Perfluorodecanoic acid enhances the formation of oleic acid in rat liver

Aya YAMAMOTO* and Yoichi KAWASHIMA†

Faculty of Pharmaceutical Sciences, Josai University, Keyakidai, Sakado, Saitama 350-02, Japan

The feeding of perfluorodecanoic acid (PFDA) to male rats at a dietary concentration of 0.005 % (w/w) for 7 days resulted in a marked increase in the activity of microsomal stearoyl-CoA desaturation in the liver. This increase in the overall desaturation activity was due to the induction of terminal desaturase among the components comprising the desaturation system. In contrast, PFDA inhibited desaturation *in vitro*, seemingly due to interference with electron transport through the desaturation system. Accordingly, PFDA can be an inducer and also an inhibitor of Δ^9-desaturation. PFDA feeding enhanced the conversion of radioactive stearic acid into oleic acid in the liver *in vivo*, indicating that the induction of Δ^9-desaturase by PFDA functions *in vivo*. PFDA feeding increased the mass of octadecenoic acid ($C_{18:1}$) in the liver and the proportion of $C_{18:1}$ in microsomal lipid. A highly significant linear correlation existed between the microsomal desaturase activity and the proportion of $C_{18:1}$ in microsomal lipid when compared using rats in five different physiological states: control, PFDA-fed, *p*-chlorophenoxyisobutyric acid (clofibric acid)-fed, starved and starved/refed. These results suggest that the increase in the hepatic level of $C_{18:1}$ caused by feeding of PFDA to rats can be explained by the common concept of regulation, i.e. the hepatic level of $C_{18:1}$ is under the control of Δ^9-desaturase. The dietary administration of PFDA also increased the content of cytochrome *P*-450 and the activity of 7-ethoxycoumarin *O*-de-ethylase in the liver.

INTRODUCTION

The levels of octadecenoic acid ($C_{18:1}$) in the liver are known to fluctuate in response to changes in the nutritional and hormonal state of the animals. Since $C_{18:1}$ is formed from saturated fatty acids by Δ^9-desaturation, the fluctuation in the hepatic level of $C_{18:1}$ is considered to be related to changes in the activity of Δ^9-desaturase (stearoyl-CoA desaturase; EC 1.14.99.5). In addition to these physiological alterations, Δ^9-desaturase has been demonstrated to be induced by *p*-chlorophenoxyisobutyric acid (clofibric acid) and several other peroxisome proliferators, regardless of their chemical structures [1,2].

Most peroxisome proliferators have been demonstrated to induce not only peroxisomal enzymes and microsomal Δ^9-desaturase, but also many enzymes that are involved in lipid metabolism [3–6]. Perfluoro-octanoic acid, a peroxisome proliferator, causes a considerable increase in the proportion of $C_{18:1}$ in hepatic lipid by inducing Δ^9-desaturase [7], as has been seen with other types of peroxisome proliferators [1,2]. Despite the similarity in their chemical structures, however, the biological effects of perfluorodecanoic acid (PFDA) have been shown to be different from those of perfluoro-octanoic acid. Thus a single administration of PFDA to rats at high doses increased the proportion of $C_{18:1}$ in the liver [8,9]; nevertheless, the activity of Δ^9-desaturase was diminished following treatment [10]. The increase in the hepatic level of $C_{18:1}$ on treatment of rats with PFDA was found to be due to the mobilization of $C_{18:1}$ from peripheral stores [8,9]. These findings are very different from the results obtained so far with other peroxisome proliferators, including perfluoro-octanoic acid [7], and are inconsistent with the generally accepted understanding that the hepatic level of $C_{18:1}$ is regulated by Δ^9-desaturase activity. This stimulated our interest in examining whether the hepatic level of $C_{18:1}$ is under the control of Δ^9-desaturase in animals given PFDA. In this context, the present work was undertaken to study the responses of the Δ^9-desaturation system to PFDA both *in vivo* and *in vitro* in relation to regulation of the hepatic level of $C_{18:1}$.

