fig. 7B). To examine the relationship between the activities of LPCAT and hepatic concentrations of PFCA, linear regression analyses were carried out for the data obtained from PFCA concentrations below 550 nmol/g liver. The correlation between the two parameters was significant, with $r=0.899$ ($p<0.001$, solid line in Fig. 7B). When the data of mice treated with PFHeA and PFHA were excluded from the analyses, the correlations become more highly significant, with $r=0.958$ ($p<0.001$, dotted line in Fig. 7B).

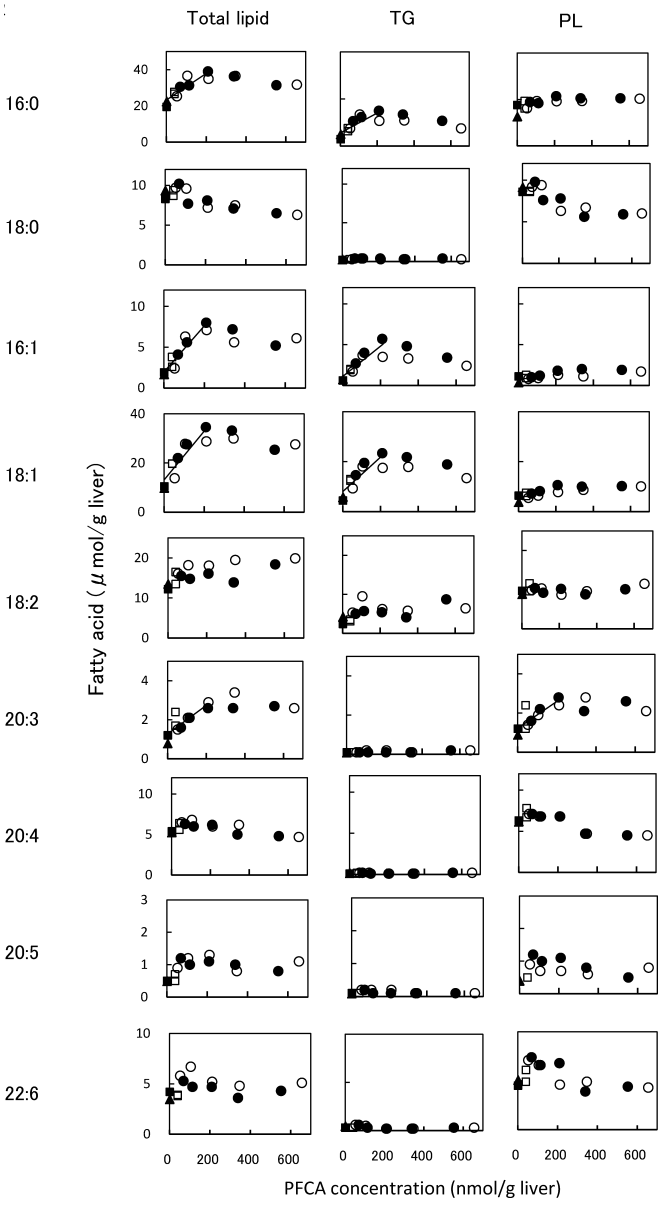

Fig. 2. Relationship between the Concentration of PFCAs and the Contents of Fatty Acids in Total Lipid, TG and PL in the Liver of Mice

▲, control; ■, PFHeA; □, PFHA; ●, PFOA; ○, PFNA. The relationships between the concentration of PFCAs (data from Fig. 1) and the content of fatty acid (data from Table 2) were determined as $Y=0.073X+23.22$ ($r=0.890$, $p<0.001$) for 16 : 0 in total lipid; $Y=0.028X+1.965$ ($r=0.948$, $p<0.001$) for 16 : 1 in total lipid; $Y=0.099X+13.11$ ($r=0.904$, $p<0.001$) for 18 : 1 in total lipid; $Y=0.007X+1.276$ ($r=0.851$, $p<0.01$) for 20 : 3 in total lipid; $Y=0.042X+5.596$ ($r=0.825$, $p<0.01$) for 16 : 0 in TG; $Y=0.018X+1.096$ ($r=0.894$, $p<0.001$) for 16 : 1 in TG; $Y=0.069X+7.992$ ($r=0.866$, $p<0.001$) for 18 : 1 in TG; and $Y=0.006X+1.224$ ($r=0.820$, $p<0.01$) for 20 : 3 in PL. The regression lines are shown as the solid lines.

