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The mechanism underlying the hypolipemic effect of perfluorooctanoic acid (PFOA), perfluorooctane sulphonic acid (PFOSA) and clofibric acid

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The influence of the peroxisomal proliferators perfluorooctanoic acid (PFOA), perfluorooctane sulphonic acid (PFOSA) and clofibric acid on lipid metabolism in rats was studied. Dietary treatment of male Wistar rats with these three compounds resulted in rapid and pronounced reduction in both cholesterol and triacylglycerols in serum. The concentration of liver triacylglycerols was increased by about 300% by PFOSA. Free cholesterol was increased by both perfluoro compounds. Cholesteryl ester was reduced to 50% by PFOSA as well by clofibrate. In hepatocytes from fed rats, all the compounds resulted in reduced cholesterol synthesis from acetate, pyruvate and hydroxymethyl glutarate, but there was no reduction of synthesis from mevalonic acid. The oxidation of palmitate was also increased in all groups. The perfluoro compounds, but not clofibrate, caused some reduction in fatty acid synthesis. The activity of liver HMG-CoA reductase was reduced to 50% or less in all treatment groups and all three compounds led to lower activity of acyl-CoA:cholesterol acyltransferase (ACAT). Changes in other enzymes related to lipid metabolism were inconsistent. The present data suggest that the hypolipemic effect of these compounds may, at least partly, be mediated via a common mechanism: impaired production of lipoprotein particles due to reduced synthesis and esterification of cholesterol together with enhanced oxidation of fatty acids in the liver.

Introduction

Many hypolipemic drugs cause proliferation of peroxisomes and increase the activity of the peroxisomal β -oxidation in rats [1-3]. Chemically, these drugs constitute a heterogeneous group including clofibrate, tibric acid, niadenate and long-chain acylthioacetic acids, tiadenol, long-chain thia acids and MEDICA 16 [1-6]. It has been suggested that the increase in the fatty acyl-CoA oxidizing system contributes to the hypolipemic effect of these drugs [7,8]. The dominating mechanism underlying reduction of serum triacylglycerols and cholesterol by these drugs is, however, uncertain. Other mechanisms which may be important for the hypolipemic effect include; reduced hepatic synthesis of fatty acids and cholesterol [9-12]; reduced

triacylglycerol release by the liver [13,14]; increased rate of VLDL degradation [6]; increased uptake or reduced release of fatty acids by adipose tissue [15,16] and increased excretion of cholesterol into bile and feces [17].

Ikeda et al. [18,19] observed that perfluorooctanoic acid (PFOA) and perfluorooctane sulphonic acid (PFOSA) efficiently induced the peroxisomal β -oxidation in rats that were fed these compounds (0.02% in the diet). Just et al. [20] reported that the perfluorocarboxylic acids alter hepatic lipid metabolism and reduce serum lipid levels showing that these compounds also belong to the group of peroxisomal proliferators with hypolipemic effect. The perfluorinated compounds are particularly interesting since they obviously are not subject to ordinary metabolic modifications. The effects of these compounds must, therefore, be due to effects of the compounds per se.

Peroxisomal inducers may bring about the hypolipemic effect by affecting different steps in lipid metabolism. However, it seems likely that some important steps in lipid metabolism are common targets for these compounds. The unphysiological character of the perfluoro compounds makes it possible that their effect

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Abbreviations: PFOA, perfluorooctanoic acid; PFOSA, perfluorooctane sulphonic acid; ACAT, acyl-CoA:cholesterol acyltransferase; HMG, hydroxymethyl glutaric acid; T.T.A, tetra-decylthioacetic acid; VLDL, very-low-density lipoproteins.

on lipid metabolism follows a pattern that would make it easier to understand possible mechanism for the lipid reduction effect of peroxisomal proliferators.

In the search for a possible common mechanism underlying the hypolipemic effect of peroxisomal inducers, we have compared hepatic fatty acid metabolism, cholesterol synthesis and the activities of enzymes related to these metabolic processes in the liver of rats fed perfluorooctane sulphonic acid (PFOSA), perfluorooctanoic acid (PFOA) and clofibrate.