MATERIALS AND METHODS

Materials

[1-^{14}C]Stearic acid (58 Ci/mol) was obtained from Dupont-New England Nuclear (Boston, MA, U.S.A.). Stearoyl-CoA, clofibric acid, BSA and cytochrome *c* were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.); PFDA was from Aldrich (Milwaukee, WI, U.S.A.); and CoA, NADH and NADPH were from Oriental Yeast Co. (Tokyo, Japan). [1-^{14}C]Stearoyl-CoA was prepared as described by Bergstrom and Reitz [11]. Standard diet (CE-2) and fat-free diet were obtained from Clea Japan (Tokyo, Japan).

Treatments of animals and preparation of microsomes

Male Wistar rats (5 weeks old) were obtained from SLC (Hamamatsu, Japan). After acclimatization for 1 week, the rats (130–140 g) were fed on either a standard diet or a diet containing 0.005 % (w/w) PFDA for 7 days. In some experiments, rats were fed a control diet or a diet that contained 0.5 % (w/w) clofibric acid for 7 days; some rats were starved for 2 days (starved rats) and then refed a fat-free diet for 2 days (starved/refed rats). Rats were decapitated and livers were isolated. The livers were perfused

Abbreviations used: clofibric acid, *p*-chlorophenoxyisobutyric acid; PFDA, perfluorodecanoic acid; $C_{18:1}$, octadecenoic acid.

* Present address; Faculty of Pharmaceutical Sciences, Science University of Tokyo, 12 Ichigaya Funagawara-machi, Shinjuku-ku, Tokyo 162, Japan.

† To whom correspondence should be addressed.

with ice-cold 0.9 % (w/v) NaCl and then homogenized in 4 vol. of 0.25 M sucrose/1 mM EDTA/10 mM Tris/HCl, pH 7.4. The homogenates were centrifuged at 18000 ***g*** for 20 min, and the supernatant was re-centrifuged under the same conditions. The resulting supernatant was centrifuged at 105000 ***g*** for 60 min. The pellet was resuspended in 0.25 M sucrose/10 mM Tris/HCl, pH 7.4, and the suspension was re-centrifuged under the same conditions. The microsomal pellet obtained was resuspended in a small volume of 0.25 M sucrose/10 mM Tris/HCl buffer, pH 7.4. All operations were carried out at 0–4 °C. Concentrations of protein were determined by the method of Lowry et al. [12] with BSA as standard.

Enzyme assays

Δ^9-Desaturation activity was measured essentially as described by Oshino et al. [13]. The reaction mixture contained 27 nmol of [1-^{14}C]stearoyl-CoA (30 nCi), 1 μmol of NADH, 50 μmol of Tris/HCl buffer (pH 7.4) and 400 μg of microsomal protein in a final volume of 0.5 ml. The mixture was incubated at 37 °C for 4 min, and the reaction was stopped by the addition of 1 ml of 10 % (w/v) KOH in 90 % methanol, followed by saponification at 80 °C for 40 min. After acidification with 6 M HCl, fatty acids were extracted and methylated with BF_3/methanol. Saturated and mono-unsaturated esters were separated by TLC on $AgNO_3$-impregnated silica gel G, which was developed with n-hexane/diethyl ether (9:1, v/v). The spots were detected under UV light after spraying with 0.05 % (w/v) Rhodamine B in 95 % ethanol. The areas corresponding to authentic methyl stearate and methyl oleate were scraped off the plate, and methyl esters were extracted with toluene. The radioactivity was measured by scintillation counting.

