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EFFECT OF GEMFIBROZIL ON LIPID BIOSYNTHESIS FROM ACETYL-CoA DERIVED FROM PEROXISOMAL β-OXIDATION

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Abstract—The effect of gemfibrozil, a peroxisome proliferator, on lipid biosynthesis from acetyl-CoA derived from peroxisomal β-oxidation was studied. The specific activity of the peroxisomal fatty acyl-CoA β-oxidation system of rats fed a chow containing 0.2% gemfibrozil for 2 weeks was approximately five times higher than that of control rats. When [1-^{14}C]lignoceric acid, a very-long-chain fatty acid which is degraded exclusively by the peroxisomal β-oxidation system at first, was injected into rats treated with gemfibrozil, radioactivity and content of bile acid in the bile were enhanced to approximately 2.2 and 3.5 times the control, respectively. Gemfibrozil increased the radioactivity and content of chenodeoxycholic acid more than that of cholic acid. The incorporation of radioactivity into cholesterol in the bile was as much as 4.5 times greater than the control, and content was 2.6 times greater. In the liver, incorporation of [^{14}C]lignoceric acid into the simple lipids phosphatidylethanolamine and phosphatidylcholine was unaffected by gemfibrozil. The radioactivity and content of cholesterol separated from the simple lipids were also virtually unaffected. However, the specific activities of 3-hydroxy-3-methylglutararyl-CoA reductase (rate-limiting enzyme of cholesterol synthesis) of peroxisomes and microsomes were remarkably stimulated by gemfibrozil treatment. These results suggest that biosyntheses of cholesterol and bile acid from acetyl-CoA derived from peroxisomal β-oxidation are stimulated by gemfibrozil, due at least in part to activation of the peroxisomal β-oxidation system and 3-hydroxy-3-methylglutaryl-CoA reductase of peroxisomes and/or microsomes. Most peroxisomal proliferators (e.g. clofibrate) have been known to inhibit 3-hydroxy-3-methylglutaryl-CoA reductase activity. Therefore, gemfibrozil is expected to be a very useful tool for elucidating the relationship between peroxisomes and the biosyntheses of cholesterol and bile acid.

Key words: gemfibrozil; peroxisome; cholesterol; bile acid; 3-hydroxy-3-methylglutaryl-CoA reductase; fatty acid β-oxidation

Since the finding of peroxisomal β-oxidation by Lazarow and de Duve [1], there has been much interest in its physiological significance. It has been established that the peroxisomal β-oxidation system specifically degrades long- to very-long-chain fatty acids [2, 3]. Dicarboxylic acids [4, 5], branched-chain fatty acids [6] and trihydroxycholestanoic acid [7] can also be degraded. The peroxisomal β-oxidation system is insensitive to KCN and is not coupled with the electron transfer system. It is therefore very different from the mitochondrial β-oxidation system, which is an energy producer, but its physiological significance remains to be fully elucidated.

We have investigated the significance of the peroxisomal β-oxidation system, and showed that acetyl-CoA supplied from peroxisomes is more readily utilized for bile acid biosynthesis than acetyl-CoA from mitochondria [8]. Clofibrate, a standard hypolipidemic agent, is known to proliferate peroxisomes while inhibiting HMG-CoA† reductase, the rate-limiting enzyme for cholesterol synthesis [9]. When HMG-CoA reductase is inhibited by administration of this agent, acetyl-CoA derived from peroxisomes is utilized preferentially not for bile acid synthesis, but for phospholipid synthesis [10]. Furthermore, the phospholipids formed are predominantly plasmalogens [11]. Thus, it appears that the peroxisomal β-oxidation system decomposes fatty acids in order to provide precursors for the production of functional lipids such as bile acids and phospholipids. Peroxisomal β-oxidation may thus have an anabolic significance through its ability to supply acetyl-CoA.

Gemfibrozil is a hypolipidemic agent whose structure is similar to that of clofibrate, but its effect on peroxisomes has not been thoroughly investigated. Like clofibrate, gemfibrozil proliferates peroxisomes and induces catalase, a typical peroxisomal enzyme [12–15]. Gemfibrozil is reported to increase incorporation of [^{14}C]acetate into cholesterol [16]. Unlike clofibrate, gemfibrozil does not seem to inhibit HMG-CoA reductase.

