Effects of Chronic Administration of Perfluorooctanoic Acid on Fatty Acid Metabolism in Rat Liver: Relationship among Stearoyl-Coenzyme A Desaturase, 1-Acylglycerophosphocholine Acyltransferase, and Acyl Composition of Microsomal Phosphatidylcholine

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Male and female rats were fed on a diet containing 0.01% (w/w) perfluorooctanoic acid (PFOA) for 2, 22, or 26 weeks and effect of PFOA on activities of microsomal 1-acylglycerophosphocholine acyltransferase, microsomal stearoyl-coenzyme A (CoA) desaturase, peroxisomal β -oxidation and on acyl composition of microsomal phosphatidylcholine in liver were studied. The treatment of male rats with PFOA for 2 weeks caused increases in the activities of stearoyl-CoA desaturase, 1-acylglycerophosphocholine acyltransferase, and peroxisomal β -oxidation. The elevated activities of microsomal 1-acylglycerophosphocholine acyltransferase and peroxisomal β -oxidation were unchanging throughout the long-term treatment. The induced activity of microsomal 1-acylglycerophosphocholine acyltransferase was found to be highly correlated with the induced activity of peroxisomal β -oxidation. In contrast to these two enzymes, the increased activity of stearoyl-CoA desaturase by the short-term treatment of rats with PFOA did not last for 26 weeks, although the activity in rats treated for the long-term was higher than that of age-matched controls. The treatment of male rats with PFOA caused great alterations in the acyl composition of microsomal phosphatidylcholine. The high correlation seen between proportion of 18:1 in the C-2 position of phosphatidylcholine and activities of both stearoyl-CoA desaturase and 1-acylglycerophosphocholine acyltransferase suggest that these two enzymes participate actively in the regulation of acyl composition of phosphatidylcholine in rat liver. The present results show that hepatic responses to PFOA remain consistent throughout the period of the administration, but the elevated activities of the hepatic enzymes and the altered acyl composition of microsomal phosphatidylcholine returned to control levels within 4 weeks after PFOA was withdrawn from the diet. Even after the chronic administration of PFOA, these parameters of female rats responded only slightly to the challenges by the chemical, which indicates a marked sex-related difference being still apparent in the response of rat liver to PFOA.

Keywords — perfluorooctanoic acid; long-term administration; 1-acylglycerophosphocholine acyltransferase; stearoyl-CoA desaturase; acyl composition; phosphatidylcholine; rat liver

Introduction

The inductions of enzymes and the alterations of acyl composition of hepatic phosphatidylcholine in rodents by peroxisome proliferators have been the subject of our investigations.¹⁻⁶⁾ Among the peroxisome proliferators, of interest is PFOA, which elicits interesting biochemical responses in both sexes of the rat. Since PFOA is now widely used as an industrial material in various commercial products and the risk for humans to be exposed to this chemical seems to be

increasing recently, biochemical understanding of the chronic effects of PFOA to lipid metabolism may be of importance. Short-term treatment of rats with PFOA caused inductions of hepatic enzymes and alterations in acyl composition of hepatic phosphatidylcholine,⁶⁻⁹⁾ with an extreme sex-related difference being seen in these responses of rats to PFOA.^{6,9)} These findings from the studies on the short-term treatment with PFOA stimulated our interest to examine the following questions: 1) Are there differences in the responses of hepatic enzymes

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Abbreviations used: In the short notation of fatty acids, the first number indicates the chain length and the second number represents the number of double bond; 16:0, palmitic; 16:1, palmitoleic; 18:0, stearic; 18:1, oleic; 18:2, linoleic; 20:3 (*n*-6), 6,9,12-eicosatrienoic; 20:3 (*n*-9), 5,8,11-eicosatrienoic; 20:4, arachidonic; 22:6, 4,7,10,13,16,19-docosahexaenoic acid. PFOA, perfluorooctanoic acid; GPC, glycerophosphocholine.

to various periods of treatment with PFOA? 2) Do the changes in the fatty acyl composition of hepatic lipid correlate with the changes in the activities of hepatic enzymes even after chronic treatment with PFOA? 3) Are there changes in the activities of hepatic enzymes or modification in the fatty acyl composition of hepatic lipid when PFOA is withdrawn after chronic treatments of animals? 4) Is the sex-related difference in the response to short-term treatment with PFOA still evident after prolonged administration of PFOA? To address these questions, in the present study, male and female rats were chronically administered PFOA with a diet and the relationship among microsomal 1-acyl-GPC acyltransferase, stearyl-coenzyme A (CoA) desaturase, and the acyl composition of microsomal phosphatidylcholine were investigated.

Materials and Methods

Materials — PFOA was purchased from Aldrich (Milwaukee, WI, U.S.A.); stearyl-CoA, palmitoyl-CoA, oleoyl-CoA, cytochrome c, bovine serum albumin, and snake venom (*Crotalus adamanteus*) were from Sigma Chemical Co., (St. Louis, MO, U.S.A.); 1-acyl-GPC was from Avanti Polar Lipid (Pelham, Al. U.S.A.); CoA, nicotinamide adenine dinucleotide (NAD^+), NADH, and reduced nicotinamide adenine dinucleotide phosphate (NADPH) were from Oriental Yeast Co., (Tokyo, Japan). All other chemicals were of analytical grade.

