Induction of 1-Acylglycerophosphocholine Acyltransferase Genes by Fibrates in the Liver of Rats

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The effect of fibrates (clofibric acid, bezafibrate and fenofibrate) on the gene expression and activity of 1-acylglycerophosphocholine acyltransferase (LPCAT) was investigated. The administration of 0.1% (w/w) clofibric acid, bezafibrate or fenofibrate in diet for 14 d to rats induced LPCAT activity in hepatic microsomes in the following order: fenofibrate>bezafibrate>clofibric acid. The LPCAT induced by fenofibrate preferred to arachidonoyl-CoA and linoleoyl-CoA to a greater extent than did LPCAT in control microsomes. The treatment with the fibrates resulted in upregulation of the relative expression of mRNAs encoding LPCAT3 and LPCAT4 in the following order: fenofibrate>bezafibrate>clofibric acid. The administration of fibrates did not change the expression of genes encoding either LPCAT1 or LPCAT2. The treatment with fibrates elevated relative levels of both mRNAs encoding Δ6 desaturase (Fads2) and Δ5 desaturase (Fads1) in the order of fenofibrate>bezafibrate>clofibric acid, and the extent of the increase in the level of Δ6 desaturase mRNA was greater than that of Δ5 desaturase. Fatty acid profile in hepatic phosphatidylcholine (PC) was significantly changed by the treatments with fibrates. These results suggest (i) that fibrates induce LPCAT activity in hepatic microsomes by elevating the expression of genes encoding LPCAT3 and LPCAT4, (ii) that the changes in fatty acid profile of hepatic PC are, in part, due to the elevated expression of two isoforms, LPCAT3 and LPCAT4, and (iii) that the ability of fibrates to induce these changes are in the order of fenofibrate>bezafibrate>clofibric acid.

Key words 1-acylglycerophosphocholine acyltransferase; clofibric acid; bezafibrate; fenofibrate; rat liver

2-(4-Chlorophenoxy)-2-methylpropionic acid (clofibric acid), a hypolipidemic drug, is well known to enhance fatty acid degradation through inducing the proliferation of peroxisomes and fatty acid *β*-oxidation in the liver.[1)] In addition to lipid degradation, clofibric acid (or clofibrate) affects lipid biosynthesis of animals through the enzymes such as stearoyl-CoA desaturase (SCD),[2,3)] palmitoyl-CoA elongase,[4,5)] acyl-CoA synthetase,[5)] glycerophosphate acyltransferase,[6)] 1-acylglycerophosphate acyltransferase,[5)] CoA-dependent transacylase[7)] and 1-acylglycerophosphocholine acyltransferase (LPCAT).[8,9)] Of these enzymes, LPCAT is of particular interest in relation to the acyl composition of membrane phospholipid. Namely, the primary physiological role of LPCAT is considered to be generation of phosphatidylcholine (PC) having an unsaturated fatty acid at the *sn*-2 position.[10)] A previous study showed that the treatment of rats with clofibric acid caused a marked alteration in acyl composition of PC[5)] and, consequently, in the composition of molecular species of PC in the liver.[11)] The clofibric acid-caused changes in acyl composition of hepatic PC were demonstrated to be the result of the inductions of SCD and LPCAT in the liver.[5,12,13)] Although studies focusing on the effect of clofibric acid on the induction of LPCAT (gene expression and enzymatic activity) have been reported, little information is available about the effect of other fibrates such as bezafibrate and fenofibrate on the alteration of LPCAT gene expression. The present study aimed to estimate the effect of three fibrates, clofibric acid, bezafibrate and fenofibrate, on the induction of LPCAT genes in the liver of rats.

MATERIALS AND METHODS

Materials Bezafibrate, clofibric acid, fenofibrate, arachidonoyl-CoA, linoleoyl-CoA and oleoyl-CoA were obtained from Sigma (St. Louis, MO, U.S.A.), 1-acylglycerophosphocholine (LPC) was from Avanti Polar Lipids Inc. (Alabaster, AL, U.S.A.). All other chemicals were of analytical grade.

Animals All animal studies complied with the recommendations of the Institutional Board for Animal Studies, Josai University. Seven-week-old male Wistar rats were obtained from SLC (Hamamatsu, Japan). After acclimatization, rats were fed on a standard diet (CE-2, Clea Japan) or a diet admixed with 0.1% (w/w) clofibric acid, bezafibrate or fenofibrate for 14 d. Under diethyl ether anesthesia, livers were excised. One part of the liver was frozen in liquid nitrogen and stored at −80°C for the determination of mRNA. The other part of the liver was perfused with ice-cold saline and homogenized with 4 volumes of 0.25 M sucrose–1 mM ethylenediaminetetraacetic acid–10 mM Tris–HCl (pH 7.4), and was used for preparing microsomes. Microsomes from the liver were prepared by differential centrifugations as described previously.[14)] Protein concentrations were determined by the method of Lowry *et al.*[15)] with bovine serum albumin (Sigma) as a standard.