Terminal desaturase activity was assayed spectrophotometrically by the method of Oshino et al. [14], as the stearoyl-CoA-stimulated re-oxidation of NADH-reduced cytochrome b_5. The rates of cytochrome b_5 oxidation were measured with a Shimadz UV-300 spectrophotometer by recording the changes in absorbance between 424 and 409 nm at 30 °C. The cuvette contained 0.9 mg of microsomal protein and 300 μmol of Tris/HCl (pH 7.4) in a final volume of 3.0 ml. Microsomal cytochrome b_5 was reduced by 2 nmol of NADH, and re-oxidation was recorded. When the re-oxidation was complete, 20 nmol of stearoyl-CoA was added and cytochrome b_5 was reduced again by 2 nmol of NADH. The first-order constant for the re-oxidation of NADH-reduced cytochrome b_5 was calculated as described by Oshino and Sato [15]. The rate constant for the re-oxidation of NADH-reduced cytochrome b_5 was measured in the presence (k) and in the absence (k^-) of stearoyl-CoA; the rate constant for Δ^9-desaturation was given by $k^+ = k - k^-$. Desaturation activity was also calculated from the difference between the times required to oxidize NADH in the presence and in the absence of stearoyl-CoA, indicated by the onset of cytochrome b_5 re-oxidation [16].

NADH:ferricyanide reductase activity in microsomes was assayed as described by Rogers and Strittmatter [17]. The microsomal activities of NADH:cytochrome *c* reductase and NADPH–cytochrome *c* reductase were estimated by the methods of Oshino et al. [13] and Takesue and Omura [18] respectively. The microsomal contents of cytochrome b_5 and cytochrome *P*-450 were determined by the methods of Omura and Sato [19]. The microsomal activity of aniline *p*-hydroxylase was evaluated as described by Imai et al. [20]. The activities of 7-ethoxycoumarin *O*-de-ethylase and aminopyrine *N*-demethylase in microsomes were determined by the methods of Aitio [21] and Cochin and Axelrod [22] respectively.

Desaturation of stearic acid *in vivo*

The injection solution of [^{14}C]stearic acid was prepared essentially as described previously [5]. [^{14}C]Stearic acid was taken to dryness by flushing with nitrogen and dissolved in a very small volume of ethanol to which an equivalent amount of KOH was added. To this solution was added 50 vol. of rat serum that had been filtered through a Millipore filter (0.22 μm), and the solution was mixed and allowed to stand at room temperature for 10 min. Under light diethyl ether anaesthesia, 0.2 ml of the injection solution containing 10 μCi of [^{14}C]stearic acid was injected into the exposed right jugular veins of rats (130–140 g) which had been fed either a control diet or a diet containing 0.005 % (w/w) PFDA for 7 days. At 10 min after the injection, rats were decapitated and the livers were isolated. Blood remaining in the livers was washed out with ice-cold 0.9 % NaCl, and the livers were rinsed with ice-cold 0.9 % NaCl. The livers were frozen in liquid nitrogen and stored at −80 °C until use. Lipid was extracted from the livers by the method of Bligh and Dyer [23], and the lipid extract was treated with BF_3/methanol to convert fatty acids into methyl esters. The radioactivity in saturated and mono-unsaturated esters was measured as described above.

Analysis of fatty acids

After the addition of a known amount of pentadecanoic acid (as an ethanol solution) as an internal standard when required, lipid was extracted from livers and microsomes by the method of Bligh and Dyer [23]. The lipid extracted was converted into methyl esters of fatty acids with BF_3/methanol, and methyl esters were separated and quantified by GLC as described previously [24].

Statistical analysis

The statistical significance of the difference between two means was estimated by Student's *t*-test. Linear regression analysis was performed to evaluate the correlation between two parameters.

RESULTS

On the basis of the results of our previous study [25], the feeding of PFDA to male rats at a concentration of 0.005 % (w/w) for 7 days was employed as a protocol for the treatments of animals in the present study. It was confirmed, in the previous study, that this treatment markedly induces hepatomegaly and peroxisome proliferator-responsive enzymes (peroxisomal β-oxidation, cytosolic long-chain acyl-CoA hydrolase and microsomal 1-acylglycerophosphocholine acyltransferase), but has little effect on growth, body weight or food intake. In accordance with our previous results [25], the feeding of PFDA to rats caused a 45 % increase in liver weight (control, 8.89 ± 0.64 g; PFDA-fed, 12.93 ± 0.43 g) and greatly induced the hepatic activity of peroxisomal β-oxidation, measured spectrophotometrically by the method of Lazarow and de Duve [26] as the cyanide-insensitive oxidation of palmitoyl-CoA (control 5.48 ± 1.04 nmol/min per mg of protein; PFDA-fed, 40.22 ± 4.38 nmol/min per mg).