Animals and Treatment — Male (120–150 g) and female (100–130 g) rats of Wistar strain, aged 5 weeks at the initiation of the treatment, were fed *ad libitum* on a diet containing 0.01% (w/w) PFOA for 2, 22 or 26 weeks. Some male rats were fed on the diet containing PFOA for 22 weeks and then PFOA was withdrawn from the diet by replacing it with the control diet; these rats were fed on the control diet for another 4 weeks until the 26th week after the initiation of the treatment with PFOA. PFOA at the dietary concentration of 0.01% were given to female rats for 2 and 26 weeks before being killed. Non-treated control male and female rats were fed on a control diet for 2 weeks (as young control rats)

or for 26 weeks (as age-matched control rats). The control diet or the diet containing 0.01% (w/w) PFOA were supplied by Clea Japan (Tokyo, Japan).

At the end of the treatments, the animals were killed by decapitation. Livers were isolated and perfused with ice-cold 0.9% NaCl and chilled in 0.25 M-sucrose on ice. A part of the liver was frozen at -30°C until assayed for peroxisomal β -oxidation; post-nuclear supernatant was prepared as described previously.⁹⁾ The other part of the liver was homogenized in 0.25 M-sucrose and microsomes were prepared by differential centrifugation as described previously.⁹⁾

Enzyme Assays — Stearyl-CoA desaturase was assayed by the method of Oshino *et al.*¹⁰⁾ as described previously¹⁾ and the activity presented as the rate constant (k^+) for stearyl-CoA-stimulated-reoxidation of NADH-reduced cytochrome b_5 .¹¹⁾ NADH-ferricyanide and NADH-cytochrome c reductases were assayed by the methods of Rogers and Strittmatter¹²⁾ and Oshino *et al.*¹³⁾ respectively. The microsomal content of cytochrome b_5 was determined by the method of Omura and Sato.¹⁴⁾ Microsomal 1-acyl-GPC acyltransferase was assayed with oleoyl-CoA as a substrate by the method of Lands and Hart,¹⁵⁾ as described previously.²⁾ The activity of cyanide-insensitive palmitoyl-CoA oxidation was measured by the method of Lazarow and de Duve¹⁶⁾ with some minor modifications,¹⁷⁾ using post-nuclear supernatant as an enzyme source.

Lipid Analyses — Lipids were extracted from microsomal suspension by the method of Bligh and Dyer.¹⁸⁾ Phosphatidylcholine was isolated by thin-layer chromatography as described by Skipski *et al.*¹⁹⁾ and was recovered from silica gel as described previously.³⁾ To analyse the acyl composition in C-1 and C-2 positions of phosphatidylcholine, an aliquot of phosphatidylcholine was hydrolyzed by phospholipase A_2 from snake venom according to Lands and Merkl.²⁰⁾ Free fatty acid and 1-acyl-GPC formed by the hydrolysis were separated by thin-layer chromatography as described previously³⁾ and were converted to fatty acid methyl esters by the method of Morrison and Smith.²¹⁾ Fatty acid methyl esters were analyzed by gas-liquid

Fig. 1. Effect of Prolonged Treatment with PFOA on the Growth of Male and Female Rats

Values are means $\pm$ S.D. for four to six rats. Male and female rats were fed on a control diet or a diet containing 0.01% (w/w) PFOA. $\square$, control male rats; $\blacksquare$, male rats fed 0.01% PFOA; $\circ$, control female rats; $\bullet$, female rats fed 0.01% PFOA.

chromatography as described previously.⁶⁾

Other Procedures — Protein concentration was determined according to Lowry *et al.*²²⁾

with bovine serum albumin as a standard. Statistical significance between two means was determined by Student's *t* test; linear or multiple regression analyses were performed to evaluate the correlations between two or three parameters.²³⁾

Results

Male and female Wistar rats were fed on a diet containing PFOA at the concentration of 0.01% (w/w) PFOA for 26 weeks. Changes in body weight of rats during the long-term treatment with dietary PFOA were presented in Fig. 1. There was no significant difference in the weight gain between male control and PFOA-treated male rats. Similarly, PFOA-treated female rats showed a growth curve which is the same as control female rats. All animals survived throughout the 26-week-treatment with PFOA without outward signs of untoward response.

Effects of PFOA on Microsomal 1-Acyl-GPC

TABLE I. Effects of Prolonged Administration of PFOA on the Activities of Microsomal 1-Acyl-GPC Acyltransferase in Male and Female Rats

Treatment of animals	1-Acyl-GPC acyltransferase	NADPH-cytochrome c reductase (nmol/min/mg protein)	Peroxisomal β -oxidation
Young male rats			
Control	67.4 $\pm$ 6.4	127 $\pm$ 18	5.5 $\pm$ 0.2
PFOA 2 weeks	220.1 $\pm$ 32.1 ^{c)}	155 $\pm$ 24	21.6 $\pm$ 2.5 ^{c)}
PFOA 22 weeks	229.8 $\pm$ 29.8 ^{c, f)}	159 $\pm$ 12 ^{a, f)}	21.9 $\pm$ 6.1 ^{b, e)}
PFOA 26 weeks	221.9 $\pm$ 12.7 ^{c, f)}	134 $\pm$ 6 ^{f)}	20.9 $\pm$ 5.1 ^{b, f)}
PFOA-withdrawal	84.6 $\pm$ 7.3 ^{a, d, h)}	125 $\pm$ 1 ^{d)}	3.0 $\pm$ 0.2 ^{c, f, g)}
Age-matched male rats			
Control	70.8 $\pm$ 6.4	108 $\pm$ 6	4.9 $\pm$ 1.4
PFOA 2 weeks	140.2 $\pm$ 6.4 ^{f)}	122 $\pm$ 6 ^{d)}	13.0 $\pm$ 0.6 ^{d)}
Young female rats			
Control	68.9 $\pm$ 6.4	76 $\pm$ 3	6.2 $\pm$ 0.4
PFOA 2 weeks	100.0 $\pm$ 1.9 ^{c)}	111 $\pm$ 8 ^{c)}	6.1 $\pm$ 0.7
PFOA 26 weeks	83.2 $\pm$ 4.6 ^{a, e)}	91 $\pm$ 9 ^{e)}	5.5 $\pm$ 1.4 ^{f)}
Age-matched female rats			
Control	62.2 $\pm$ 6.9	62 $\pm$ 8	3.9 $\pm$ 0.9
PFOA 2 weeks	105.3 $\pm$ 22.3 ^{e)}	91 $\pm$ 4 ^{f)}	7.3 $\pm$ 0.9 ^{e)}