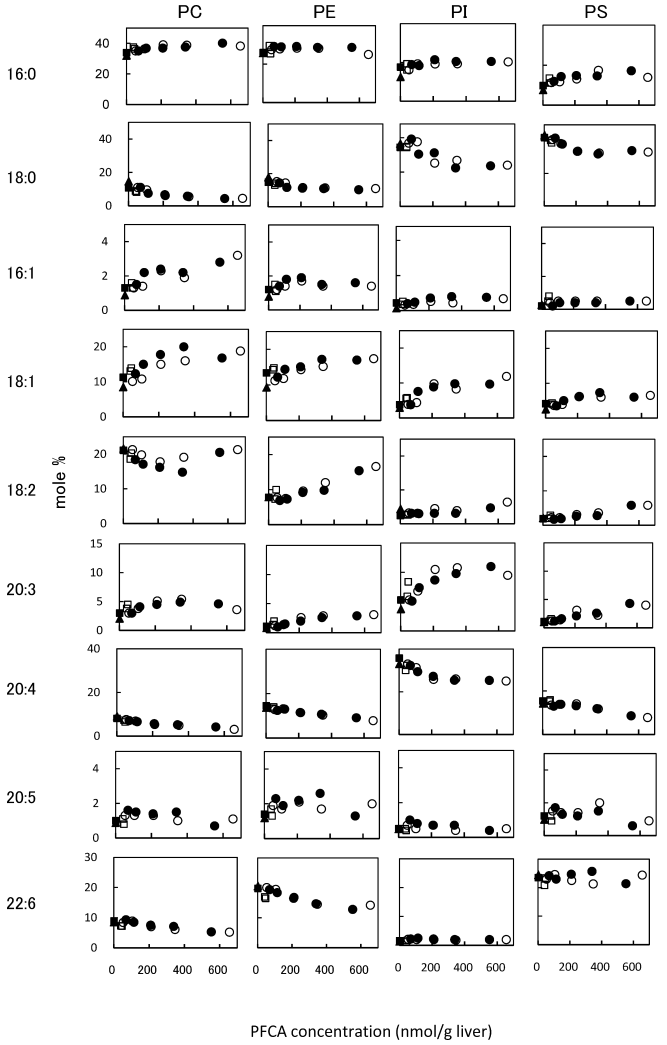

Fig. 3. Relationship between the Concentration of PFCAs and the Proportions of Fatty Acids in Microsomal PLs in the Liver of Mice

The sets of data were derived from the data of Fig. 1 (the concentration of PFCAs) and those of Table 1(the content of fatty acid). ▲, control; ■, PFHeA; □, PFHA; ●, PFOA; ○, PFNA.

DISCUSSION

PFCA-Mediated Alterations in Fatty Acid Profile in Hepatic Lipids The present study showed that PFCAs with a chain length of 6—9 carbons markedly changed the fatty acid profile of hepatic lipids. The most characteristic alterations caused by PFCAs in fatty acid profile were an increase in the total content of fatty acids, originating from the increases in the contents and proportions of 16 : 1 and 18 : 1, and the content of 16 : 0 in TG. It should be noted here that clofibrate, a typical peroxisome proliferator, decreases the hepatic content of TG,[35] in contrast to PFCAs. The content and proportion of 20 : 3 were considerably increased by PFCAs in hepatic PL, to a lesser extent than those of 16 : 1 and 18 : 1 in TG. The changes in proportions of 20 : 3 were well reflected in the elevated proportions of 20 : 3 in microsomal PC and PI. Importantly, the changes in the contents and proportions of these fatty acids were elucidated as a rough estimates by hepatic concentration of PFCA regardless of their carbon chain length, implying that the alterations observed in hepatic lipids depend on simply the number of