This experiment studied the effect of gemfibrozil on lipid synthesis from acetyl-CoA derived from peroxisomes. [^{14}C]Lignoceric acid, a very-long-chain fatty acid, is degraded exclusively by the peroxisomal

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† Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; TLC, thin-layer chromatography; HDL, high-density lipoprotein.

fatty acid β-oxidation system [17, 18], and so was used as a source of peroxisomal acetyl-CoA. In previous experiments, the acetyl-CoA derived from peroxisomes had been utilized for phospholipid synthesis, when the pathway of bile acid biosynthesis was blocked through the inhibition of HMG-CoA reductase by clofibrate [10]. Gemfibrozil is expected to proliferate peroxisomes and not to block the pathway for bile acid synthesis. We were interested in the destination of acetyl-CoA generated under these conditions. The data presented here show that gemfibrozil markedly stimulates the peroxisomal fatty acid β-oxidation system and the HMG-CoA reductase activity of peroxisomes and/or microsomes, and hence increases biosyntheses of cholesterol and bile acid of acetyl-CoA supplied from peroxisomes. The increased acetyl-CoA seems to be utilized mainly for sterol rather than phospholipid synthesis.

MATERIALS AND METHODS

Materials. [^{14}C]Lignoceric acid (52 mCi/mmole, 1924 MBq/mmole) was obtained from Isotopchim (France). DL-3-[Glutaryl-3-^{14}C]hydroxy-3-methyl-glutaryl-CoA (57.7 mCi/mmole, 2.1 GBq/mmole) and [RS]-[5-^{3}H]-mevalonolactone (33.0 Ci/mmole, 1221.0 GBq/mmole) were purchased from New England Nuclear (U.S.A.). Gemfibrozil, bile acids, cholesterol, HMG-CoA, mevalonolactone and Nycodenz were obtained from Sigma (U.S.A.). All other reagents were of analytical grade from Wako Pure Chemicals (Japan).

Treatment of the rats. Male Wistar rats (250–300 g) were fed *ad lib* standard chow CE-2 (Clea Japan, Japan) and held on a 12-hr light–dark cycle. The treated rats were fed chow containing 0.2% gemfibrozil (w/w) for 2 weeks. At 4 hr into their light cycle, serum was collected under anesthesia through the abdominal aorta of the rats after overnight starvation. The rats were killed and the livers excised after perfusion with cold saline.

When [^{14}C]lignoceric acid was used, bile-duct-fistula operations were carried out 4 hr into the light cycle in order to exhaust accumulated secondary bile acids and the cholesterol pool via PE-10 tubing. The rats were placed in Bollman restraining cages and starved. [1-^{14}C]Lignoceric acid (0.9 μCi, 33.3 kBq) dissolved in 0.2 ml of 3% Tween 80-saline solution was intravenously injected into a thigh vein 24 hr after the operation. The bile was subsequently collected and pooled into two fractions: 0–3 hr and 3–6 hr after the injection. The livers were excised after perfusion.

Preparation of enzyme samples. Livers were homogenized in a medium containing 0.25 M sucrose, 1 mM EDTA, 0.1% ethanol and 5 mM HEPES, pH 7.4. The 10% (w/v) homogenates were fractionated by differential centrifugation according to de Duve *et al.* [19], and the light mitochondrial and microsomal fractions were then obtained. For the assay of peroxisomal HMG-CoA reductase, the light mitochondrial fraction was subjected to Nycodenz gradient centrifugation as described previously [20]. In the previous experiment, isolated peroxisomes were 90–95% pure as determined by marker enzyme distribution (marker enzymes: peroxisomes, D-amino acid oxidase; microsomes, esterase; mitochondria, cytochrome C oxidase; lysosomes, acid phosphatase) and contained < 3% mitochondrial contamination and < 5% microsomal protein [20]. The enzyme preparations were stored at −20° until the assay.

Enzyme and protein assays. The activity of the peroxisomal fatty acyl-CoA oxidizing system was determined by the method of Lazarow and de Duve [1] with a slight modification [21].

Catalase is the main enzyme contained in peroxisomes, and activity was determined according to the method of Leighton *et al.* [22] except that the procedure was performed manually [23].

D-Amino acid oxidase (a peroxisomal marker) and urate oxidase were assayed as described previously [24].

Esterase (a microsomal marker) was measured using *o*-nitrophenyl acetate as a substrate according to Beaufay *et al.* [25].

Cytochrome c oxidase (a mitochondrial marker) activity was determined by the method of Wharton and Tzagoloff [26].

Acid phosphatase (a lysosomal marker) was assayed as described previously [23].