Effects of PFDA feeding on the Δ^9-desaturation system

The activity of Δ^9-desaturation in the hepatic microsomes of PFDA-fed rats was 2.4-fold greater than in control rats when

Table 1 Effects of PFDA feeding on the Δ^9-desaturation system in rat liver

Rats were fed on either a control diet or a diet containing 0.005% (w/w) PFDA for 7 days. The rate constant for the re-oxidation of NADH-reduced cytochrome b_5 was measured with (k) and without (k^-) stearoyl-CoA; the rate constant (k^+) for desaturation is given by $k^+ = k - k^-$. Stearoyl-CoA desaturation was assayed by measuring the conversion of [^{14}C]stearoyl-CoA into [^{14}C]oleic acid by microsomes. Each value represents the mean ± S.D. for four rats. Significant differences from controls: $^*P < 0.05$; $^{***}P < 0.001$.

Dietary treatment	Stearoyl-CoA desaturation (nmol/min per mg of protein)	NADH:cytochrome b_5 reductase (μmol/min per mg of protein)		Cytochrome b_5 content (nmol/mg of protein)	Rate constants for cytochrome b_5 re-oxidation (min^{-1})		
		Ferricyanide	Cytochrome c		k	k^-	k^+
Control	0.63 ± 0.11	7.54 ± 0.59	2.07 ± 0.15	0.84 ± 0.08	2.62 ± 0.15	1.22 ± 0.07	1.40 ± 0.13
PFDA	1.54 ± 0.28***	6.73 ± 0.28*	0.75 ± 0.10***	0.75 ± 0.06	4.74 ± 0.32***	0.92 ± 0.06***	3.81 ± 0.29***

assayed with [^{14}C]stearoyl-CoA as substrate (Table 1). To examine the effects of PFDA feeding on individual components, namely NADH:cytochrome b_5 reductase, cytochrome b_5 and terminal desaturase, which comprise the Δ^9-desaturation system, the activities or the contents of these components were measured individually (Table 1). In contrast with the marked increase in the overall desaturation activity, PFDA had little effect on the activity of NADH:cytochrome b_5 reductase, measured with ferricyanide as a non-physiological electron acceptor. In fact, the activity of the reductase was suppressed by the treatment when utilizing cytochrome c as an artificial electron acceptor. Moreover, PFDA feeding did not change the microsomal content of cytochrome b_5. On the other hand, the treatment of rats with PFDA markedly increased the rate constant (k) for the re-oxidation of NADH-reduced cytochrome b_5 when measured in the presence of stearoyl-CoA, but lowered slightly the rate constant (k^-) measured in the absence of stearoyl-CoA. Consequently, the activity of terminal desaturase measured as the rate constant (k^+) for the stearoyl-CoA-stimulated re-oxidation of NADH-reduced cytochrome b_5 was increased 2.7-fold by the treatment of rats with PFDA.

The effect of PFDA feeding on the time required to oxidize a fixed amount of NADH by the Δ^9-desaturation system was examined in the absence and the presence of excess amounts of stearoyl-CoA, as described by Strittmatter et al. [16]. The treatment of rats with PFDA decreased slightly, but significantly, the rate of oxidation of NADH, but only when measured in the absence of stearoyl-CoA (time taken: control, 4.08 ± 0.16 min; PFDA-fed, 4.88 ± 0.13 min). In contrast, the treatment increased the rate of NADH oxidation when assayed in the presence of stearoyl-CoA (time taken: control, 2.44 ± 0.25 min; PFDA-fed, 1.46 ± 0.15 min). The activity of stearoyl-CoA desaturation, which was calculated from the difference between the time required to oxidize NADH in the presence and in the absence of stearoyl-CoA and using the assumption that 1 mol of NADH is required for the formation of 1 mol of oleoyl-CoA, was 2.8-fold higher in PFDA-fed rats (control, 0.58 ± 0.12 nmol/min per mg of protein; PFDA-fed, 1.62 ± 0.18 nmol/min per mg).