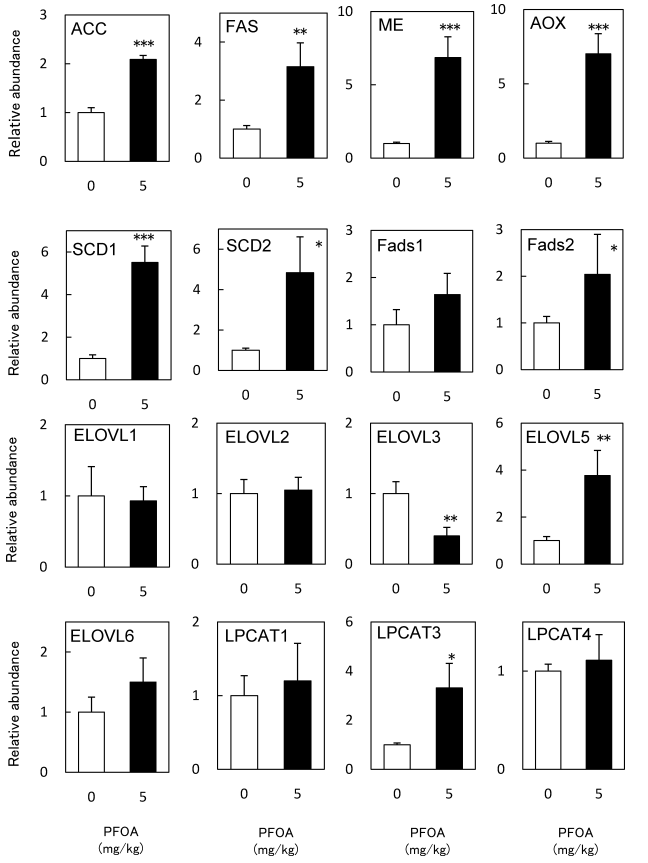

Fig. 4. Effects of PFOA on the Levels of mRNA Encoding Enzymes Involved in Fatty Acid Synthesis and LPCAT

Mice were intraperitoneally injected with PFOA at a dose of 5 mg/kg of body weight once a day for 5 d. Values are mean±S.D. for four mice. Significantly different from the control at $*p<0.05$; $**p<0.01$; $***p<0.001$.

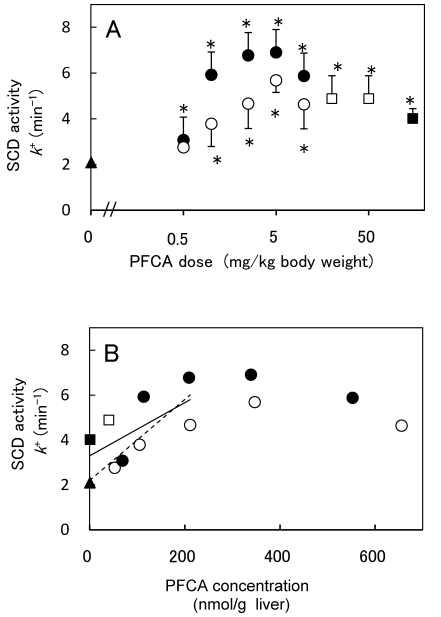

Fig. 5. Induction of SCD by PFCAs in the Liver

Mice were intraperitoneally injected with PFHeA, PFHA, PFOA or PFNA once a day for 5 d. ▲, control; ■, PFHeA; □, PFHA; ●, PFOA; ○, PFNA. Values are mean±S.D. for four mice. * Significantly different from the control at $p<0.05$. (A) The dose-dependent induction of SCD by PFCAs. (B) The relationships between the concentration of PFCAs and the activity of SCD were determined as $Y=0.011X+3.294$ (solid line; $r=0.626$, $p>0.05$) when all the data below 200 nmol/g liver were employed for the determination; and $Y=0.018X+2.202$ (dotted line; $r=0.825$, $p<0.01$) when the data from PFHeA and PFHA were excluded from the regression analysis.