Male and female rats were fed on a diet containing 0.01% (w/w) PFOA for 2, 22, and 26 weeks. A group of male rats that were fed on a diet containing 0.01% (w/w) PFOA for 22 weeks and then on a control diet for 4 weeks (PFOA-withdrawal). Values are means $\pm$ S.D. for four or five rats. *p* values for results significantly differed from young control data at a) *p* < 0.05, b) *p* < 0.01, c) *p* < 0.001, from age-matched control data at d) *p* < 0.05, e) *p* < 0.01, f) *p* < 0.001, and from PFOA 26 weeks data at g) *p* < 0.05, h) *p* < 0.001.

Acyltransferase and Peroxisomal β -Oxidation

The effects of prolonged administration of PFOA to rats on microsomal 1-acyl-GPC acyltransferase, microsomal NADPH-cytochrome c and peroxisomal β -oxidation in livers was examined (Table I). A marked increase in the activity of microsomal 1-acyl-GPC acyltransferase was brought about in young male rats treated with PFOA for 2 weeks and the high level of the activity was unchanging throughout the treatment of male rats with PFOA for 26 weeks. The treatment of age-matched male rats with PFOA for 2 weeks led to a substantial increase in the activity of microsomal 1-acyl-GPC acyltransferase, but to a lesser extent than those seen in young male rats. Upon the withdrawal of PFOA from the diet for 4 weeks, the induced activity of microsomal 1-acyl-GPC acyltransferase was almost reversed. A slight increase in the activity of this enzyme was observed in female rats treated with PFOA. In contrast to microsomal 1-acyl-GPC acyltransferase, NADPH-cytochrome c reductase, a marker enzyme of microsomes, was not much affected by

Fig. 2. Correlation between Microsomal 1-Acyl-GPC Acyltransferase and Peroxisomal β -Oxidation in Rat Liver

Regression analysis was performed on twelve mean data from Table I. Peroxisomal β -oxidation activity *versus* microsomal 1-acyl-GPC acyltransferase activity. $Y = 33.534 + 8.772X$, $r = 0.9833$, $p < 0.001$

the administration of PFOA to either male or female rats. Activities of peroxisomal β -oxidation in livers of both young and age-matched rats were induced in the same manner, as was seen

TABLE II. Effects of Prolonged Administration of PFOA on the Activities of Microsomal Components of Stearoyl-CoA Desaturation System in Livers of Male and Female Rats

Treatment of animals	NADH-ferricyanide reductase ($\mu\text{mol/min/mg protein}$)	NADH-cytochrome c reductase	Cytochrome b_5 (nmol/mg protein)	Desaturase k^+ (min^{-1})
Young male rats				
Control	6.82 ± 0.50	1.80 ± 0.33	0.55 ± 0.04	1.39 ± 0.16
PFOA 2 weeks	7.95 ± 0.80	$0.89 \pm 0.14^{b)}$	$0.63 \pm 0.03^{a)}$	$2.56 \pm 0.22^{c)}$
PFOA 22 weeks	$8.70 \pm 0.43^{b,e)}$	$1.26 \pm 0.09^{a,f)}$	$0.80 \pm 0.03^{c)}$	$2.08 \pm 0.68^{d)}$
PFOA 26 weeks	$8.14 \pm 0.43^{b,d)}$	$1.19 \pm 0.11^{a,f)}$	$0.77 \pm 0.07^{b)}$	$1.59 \pm 0.19^{f)}$
PFOA-withdrawal	$7.66 \pm 0.41^{a)}$	$2.24 \pm 0.31^{h)}$	$0.79 \pm 0.03^{c)}$	$0.75 \pm 0.26^{b,g)}$
Age-matched male rats				
Control	7.41 ± 0.22	2.29 ± 0.16	0.78 ± 0.02	0.78 ± 0.09
PFOA 2 weeks	$9.69 \pm 1.23^{d)}$	$1.77 \pm 0.34^{d)}$	0.75 ± 0.08	$1.58 \pm 0.30^{e)}$
Young female rats				
Control	7.07 ± 1.18	1.63 ± 0.46	0.49 ± 0.06	1.78 ± 0.31
PFOA 2 weeks	8.27 ± 0.59	$2.39 \pm 0.20^{a)}$	0.57 ± 0.06	2.03 ± 0.65
PFOA 26 weeks	$9.05 \pm 0.27^{a)}$	$3.42 \pm 0.13^{c)}$	$0.71 \pm 0.04^{c)}$	1.51 ± 0.36
Age-matched female rats				
Control	8.57 ± 0.75	3.24 ± 0.26	0.68 ± 0.02	2.06 ± 0.68
PFOA 2 weeks	7.58 ± 1.10	2.93 ± 0.35	0.67 ± 0.07	1.92 ± 0.42

The treatments of animals were the same as in Table I. Values are means $\pm$ S.D. for four or five rats. p values for results significantly differed from young control data at $a) p < 0.05$, $b) p < 0.01$, $c) p < 0.001$, from age-matched control data at $d) p < 0.05$, $e) p < 0.01$, $f) p < 0.001$, and from PFOA 26 weeks data at $g) p < 0.01$, $h) p < 0.001$.

with microsomal 1-acyl-GPC acyltransferase.