The activity of HMG-CoA reductase, which catalyses the formation of mevalonate from HMG-CoA, was determined by the method of Keller *et al.* using [3-^{14}C]HMG-CoA and [5-^{3}H]mevalonolactone as a substrate and an internal standard, respectively [27]. Enzyme samples were diluted in 50 mM potassium phosphate buffer, pH 7.4, containing 30 mM EDTA, 200 mM NaCl and 10 mM dithiothreitol. Approximately 50 μg of protein was used.

Protein was measured according to Lowry *et al.* [28]. Since Nycodenz interferes with the determination of protein, it was removed by trichloroacetic acid co-precipitation of the protein with deoxycholate before the Lowry protein assay [28].

Extraction of bile acid and cholesterol from bile. A bile sample was diluted with nine volumes of 0.5 M phosphate buffer (pH 7.0) and passed through a Sep-Pak C_{18} cartridge (Waters, U.S.A.). After successive washing of the cartridge with water (2 mL) and 1.5% ethanol (2 mL), bile acid and cholesterol were eluted with 90% ethanol (4 mL) [29]. Bile acid and cholesterol were separated by TLC chromatography (reversed phase $KC_{18}F$, 20 × 20 cm, Whatman, U.S.A.) with *n*-hexane/diethyl ether/formic acid (80:20:2) as described previously [30]. For the assay of primary bile acids, the eluate with 90% ethanol was dried and hydrolysed in 10% KOH–50% ethanol solution at 121° for 4 hr. The hydrolysate was acidified and extracted with ethyl acetate. Bile acid extracted from the hydrolysate was injected on a Lichrosorb RP 18 column (250 × 10 mm, Merck, Germany) and eluted with 90% (v/v) methanol at 40° at a flow rate of 1 mL/min. The differential refraction was monitored. The fractions corresponding to standard cholic acid and chenodeoxycholic acid were collected and dried. Radioactivity was measured with Liquifluor (New England Nuclear) as a scintillator.

Extraction of lipids from liver. Lipids were extracted with Folch's solution from liver After

washing with saline, the chloroform layer was evaporated to dryness and the residue taken up in 2 mL of chloroform/acetic acid (100:1). This solution was applied to a silica Sep-Pak column (Waters, U.S.A.). The vessel containing the lipid extracts was washed once with 2 mL chloroform/acetic acid (100:1) and the washing was added to the Sep-Pak column. The column was eluted with 12 mL chloroform/acetic acid (100:1). Simple lipids were eluted under these conditions. Further elution with 5 mL methanol/chloroform (2:1) yielded phosphatidylethanolamine. Phosphatidylcholine was eluted with an additional 5 mL of methanol/chloroform/water (2:1:0:8) [31]. Cholesterol was separated from the simple lipid fraction by TLC chromatography (silicagel G, 20 × 20 cm, Analtech, Japan) with petroleum ether/diethyl ether/acetic acid (90:10:1). The areas corresponding to the positions of standard free cholesterol and cholesterol ester were scraped from the plate and extracted. Radioactivities of the lipid fractions were determined as stated above.

Colorimetric methods for assaying lipids. Colorimetric estimation of bile acids was performed according to Eastwood *et al.* using cholic acid as a standard [32]. Cholesterol content was determined according to Zlatkis and Zak [33]. Triglyceride content was assayed by the method of Sardesai and Manning [34] using the triglyceride test Wako clinical assay kit.

RESULTS

Effect of gemfibrozil, a peroxisome proliferator, on liver weight and serum lipids

The liver weight and serum lipid content of rats treated with 0.2% gemfibrozil for 2 weeks were determined. Gemfibrozil is known to cause hepatomegaly [12–14]. As shown in Table 1, body weight was not changed by gemfibrozil, but liver weight was clearly increased to approx. 1.5 times the control. Cholesterol concentration in the serum was hardly changed by gemfibrozil, while triglyceride was drastically decreased to approx. 28% of the control. These values are in reasonable agreement with the data reported previously [12, 14].

Effect of gemfibrozil treatment on typical peroxisomal enzymes

Specific enzyme activities of the fatty acyl-CoA β-oxidation system of rat liver were remarkably enhanced by gemfibrozil, and catalase was significantly increased to approx. 1.3 times the control. D-Amino acid oxidase and urate oxidase showed small increases in specific activities, but the changes were not statistically significant. Protein was increased approx. twice over the control, indicating that peroxisome proliferation is induced by gemfibrozil (Table 2).