Methods and Materials

Animals

Male Wistar rats were used. They were divided into five groups. One group was allowed normal food ad libitum (control rats). Three other groups were given food which contained 0.3% clofibric acid, 0.02% perfluorooctanoic acid or 0.02% perfluorooctane sulphonic acid, respectively. The diets were prepared by soaking standard food (in pellet form) in diethylether in which the compounds had been dissolved. The fifth group was restricted in food intake to that consumed by the PFOSA group. The stock diet was used for these paired feeding experiments. The average body weight when the experiments started was 269 g and the average daily food consumption per rat in the different groups were; control: 23.7 g, clofibrate: 23.1 g, PFOA: 22.7 g, PFOSA: 20.0 g.

Materials

[2-¹⁴C]Mevalonic acid, [1-¹⁴C]pyruvic acid and [2-¹⁴C]pyruvic acid were from New England Nuclear. [1-¹⁴C]acetic acid was obtained from Amersham (UK). Clofibrate was from Fluka (Buchs, Switzerland). Perfluorooctanoic acid was purchased from Aldrich-Chemie (Steinheim, Germany) and Perfluoro-octane sulphonic acid was from Fluorochem (Old Glossop, UK). Other chemicals were from Sigma (St. Louis, MO, USA).

Preparation of hepatocytes

The effects of dietary treatment were studied in hepatocytes isolated from rats fed the diets for 1 week and for the study of direct effects of the compounds, hepatocytes were isolated from rats fed the standard diet. Isolation of hepatocytes was performed by perfusion with collagenase, according to Berry and Friend [21], with the modifications described by Seglen [22].

Oxidation of palmitate and conversion of labelled substrate into lipids

Fatty acid oxidation was measured according to Christiansen et al. [23] with 0.5 mM palmitate as substrate. Fatty acids and cholesterol synthesized from radioactive precursors were extracted from the cell suspension after 90 min incubation. The reaction was stopped by the addition of 5% saturated KOH in ethanol and the mixture was heated at 90°C for 1 h. The nonsaponifiable lipids were extracted with petroleum ether. The suspension was then acidified with HCl. The extracts were evaporated to dryness and the radioactive lipid residue was dissolved in 100 μ l hexane. The lipid extracts were chromatographed with hexane/diethyl ether/acetic acid (80:20:1) on silica gel thin-layer plates. The spots corresponding to cholesterol and fatty acids were identified by standards and were then isolated for measurement of radioactivity.

Enzyme assays and measurements of DNA, protein and lipids

Pyruvate dehydrogenase was estimated by measuring the ¹⁴CO₂ liberated when hepatocytes were incubated with 5 mM [1-¹⁴C]pyruvate for 30 min at 37°C. Acetate thiokinase was measured as described by Jones and Lipman [24]. Liver microsomes were prepared according to Easom and Zammit [25], and HMG-CoA reductase was measured according to Drevon et al. [26]. CDPcholine:1,2-diacylglycerol cholinephosphotransferase, EC 2.7.8.2 and lysolecithin acyltransferase

TABLE I

Body and liver weights and liver lipid content in rats fed different diets for 7 days

Clofibrate was given as 0.3% (w/w) and PFOA and PFOSA as 0.02% in the diet. Values are given as means $\pm$ S.E. There were four observations in each group. Fisher's *P*-values are given; * *P* < 0.05; ** *P* < 0.01 vs. control group.

	Control	Clofibrate	PFOA	PFOSA	Stock diet limited fed
Body weight (g)	305 $\pm$ 2	298 $\pm$ 3	288 $\pm$ 6 *	275 $\pm$ 6 **	282 $\pm$ 7 **
Liver weight (g)	11.3 $\pm$ 0.4	16.1 $\pm$ 0.8 **	18.8 $\pm$ 0.7 **	15.9 $\pm$ 0.7 **	
Liver triacylglycerols (μ mol/g liver)	4.1 $\pm$ 0.5	4.0 $\pm$ 0.7	3.9 $\pm$ 0.3	13.8 $\pm$ 18 **	4.1 $\pm$ 0.6
Liver non-esterified cholesterol (μ mol/g liver)	4.4 $\pm$ 0.4	4.5 $\pm$ 0.3	6.5 $\pm$ 0.4 *	7.6 $\pm$ 0.3 **	
Liver cholesterol ester (μ mol/g liver)	0.58 $\pm$ 0.08	0.24 $\pm$ 0.02 **		0.27 $\pm$ 0.03 *	

(EC 2.3.1.23) was measured as described by Parthasarathy et al. [27]. Acyl-CoA:cholesterol acyltransferase (ACAT) was measured according to Rustan et al. [28] and the synthesis of phosphatidylserine, phosphatidylethanolamine and phosphatidylcholine was measured as described by Vance [29]. Other enzymes were measured as described earlier [30]. DNA was measured by the method of Labarca and Paigen [31] and protein was estimated by the biuret method or by the method of Lowry et al. [32].