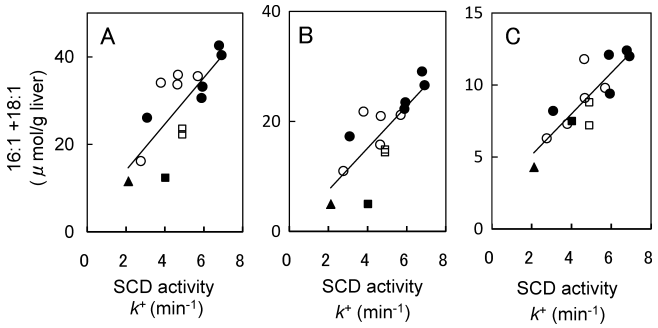

Fig. 6. Relationships between the Activity of SCD and the Contents of Monounsaturated Fatty Acids (16 : 1+18 : 1) in Total Lipid, TG and PL in the Liver of Mice

▲, Control; ■, PFHeA; □, PFHA; ●, PFOA, ○, PFNA. The relationships between the contents of 16 : 1+18 : 1 (data from Fig. 2) and the activity of SCD (data from Fig. 5A) were determined as $Y=5.286X+3.515$ ($r=0.771$, $p<0.05$) for total lipid (A); $Y=3.819X-0.118$ ($r=0.784$, $p<0.01$) for TG (B); and $Y=1.428X+2.275$ ($r=0.853$, $p<0.001$) for PL (C).

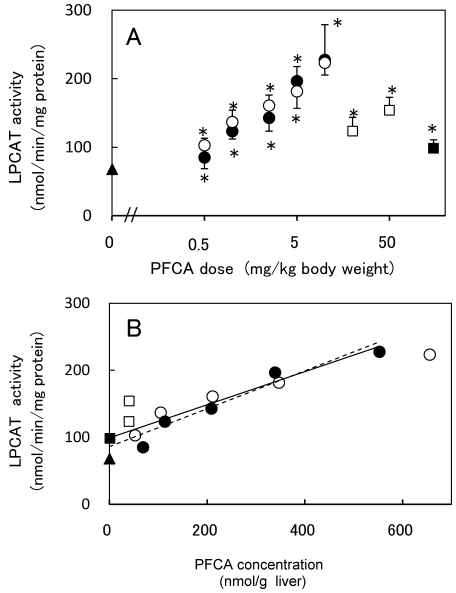

Fig. 7. Induction of LPCAT by PFCAs in the Liver

Mice were intraperitoneally injected with PFHeA, PFHA, PFOA or PFNA once a day for 5 d. ▲, Control; ■, PFHeA; □, PFHA; ●, PFOA; ○, PFNA. Values are mean±S.D. for four mice. * Significantly different from the control at $p<0.05$. (A) The dose-dependent induction of LPCAT by PFCAs. (B) The relationships between the concentration of PFCAs and the activity of LPCAT were determined as $Y=0.247X+99.00$ (solid line; $r=0.899$, $p<0.001$) when all the data below 550 nmol/g liver were employed for the determination; and $Y=0.283X+85.95$ (dotted line; $r=0.958$, $p<0.001$) when the data from PFHeA and PFHA were excluded from the regression analysis.

PFCA molecules in a hepatocyte. Accordingly, we estimated the effects of PFCA on the expression of enzymes that participated in fatty acid metabolism by employing PFOA.