Influence of gemfibrozil on bile lipids in rat after administration of [^{14}C]lignoceric acid

[^{14}C]Lignoceric acid was administered to bile-duct fistula rats 24 hr after the operation, when the secondary bile acid had almost completely disappeared from biliary lipids [8]. Bile was then collected from the rats up to 6 hr. As shown in Fig. 1, bile acids were linearly excreted up to 6 hr into the bile of both control and gemfibrozil-treated rats. The cumulative amount of bile acids of treated rats up to 6 hr was 2.8 times the control.

Figure 2 illustrates the radioactivity excreted into the bile. Excretion rates of radioactivity for the initial 3 hr in both control and treated rats were twice those of the next 3 hr. The cumulative amounts of radioactivity up to 3 hr and 6 hr from gemfibrozil-treated animals were approx. 1.6 times the control.

These results show that biosynthesis of bile acids was stimulated by gemfibrozil.

The bile acid and cholesterol were extracted from the pooled bile up to 3 hr after administration of [^{14}C]lignoceric acid (Fig. 3). The amount of bile acid was increased to 3.5 times the control by gemfibrozil, and incorporation from [^{14}C]lignoceric acid into bile acid was 2.2 times higher than that of control rats. In the case of cholesterol, the amount and radioactivity were increased to 2.6 and 4.5 times the control, respectively.

Therefore, biosyntheses of both bile acid and cholesterol seem to be stimulated by gemfibrozil.

Figure 4 shows the effect of gemfibrozil on the primary bile acids biosynthesized from [^{14}C]lignoceric acid. The incorporation of radioactivity into cholic acid was much higher than that into chenodeoxycholic acid. The amount and radioactivity of cholic acid were enhanced by gemfibrozil to approx. 2.0 and 1.5 times the control, respectively. On the other hand, the amount and radioactivity of chenodeoxycholic acid were increased to 8.1 and 4.9 times the control, respectively, indicating that

Table 1. Effect of gemfibrozil on liver weight and serum lipids of rat

	Control	Gemfibrozil	Gemfibrozil/control ratio
Body weight (g)	308 ± 32	317 ± 23	1.03 ± 0.07
Liver weight (g/100 g body weight)	3.80 ± 0.35	5.66 ± 0.58	1.49 ± 0.15**
Serum			
Cholesterol (mg/dL)	111.8 ± 26.5	128.6 ± 37.1	1.15 ± 0.33
Triglyceride (mg/dL)	79.78 ± 19.92	22.04 ± 5.36	0.276 ± 0.067*

Rats were fed laboratory chow containing or not containing 0.2% gemfibrozil for 14 days. Data are means values ± SD of five animals; * and ** indicate significant differences: * $P < 0.001$; ** $P < 0.02$.

Table 2. Effect of gemfibrozil on specific activities of typical peroxisomal enzymes and protein content

	Control	Gemfibrozil	Gemfibrozil/ control ratio
Fatty acyl-CoA β-oxidation system (U/mg)	3.005 ± 0.547	14.45 ± 2.10	4.81 ± 0.67*
Catalase (U/mg)	52.69 ± 7.61	69.57 ± 8.98	1.32 ± 0.17**
D-amino acid oxidase (mU/mg)	6.848 ± 0.665	7.940 ± 2.071	1.16 ± 0.30
Urate oxidase (mU/mg)	34.35 ± 4.97	41.11 ± 4.56	1.20 ± 0.13
Protein (mg/g liver)	22.35 ± 2.46	43.38 ± 4.34	1.94 ± 0.19*

After treatment with 0.2% gemfibrozil for 2 weeks, rats were killed and livers excised. Light mitochondrial fraction was prepared from the liver homogenate, and activities of marker enzymes and protein content were then assayed. Data are mean values ± SD of five animals. * and ** represent significant changes: * $P < 0.001$; ** $P < 0.02$.

gemfibrozil stimulates the formation of chenodeoxycholic acid much more so than that of cholic acid. These differences may imply that the pathway of biosynthesis of chenodeoxycholic acid differs in some respects from that of cholic acid, the predominant component of rodent bile acids [35].

Effect of gemfibrozil on hepatic lipid synthesis from [^{14}C]lignoceric acid

Lipids were extracted from the liver 6 hr after the administration of [^{14}C]lignoceric acid. The uptakes of radioactivity into phosphatidylethanolamine and phosphatidylcholine were approx. 15% and 10%, respectively, of the total radioactivity incorporated into the liver (Fig. 5). Gemfibrozil hardly influenced radioactivity in the phospholipids. Radioactivity incorporated into the simple lipid fraction was also unaffected by gemfibrozil.