Measurement of LPCAT Activity LPCAT activity was measured spectrophotometrically on the basis of the reaction of liberated CoA with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) by utilizing oleoyl-CoA, as described previously.[9)] The reaction mixture for LPCAT assay contained 20–30 nmol oleoyl-CoA, 150 nmol LPC, 1 μmol DTNB, 100 μmol Tris–HCl buffer (pH 7.4) and 50–75 μg (depending on microsomal activity) of microsomal protein in a final volume of 1 mL. After the

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preincubation for 2 min in the absence of oleoyl-CoA, the incubation was initiated by adding oleoyl-CoA, and the increase in absorbance at 412 nm was followed at 30°C. Control value without LPC was subtracted to obtain net acyl transfer rate.

Lipid Analyses Total lipid was extracted from the liver by the method of Bligh and Dyer.[16] Phospholipids were separated by thin-layer chromatography on silica gel G plates (Merck, Darmstadt, Germany), which were developed with a solvent system comprised of chloroform–methanol–acetic acid–water (50 : 37.5 : 3.5 : 2, v/v). After visualization by spraying 0.001% (w/v) primuline in acetone, the regions on the plates that corresponded to PC were scraped off and transferred to tubes. The lipids were extracted from silica gel with chloroform–methanol–0.1 M HCl (4 : 4 : 1, v/v).[14] Methyl esters of fatty acids were prepared from each extract using sodium methoxide in methanol. All solvents employed for lipid analyses contained 0.005% (w/v) butylated hydroxytoluene. The composition of the fatty acid methyl esters was determined by gas–liquid chromatography (Shimadzu GC-2014) equipped with a frame ionization detector by using a flexible fused silica capillary column (SLB-IL100, 30 m×0.32 mm internal diameter: film thickness 0.26 μm, Sigma-Aldrich) with helium gas as a carrier gas. Initial column temperature was 120°C for 5 min, after which the temperature was increased 3°C per min to a final temperature of 230°C. The injection port temperature was 240°C and a flame ionization detector was used at 240°C.

Reverse Transcription and Quantitative Real-Time Polymerase Chain Reaction (Real-Time PCR) Total RNA was prepared from the liver using QIAzol reagent and RNeasy kit (QIAGEN, Hilden, Germany). cDNA was synthesized from 500 ng of total RNA with avian myeloblastosis virus reverse transcriptase (Takara, Shiga, Japan). Real-time PCR experiments were carried out using SYBR premix ExTaq (TaKaRa). The amplification and detection were performed with an ABI PRISM 7500 sequence detection system (Applied Biosystems, Foster City, CA, U.S.A.). The thermal cycling program was as follows: 10 s denaturation step at 95°C followed by 50 cycles of 5 s denaturation at 95°C, 34 s annealing and extension at 60°C. After the reaction, dissociation curve analyses were carried out to confirm the amplification of a single PCR product.

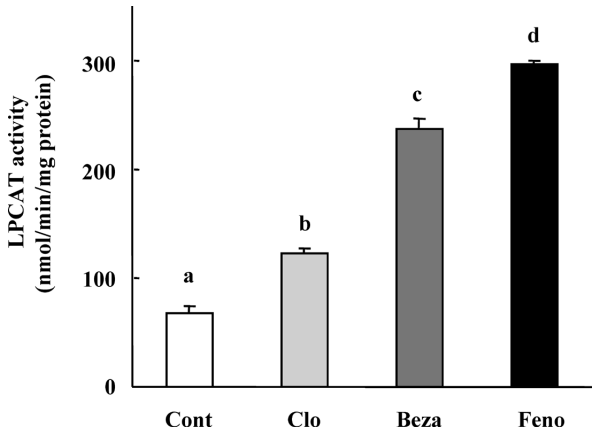

Fig. 1. Effects of Fibrates on the LPCAT Activity in the Liver of Rats

Rats were fed on a standard diet or a diet admixed with 0.1% (w/w) clofibric acid, bezafibrate or fenofibrate for 14 d. LPCAT activities were assayed by using oleoyl-CoA as a substrate. Cont, control; Clo, clofibric acid; Beza, bezafibrate; Feno, fenofibrate. Values are mean±S.D. for four rats. [a–d] Differences in the mean without a common superscript are statistically significant ($p<0.05$).