The present results pertaining to mass analyses of fatty acids strongly suggest the increased supply of 16 : 0, 16 : 1, 18 : 1 and 20 : 3 by PFCAs. Of these fatty acids, 16 : 1 and 18 : 1 are synthesized from 16 : 0 that is synthesized *de novo* or from dietary 16 : 0 or 18 : 0. The present study showed that PFOA increased the levels of mRNAs encoding FAS, ACC and ME that are the enzymes involved in *de novo* synthesis of 16 : 0, SCD1 encoding SCD.[36] These results strongly suggest that PFCAs up-regulate the formation of 16 : 1 and 18 : 1 in the liver. These monounsaturated fatty acids are considered to be preferentially incorporated into TG, which is synthesized from glycerol-3-phosphate *via* acylglycerophosphate, phosphatidic acid and diacylglycerol. This biosynthetic process is mediated by glycerol-3-phosphate acyltransferase, acylglycerophosphate acyltransferase, phosphatidate phosphatase, and diacylglycerol acyltransferase; these three acyltransferases possess a broad acyl-donor specificity utilizing fatty acyl-CoAs that have a chain length of 16 or 18 carbons and 0—3 double bonds.[37—40] Accordingly, under the conditions that the supply of 18 : 1 and 16 : 1 are elevated, these monounsaturated fatty acids are considered to be incorporated into TG by the action of these acyltransferases. In this context, the SCD that is induced by PFCAs may play a central role in the changes in fatty acid profile in hepatic lipids.

On the other hand, 20 : 3 is one of the intermediates of 20 : 4 formation; 20 : 4 is considered to be synthesized from 18 : 2 *via* the pathway of 18 : 2→6,9,12-octadecatrienoic acid (18 : 3)→20 : 3→20 : 4. The process of 18 : 2→18 : 3, which is catalyzed by Δ6 desaturase, is the rate-limiting step of 20 : 4 synthesis in the liver of physiologically normal states.[41] The present study showed that PFOA elevated the expressions of Fads2 encoding Δ6 desaturase[42] and of ELOVL5 encoding chain elongase responsible for the conversion of 18 : 3 to 20 : 3,[43] but not of Fads1 encoding Δ5 desaturase that catalyzes the final step of 20 : 4 formation.[42] It seems likely, therefore, that the rate-limiting step of 20 : 4 formation is changed from Δ6 desaturation (18 : 2→18 : 3) to Δ5 desaturation (20 : 3→20 : 4) as a result of the up-regulations mediated by PFCAs of both Fads2 (Δ6 desaturase for 18 : 3→20 : 3) and ELOVL5 (the chain elongase for 18 : 3→20 : 3), because the difference between the activities of Δ6 desaturation and Δ5 desaturation is not significantly large in the liver.[41] Thus, the supply of 20 : 3 seems to be increased by PFCAs. PLs (PC, PE, PI and PS) are synthesized from the pre-existing phosphatidic acid. The initial biosynthesis of certain PL molecular species is followed by the programmed redistribution of acyl chains into different combinations; the processes are mediated by fatty acid remodeling system that consists of phospholipases A_2 and A_1, and acyl-CoA : lysophospholipid acyltransferases. In general, 1-acyl-2-lysophospholipid acyltransferases preferentially transfer polyunsaturated fatty acids.[44—48] Since, in particular, 1-acylglycerophosphoinositol acyltransferase prefers 20 : 4-CoA as an acyl-donor,[47] it seems likely that 20 : 3 is preferentially incorporated into PI under the conditions that the supply of 20 : 3 is increased by PFCAs, as the present study.

Mechanism Underlying the Increase in Monounsaturated Fatty Acids The present study clearly showed that all the PFCAs with a chain length of 6—9 carbons induced SCD and that there were significant correlations between SCD activity and the content of 16 : 1+18 : 1 in hepatic lipids. Moreover, at a rough estimate, the increase in the activity of SCD depended on the hepatic concentration of PFCAs regardless of their carbon chain length. These results suggest that the induction of SCD by PFCAs is one of the crucial factors by which the PFCAs change the fatty acid composition of hepatic lipids.