Liver lipids were extracted with chloroform/methanol (2:1, v/v). Triacylglycerols was determined directly on the dried extract with a kit method (Nycotest, Oslo, Norway). Free and esterified cholesterol was determined by Nycotest kit method for cholesterol (Nycotest) after separation of the extract on thin-layer chromatography. Serum cholesterol and triacylglycerols was measured directly by the kit methods.

Results

Table I shows that 0.02% PFOA or PFOSA in the diet resulted in a lower body weight after 7 days of feeding as compared with the control group. The clofibrate diet (0.3%) did not affect the rat weight. The group with restricted food intake to that of the PFOSA group had about the same weight as the PFOSA group. All the compounds resulted in a 40–60% increased liver weight. Similar effect on the liver weight has been observed earlier in rats fed clofibrate [9,10,33].

Changes in serum and liver lipids by clofibrate, PFOA and PFOSA

Fig. 1A shows that all three diets significantly reduced serum cholesterol. In all treatment groups, cholesterol was significantly reduced (to 50–70% of control) after 24 h. Dietary treatment for 2 weeks resulted in further cholesterol reductions by 70% or more. In agreement with other observations, fasting for 2 days did not bring about significant cholesterol changes.

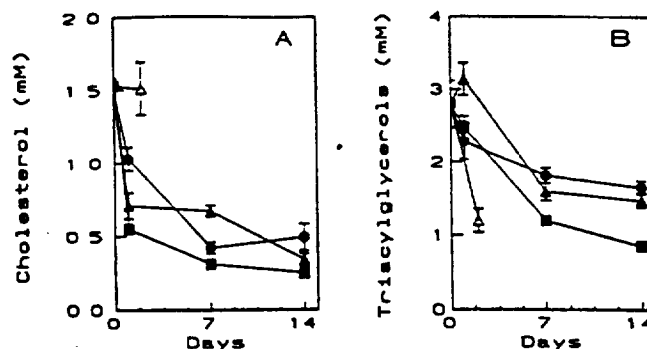


Fig. 1. Effect of clofibrate, PFOA and PFOSA on the serum lipids. (A) Cholesterol; (B) triacylglycerols; Δ, fasted; ●, 0.3% clofibrate; ▲, 0.02% PFOA; ■, 0.02% PFOSA. The data represent mean $\pm$ S.E. of four observations.

Fig. 1B shows the effects of clofibrate, PFOA and PFOSA on triacylglycerols in the rat serum. None of the compounds resulted in significant changes after 1 day of treatment. After 7 days, clofibrate and PFOA resulted in reduction to about 60% of control value and no further reduction was obtained with further treatment. PFOSA reduced triacylglycerols to about 50% and 30% of control value after 1 and 2 weeks of treatment. The significant reduction obtained by fasting for 2 days was as expected. In the rats fed stock diet but restricted to that consumed by the PFOSA group, the serum triacylglycerols was 2.43 ± 0.13 mM after 1 week of treatment (not shown) which is not significantly lower than observed in the control group (fed stock diet ad libitum).

Table I shows that PFOSA increased the liver triacylglycerols content to more than 3-times the control value. This effect of PFOSA on triacylglycerols was clearly in contrast to the effect of clofibrate and PFOA which did not affect the content of triacylglycerols and clearly indicates that PFOSA inhibits the excretion of triacylglycerols from the liver. Both PFOA and in particular PFOSA increased the liver content of non-esterified cholesterol. In contrast to this, a significant

TABLE II

Conversion of labelled substrates into ^{14}C -labelled cholesterol by hepatocytes of rats fed different diets for 7 days

Clofibrate was given as 0.3% (w/w) and PFOA and PFOSA as 0.02% in the diet. The concentration of $[2-^{14}\text{C}]$ mevalonate and $[3-^{14}\text{C}]$ hydroxy-methylglutarate was 0.5 mM. Incubations with $[1-^{14}\text{C}]$ acetate and $[2-^{14}\text{C}]$ pyruvate were conducted with 5 mM of the labelled substrates and included also unlabelled glucose (10 mM). Values are means $\pm$ S.E. There were four observations in each group. Fisher's P values are given * $P < 0.05$; ** $P < 0.01$.