As shown in Fig. 3, gemfibrozil increased the radioactivity of cholesterol in the bile, leading us to study its effect on hepatic cholesterol. Cholesterol was separated by TLC chromatography from the simple lipid fraction obtained in the experiment shown in Fig. 5 and determined. However, gemfibrozil hardly affected the content or radioactivity of hepatic cholesterol (Table 3).

Influence of gemfibrozil on HMG-CoA reductase activity of peroxisomes and microsomes

A key regulatory enzyme in the biosynthesis of cholesterol, HMG-CoA reductase had been believed until recently to reside exclusively in the endoplasmic reticulum of mammalian cells [36]. However, a recent publication showed that the enzyme is present not only in the endoplasmic reticulum but also within peroxisomes [27]. In order to study the mechanism

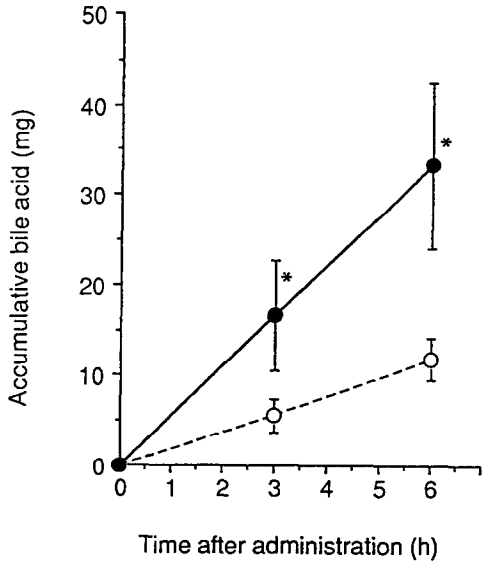

Fig. 1. Effect of gemfibrozil on biliary excretion of bile acid. One day after implantation of a bile duct fistula, 0.9 mCi (33.3 kBq) of [1-^{14}C]lignoceric acid was injected i.v. and the bile then collected. The amount of bile acid was determined as described in the text. (●) Gemfibrozil-treated rats; (○) control rats. Data are mean values ± SD of five experiments; * indicates a significant difference ($P < 0.01$).

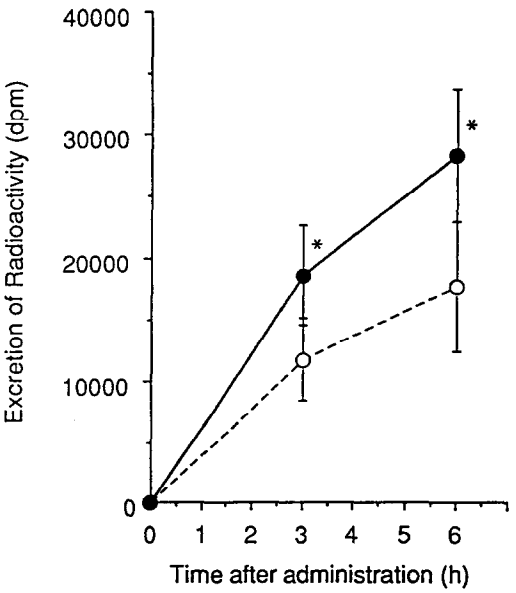

Fig. 2. Effect of gemfibrozil on biliary excretion of radioactivity after administration of [^{14}C]lignoceric acid. Radioactivity in the bile collected in the experiment of Fig. 1 was determined. (●) Gemfibrozil-treated rats; (○) control rats. Data are mean values ± SD of five experiments; * indicates a significant difference ($P < 0.02$).