Effect of PFDA on Δ^9-desaturation *in vitro*

To examine the effects of PFDA on Δ^9-desaturation *in vitro*, the activity of NADH:cytochrome b_5 reductase and the time required to oxidize a fixed amount of NADH were measured after the preincubation of microsomes with PFDA (Figure 1). In these experiments, microsomes isolated from the livers of starved/refed rats were employed. Although no changes were observed in the activity of NADH:cytochrome b_5 reductase when assaying with either ferricyanide or cytochrome c (Figure 1, top panel), PFDA appreciably prolonged, in a concentration-dependent manner, the time required for NADH oxidation measured both in the absence and in the presence of stearoyl-CoA (Figure 1, middle panel). The activity of stearoyl-CoA desaturation calculated from the difference between the time required to oxidize NADH in the presence and in the absence of stearoyl-CoA was markedly inhibited by PFDA, in a concentration-dependent manner (Figure 1, bottom panel).

Effects of PFDA feeding on the formation of oleic acid *in vivo*

To examine whether PFDA enhances or suppresses stearoyl-CoA desaturation *in vivo*, [1-^{14}C]stearic acid was injected intravenously into control and PFDA-fed rats, and the formation of radiolabelled oleic acid at 10 min after the injections was measured (Table 2). The conversion of radioactive stearic acid into oleic acid was enhanced 1.5-fold by the treatment of rats with PFDA, whereas there was no difference in total radioactivity incorporated into hepatic lipid between control and PFDA-fed rats. Consequently, the formation of oleic acid in the livers of PFDA-fed rats was 1.5-fold higher than that in controls.

Alterations caused by PFDA feeding in the hepatic fatty acid composition

The administration of PFDA to rats caused a marked change in the hepatic fatty acid composition (Table 3). Treatment with PFDA increased by 2.3-fold the proportion of $C_{18:1}$, but, in contrast, decreased the proportions of stearic acid, linoleic acid and arachidonic acid. Nevertheless, the contents of the latter fatty acids did not change on the basis of liver weight. Upon PFDA feeding, the hepatic content of total fatty acids on the basis of per g of liver and total liver was increased 1.6- and 2.4-fold respectively, whereas the content of $C_{18:1}$ on the basis of per g of liver and total liver in PFDA-fed rats was increased 3.7- and 5.4-fold respectively compared with the controls.

Table 4 shows the relationship between the activity of Δ^9-desaturase and the proportion of $C_{18:1}$ in the microsomal lipid of livers from rats in five different physiological states. Starving the rats suppressed Δ^9-desaturase activity markedly, and refeeding of starved rats caused a marked increase in the activity of the desaturase. The administration of either clofibric acid or PFDA considerably increased the activity of Δ^9-desaturase. The level of $C_{18:1}$ in microsomal lipid was higher in the starved/refed, clofibric acid-fed and PFDA-fed rats, but lower in the starved rats, compared with controls. To examine the relationship between the activity of Δ^9-desaturase and the proportion of $C_{18:1}$ in microsomal lipid, a linear regression analysis was carried out. This revealed that the correlation between the two parameters was significant, with $r = 0.976$ ($P < 0.01$).