Substrate	Formation of ^{14}C -labelled cholesterol (nmol substrate carbon $\cdot$ mg DNA $^{-1} \cdot$ h $^{-1}$)			
	control	clofibrate	PFOA	PFOSA
$[1-^{14}\text{C}]$ Acetate	147.6 $\pm$ 21.4	59 $\pm$ 13 **	37.6 $\pm$ 16.5 *	21.2 $\pm$ 7.4 **
$[2-^{14}\text{C}]$ Pyruvate	78.2 $\pm$ 14.6	46 $\pm$ 9.5 **	35.2 $\pm$ 9.7 *	26.5 $\pm$ 5.4 **
$[3-^{14}\text{C}]$ Hydroxy-methylglutarate	17.9 $\pm$ 3.3	3.6 $\pm$ 0.9 **	3.6 $\pm$ 1.3 **	5.0 $\pm$ 1.1 **
$[2-^{14}\text{C}]$ Mevalonate	2280 $\pm$ 150	2160 $\pm$ 280	1730 $\pm$ 240	2030 $\pm$ 310

TABLE III

Conversion of labelled substrates into ^{14}C -labelled fatty acids and oxidation of $[\text{U-}^{14}\text{C}]$ palmitate by hepatocytes of rats fed different diets for 7 days

The concentrations of compounds in the diet and the concentrations of $[\text{1-}^{14}\text{C}]$ acetate and $[\text{2-}^{14}\text{C}]$ pyruvate were as described in Table II. The concentration of $[\text{U-}^{14}\text{C}]$ palmitate was 0.5 mM. Values are means $\pm$ S.E. There were 4 observations in each group. Fisher's P values are given: * $P < 0.05$; ** $P < 0.01$ vs. control group.

Substrate	^{14}C -Labelled fatty acid formation (nmol substrate carbon $\cdot$ mg DNA $^{-1} \cdot$ h $^{-1}$)			
	control	clofibrate	PFOA	PFOSA
$[\text{1-}^{14}\text{C}]$ Acetate	252 $\pm$ 28	180 $\pm$ 28	90 $\pm$ 54 *	104 $\pm$ 28 **
$[\text{2-}^{14}\text{C}]$ Pyruvate	221 $\pm$ 21	322 $\pm$ 23 *	182 $\pm$ 55	128 $\pm$ 21 *
$[\text{U-}^{14}\text{C}]$ Palmitate oxidation (nmol substrate oxidized $\cdot$ mg DNA $^{-1} \cdot$ h $^{-1}$)				
	control	clofibrate	PFOA	PFOSA
	502 $\pm$ 50	726 $\pm$ 36 *	564 $\pm$ 63	861 $\pm$ 102 *

reduction (to approx. 50%) in esterified cholesterol in the treated rats (PFOA-treated rats not measured) was found.

Conversion of labelled substrates into cholesterol by hepatocytes of rats fed different diets

Table II shows the incorporation of labelled carbon atoms from differently labelled acetate, pyruvate, mevalonate and hydroxymethylglutaric acid (HMG). The major point emerging from this table is that the rate of cholesterol synthesis was significantly reduced from all substrates which are proximal to the HMG-CoA dehydrogenase step in all treatment groups. In contrast, cholesterol synthesis from mevalonate was not reduced in any of the groups.

Table III shows the synthesis of fatty acids from pyruvate and acetate. Both PFOA and PFOSA treat-

TABLE IV

Conversion of $[\text{1-}^{14}\text{C}]$ acetate into ^{14}C -labelled fatty acids and cholesterol by hepatocytes in the presence of hypolipemic compounds

The concentration of $[\text{1-}^{14}\text{C}]$ acetate was 5 mM and the incubations also included 10 mM glucose. Each incubation flask contained 4 mg cell protein and 1.5% albumin in 3 ml Krebs-Henseleit bicarbonate buffer. The concentration of hypolipemic compounds was 1 mM. Values are means $\pm$ S.E. There were four observations in each group. Fisher's P values are given ** $P < 0.01$ vs. control group.