Changes in gene expression were calculated by using the comparative threshold cycle (C_t) method. C_t values were first normalized by subtracting the C_t value obtained from β-actin (control). The sequences of primers used in this study are listed in Table 1.

Statistical Analysis Homogeneity of variance was established using one-way analysis of variance. When a difference was significant ($p<0.05$), Scheffé's multiple range test was used as a *post hoc* test. The results were considered to be significant if the value of p was <0.05.

RESULTS

Elevation of Hepatic LPCAT Activity by Fibrates LPCAT activities in hepatic microsomes of rats that were fed on diets containing 0.1% (w/w) fibrates for 14 d were estimated (Fig. 1). The three fibrates significantly increased LPCAT activities, with the magnitude of 4.4-fold for fenofibrate, 3.5-fold for bezafibrate and 1.8-fold for clofibric acid.

Induction of mRNA for LPCAT Isoforms by Fibrates

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

	Primer sequence (5′–3′)	Accession No.
AOX	F: TTCGTGCAGCCAGATTGGTAG	NM_017340
	R: CGGCTTTGTCTTGAATCTTGG	
Fads1	F: TACAGGCAACCTGCAACGTTC	NM_053445
	R: GGTGCCACCTTGTGGTAGTTGT	
Fads2	F: GCCACTTAAAGGGTGCCTCC	BC081776
	R: TGCAGGCTCTTTATGTCGGG	
LPCAT1	F: CTCCTGAGGATGGCAGCATAGA	NM_001100735
	R: TCAATAGCCTGGAACAAGTCGG	
LPCAT2	F: TTTCATCCAGCTGTGTGTGCTC	XM_001064713
	R: TGCTTGCACAGGCATAAACTCA	
LPCAT3	F: TTTCTGGTTCCGCTGCATGT	BC089869
	R: CCGACAGAATGCACACTCCTTC	
LPCAT4	F: TTCGGTTTCAGAGGATACGACAA	NM_001108016
	R: AATGTCTGGATTGTCGGACTGAA	
β-Actin	F: TGCAGAAGGAGATTACTGCC	V01217
	R: CGCAGCTCAGTAACAGTCC	