To gain insight into the mechanism underlying SCD induction by PFCA molecules, the present study focused on the relationship between PFCAs and inductions of enzymes, AOX and LPCAT, through the activation of PPARa, because

SCD1, which is one of the genes encoding SCD in the liver, is known to contain a peroxisome proliferator responsive element in its regulatory region.[36] In previous studies, we obtained evidence suggesting that the molecular potentials of the PFCAs to induce AOX are essentially the same, but not dependent on their carbon chain length, and that the difference in the potency *in vivo* to induce AOX among the PFCAs with different carbon chain lengths is due mainly to differences in the toxicokinetics of the PFCAs.[22—24,49] Moreover, PPARα was shown to recognize a peroxisome proliferator responsive element located upstream of AOX gene,[50] and PFOA has been demonstrated to activate PPARα.[51,52] Previous studies demonstrated that microsomal LPCAT was induced by the treatment of rats with peroxisome proliferators having diverse chemical structures[31] and that the induction of LPCAT by PFCAs significantly correlated with the induction of AOX.[22] Subsequently, our study revealed that the gene responsible for hepatic LPCAT under the control of PPARα is LPCAT3,[53] one of several isoforms of LPCAT.[54] Moreover, LPCAT activity is known to be hardly affected by neither nutritional nor hormonal states.[55,56] In the present study, one linear regression line was confirmed between the activities of microsomal LPCAT and hepatic concentrations of PFCAs at a concentration up to 550 nmol/g liver, as was previously observed with AOX.[22,23] These findings suggest that LPCAT is induced through a PPARα-mediated mechanism as well as AOX. It is noteworthy, therefore, that microsomal LPCAT can be considered to be a microsomal parameter to estimate the response of the liver to challenge with peroxisome proliferators.

The present study showed that the treatment of mice with PFOA elevated the mRNA level of SCD1 in the liver, suggesting strongly that PFOA induces SCD through a PPARα-mediated mechanism. These findings, taken together, led us to the idea that PFCAs other than PFOA are also ligands for PPARα and induce SCD through activating PPARα, whereas little information is available about how potent the PFCAs are as ligands for PPARα. In the present study, however, a significant correlation was found between the activity of SCD and the hepatic concentrations of PFCAs with a chain length of 8 and 9 carbons at the limited range of concentration below 200 nmol/g liver, which differed from the cases of PFCA and LPCAT as well as AOX for which a significant correlation was observed at a wide range of PFCA concentrations up to approximately 500 nmol/g liver.[22] Although the precise mechanism by which PFCAs with a chain length of 6—9 carbons induce SCD remains to be resolved, three possibilities are conceivable with regard to the difference in response to PFCAs between SCD and LPCAT as follows. (i) PFCAs, in particular PFNA and PFOA at higher concentrations, disturbed the membrane properties of the endoplasmic reticulum; as a result, SCD but not LPCAT was suppressed because stearoyl-CoA desaturation system, which consists of NADH-cytochrome b_5 reductase, cytochrome b_5 and SCD,[29] is structurally complex. In this case, SCD activity may become lower than that expected at higher concentrations of PFCAs. (ii) The induction of SCD by PFCAs can be elucidated, in part, by a PPARα-mediated mechanism. With regard to the regulation of SCD, more than one proposal including PPARα-mediated regulation has been put forward.[43,57,58] It is interesting, therefore, to speculate that the induction of SCD by PFCAs is mediated through not only PPARα activation, but also other regulatory factors such as sterol regulatory element-binding protein. (iii) Molecular potentials of individual PFCAs to induce SCD are somewhat different, and those to induce LPCAT are almost the same.

In summary, from a toxicological point of view, it is important to know whether PFCAs having different carbon chain lengths differently affect the fatty acid profile in hepatic lipids. The present results led us to conclude that the difference in the potency *in vivo* to cause changes in the fatty acid profile among PFCAs with a chain length of 6—9 carbons is due essentially to the differences in the accumulation in the liver and, in part, to the molecular potential of individual PFCA to alter the activity of fatty acid metabolizing enzymes, especially SCD.