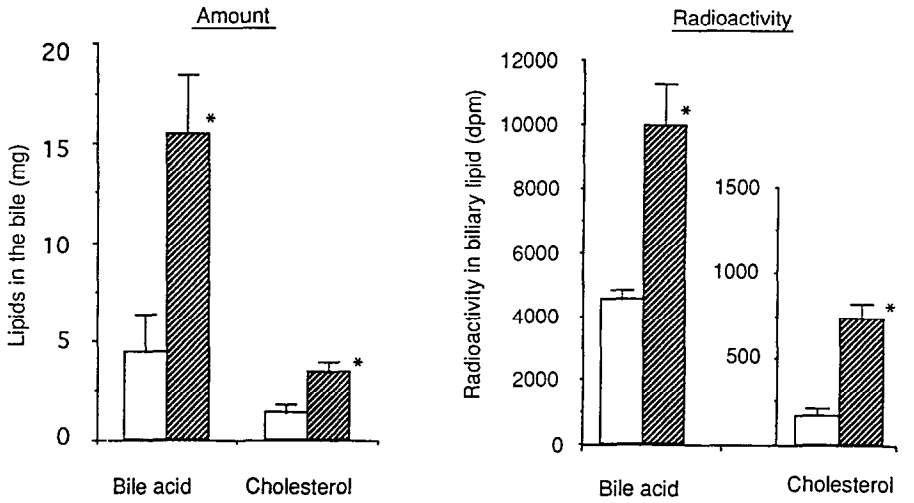

Fig. 3. Influence of gemfibrozil on the synthesis of bile acid and cholesterol after administration of [^{14}C]-lignoceric acid. Bile acid fraction and cholesterol fraction were obtained by Sep-Pak C_{18} and TLC chromatography from the bile collected at 0–3 hr in the experiment of Fig. 1. The lipid content and radioactivity of each fraction were determined. Data are mean values ± SD of five experiments. The amounts and radioactivities of both lipids in the bile from control rats (open bars) were significantly different from those of gemfibrozil-treated rats (oblique bars); * indicates a significant difference ($P < 0.001$).

of activation of syntheses of cholesterol and bile acid by gemfibrozil, we determined the HMG-CoA reductase activity of both peroxisomes and microsomes. As shown in Table 4, HMG-CoA reductase activities of peroxisomes and microsomes were markedly stimulated by *in vivo* treatment with gemfibrozil. This indicates that the mechanism of the activation of syntheses of cholesterol and bile acid by gemfibrozil involves stimulation of HMG-CoA reductase activity of peroxisomes and/or microsomes.

DISCUSSION

We investigated the effect of gemfibrozil on biosyntheses of lipids (mainly steroids and phospholipids) from acetyl-CoA derived from peroxisomal fatty acid β-oxidation.

The influence of gemfibrozil on serum lipids (Table 1) is in agreement with earlier reports concerning rats [12, 14]. Vazquez *et al.* reported that gemfibrozil decreased cholesterol content in serum of guinea pig, but did not affect triglyceride content [37]. In

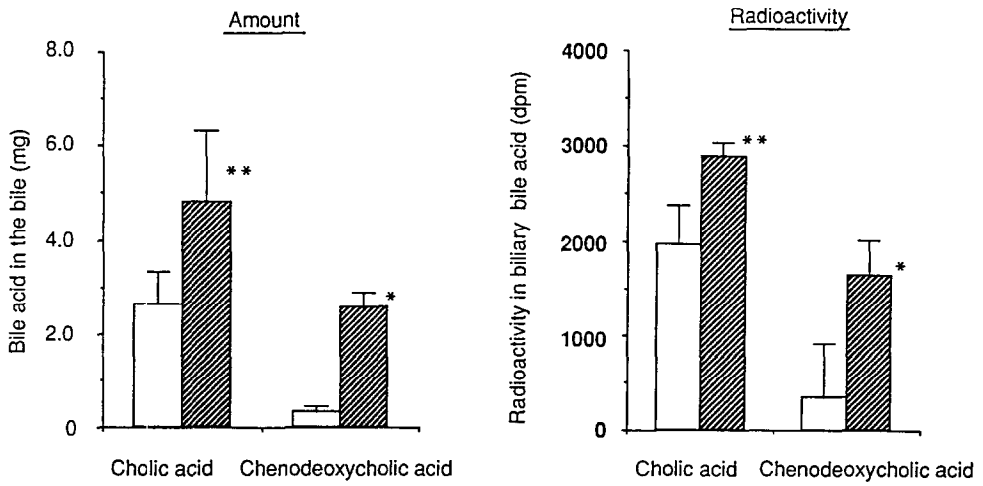

Fig. 4. Effect of gemfibrozil on components of bile acids in bile after administration of [^{14}C]lignoceric acid. Bile acids obtained by Sep-Pak C_{18} chromatography in the experiment shown in Fig. 3 were hydrolysed, and then subjected to HPLC for fractionation into cholic acid and chenodeoxycholic acid. Data are mean values ± SD of five experiments. The amounts and radioactivities of both bile acids in the bile from control rats (open bars) were significantly different from those of gemfibrozil-treated rats (oblique bars); * indicates a significant difference at $P < 0.01$ and ** at $P < 0.02$.