To determine whether there is a relationship between the activities of microsomal 1-acyl-GPC acyltransferase and peroxisomal β -oxidation, a linear regression analysis was carried out. A high correlation between the activities of these two enzymes was confirmed ($r=0.9833$, $p<0.001$) (Fig. 2).

Effects of PFOA on Microsomal Stearoyl-CoA Desaturation System

Table II shows the effect of prolonged administration of PFOA to rats on the components of stearoyl-CoA desaturation system in hepatic microsomes. The administration of PFOA to young male rats for 2 weeks resulted in an increase in the activity of terminal desaturase. Although the increased level of the activity did not last until the 26th week of the treatment with PFOA, the activity of terminal desaturase in male rats treated for 26 weeks was the same as

TABLE III. Effects of Prolonged Administration of PFOA on Fatty Acyl Composition of Liver Microsomal Phosphatidylcholine

Treatment of animals	16:0	16:1	18:0	18:1	18:2 (mol%)
Young male rats					
Control	25.6 $\pm$ 0.4	1.5 $\pm$ 0.1	20.3 $\pm$ 0.9	8.2 $\pm$ 0.4	17.2 $\pm$ 0.6
PFOA 2 weeks	27.0 $\pm$ 0.3 ^{b)}	2.2 $\pm$ 0.1 ^{c)}	19.6 $\pm$ 1.2	14.5 $\pm$ 2.0 ^{b)}	8.0 $\pm$ 1.3 ^{c)}
PFOA 26 weeks	26.3 $\pm$ 0.8	1.2 $\pm$ 0.1 ^{b)}	20.7 $\pm$ 1.0 ^{d)}	9.1 $\pm$ 0.2 ^{b,d)}	12.3 $\pm$ 0.5 ^{c,f)}
PFOA-withdrawal	25.1 $\pm$ 0.6	1.1 $\pm$ 0.3 ^{a)}	21.1 $\pm$ 1.0 ^{e)}	8.0 $\pm$ 0.3 ^{h)}	15.8 $\pm$ 0.9 ^{a,h)}
Age-matched male rats					
Control	25.2 $\pm$ 0.9	1.1 $\pm$ 0.3	19.0 $\pm$ 0.5	8.3 $\pm$ 0.5	16.4 $\pm$ 0.6
Young female rats					
Control	16.9 $\pm$ 1.0	0.9 $\pm$ 0.1	33.9 $\pm$ 2.2	6.4 $\pm$ 0.4	14.4 $\pm$ 0.9
PFOA 2 weeks	17.8 $\pm$ 1.2	1.2 $\pm$ 0.2	32.4 $\pm$ 1.0	9.5 $\pm$ 1.1 ^{b)}	8.5 $\pm$ 0.6 ^{c)}
PFOA 26 weeks	19.4 $\pm$ 1.1 ^{a)}	1.0 $\pm$ 0.4	29.8 $\pm$ 1.0 ^{a)}	6.6 $\pm$ 0.6	12.4 $\pm$ 0.9 ^{a,d)}
Age-matched female rats					
Control	19.1 $\pm$ 0.4	1.0 $\pm$ 0.2	29.6 $\pm$ 0.7	6.7 $\pm$ 0.4	14.7 $\pm$ 0.8

Treatment of animals	20:3 (n-9)	20:3 (n-6)	20:4	22:6
Young male rats				
Control	0	1.5 $\pm$ 0.1	20.5 $\pm$ 0.7	5.2 $\pm$ 0.5
PFOA 2 weeks	1.3 $\pm$ 0.8 ^{a)}	3.1 $\pm$ 0.3 ^{c)}	20.6 $\pm$ 0.5	3.7 $\pm$ 0.4 ^{b)}
PFOA 26 weeks	0.2 $\pm$ 0.1 ^{c,f)}	3.2 $\pm$ 0.1 ^{c,f)}	22.7 $\pm$ 0.4 ^{b)}	4.2 $\pm$ 0.3 ^{b,e)}
PFOA-withdrawal	0	1.3 $\pm$ 0.2 ^{d,h)}	22.7 $\pm$ 0.5 ^{b)}	5.0 $\pm$ 0.6 ^{g)}
Age-matched male rats				
Control	0	0.9 $\pm$ 0.2	24.3 $\pm$ 1.5	5.0 $\pm$ 0.6
Young female rats				
Control	0	1.7 $\pm$ 0.4	20.9 $\pm$ 2.0	4.9 $\pm$ 0.5
PFOA 2 weeks	0	2.4 $\pm$ 0.5	21.4 $\pm$ 1.0	6.7 $\pm$ 0.6 ^{b)}
PFOA 26 weeks	0	1.7 $\pm$ 0.1 ^{f)}	21.8 $\pm$ 1.3	7.3 $\pm$ 0.9 ^{b)}
Age-matched female rats				
Control	0	0.9 $\pm$ 0.1	20.9 $\pm$ 0.7	7.0 $\pm$ 0.2

Male and female rats were fed on a diet containing 0.01% (w/w) PFOA for 2 and 26 weeks. PFOA-withdrawal group were treated as in Table I. Lipids were extracted from microsomal suspension and phosphatidylcholine was isolated as described in Materials and Methods. Values are means $\pm$ S.D. for four rats. p values for results significantly differed from young control data at a) $p<0.05$, b) $p<0.01$, c) $p<0.001$, and from age-matched control data at d) $p<0.05$, e) $p<0.01$, f) $p<0.001$, and from PFOA 26 weeks data at g) $p<0.05$, h) $p<0.001$.

that in age-matched male rats treated with PFOA for 2 weeks. This activity was two times as high as that of age-matched control. The increased activity seen with young male rats treated with PFOA for 22 weeks was decreased to the level of age-matched control rats following the withdrawal of PFOA from the diet for 4 weeks. No significant change was brought about in the activity of terminal desaturase in young female rats treated with PFOA for either 2 or 26 weeks, as was observed in age-matched female rats treat-

ed with PFOA for 2 weeks.