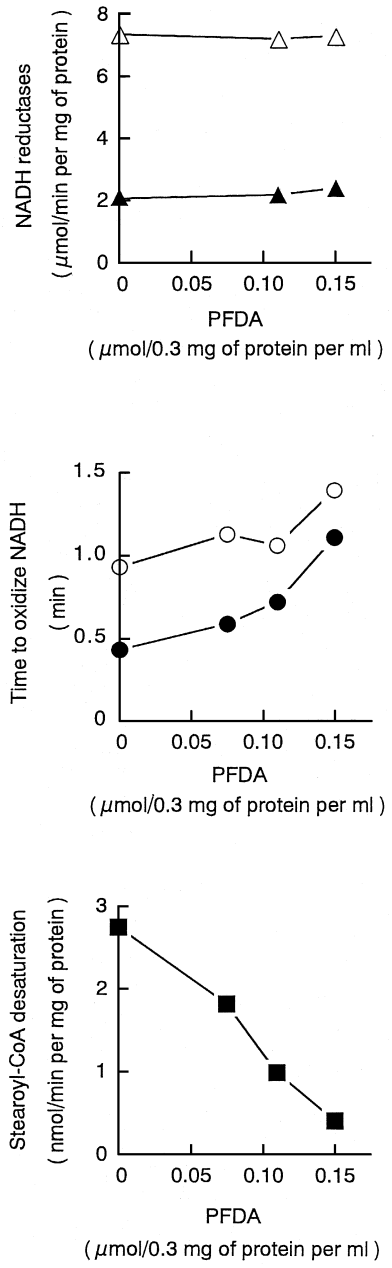

Figure 1 Effects of PFDA on the Δ^9-desaturation system *in vitro*

Microsomes isolated from starved/refed rats were diluted with 0.1 M Tris/HCl buffer (pH 7.4) to a concentration of 0.3 mg of protein/ml. To this microsomal suspension was added (using a microsyringe) PFDA dissolved in acetone, and then the mixture was preincubated at 30 °C for 2 min. The final concentration of acetone in the preincubation mixture was always 1% (v/v). NADH:cytochrome *c* reductase (▲) and NADH:ferricyanide reductase (△) activities were assayed employing 5 μg and 20 μg respectively of the microsomal protein in a final volume of 1 ml (top panel). To measure the time required to oxidize 2 nmol of NADH in the absence (○) and in the presence (●) of stearoyl-CoA (20 nmol), 0.9 mg of microsomal protein was used in a final volume of 3 ml (middle panel). Desaturation activity (bottom panel) was calculated from the difference between the times required to oxidize NADH in the presence and in the absence of stearoyl-CoA.

Table 2 Effects of PFDA feeding on the hepatic formation of oleic acid from [^{14}C]stearic acid *in vivo*

Rats were fed on either a control diet or a diet containing 0.005% (w/w) PFDA for 7 days. Rats were injected intravenously with 10 μCi of [^{14}C]stearic acid, and the livers were isolated 10 min after the injections. Each value represents the mean ± S.D. for three or four animals. Significant difference from controls: $^{***}P < 0.001$.

Dietary treatment	$10^{-6} \times$ ^{14}C incorporated into hepatic lipid (d.p.m./liver)	Conversion of stearic acid into oleic acid (%)	$10^{-6} \times$ Oleic acid formed (d.p.m./liver)
Control	13.5 ± 0.3	8.6 ± 0.5	1.15 ± 0.08
PFDA	14.0 ± 0.9	12.7 ± 0.9***	1.78 ± 0.13***

Table 3 Effects of PFDA feeding on the fatty acid composition of hepatic lipids

Rats were fed on either a control diet or a diet containing 0.005% (w/w) PFDA for 7 days. Each value represents the mean ± S.D. for four animals. Significant differences from controls: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Fatty acid	Content (mol/100 mol)	
	Control	PFDA-fed
$C_{16:0}$	28.8 ± 1.9	31.4 ± 1.1
$C_{16:1}$	3.1 ± 0. 5	5.8 ± 1.1**
$C_{18:0}$	17.1 ± 2.4	11.9 ± 0.2*
$C_{18:1}$	9.4 ± 1.5	21.5 ± 1.4***
$C_{18:2}$	17.7 ± 1.0	12.0 ± 1.7**
$C_{20:3,n-9}$	0.1 ± 0.0	0.2 ± 0.1
$C_{20:3,n-6}$	0.9 ± 0.1	2.0 ± 0.2***
$C_{20:4,n-6}$	13.6 ± 2.3	9.9 ± 0.7*
$C_{20:5,n-3}$	1.3 ± 0.3	0.9 ± 0.1*
$C_{22:5,n-3}$	1.1 ± 0.2	0.5 ± 0.0**
$C_{22:6,n-3}$	5.0 ± 0.9	2.8 ± 0.2**
Total (μmol/g of liver)	58.1 ± 7.4	95.1 ± 3.3***
Total (μmol/liver)	515 ± 61	1228 ± 59***