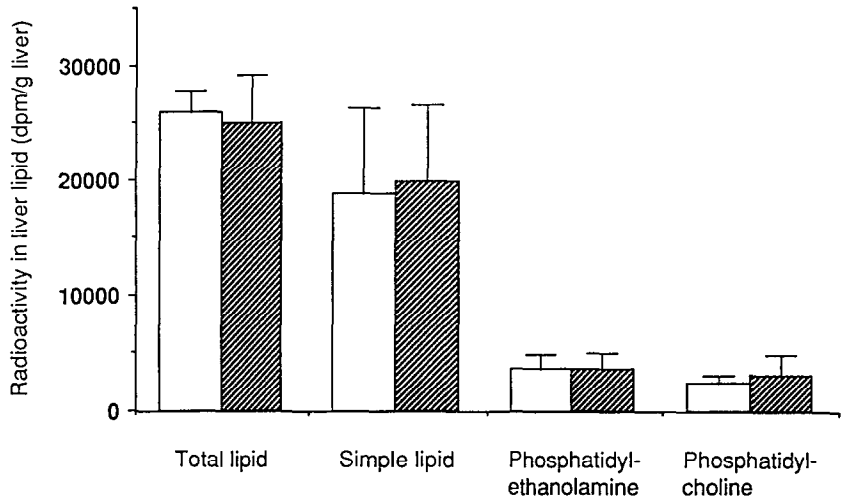

Fig. 5. Influence of gemfibrozil on the synthesis of hepatic lipids from [^{14}C]lignoceric acid. Rats were fed laboratory chow containing or not containing 0.2% gemfibrozil. The livers were excised from the bile-duct-fistula rats 6 hr after administration of [^{14}C]lignoceric acid. The total lipid extracts of the livers were fractionated into the indicated lipid fractions by Sep-Pak silica chromatography. Data are mean values ± SD of five experiments. The radioactivities of lipids in the livers from control rats (open bars) were not significantly different from those of gemfibrozil-treated rats (oblique bars).

man, oral administration of gemfibrozil was reported to reduce triglyceride content and total cholesterol in the serum, whereas HDL cholesterol was increased [38]. Therefore, there seems to be a species difference in the effect of gemfibrozil on serum lipids.

In the experiments of Figs 1–4, [^{14}C]lignoceric acid was injected into bile-duct-fistula rats from which secondary bile acids had been exhausted for 24 hr. Therefore, almost all of the bile acids contained in this bile are primary bile acids such as cholic acid and chenodeoxycholic acid, and cholesterol should be newly synthesized sterol. That is, the increases in content and radioactivity of bile acid and cholesterol indicate that biosynthesis of bile acid and cholesterol is stimulated by gemfibrozil.

Lalwani *et al.* reported that gemfibrozil increased

Table 3. Influence of gemfibrozil on hepatic cholesterol of rats injected with [^{14}C]lignoceric acid

	Control	Gemfibrozil	Gemfibrozil/ control ratio
Cholesterol			
Radioactivity (dpm/g liver)	789 ± 49	784 ± 184	0.99 ± 0.23
Content (mg/g liver)	3.467 ± 0.274	3.224 ± 0.440	0.93 ± 0.13

Cholesterol was separated by TLC from the simple lipid fraction of the livers obtained in the experiment shown in Fig. 5. Data are mean values ± SD of total cholesterol from five experiments.

Table 4. Effect of gemfibrozil on HMG-CoA reductase activity of peroxisomes and microsomes

	HMG-CoA reductase (pmol/min/mg protein)		
	Control	Gemfibrozil	Gemfibrozil/ control ratio
Peroxisomes	9.47 ± 3.94	70.90 ± 24.88	7.49 ± 2.63*
Microsomes	65.2 ± 16.0	1491.3 ± 263.0	22.9 ± 4.0*

Rats were fed chow containing or not containing 0.2% gemfibrozil for 2 weeks. Peroxisomes and microsomes were prepared from the rat livers, and HMG-CoA reductase activity was assayed as described in the text. Data are means ± SD of five animals; * indicates a significant difference ($P < 0.001$).

fatty acid β-oxidation to the same extent as catalase [39], but we found that gemfibrozil activates peroxisomal fatty acid β-oxidation more so than catalase (Table 2). We suggest that gemfibrozil enhances the supply of acetyl-CoA from peroxisomes through activation of the β-oxidation system, and hence stimulates biosyntheses of cholesterol and bile acid.