Addition	Formation of ^{14}C -labelled cholesterol	Formation of ^{14}C -labelled fatty acid
	(nmol substrate carbon mg DNA $^{-1} \cdot$ h $^{-1}$)	
None	114 $\pm$ 8	313 $\pm$ 14
TTA	1.4 $\pm$ 0.07 **	7.5 $\pm$ 1.3 **
Clofibric acid	57 $\pm$ 0.7 **	243 $\pm$ 8 **
PFOA	63 $\pm$ 11 **	611 $\pm$ 20 **
PFOSA	65 $\pm$ 7 **	1908 $\pm$ 29 **

ment reduced lipid synthesis (not significant from pyruvate in the PFOA group). No reduction in fatty acid synthesis was found in hepatocytes from clofibrate-treated animals. The table further shows that the peroxisomal inducers, as expected, resulted in an increased rate of palmitate oxidation (although PFOA did not result in a significant increase). The experiments do not exclude the possibility that intracellular content of the tested compounds might have a direct (reversible) effect on fatty acid and cholesterol synthesis *in vivo*. Such effects could have escaped our detection since the compounds might have been washed out during the cell isolation procedure. To test possible direct inhibitory effects of these drugs, the synthesis of fatty acids and cholesterol in hepatocytes with additions of the compounds to the incubation medium were performed (Table IV). The table shows that 1 mM of clofibric acid, PFOA and FPOSA inhibited the chole-

TABLE V

Activity of enzymes related to the synthesis of cholesterol and fatty acids in liver of rats fed different diets for 7 days

The concentrations of compounds in the diet were as described in Table I. Values are given as means $\pm$ S.E. There were four observations in each group. * $P < 0.05$; ** $P < 0.05$; *** $P < 0.01$ vs. control group.

Enzyme	Activity ($\mu\text{mol} \cdot (\text{mg DNA}^{-1} \cdot \text{min}^{-1})$)			
	control	clofibrate	PFOA	PFOSA
Pyruvate dehydrogenase	0.59 $\pm$ 0.05	0.58 $\pm$ 0.04	0.42 $\pm$ 0.08	0.36 $\pm$ 0.04 *
Citrate synthase	0.97 $\pm$ 0.12	0.94 $\pm$ 0.06	0.80 $\pm$ 0.05	1.10 $\pm$ 0.29
ATP-citrate lyase	1.54 $\pm$ 0.34	0.78 $\pm$ 0.38	0.75 $\pm$ 0.17 *	0.24 $\pm$ 0.06 **
Acetate thiokinase	8.7 $\pm$ 0.64	5.7 $\pm$ 0.85	3.5 $\pm$ 0.5 **	5.7 $\pm$ 0.28 **
Malate dehydrogenase (NADP) (decarboxylating)	0.36 $\pm$ 0.05	0.83 $\pm$ 0.05 **	1.34 $\pm$ 0.05 **	0.21 $\pm$ 0.01
Malate dehydrogenase	27.1 $\pm$ 3.2	18.4 $\pm$ 2.4 *	20.7 $\pm$ 2.6	19.6 $\pm$ 3.0
Glucose-6-phosphate dehydrogenase	0.50 $\pm$ 0.10	0.17 $\pm$ 0.03 *	0.23 $\pm$ 0.04 *	0.11 $\pm$ 0.01 **
Isocitrate dehydrogenase (NADP)	2.44 $\pm$ 0.35	2.71 $\pm$ 0.56	2.73 $\pm$ 0.19	3.23 $\pm$ 0.13
	(nmol $\cdot$ $\mu\text{g protein}^{-1} \cdot \text{min}^{-1}$)			
HMG-CoA reductase	0.31 $\pm$ 0.03	0.16 $\pm$ 0.04 *	0.15 $\pm$ 0.05 *	0.11 $\pm$ 0.01 **