Table 4 Relationship between Δ^9-desaturase activity and the proportion of $C_{18:1}$ in hepatic microsomal lipids

The dietary treatments are described in the Materials and methods section. The activity of Δ^9-desaturase was assayed as a rate constant (k^+) for the stearoyl-CoA-stimulated re-oxidation of NADH-reduced cytochrome b_5. The proportion of $C_{18:1}$ in microsomal lipid was measured by GLC. Each value represents the mean ± S.D. for three or six animals. Regression analysis was performed on the five paired data sets shown, i.e. the proportion of $C_{18:1}$ in microsomal lipid against the activity of Δ^9-desaturase: $y = 6.71 + 1.41x$ ($r = 0.976$; $P < 0.01$).

Physiological state	k^+ for Δ^9-desaturase (min^{-1})	Proportion of $C_{18:1}$ (mol/100 mol)
Control	1.24 ± 0.34	7.4 ± 0.3
Starved	0.18 ± 0.11	6.4 ± 0.2
Starved/refed	10.82 ± 2.76	20.9 ± 1.7
Clofibric acid-fed	5.04 ± 0.50	15.8 ± 0.5
PFDA-fed	3.74 ± 0.29	12.6 ± 0.5

Effects of PFDA feeding on microsomal drug-metabolizing enzyme systems

The effects of PFDA feeding on another electron-transport system in hepatic microsomes were examined (Table 5). The level of cytochrome *P*-450, determined spectrophotometrically as the ferrous–CO complex, was increased 1.7-fold upon feeding with PFDA, although the activity of NADPH–cytochrome *c* (*P*-450) reductase was not changed substantially. To ascertain whether or

Table 5 Effects of PFDA feeding on the levels of microsomal drug-metabolizing enzyme systems in the liver

Rats were fed on either a control diet or a diet containing 0.005% (w/w) PFDA for 7 days. Each value represents the mean ± S.D. for four rats, and is expressed per mg of protein. Significant differences from controls: $^*P < 0.05$; $^{***}P < 0.001$.

		Activity (nmol/min per mg)			
Dietary treatment	Cytochrome *P*-450 content (nmol/mg)	NADPH:cytochrome *c* reductase	7-Ethoxycoumarin *O*-de-ethylase	Aminopyrine *N*-demethylase	Aniline *p*-hydroxylase
Control	1.23 ± 0.12	220 ± 20	2.02 ± 0.39	17.9 ± 4.1	0.72 ± 0.11
PFDA	2.07 ± 0.17***	260 ± 20*	3.33 ± 0.12***	19.2 ± 2.0	0.59 ± 0.03

not specific forms of cytochrome *P*-450 were affected by PFDA feeding, the metabolism of various model substrates was also assessed. The substrates studied were 7-ethoxycoumarin, aminopyrine and aniline. Feeding with PFDA increased by 1.6-fold the activity of 7-ethoxycoumarin *O*-de-ethylase, but did not affect the activities of either aminopyrine *N*-demethylase or aniline *p*-hydroxylase.