Considering that gemfibrozil is a hypolipidemic agent, the remarkable stimulation of HMG-CoA reductase activities of both peroxisomes and microsomes (Table 4) is unexpected. However, Maxwell *et al.* also reported that cholesterol synthesis from [^{14}C]acetate and [^{14}C]octanoate in rat liver was stimulated by *in vivo* treatment with gemfibrozil [16, 40]. Thompson and Krisans stated as unpublished data that gemfibrozil induces peroxisomal HMG-CoA reductase [41]. From these data, the elevation in the biosyntheses of bile acid and cholesterol may be due not only to the increase in the supply of acetyl-CoA produced by peroxisomal β-oxidation, but also to the activation of HMG-CoA reductase of peroxisomes and/or microsomes.

Previously, we reported that 4α-methyl-5α-cholest-7-en-3β-ol and 4,4-dimethyl-5α-cholest-8-en-3β-ol, intermediates of cholesterol synthesis, are accumulated in peroxisomes and microsomes after administration of aminotriazole to rats, and their contents are increased by gemfibrozil pretreatment [20]. These results, in conjunction with the present study, indicate that the increased accumulation of the intermediate sterols induced by gemfibrozil may be caused not only by the proliferation of peroxisomes but also by the activation of HMG-CoA reductase. These results confirm that gemfibrozil stimulates cholesterol synthesis in both peroxisomes and microsomes.

Stange *et al.* reported that activity of HMG-CoA reductase of human mononuclear cells did not change on *in vitro* treatment with gemfibrozil, but was reduced by *in vivo* treatment [42]. Thus, there appear to be species differences in the effects of gemfibrozil on HMG-CoA reductase.

The greater increase in the biosynthesis of chenodeoxycholic acid over that of cholic acid (Fig. 4) implies that gemfibrozil directly activated the enzyme(s) related to biosynthesis of chenodeoxycholic acid, or more strongly stimulated the synthetic pathway for chenodeoxycholic acid owing to saturation of the substrate for sterol 12α-hydroxylase, which works at the branch point to the pathway for cholic acid synthesis.

From the previous data gathered in our laboratory [8, 10], we had expected that if the supply of acetyl-CoA is elevated because of activation of peroxisomal β-oxidation by gemfibrozil, the acetyl-CoA may be utilized for synthesis of phospholipids as well as bile acid. However, the results in Fig. 5 were not as expected. Therefore, it is likely that the pathway for syntheses of cholesterol and bile acid is more stimulated than that for synthesis of phospholipids owing to the remarkable activation of HMG-CoA reductase; hence no significant increase in phospholipid synthesis could be detected.

The different changes in the content and radioactivity of cholesterol between bile and liver (Fig. 3 and Table 3) may be the result of the small size of the pool of cholesterol derived from peroxisomal acetyl-CoA; thus only a small amount of cholesterol could presumably be accumulated in the liver and a large amount was excreted into the bile.

The present study has revealed many differences between the effects of clofibrate and gemfibrozil on lipid biosynthesis. For example, biosyntheses of cholesterol and bile acid were elevated by gemfibrozil; moreover, the syntheses from acetyl-CoA derived from peroxisomal β-oxidation were stimulated. *In vivo* treatment with gemfibrozil remarkably enhanced the HMG-CoA reductase activity of peroxisomes and microsomes. Phospholipid synthesis was unchanged by gemfibrozil; the increased acetyl-CoA owing to the activation of peroxisomal β-oxidation by gemfibrozil is less effectively utilized for fatty acid synthesis of phospholipids than for sterol synthesis.

Many hypolipidemic agents such as clofibrate are known to proliferate peroxisomes and enhance peroxisomal fatty acid β-oxidation. Many of the enzymes related to syntheses of cholesterol and bile acid are present in peroxisomes [41, 43–45]. However, almost all currently available hypolipidemic agents inhibit the syntheses of cholesterol and bile acid. Therefore, it is impossible to use these compounds in order to clarify the relationship between peroxisomal proliferation and the syntheses of cholesterol and bile acid *in vivo*.

In the present study, using gemfibrozil first enabled us to elucidate that peroxisomal proliferation parallels the biosyntheses of cholesterol and bile acid, and further that acetyl-CoA derived from peroxisomal β-oxidation is preferentially utilized for the syntheses of cholesterol and bile acid. These results indicate that gemfibrozil will serve as a very useful tool for studies on the relationship between peroxisomes and the syntheses of cholesterol and bile acid.

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