In contrast to terminal desaturase, the activity of NADH-cytochrome b_5 reductase assayed with ferricyanide as an electron acceptor and microsomal content of cytochrome b_5 were not considerably changed by the long-term treatment of rats with PFOA. The activity of NADH-cytochrome b_5 reductase was decreased by the administration of PFOA to male rats, regardless of the duration of the treatments, when measured with cytochrome c as an electron acceptor.

TABLE IV. Effects of Prolonged Administration of PFOA on Fatty Acyl Composition at C-2 Position of Phosphatidylcholine in Hepatic Microsomes

Treatment of animals	16:0	16:1	18:0	18:1	18:2 (mol%)
Young male rats					
Control	1.6 ± 0.3	1.7 ± 0.3	0.5 ± 0.1	7.9 ± 0.3	34.0 ± 1.3
PFOA 2 weeks	0.9 ± 0.3 ^{a)}	2.1 ± 0.2	0.4 ± 0.2	23.0 ± 3.4 ^{c)}	16.2 ± 2.3 ^{c)}
PFOA 26 weeks	1.0 ± 0.1 ^{a,e)}	0.9 ± 0.1 ^{c)}	0.4 ± 0.1	13.4 ± 0.6 ^{c,f)}	24.4 ± 0.7 ^{c,f)}
PFOA-withdrawal	2.1 ± 0.4 ^{g)}	1.1 ± 0.3 ^{a)}	0.7 ± 0.3	7.6 ± 0.5 ^{d,g)}	31.9 ± 1.8 ^{g)}
Age-matched male rats					
Control	1.9 ± 0.4	0.9 ± 0.4	0.6 ± 0.2	6.4 ± 0.5	31.5 ± 1.0
Young female rats					
Control	2.6 ± 0.6	1.3 ± 0.3	1.2 ± 0.5	9.3 ± 1.0	30.4 ± 2.2
PFOA 2 weeks	2.1 ± 0.5	1.8 ± 0.4	1.0 ± 0.4	15.7 ± 2.4 ^{b)}	17.8 ± 1.6 ^{c)}
PFOA 26 weeks	2.0 ± 0.2 ^{b)}	1.1 ± 0.4	0.9 ± 0.3 ^{d)}	7.8 ± 0.9	26.2 ± 2.8
Age-matched female rats					
Control	3.5 ± 0.5	1.4 ± 0.4	1.8 ± 0.4	7.0 ± 0.5	30.8 ± 0.7

Treatment of animals	20:3 (n-9)	20:3 (n-6)	20:4	22:6
Young male rats				
Control	0	3.3 ± 0.2	42.0 ± 0.9	9.1 ± 0.8
PFOA 2 weeks	2.6 ± 1.4 ^{a)}	6.4 ± 0.7 ^{c)}	42.2 ± 0.8	6.3 ± 0.8 ^{b)}
PFOA 26 weeks	0.4 ± 0.1 ^{c,f)}	6.5 ± 0.2 ^{c,f)}	46.1 ± 0.8 ^{c,d)}	6.9 ± 0.7 ^{b)}
PFOA-withdrawal	0	2.6 ± 0.5 ^{d,g)}	47.5 ± 1.6 ^{c)}	6.5 ± 1.9 ^{a)}
Age-matched male rats				
Control	0	1.8 ± 0.4	49.1 ± 1.8	7.9 ± 0.5
Young female rats				
Control	0	4.1 ± 0.6	44.3 ± 3.3	6.9 ± 0.9
PFOA 2 weeks	1.0 ± 0.4 ^{b)}	5.1 ± 0.8	44.6 ± 2.8	11.0 ± 2.1 ^{a)}
PFOA 26 weeks	0	3.5 ± 0.6 ^{d)}	46.4 ± 2.5	12.1 ± 3.1 ^{a)}
Age-matched female rats				
Control	0	2.2 ± 0.4	43.7 ± 1.2	9.5 ± 0.8

The treatments of animals were the same as in Table III. Fatty acyl composition at C-2 position of phosphatidylcholine was determined as described in Materials and Methods. Values are means ± S.D. for four rats. *p* values for result significantly differed from young control data at *a)* *p* < 0.05, *b)* *p* < 0.01, *c)* *p* < 0.001, from age-matched control data at *d)* *p* < 0.05, *e)* *p* < 0.01, *f)* *p* < 0.001, and from PFOA 26 weeks data at *g)* *p* < 0.001.

Since the activity of NADH-cytochrome c reductase, even after its decrease, seems to be much higher than that of over-all desaturation, microsomal transport chain may provide enough electron to terminal desaturase in male rats treated with PFOA. In young and age-matched female rats, PFOA little changed any of the microsomal components of the stearyl-CoA desaturation system.