terol synthesis to the same extent (approx. 50%). Surprisingly, tetradecylthioacetic acid (TTA), another inducer of peroxisomal β -oxidation with hypolipemic effect in rats [3,4], almost completely inhibited cholesterol synthesis. The pronounced inhibitory effect of TTA on the fatty acid synthesis observed by Skrede et al. [34] was also confirmed. Clofibric acid reduced the fatty acid production by about 20%. However, a direct inhibitory effect on the fatty acid synthesis is not a property shared by all the tested compounds. Both perfluorinated compounds unexpectedly stimulated the rate of fatty acid synthesis strongly. It is unlikely that this was due to an inhibition of Krebs cycle with a concomitant increase in lipogenic precursors, since these compounds inhibited the cholesterol synthesis. It seems more likely that the compounds stimulate a rate-limiting enzyme, e.g., acetyl-CoA carboxylase. At lower concentrations (0.5 and 0.1 mM), there were very small inhibitory effects of clofibric acid, PFOA and PFOSA (data not shown).

Enzymes related to the synthesis of cholesterol and fatty acids from pyruvate and acetate

Table V shows that pyruvate dehydrogenase, citrate synthase and acetate thiokinase were only slightly affected by treatment with the three compounds. ATP-citrate lyase activity was reduced to about 15% of control values by PFOSA. PFOA reduced the activity of this enzyme significantly to 50%. The effect of the compounds on three NADPH-generating enzymes shows a remarkable pattern. All three compounds significantly reduced the activity of glucose-6-phosphate dehydrogenase. PFOSA reduced the activity to 20% of control values. In contrast, isocitrate dehydrogenase was unaffected by all three compounds. The activity of malic enzyme was increased 2- and 3.5-fold by clofibrate and PFOA, respectively. Malate dehydrogenase, which is not specifically involved in lipid synthesis, was virtually unchanged by any of the compounds. The

activity of HMG-CoA reductase, the rate-limiting and regulated step in cholesterol synthesis, was reduced to 50% or less in all three treatment groups.

Enzymes related to the synthesis of cholesteryl ester and phospholipids in rats fed different diets

Table VI shows that acyl-CoA: cholesterol acyltransferase (ACAT), was significantly downregulated by all three compounds. PFOSA, which had the strongest effect, reduced the activity to one third of control. The downregulation of this enzyme is in keeping with the reduction of liver cholesterol ester (Table I). The table further shows that the activities of two enzymes important in the phospholipid turnover, acyl-CoA:1-acylglycerol-3-phosphocholine acyltransferase and CDP-choline:1,2-diacylglycerol cholinephosphotransferase, were not significantly altered in the rats fed any of the hypolipemic drugs. The activity of phosphatidylserine synthase, phosphatidylethanolamine synthase and phosphatidylcholine synthase were also unaffected by any of the dietary regimes utilized (not shown).

Activities of enzymes related to phospholipid synthesis in the presence of hypolipemic drugs

Table VII shows that at 0.5 mM clofibric acid had little effect on activities of the enzymes listed in the table. The five enzymes were all inhibited by both perfluorinated compounds. PFOA had a particular inhibitory effect on phosphatidylserine synthase activity which was reduced to 18% of normal with 0.5 mM PFOA. PFOSA had strongest inhibitory effect on the activity of CDP-choline:1,2-diacylglycerol cholinephosphotransferase, phosphatidylserine synthase and phosphatidylethanolamine synthase, which were reduced to 14%, 13% and 28% of control, respectively with 0.5 mM PFOSA. At lower concentration (0.1 mM) the inhibitory effects of the perfluorinated compounds were very moderate (data not shown). Clofibric acid had no significant effect at 1 mM concentration.

TABLE VI

Activity of enzymes related to synthesis of cholesteryl esters and phospholipids in livers of rats fed hypolipemic drugs for 7 days

The concentrations in the diet were as described in Table I. Values are given as means $\pm$ S.E. There were four observations in each group.