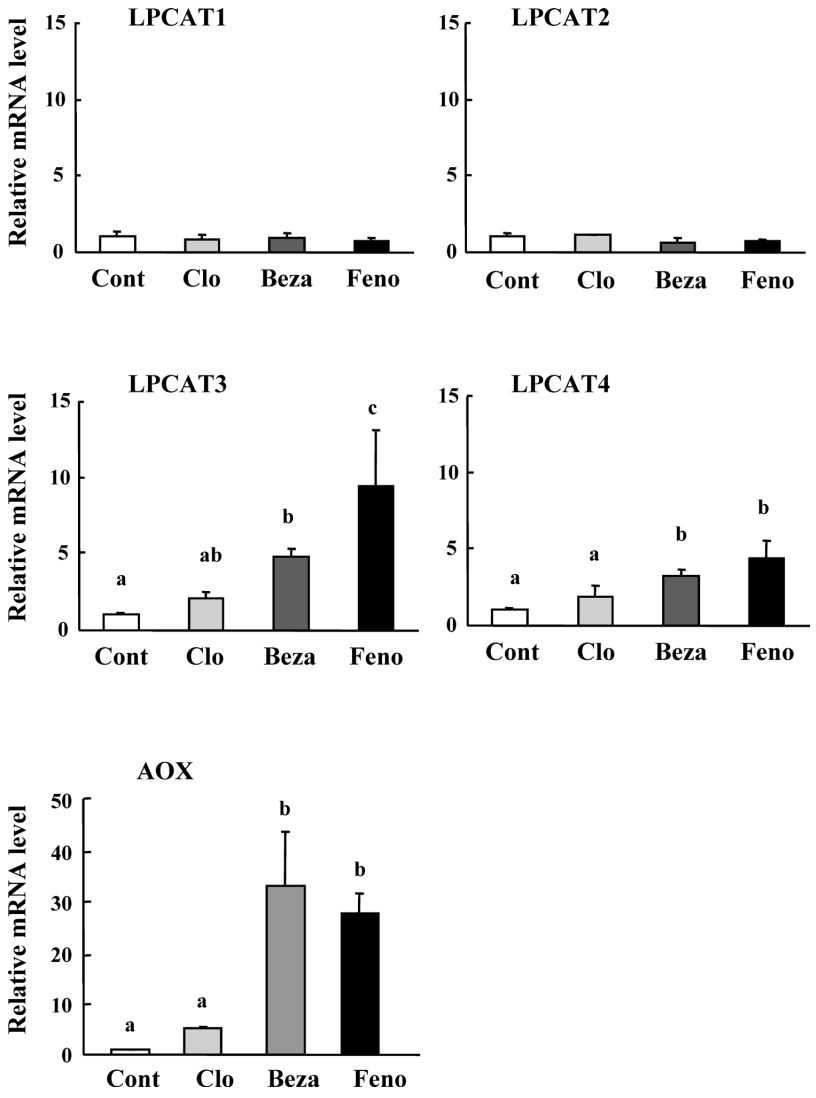

Fig. 2. Effects of Fibrates on the Levels of mRNAs Encoding LPCATs in the Liver of Rats

Rats were fed on a standard diet or a diet containing 0.1% (w/w) clofibric acid, bezafibrate or fenofibrate for 14 d. Cont, control; Clo, clofibric acid; Beza, bezafibrate; Feno, fenofibrate. Values are mean±S.D. for four rats. [a–c] Differences in the mean without a common superscript are statistically significant ($p<0.05$).

We next determined the mRNA levels of LPCAT isoforms in the liver of rats treated with the fibrates (Fig. 2). Treatments of rats with fenofibrate increased the relative expression of mRNA encoding LPCAT3 by 9.5-fold. The treatment with bezafibrate also increased LPCAT3 mRNA expression by 4.8-fold. The administration of fenofibrate and bezafibrate induced increases of 4.4-fold and 3.2-fold, respectively, of the levels of mRNA encoding LPCAT4. Although the treatment with clofibric acid tended to induce both LPCAT3 and LPCAT4 mRNA expression, the increases were not significant. The fibrates used in this study did not affect the relative expression levels of mRNAs encoding either LPCAT1 or LPCAT2. Fenofibrate and bezafibrate markedly increased mRNA levels for acyl-CoA oxidase (AOX), whereas clofibric acid less effectively induced the expression of AOX gene. The relationships of relative expression levels of mRNAs between AOX and LPCATs were examined (Fig. 3). No significant correlations were observed between AOX and either LPCAT3 or LPCAT4 (Figs. 3A, B). On the other hand, significant correlation was observed between the relative expression levels of mRNAs between LPCAT3 and LPCAT4 (Fig. 3C).

Effects of Fibrates on the Fatty Acid Profile of PC in the Liver The effects of fibrates on fatty acid composition in PC in the liver of rats treated with fibrates were estimated (Table 2). The treatment with the three fibrates increased the proportions of oleic (18 : 1n-9) and 8,11,14-eicosatrienoic (20 : 3n-6) acids and decreased the proportions of linoleic (18 : 2n-6) and *cis*-vaccenic (18 : 1n-7) acids. Moreover, the increase in the proportion of palmitic acid (16 : 0) and the decrease in that arachidonic acid (20 : 4n-6) were brought about to a lesser extent than those observed with 18 : 1n-9 and 18 : 2n-6. These alterations in fatty acid profile in PC were common to the three fibrates, but the extent of the changes produced by fenofibrate was similar to that by bezafibrate and was greater than that by clofibric acid (Fig. 4).

Maximal reaction velocities of LPCAT for linoleoyl-CoA and arachidonoyl-CoA relative to that for oleoyl-CoA in microsomes of fenofibrate-treated rats were 1.46 and 2.09 fold, respectively, higher than those of control rats (Table 3). Moreover, the treatment of rats with fenofibrate and bezafibrate elevated the relative levels of fatty acid desaturase (Fads)1 mRNA encoding Δ5 desaturase, which converts 20 : 3n-6 to