Effects of PFOA Administration on Acyl Composition of Phosphatidylcholine in Hepatic Microsomes

Effects of prolonged administration of PFOA on acyl composition of liver microsomal phosphatidylcholine were studied (Table III). The administration of PFOA to young male rats for 2 weeks caused great increases in proportions of 18:1, 20:3 (*n*-9) and 20:3 (*n*-6) and an evident decrease in the proportion of 18:2 in phosphatidylcholine. Although alterations in the proportions of 18:1 and 18:2 in male rats treated for 26 weeks were seen, the changes in acyl composition of phosphatidylcholine in the long-term treated group were less pronounced compared to those seen in rats treated for the short-term. The administration of PFOA to female rats for either 2 or 26 weeks caused no appreciable alterations in acyl composition of phosphatidyl-

choline, except for the proportion of 18:2.

Since 18:1 is known to be distributed to both C-1 and C-2 positions of phosphatidylcholine, acyl composition of C-1 and C-2 positions of phosphatidylcholine were separately analysed. Table IV shows the effects of prolonged administration of PFOA on fatty acyl composition at C-2 position of phosphatidylcholine. The treatment of young male rats with PFOA for 2 weeks caused a marked increase in the proportion of 18:1 and a great decrease in the proportion of 18:2 to almost a half of the control. A similar, but to a lesser extent, alterations in the proportions of 18:1 and 18:2 were seen in male rats treated with PFOA for 26 weeks. This increase, however, was higher than those observed in age-matched male rats treated with PFOA for 2 weeks. In female rats fed PFOA-diet for 2 weeks, an appreciable increase in the proportion of 18:1 and a considerable decrease in the proportion of 18:2 were brought about, whereas no significant alterations were observed in either age-matched female rats treated with PFOA for 2 weeks or female rats treated with PFOA for 26 weeks.

In contrast to acyl composition of C-2 position of phosphatidylcholine, proportion of 18:1 in C-1 position was not markedly changed by the treatment of rats with PFOA (Table V). It is

TABLE V. Effects of Prolonged Administration of PFOA on Fatty Acyl Composition at C-1 Position of Phosphatidylcholine in Hepatic Microsomes

Treatment of animals	16:0	16:1	18:0	18:1	18:2
Young male rats					
Control	46.8 ± 0.8	1.1 ± 0.1	41.9 ± 1.6	8.8 ± 0.9	1.4 ± 0.1
PFOA 2 weeks	51.0 ± 1.3 ^{b)}	1.8 ± 0.3 ^{b)}	40.4 ± 1.8	6.2 ± 0.8 ^{b)}	0.7 ± 0.1 ^{c)}
PFOA 26 weeks	49.1 ± 1.8	1.4 ± 0.1 ^{a)}	42.8 ± 2.1 ^{d)}	5.4 ± 0.6 ^{c,f)}	1.3 ± 0.2 ^{d)}
PFOA-withdrawal	44.8 ± 0.9 ^{a,d,h)}	1.1 ± 0.1 ^{g)}	43.4 ± 1.5 ^{e)}	9.3 ± 0.5 ⁱ⁾	1.5 ± 0.4
Age-matched male rats					
Control	46.8 ± 1.2	1.3 ± 0.1	39.3 ± 1.1	10.7 ± 1.5	2.0 ± 0.3
Young female rats					
Control	28.9 ± 2.0	0.6 ± 0.2	66.0 ± 2.4	3.7 ± 0.3	0.8 ± 0.2
PFOA 2 weeks	30.8 ± 1.9	0.7 ± 0.1	63.7 ± 2.1	4.3 ± 0.6	0.5 ± 0.1
PFOA 26 weeks	33.9 ± 1.3 ^{a)}	0.6 ± 0.04	59.0 ± 1.2 ^{b)}	5.7 ± 0.2 ^{c,d)}	0.8 ± 0.1
Age-matched female rats					
Control	33.4 ± 0.4	0.8 ± 0.1	57.9 ± 1.0	7.1 ± 0.6	0.9 ± 0.2

The treatments of animals were the same as in Table III. Fatty acyl composition at C-1 position of phosphatidylcholine was determined as described in the Materials and Methods. Values are means ± S.D. for four rats. *p* values for results significantly differed from young control data at a) *p* < 0.05, b) *p* < 0.01, c) *p* < 0.001, from age-matched control data at d) *p* < 0.05, e) *p* < 0.01, f) *p* < 0.001, and from PFOA 26 weeks data at g) *p* < 0.05, h) *p* < 0.01, i) *p* < 0.001.

noteworthy that the proportion of 18:1 in C-1 position of phosphatidylcholine from age-matched male control was approximately 63 percent higher than that from male rats treated with PFOA for 26 weeks.

Upon the withdrawal of PFOA from diet, the altered acyl composition of phosphatidylcholine in male rats treated with PFOA for 22 weeks was reversed to the acyl composition which is comparable to age-matched male control rats within 4 weeks (Tables, III, IV, and V).

Relationship among Proportion of 18:1 in Phosphatidylcholine Stearoyl-CoA Desaturase and Microsomal 1-Acyl-GPC Acyltransferase

To confirm the relationship among the proportion of 18:1 in C-2 position of phosphatidylcholine, and the activities of stearoyl-CoA desaturase and microsomal 1-acyl-GPC acyltransferase, a multiple linear regression analysis was performed. When nine sets of data from Tables I, II, and IV were taken into calculation, it revealed a marked correlation between the proportion of 18:1 in C-2 position of phosphatidylcholine and the activities of stearoyl-CoA desaturase and microsomal 1-acyl-GPC acyltransferase ($Y = -1.564 + 4.286X_1 + 0.051X_2$, $r = 0.9037$, $p < 0.01$). The partial correlations between the proportion of 18:1 and desaturase activity and the proportion of 18:1 and 1-acyl-GPC acyltransferase were 0.693 and 0.782, respectively.