** $P < 0.01$ vs. control group.

Enzyme	Control	Clofibrate	PFOA	PFOSA
Acyl-CoA Cholesterol acyltransferase (ACAT) (nmol/mg protein per min)	660 $\pm$ 51	427 $\pm$ 18 **	323 $\pm$ 39 **	237 $\pm$ 41 **
Acyl-CoA:1-acylglycerol-3-phosphocholine acyltransferase (nmol/mg protein per min)	14.0 $\pm$ 1.3	12.4 $\pm$ 1.0	12.7 $\pm$ 1.3	11.8 $\pm$ 1.8
CDP-choline:1,2-diacylglycerol cholinephosphotransferase (nmol/mg protein per min)	10.4 $\pm$ 1.3	9.1 $\pm$ 1.2	7.6 $\pm$ 0.7	8.6 $\pm$ 1.0

TABLE VII

Activities of enzymes related to phospholipid synthesis in the presence of hypolipemic drugs

Concentrated solutions (10 mM) of clofibric acid, PFOA and PFOSA in DMSO were diluted to 0.5 mM final concentration in assay system (The controls were added the same amount of pure DMSO.) The effect of the drugs on each of the enzymes were tested four times and in each of the experiments, the activities were calculated as per cent of the control value. Values are given as means $\pm$ S.E.

	Control	Clofibric acid	PFOA	PFOSA
Acyl-CoA lysophosphatidyl transferase	100	90 $\pm$ 6	56 $\pm$ 7	61 $\pm$ 5
CDP-choline: 1,2-diacylglycerol cholinephosphotransferase	100	95 $\pm$ 5	71 $\pm$ 2	14 $\pm$ 0.6
Phosphatidylserine synthase	100	101 $\pm$ 9	18 $\pm$ 4	13 $\pm$ 1.4
Phosphatidylethanolamine synthase	100	101 $\pm$ 3	47 $\pm$ 5	28 $\pm$ 2
Phosphatidylcholine synthase	100	89 $\pm$ 3	47 $\pm$ 5	48 $\pm$ 3

The effect of the compounds on the activity of ACAT was tested only at 100 μ M and with this concentration the ACAT activity was unaffected by the compounds (not shown).

Discussion

Reduction of the steady-state levels of serum lipids may be visualized as the result of downregulation of lipid synthesis or increased clearance from plasma. The aim of this study was to evaluate the effects of three different peroxisomal proliferators on a series of enzymes involved in hepatic lipid synthesis. The most important observations with all three compounds was downregulation of HMG-CoA reductase, the rate-limiting enzyme of cholesterol synthesis and of cholesterol esterification enzyme (ACAT). The agreement between the observed alterations in the enzyme activities and production of cholesterol and lipids in intact liver cells provides some support for the assumption that such enzyme measurements do reflect real changes in metabolic activity in the intact organ and shed light on the mechanism of the hypolipemic effects of the agents investigated in this study. These compounds do not show a correlated effect on the lipogenic and cholesterologenic pathways in the *in vivo* experiments. The *in vitro* experiments indicate that there are direct, presumably reversible, effects on these pathways. These effects are different than those observed after dietary manipulation. The latter probably represent changes in enzyme concentrations since, at least, the changes in the cholesterologenic pathway are correlated with the changes in HMG-CoA reductase.

The perfluorinated compounds utilized in this study are strong local irritants. The observed actions should not be regarded as mere unspecific toxic effects since,

in addition to reduction of the activity of some enzyme systems, these compounds increase the activity of other enzymes, e.g., enzymes of the peroxisomal fatty acid β -oxidation system [18,19]. However, local irritation may explain the reduction of food intake and slower weight increase observed after feeding perfluorinated compounds. However, this probably contributes little to the reduction in serum triacylglycerols, since no significant reduction was observed in rats with restricted food intake. Increased liver weight correlates well with earlier studies on peroxisomal proliferators [35,36].

Cholesterol and fatty acid synthesis

The reduction of cholesterol synthesis from different labelled substrates fits well with the reduced activity of HMG-CoA reductase observed in this study. Three substrates, proximal to the reductase step, were incorporated into cholesterol at a reduced rate whereas no reduction from mevalonate was observed in any of the treatment groups. Lowering of HMG-CoA reductase levels by clofibrate is in accord with other observations [9,37,38]. Even though the three compounds tested in this study reduce the HMG-CoA reductase activity, this effect may not be a characteristic of all peroxisomal inducers. MEDICA 16, another compound in this group, does not act via an effect on this reductase [12,39], but rather inhibits the synthesis of cholesterol at a step distal to HMG-CoA reductase. Our observations with clofibrate are at variance with the data obtained by Azarnoff et al. [10] who found that cholesterol synthesis from mevalonic acid was reduced in livers of rats fed clofibrate.