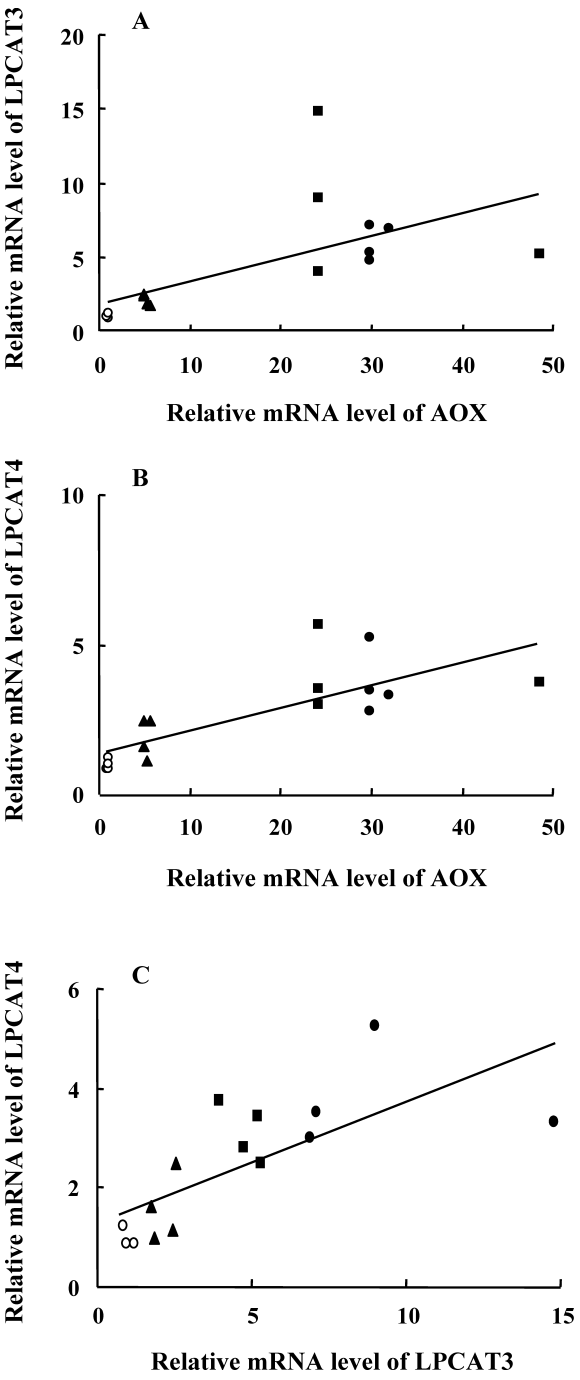

Fig. 3. Relationship between the Relative mRNA Levels of AOX, LPCAT3 and LPCAT4

Regression analysis was performed on the data from relative expression levels of mRNA encoding LPCAT3, 4 and AOX from the data in Fig. 2. (A) The mRNA levels of LPCAT3 *versus* AOX, $Y=0.1529X+1.8604$, $r^2=0.3715$; $p>0.05$. (B) The mRNA levels of LPCAT4 *versus* AOX, $Y=0.0756X+1.3865$, $r^2=0.5914$; $p>0.05$. (C) The mRNA levels of LPCAT3 *versus* LPCAT4, $Y=0.2482X+1.3147$, $r^2=0.5203$; $p<0.01$. ○, control; ▲, clofibric acid; ■, bezafibrate; ●, fenofibrate.

20 : 4n-6, by 3.6 and 2.2 fold, respectively; fenofibrate and bezafibrate increased the expressions of Fads2 mRNA encoding Δ6 desaturase, which converts 18 : 2n-6 to 18 : 3n-6, by 8.4 and 3.9 fold, respectively. Clofibric acid tended to elevate the mRNA levels of both Fads1 and Fads2.

DISCUSSION

The present study showed that the LPCAT activity in the liver of rats is upregulated by feeding on a diet admixed with 0.1% (w/w) clofibric acid, bezafibrate or fenofibrate for 14 d. Fenofibrate and bezafibrate induced LPCAT activity more strongly than did clofibric acid. Recently, through genomic efforts, the cloning of several distinctive 1-acyl-2-lysophsopholipid acyltransferases was reported.[17–21] LPCAT1 was demonstrated to have a preference for saturated of fatty acyl-CoAs and was predominantly expressed in the lung.[18,19] LPCAT2 catalyzes both PAF and PC synthesis mainly in inflammatory cells; thus, two types of glycerophospholipids (PAF and PC) are synthesized from the same precursor (lyso-PAF) by LPCAT2.[20] LPCAT3 is expressed ubiquitously and is primarily responsible for hepatic LPCAT with substrate specificities toward unsaturated fatty acyl-CoAs and 1-acylglycerophosphocholine.[17,21] LPCAT4 mRNA is highly expressed in epididymis, brain, testis and ovary, and has preferences for 18 : 1-CoA.[21] The present study showed that the relative expressions of mRNAs encoding LPCAT3 and LPCAT4 were upregulated by the treatment with fenofibrate and bezafibrate, whereas those of LPCAT1 and LPCAT2 were not changed. Moreover, treatment with clofibric acid tended to increase both LPCAT3 and LPCAT4 mRNA expressions, but this was less potent than those of bezafibrate and fenofibrate. These results strongly suggest that the elevation of LPCAT activities by fibrates is due to the increase in the expressions of genes for LPCAT3 and LPCAT4. Previous studies suggested that the expression of LPCAT3 is regulated by peroxisome proliferator-activated receptor α (PPARα)-dependent fashion.[17,22] AOX is a typical gene of which expression is upregulated by fibrates, and the induction was demonstrated to be mediated through the activation of PPARα.[23] The present study showed that although the expression of LPCAT3 gene was elevated by the treatment of rats with fibrates, there was no significant correlation between mRNA levels of LPCAT3 and those of AOX. These results suggest that LPCAT3 and AOX are PPARα-responsive genes, whereas these two genes show different expression patterns in response to fibrates. When compared with LPCAT3, less information is available about the regulation of the expression of LPCAT4 gene. The present study showed that fibrates induced mRNA for LPCAT4 and that there is no correlation between levels of mRNA for AOX and those for LPCAT4, as was the case for LPCAT3. On the other hand, significant correlation was confirmed between relative levels of mRNA for LPCAT3 and LPCAT4. These results suggest that the expression of LPCAT4 is under the regulation similar to that for LPCAT3. Moreover, the ratios of relative mRNA level of LPCAT3 to that of LPCAT4 in the liver of rats treated with clofibric acid, bezafibrate and fenofibrate are 1.11, 1.48 and 2.14, respectively. These facts indicate that fenofibrate upregulates LPCAT3 more effectively than LPCAT4, when compared with bezafibrate and clofibric acid. Collectively, it seems likely that although the expressions of genes for LPCAT3 and LPCAT4 are regulated by similar manner, the expressions of the two genes somewhat differently respond to different fibrates.