Discussion

Feeding young male rats PFOA-diet for 2 weeks increased the activities of both microsomal 1-acyl-GPC acyltransferase and peroxisomal β -oxidation and the activities remained elevated throughout the administration of PFOA for 26 weeks. A high correlation between the activities of microsomal 1-acyl-GPC acyltransferase and peroxisomal β -oxidation in rat liver may support the hypothesis that microsomal 1-acyl-GPC acyltransferase is a good parameter capable of responding to the challenges by peroxisome proliferators and that a common regulatory process operates possibly at the step of transcriptional regulation of their genes.²⁴⁾ The activity of stearoyl-CoA desaturase was considerably in-

duced by the short-term treatment of male rats with PFOA. Although this high activity did not last for 26 weeks, the activity of desaturase in rats treated with PFOA for the long-term was significantly higher compared with that in age-matched male controls. The increase in desaturase activity seemed to be attributable to the changes in terminal desaturase activity itself, but not to the changes in either NADH-cytochrome b_5 reductase activity or cytochrome b_5 content. In contrast to these two microsomal enzymes, the activity of NADPH-cytochrome c reductase was little changed by various treatments with PFOA of rats. These results suggest that the inductions of microsomal 1-acyl-GPC acyltransferase and stearoyl-CoA desaturase are not nonspecific.

Phosphatidylcholine is the most abundant phospholipid in hepatic microsomes of rats and the one containing high proportions of 18:1 and 18:2. Our previous study⁶⁾ showed that the proportion of 18:1 in hepatic phosphatidylcholine of male rats responded to the short-term challenges by PFOA more sensitively than that of phosphatidylethanolamine. On the other hand, the present study showed that the long-term administration of PFOA did not change the proportion of 18:1 in phosphatidylcholine so markedly as did the short-term administration. However, the distinct analysis of acyl composition of C-1 and C-2 positions of phosphatidylcholine revealed that great changes were brought about in both C-2 position and C-1 position of phosphatidylcholine following long-term treatment with PFOA when compared with age-matched control. Furthermore, a multiple linear regression analysis showed that the proportion of 18:1 in C-2 position of phosphatidylcholine highly correlated with the activities of both stearoyl-CoA desaturase and 1-acyl-GPC acyltransferase. These results, taken together with our previous finding,⁴⁾ may support the concept that the increased activities of stearoyl-CoA desaturase and microsomal 1-acyl-GPC acyltransferase participate actively in the regulation of the acyl composition of phosphatidylcholine in rat liver.

In this study, we found that the induced activities of stearoyl-CoA desaturase, microsomal

1-acyl-GPC acyltransferase, and peroxisomal β -oxidation were maintained throughout the administration of PFOA for at least 26 weeks. However, the withdrawal of PFOA from the diet after a 22 week-treatment restored the elevated activities of these enzymes and the alterations in acyl composition of microsomal phosphatidylcholine. These results strongly indicate that the response of rat liver to PFOA is reversible when the treatment is discontinued.

Many studies²⁵⁻²⁹⁾ provided evidence that a sex-related difference in the response of liver to peroxisome proliferators is in common when rats were used as experimental animals. Nevertheless, our previous study^{6,9)} showed the extent of sex-related difference, which was observed with rats in the response to PFOA, was extremely marked compared with those of other peroxisome proliferators. The subsequent study³⁰⁾ showed that the treatments of female rats with PFOA for 26 weeks did not considerably alter the activity of peroxisomal β -oxidation in liver. In the present study, similar results to those of peroxisomal β -oxidation were obtained with microsomal 1-acyl-GPC acyltransferase and stearoyl-CoA desaturase, strongly indicating a marked sex-related difference in rats to PFOA being still apparent even after the long-term treatment. Recently, it was reported that the urinary excretion of PFOA was strongly dependent on oestradiol in rats.³¹⁾ Our present study might suggest that this kind of sex-hormone dependent excretion of PFOA still actively functions even after the long-term treatment.