Acknowledgments This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES

1) Jump D. B., *Curr. Opin. Lipidol.*, **19**, 242—247 (2008).
2) Bordoni A., Di Nunzio M., Danesi F., Biagi P. L., *Genes Nutr.*, **1**, 95—106 (2006).
3) Chakravarthy M. V., Lodhi I. J., Yin L., Malapaka R. R. V., Xu H. E., Turk J., Semenkovich C. F., *Cell*, **138**, 476—488 (2009).
4) Karaskov E., Scott C., Zhang L., Teodoro T., Ravazzola M., Volchuk A., *Endocrinology*, **147**, 3398—3407 (2006).
5) Maedler K., Spinas G. A., Dyntar D., Moritz W., Kaiser N., Donath M. Y., *Diabetes*, **50**, 69—76 (2001).
6) Diakogiannaki E., Welters H. J., Morgan N. G., *J. Endocrinol.*, **197**, 553—563 (2008).
7) Wei Y., Wang D., Topczewski F., Pagliassotti M. J., *Am. J. Physiol. Endocrinol. Metab.*, **291**, E275—E281 (2006).
8) Malhi H., Bronk S. F., Werneburg N. W., Gores G. J., *J. Biol. Chem.*, **281**, 12093—12101 (2006).
9) Ota T., Gayet C., Ginsberg H. N., *J. Clin. Invest.*, **118**, 316—332 (2008).
10) Enser M., Roberts J. L., *Biochem. J.*, **206**, 561—570 (1982).
11) Enser M., Ashwell M., *Lipids*, **18**, 776—780 (1983).
12) Muoio D. M., Koves T. R., "Novartis Foundation Symposium 286—Fatty Acids and Lipotoxicity in Obesity and Diabetes," ed. by Bock G., Goode J., Wiley InterScince, 2007.
13) Cao H., Gerhold K., Mayers J. R., Wiest M. M., Watkins S. M., Hotamisligil G. S., *Cell*, **134**, 933—944 (2008).
14) Kawashima Y., Mizuguchi H., Kozuka H., *Biochim. Biophys. Acta*, **1210**, 187—194 (1994).
15) Guenthner R. A., Vietor M. L., *Ind. Eng. Chem. Prod. Res. Dev.*, **1**, 165—169 (1962).
16) Olsen G. W., Burris J. M., Burlew M. M., Mandel J. H., *J. Occup. Environ. Med.*, **45**, 260—270 (2003).
17) Kannan K., Corsolini S., Falandysz J., Fillmann G., Kumar K. S., Loganathan B. G., Mohd M. A., Olivero J., Van Wouwe N., Yang J. H., Aldoust K. M., *Environ. Sci. Technol.*, **38**, 4489—4495 (2004).
18) Olsen G., Hansen K. J., Stevenson L. A., Burris J. M., Mandel J. H., *Environ. Sci. Technol.*, **37**, 888—891 (2003).
19) Kudo N., Kawashima Y., *J. Toxicol. Sci.*, **28**, 49—57 (2003).
20) Kudo N., Sakai A., Mitsumoto A., Hibino Y., Tsuda T., Kawashima Y., *Biol. Pharm. Bull.*, **30**, 1535—1540 (2007).
21) Takagi A., Sai K., Umemura T., Hasegawa R., Kurokawa Y., *J. Environ. Pathol. Toxicol. Oncol.*, **11**, 145—149 (1992).
22) Kudo N., Suzuki-Nakajima E., Mitsumoto A., Kawashima Y., *Biol. Pharm. Bull.*, **29**, 1952—1957 (2006).
23) Kudo N., Bandai N., Suzuki E., Katakura M., Kawashima Y., *Chem. Biol. Interact.*, **124**, 119—132 (2000).
24) Kudo N., Kawashima Y., *Biol. Pharm. Bull.*, **26**, 47—51 (2003).
25) Dreyer C., Krey G., Keller H., Givel F., Helftenbein G., Wahli W., *Cell*, **68**, 879—887 (1992).