DISCUSSION

Microsomal stearoyl-CoA desaturation is known to be catalysed by a membrane-bound multicomponent enzyme system consisting of three components; this system requires molecular oxygen and electrons, which are transferred from NADH through cytochrome b_5 to a terminal desaturase [27]. It has been reported previously that the administration of a single high dose of PFDA resulted in an increase in the time required for the oxidation of a fixed amount of NADH by the microsomal desaturation system, leading to a marked suppression of stearoyl-CoA desaturation [10]. The present study shows that PFDA induced *in vitro* a marked prolongation of the time required for the oxidation of NADH by the desaturation system and for electron transport from NADH to cytochrome b_5. Thus PFDA appears to interfere with the interactions of the terminal desaturase with cytochrome b_5, stearoyl-CoA or molecular oxygen, so that Δ^9-desaturation is suppressed. To our knowledge, inhibition of Δ^9-desaturation by peroxisome proliferators (except for PFDA) has not been found previously. In contrast with the previous findings [10], however, the present results on the effects of PFDA *in vivo* on the Δ^9-desaturation system demonstrated that PFDA markedly increased overall desaturation activity by inducing the terminal desaturase, as was demonstrated previously for other peroxisome proliferators [2]. Thus the effects of PFDA on Δ^9-desaturation *in vivo* and *in vitro* were markedly different.

This discrepancy raised a question as to whether the conversion of stearic acid into oleic acid by Δ^9-desaturase *in vivo* in the livers of rats is enhanced by PFDA. The present study demonstrated that the increased formation of oleic acid from radiolabelled stearic acid took place in the livers of PFDA-fed rats, even though PFDA was inhibitory to Δ^9-desaturase *in vitro*. One possible reason for the discrepancy between the results obtained *in vivo* and *in vitro* is that PFDA entering hepatocytes may cause the induction of Δ^9-desaturase, but the concentration of PFDA near the desaturase on the endoplasmic reticulum is below the threshold capable of inhibiting it effectively. Since fatty-acid-binding protein, an abundant cytosolic protein with a high affinity for fatty acids [28], was shown to be induced by PFDA and to bind it [29], it is possible that the inhibitory effects of PFDA may be buffered by this protein within hepatocytes.

The increased level of $C_{18:1}$ in the livers of rats following the administration of a single high dose of PFDA has been suggested to be caused by mobilization from peripheral stores [8,9] and by the inhibition of peroxisomal β-oxidation [30]. In the present study, however, a linear correlation was confirmed between the proportion of $C_{18:1}$ in microsomal lipid and the activity of Δ^9-desaturase in microsomes isolated from the livers of rats in five different physiological states (control, starved, starved/refed, clofibric acid-fed and PFDA-fed). These results strongly suggest that the stearoyl-CoA desaturase induced by PFDA functions *in vivo* to supply $C_{18:1}$ to membrane lipid comprising organelles, as is observed in the other physiological states. It is unequivocal, therefore, that the hepatic level of $C_{18:1}$ is regulated by Δ^9-desaturase activity in rats treated with PFDA, as demonstrated by the nutritional manipulation of the animals.

In many studies on the biological effects of PFDA, the administration of a single high dose of PFDA to rodents has been employed [8–10,31]. Associated with PFDA treatment have been a progressive decrease in feed intake, body weight loss, hepatomegaly, alterations in hepatic lipid composition and suppression of Δ^9-desaturation and drug metabolism. In contrast with the previous findings [10,30], however, the present study showed that the dietary administration of PFDA at a relatively low dose caused the induction of not only Δ^9-desaturase, but also peroxisomal β-oxidation, cytochrome *P*-450 and 7-ethoxycoumarin *O*-de-ethylase, although these experiments cannot be compared directly with the previous studies [8–10,31] because of different protocols. Accordingly, the action of PFDA at a high concentration might not be due to a single effect, and the exact mechanisms behind the results obtained in studies employing a high dose of PFDA remain unclear.

In conclusion, PFDA, like other peroxisome proliferators, has the ability to induce Δ^9-desaturase, although unlike other peroxisome proliferators it is an inhibitor of Δ^9-desaturation. However, the induction of Δ^9-desaturase by PFDA regulates the hepatic $C_{18:1}$ content *in vivo*, which is consistent with the generally accepted understanding of the regulation by Δ^9-desaturase of the hepatic level of $C_{18:1}$.

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