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References

- 1) Y. Kawashima and H. Kozuka: Increased activity of stearoyl-CoA desaturation in liver from rat fed clofibric acid, *Biochim. Biophys. Acta*, **713**, 622—628 (1982).
- 2) Y. Kawashima, A. Hirose and H. Kozuka: Selective increase in acylation of 1-acylglycerophosphorylcholine in livers of rats and mice by peroxisome proliferators, *Biochim. Biophys. Acta*, **793**, 232—237 (1984).
- 3) Y. Kawashima, A. Hirose and H. Kozuka: Modification by clofibric acid of acyl composition of glycerolipids in rat liver, *Biochim. Biophys. Acta*, **795**, 543—551 (1984).
- 4) Y. Kawashima, A. Hirose, T. Adachi and H. Kozuka: Role of stearoyl-CoA desaturase and 1-acylglycerophosphorylcholine acyltransferase in the regulation of the acyl composition of phosphatidylcholine in rat liver, *Biochim. Biophys. Acta*, **837**, 222—229 (1985).
- 5) Y. Kawashima, A. Hirose and H. Kozuka: Alterations by peroxisome proliferators of acyl composition of hepatic phosphatidylcholine in rats, mice and guinea-pigs, *Biochem. J.*, **235**, 251—255 (1986).
- 6) Y. Kawashima, N. Uy-Yu and H. Kozuka: Sex-related differences in the enhancing effects of perfluoro-octanoic acid on stearoyl-CoA desaturase and its influence on the acyl composition of phospholipid in rat liver, *Biochem. J.*, **263**, 897—904 (1989).
- 7) T. Ikeda, K. Aiba, K. Fukuda and M. Tanaka: The induction of peroxisome proliferation in rat liver by perfluorinated fatty acids, metabolically inert derivatives of fatty acids, *J. Biochem. (Tokyo)*, **98**, 475—482 (1985).
- 8) T. P. Pastoor, K. P. Lee, M. A. Perri and P. J. Gillies: Biochemical and morphological studies of ammonium perfluorooctanoate-induced hepatomegaly and peroxisome proliferation, *Exp. Mol. Pathol.*, **47**, 98—109 (1987).
- 9) Y. Kawashima, N. Uy-Yu and H. Kozuka: Sex-related difference in the inductions by perfluoro-octanoic acid of peroxisomal β -oxidation, microsomal 1-acylglycerophosphocholine acyltransferase and cytosolic long-chain acyl-CoA hydrolase in rat liver, *Biochem. J.*, **261**, 595—600 (1989).
- 10) N. Oshino, Y. Imai and R. Sato: A function of cytochrome b_5 in fatty acid desaturation by rat liver microsomes, *J. Biochem. (Tokyo)*, **69**, 155—167 (1971).
- 11) F. L. Hoch, J. W. DePierre and L. Ernster: Thyroid control over biomembranes: Liver-microsomal cytochrome b_5 in hypothyroidism, *Eur. J. Biochem.*, **109**, 301—306 (1980).
- 12) M. J. Rogers and P. Strittmatter: Lipid-protein interactions in the reconstitution of the microsomal reduced nicotinamide adenine dinucleotide-cytochrome b_5 reductase system, *J. Biol. Chem.*, **248**, 800—806 (1973).
- 13) N. Oshino, Y. Imai and R. Sato: Electron-transfer mechanism associated with fatty acid desaturation catalyzed by liver microsomes, *Biochim. Biophys. Acta*, **128**, 13—28 (1966).
- 14) T. Omura and R. Sato: The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature, *J. Biol. Chem.*, **239**, 2370—2378 (1964).
- 15) W. E. M. Lands and P. Hart: Metabolism of glycerolipids. IV. Specificities of acyl coenzyme A: Phospholipid acyltransferases, *J. Biol. Chem.*, **240**, 1905—1911 (1965).
- 16) P. B. Lazarow and C. De Duve: A fatty acyl-CoA oxidizing system in rat liver peroxisomes; enhancement by clofibrate, a hypolipidemic drug, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 2043—2046 (1976).
- 17) Y. Kawashima, H. Katoh and H. Kozuka: Differential

- effects of altered hormonal state on the induction of acyl-CoA hydrolases and peroxisomal β -oxidation by clofibric acid, *Biochim. Biophys. Acta*, **750**, 365—372 (1983).
- 18) E. G. Bligh and W. J. Dyer: A rapid method of total lipid extraction and purification, *Can. J. Biochem. Physiol.*, **37**, 911—917 (1959).
- 19) V. P. Skipski, R. F. Peterson and M. Barclay: Quantitative analysis of phospholipids by thin-layer chromatography, *Biochem. J.*, **90**, 374—378 (1964).
- 20) W. E. M. Lands and I. Merkl: Metabolism of glycerolipids. III. Reactivity of various acyl esters of coenzyme A with α' -acylglycerophosphorylcholine, and positional specificities in lecithin synthesis, *J. Biol. Chem.*, **238**, 898—904 (1963).
- 21) W. R. Morrison and L. M. Smith: Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol, *J. Lipid Res.*, **5**, 600—608 (1964).
- 22) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall: Protein measurement with the folin phenol reagent, *J. Biol. Chem.*, **193**, 265—275 (1951).
- 23) J. H. Zar: Multiple Regression and Correlation, "Biostatistical Analysis," 2nd ed., Prentice-Hall Inc., Eaglewood Cliffs, New Jersey, 1984, pp. 328—360.
- 24) Y. Kawashima, S. Horii, T. Matsunaga, A. Hirose, T. Adachi and H. Kozuka: Co-induction by peroxisome proliferators of microsomal 1-acylglycerophosphocholine acyltransferase with peroxisomal β -oxidation in rat liver, *Biochim. Biophys. Acta*, **1005**, 123—129 (1989).
- 25) D. H. Fahimi and A. Reinicke: The short- and long-term effects of bezafibrate in the rat, *Ann. N. Y. Acad. Sci.*, **386**, 111—135 (1982).
- 26) T. Osumi and T. Hashimoto: Enhancement of fatty acyl-CoA oxidizing activity in rat liver peroxisomes by Di-(2-ethylhexyl)phthalate, *J. Biochem. (Tokyo)*, **83**, 1361—1365 (1978).
- 27) F. E. Mitchell, S. C. Price, R. H. Hinton, P. Grasso and J. W. Bridges: Time and dose-response study of the effects on rats of the plasticizer di(2-ethylhexyl) phthalate, *Toxicol. Appl. Pharmacol.*, **81**, 371—392 (1985).
- 28) D. Svoboda, H. Grady and D. Azarnoff: Microbodies in experimentally altered cells, *J. Cell Biol.*, **35**, 127—152 (1967).
- 29) R. H. Gray and F. A. de la Iglesia: Quantitative microscopy comparison of peroxisome proliferation by the lipid-regulating agent gemfibrozil in several species, *Hepatology*, **4**, 520—530 (1984).
- 30) N. Uy-Yu, Y. Kawashima and H. Kozuka: Comparative studies on sex-related difference in biochemical responses of livers to perfluorooctanoic acid between rats and mice, *Biochem. Pharmacol.*, **39**, 1492—1495 (1990).
- 31) M. Ylinen, H. Hanhijärvi, J. Jaakonaho and P. Peura: Stimulation by oestradiol of the urinary excretion of perfluorooctanoic acid in the male rat, *Pharmacol. and Toxicol.*, **65**, 274—277 (1989).