26) Kawashima Y., Uy-Yu N., Kozuka H., *Biochem. J.*, **263**, 897—904 (1989).
27) Ohya T., Kudo N., Suzuki E., Kawashima Y., *J. Chromatogr. B*, **720**, 1—7 (1998).
28) Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J., *J. Biol. Chem.*, **193**, 265—275 (1951).
29) Oshino N., Imai Y., Sato R., *J. Biochem.*, **69**, 155—167 (1971).
30) Kawashima Y., Kozuka H., *Biochim. Biophys. Acta*, **713**, 622—628 (1982).
31) Kawashima Y., Horii S., Matsunaga T., Hirose A., Adachi T., Kozuka H., *Biochim. Biophys. Acta*, **1005**, 123—129 (1989).
32) Bligh E. G., Dyer W. J., *Can. J. Biochem. Physiol.*, **37**, 911—917 (1959).
33) Imai K., Koyama M., Kudo N., Shirahata A., Kawashima Y., *Biochem. Pharmacol.*, **58**, 925—933 (1999).
34) Holub B. J., Skeaff C. M., *Methods Enzymol.*, **141**, 234—244 (1987).
35) Kawashima Y., Mizuguchi H., Musoh K., Kozuka H., *Biochim. Biophys. Acta*, **1212**, 311—318 (1994).
36) Miller C. W., Ntambi J. M., *Proc. Natl. Acad. Sci. U.S.A.*, **93**, 9443—9448 (1996).
37) Tamai Y., Lands W. E. M., *J. Biochem.*, **76**, 847—860 (1974).
38) Barden R. E., Cleland W. W., *J. Biol. Chem.*, **244**, 3677—3684 (1969).
39) Yamada K., Okuyama H., *Arch. Biochem. Biophys.*, **190**, 409—420 (1978).
40) Hosaka K., Schiele U., Numa S., *Eur. J. Biochem.*, **76**, 113—118 (1977).
41) Marcel Y. L., Christiansen K., Holman R. T., *Biochim. Biophys. Acta*, **164**, 25—34 (1968).
42) Brenna J. T., Kothapalli K. S. D., Park W. J., *Prostaglandins Leukot. Essent. Fatty Acids*, **82**, 281—285 (2010).
43) Wang Y., Botolin D., Xu J., Christian B., Mitchell E., Jayaprakasam B., Nair M., Peters J. M., Busik J., Olson L. K., Jump D. B., *J. Lipid Res.*, **47**, 2028—2041 (2006).
44) Yamashita A., Sugiura T., Waku K., *J. Biochem.*, **122**, 1—16 (1997).
45) Okuyama H., Yamada K., Ikezawa H., *J. Biol. Chem.*, **250**, 1710—1713 (1975).
46) Lands W. E. M., Inoue M., Sugiura Y., Okuyama H., *J. Biol. Chem.*, **257**, 14968—14972 (1982).
47) Holub B. J., *Lipids*, **11**, 1—5 (1976).
48) Holub B. J., *Biochim. Biophys. Acta*, **618**, 255—262 (1980).
49) Kudo N., Suzuki E., Katakura M., Ohmori K., Noshiro R., Kawashima Y., *Chem.-Biol. Interact.*, **134**, 203—216 (2001).
50) Tugwood J. D., Issemann I., Anderson R. G., Bundell K. R., McPheat W. L., Green S., *EMBO J.*, **11**, 433—439 (1992).
51) Maloney E. K., Waxman D. J., *Toxicol. Appl. Pharmacol.*, **161**, 209—218 (1999).
52) Vanden Heuvel J. P., Thompson J. T., Frame S. R., Gillies P. J., *Toxicol. Sci.*, **92**, 476—489 (2006).
53) Yamazaki T., Hirose A., Sakamoto T., Okazaki M., Mitsumoto A., Kudo N., Kawashima Y., *J. Pharmacol. Sci.*, **111**, 201—210 (2009).
54) Hishikawa D., Shindou H., Kobayashi S., Nakanishi H., Taguchi R., Shimizu T., *Proc. Natl. Acad. Sci. U.S.A.*, **105**, 2830—2835 (2008).
55) Ellinson J. S., Hill E. E., Lands W. E. M., *Biochim. Biophys. Acta*, **196**, 176—192 (1970).
56) Nakagawa S., Kawashima Y., Hirose A., Kozuka H., *Biochem. J.*, **297**, 581—584 (1994).
57) Ntambi J. M., *J. Lipid Res.*, **40**, 1549—1558 (1999).
58) Ntambi J. M., Miyazaki M., *Prog. Lipid Res.*, **43**, 91—104 (2004).