The most important role of 1-acyl-2-lysophospholipid acyltransferases to be noted is that these enzymes participate in remodeling of pre-existing phospholipid molecules.[24] Namely,

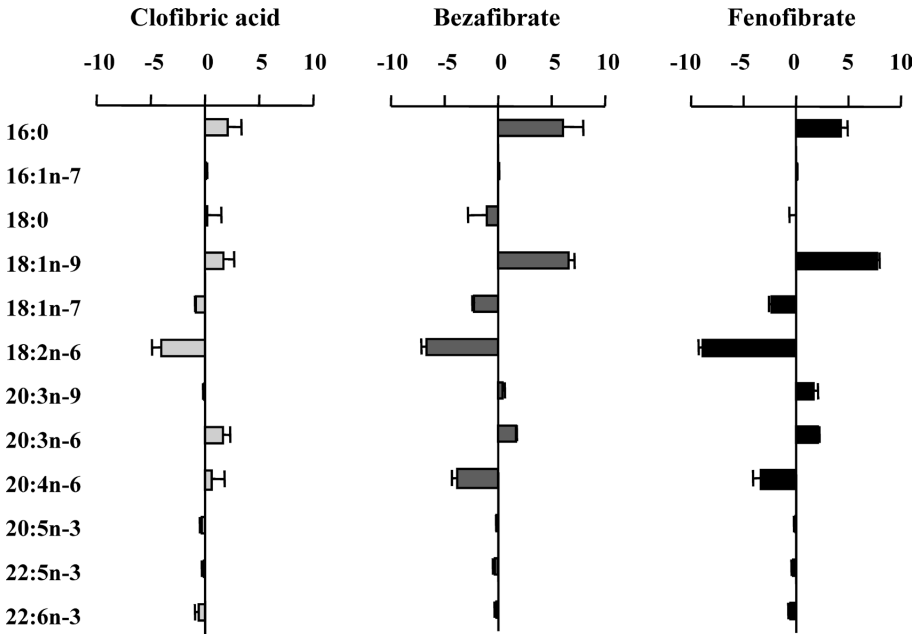

Fig. 4. Difference of Fatty Acids in Hepatic PC between Control Rats and Rats Treated with Clofibric Acid, Bezafibrate and Fenofibrate

With regard to each fatty acid, differences in proportion between the means of control rats and those of rats treated with clofibric acid, bezafibrate and fenofibrate were calculated from the data in Table 2.