In contrast to the inhibitory effect of MEDICA 16 on ATP-citrate lyase [39], clofibric acid, PFOA and PFOSA had essentially no direct effect on ATP-citrate lyase at 1 mM (data not shown), but the drugs down-regulated the enzyme after dietary administration. This effect may contribute to reduced synthesis of cholesterol *in vivo*, since the enzyme is important in the main pathway for cholesterol precursor synthesis. It is interesting that all three compounds reduced one of the NADPH generating enzymes (glucose-6-phosphate dehydrogenase), while isocitrate dehydrogenase (NADP) was unchanged. Reduced capacity for NADPH generation can therefore hardly contribute to reduction in serum lipid levels.

The synthesis of fatty acids from acetate or pyruvate was not reduced in hepatocytes from rats fed clofibrate (Table III), but was significantly reduced in the hepatocytes from rats fed PFOSA. The reduced rate of fatty acid synthesis in the PFOA and PFOSA groups is probably not related to reduction in serum triacylglycerols. In rats fed PFOSA, there was accumulation of liver triacylglycerols. The lack of reduction of fatty acid synthesis by clofibrate suggests that other pro-

cesses in lipid metabolism must be more essential for the hypolipemia. Tomarelli et al. [40] found increased synthesis of lipids from acetate in rats fed clofibric acid and also in rats fed the very potent hypolipemic compound WY-14,643.

Synthesis of cholesteryl ester and phospholipids

Liver exports lipids to other organs mainly as VLDL particles. In addition to apolipoproteins, triacylglycerols and free cholesterol, these particles consist mainly of esterified cholesterol and phospholipids. Hence, reduced hepatic synthesis of these components might lead to reduced transport of lipids from the liver.

The hepatic content of cholesteryl ester was reduced both in clofibrate and PFOSA fed rats (PFOA fed rats were not tested) even where free cholesterol was not reduced (Table I). Similar effect was obtained by Avigan et al. [41] with the hypolipemic drug MER-29. Reduced cholesteryl ester production is most likely a result of downregulation of the ACAT activity which was observed in all treatment groups. A direct inhibitory effect on the enzyme by the compounds is probably of less importance since 100 μ M of the compounds did not affect the enzyme activity (higher concentrations were not tested). Vance [29] argued that synthesis of phospholipids may be limiting for lipoprotein synthesis. In this study, we have not observed any downregulation of enzymes involved in the synthesis or metabolism of phospholipids. We have observed only small direct effects of clofibric acid on these enzymes. It seems likely, however, that reduced phospholipid synthesis plays a role in the lipid-reducing effect of the perfluorinated compounds, since these had direct inhibitory effect on several important enzymes. Parthasarathy et al. [27] suggested from their studies that inhibition of phosphatidyl-choline synthesis, particularly by the lysolecithin acyltransferase pathway, may be related to a drug's effectiveness in decreasing serum lipids. We found that the transferase was moderately inhibited by the perfluorinated compounds. We also observed that this enzyme was virtually unaffected by 0.5 mM (Table VII) and also by 1 mM of clofibric acid. This agrees with the data reported by Parthasarathy et al. [27]. Our data do not support the hypothesis that reduced activity of lysolecithine acyltransferase plays a central role in the hypolipemic effect of the compounds we have tested. Inhibition of other enzymes of phospholipid synthesis may play a role.

Reduced release of lipids from the liver

In earlier studies, it has been reported that clofibrate reduces the release of lipids from the liver [13,14]. A direct effect on the excretion process by clofibrate was not confirmed by accumulation of liver triacylglycerols in clofibrate-fed, nor in PFOA-fed animals. A

reduced release of lipids from liver was probably an effect of PFOSA, however, since the compound increased liver triacylglycerols by about 200% in spite of reduced rate of fatty acid synthesis (Table IV).

Since interference with the synthesis of cholesteryl ester may cause reduced hepatic lipid output [42], it may be concluded that reduction in cholesterol synthesis and esterification due to downregulation of HMG-CoA reductase and ACAT together with enhanced fatty acid oxidation in the liver, are effects caused by clofibric acid as well as by the perfluorinated compounds. This may reduce VLDL production by liver which plays a central role after the postprandial chylomicronemic period during which the liver is the dominating organ for delivery of lipids to serum.

In addition, the different hypolipemic drug may act by inhibiting the synthesis of other lipoprotein components such as phosphatidylcholine [27].

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