Table 2. Fatty Acid Composition of Phosphatidylcholine in the Liver

Fatty acids	Control	Clofibric acid	Bezafibrate	Fenofibrate
	(mol%)			
16:0	21.70±1.29[a]	23.77±1.34[a]	27.78±1.84[b]	25.96±0.63[b]
16:1n-7	0.55±0.22[a]	0.65±0.09[a]	0.63±0.03[a]	0.63±0.08[a]
18:0	20.90±0.77[a]	21.08±1.33[a]	19.83±1.65[a]	20.90±0.61[a]
18:1n-9	3.96±0.10[a]	5.68±0.97[b]	10.55±0.49[c]	11.66±0.32[c]
18:1n-7	3.93±0.14[a]	3.10±0.10[b]	1.71±0.14[c]	1.59±0.21[c]
18:2n-6	17.74±2.06[a]	13.71±0.86[b]	11.05±0.49[c]	8.84±0.34[c]
20:3n-9	0.47±0.20[a]	0.40±0.11[a]	0.89±0.20[a]	2.17±0.34[b]
20:3n-6	1.09±0.15[a]	2.72±0.67[b]	2.75±0.10[b]	3.19±0.15[b]
20:4n-6	24.12±2.12[a]	24.73±1.13[a]	20.27±0.48[b]	20.79±0.63[b]
20:5n-3	0.63±0.29[a]	0.33±0.07[a]	0.50±0.03[a]	0.60±0.08[a]
22:5n-3	0.60±0.07[a]	0.48±0.18[a]	0.32±0.03[b]	0.30±0.02[b]
22:6n-3	3.80±0.96[a]	3.22±0.32[a]	3.66±0.14[a]	3.28±0.18[a]

Rats were fed on a control diet or a diet containing 0.1% (w/w) clofibric acid, bezafibrate or fenofibrate for 14 d. Values represent mean±S.D. for three or four rats. [a–c] Differences in horizonal means without a common superscript are statistically significant ($p<0.05$). If no superscript appears, the differences in the means are not significant ($p>0.05$). The fatty acids are designated by the numbers of carbon atoms and double bonds; palmitic acid, 16:0; palmitoleic acid, 16:1n-7; stearic acid, 18:0; oleic acid, 18:1n-9; *cis*-vaccenic acid, 18:1n-7; linoleic acid, 18:2n-6; 5,8,11-eicosatrienoic acid, 20:3n-9; 8,11,14-eicosatrienoic acid, 20:3n-6; arachidonic acid, 20:4n-6; 5,8,11,14,17-eicosapentaenoic acid, 20:5n-3; 7,10,13,16,19-docosapentaenoic acid, 22:5n-3; 4,7,10,13,16,19-docosahexaenoic acid, 22:6n-3.

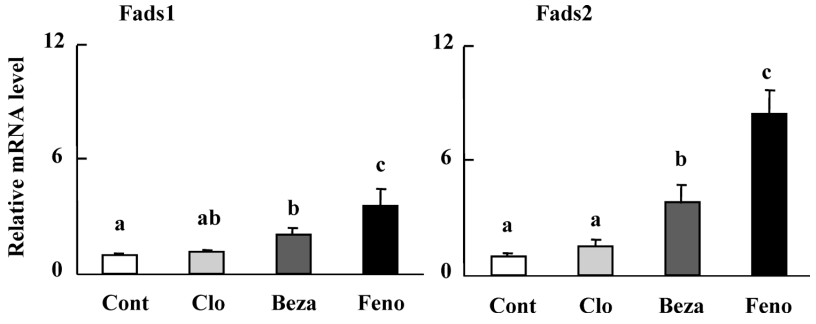

Fig. 5. Effects of Fibrates on the Levels of mRNAs Encoding Fads1 and Fads2 in the Liver of Rats

Rats were fed on a standard diet or a diet containing 0.1% (w/w) clofibric acid, bezafibrate or fenofibrate for 14 d. Cont, control; Clo, clofibric acid; Beza, bezafibrate; Feno, fenofibrate. Values are mean±S.D. for four rats. [a–c] Differences in the mean without a common superscript are statistically significant ($p<0.05$).

Table 3. Effects of Fenofibrate on Acyl-CoA Specificity of LPCAT

Substrate	Control (nmol/min/mg protein)	Fenofibrate (nmol/min/mg protein)
Oleoyl-CoA	74.6±12.6 (1.00)	229.2±9.3 (1.00)
Linoleoyl-CoA	82.4±19.4 (1.10)	333.6±57.2 (1.46)
Arachidonoyl-CoA	112.9±23.7 (1.51)	477.9±52.0 (2.09)

Rats were fed on a control diet or a diet containing 0.1% (w/w) fenofibrate for 14 d. Microsomes were prepared from the liver. LPCAT activities were assayed by using oleoyl-CoA, linoleoyl-CoA and arachidonoyl-CoA as substrates. The conditions for the assay were the same as those described in Materials and Methods, except for the use of 10–50 μg of microsomal protein, depending on the microsomal activity of LPCAT. Values represent mean±S.D. for four rats.

phospholipids are produced *de novo* and then remodeled. The remodeling pathway involves deacylation of phospholipids followed by reacylation of lysophospholipids with acyl-CoAs to attain the proper fatty acids within phospholipids. The present study demonstrated that treatment with fibrates strikingly elevated the proportions of 18 : 1n-9 and markedly decreased that of 18 : 2n-6 in hepatic PC. Previous studies showed that hepatic LPCAT, which is induced by clofibric acid, plays a crucial role in remodeling the acyl composition of *sn*-2 position of PC[6] and that the induction of LPCAT consequently leads to a striking alteration in the composition of molecular species of PC in the liver.[11] In addition, our recent studies demonstrated that fibrates induce the expression of mRNAs encoding SCD1 and SCD2 in the liver[25] and that clofibric acid increases the formation of 18 : 1n-9 and the incorporation of the newly formed 18 : 1n-9 into *sn*-2 position of PC in endoplasmic reticulum in the liver.[26] Thus, it seems likely that fibrates significantly elevated the proportion of 18 : 1n-9 of PC in the liver as the results of both the induction of LPCAT and the increase in supply of 18 : 1n-9. With regard to proportions of 18 : 2n-6, 20 : 3n-6 and 20 : 4n-6 in PC, it is considered that those proportions are regulated by the activity and substrate specificity of LPCAT and the amounts of available acyl-CoAs as substrates. The present study showed that there was difference in acyl-CoA specificity of LPCAT between control and fenofibrate-treated rats; maximal reaction velocities of fenofibrate-induced LPCAT for arachidonoyl-CoA and linoleoyl-CoA relative to that for oleoyl-CoA were considerably higher than those of control LPCAT. It seems likely, therefore, that the relative contribution of LPCAT3 gene product to LPCAT activity in microsomes of fenofibrate-treated rats is greater than that of LPCAT4, when compared with LPCAT in microsomes of control rats, because LPCAT3 prefers to arachidonoyl-CoA and linoleoyl-CoA and LPCAT4 specifically utilizes oleoyl-CoA. Despite of the possibility that fibrate-induced LPCAT transacylates polyunsaturated fatty acids from acyl-CoAs to LPC more effectively than 18 : 1n-9, the proportions of 20 : 4n-6 and 18 : 2n-6 were lowered and the proportion of 20 : 3n-6 was elevated by fibrates in PC. To elucidate these discrepancy between the substrate specificity of LPCAT and fatty acid profile of PC, effects of fibrates on the expression of genes encoding fatty acid desaturases that participate in the formation of 20 : 4n-6 were estimated. The present study showed that fenofibrate and bezafibrate significantly increased the expression of genes for both Fads1 encoding Δ5 desaturase (20 : 3n-6 to 20 : 4n-6) and Fads2 encoding Δ6 desaturase (18 : 2n-6 to 18 : 3n-6), but there were large difference in the elevation of mRNA levels between Δ5 desaturase and Δ6 desaturase. Namely, the extent of increase in the level of mRNA for Δ6 desaturase was more prominent than that for Δ5 desaturase. The conversion of 18 : 2n-6 to 18 : 3n-6 is the rate-limiting step of 20 : 4n-6 synthesis in the liver of physiologically normal rats.[27] It seems likely, however, that the rate-limiting step of 20 : 4n-6 formation is changed from Δ6 desaturation to Δ5 desaturation as a result of the large difference in upregulation mediated by fibrates between Δ 6 desaturase to Δ5 desaturase, because the difference between the activities of Δ6 desaturase and Δ5 desaturase is not significantly large in the rat liver.[27] Thus, it is plausible that 20 : 3n-6 is accumulated in hepatocytes by fibrates, leading to the increase in the supply of 20 : 3n-6 to LPCAT. With regard to 18 : 2n-6, its content in the liver was reduced to the levels less than a half of control by the treatment of rats with fenofibrate and bezafibrate (unpublished results). Taken together, 18 : 1n-9 and 20 : 3n-6 seems to be incorporated into PC more efficiently than 18 : 2n-6 and 20 : 4n-6. Recent study has suggested that the lower contents of unsaturated fatty acid in membrane phospholipid are implicated to cause unfolded protein response.[28] It is of interest, therefore, whether fibrates affect the lipotoxicity in the liver through the changes induced by LPCAT and fatty acid desaturases (SCD and Fads) in fatty acid profile of membrane phospholipid.

In conclusion, fibrates, in common, induced the expression of genes for LPCAT3 and LPCAT4 and markedly changed the fatty acid profile of hepatic PC, in concert with SCD and Fads. The ability of fibrates to induce these alterations was in the order of fenofibrate>bezafibrate>clofibric acid.

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