Biochemical Pharmacology, Vol. 46, No. 8, pp 1515–1518, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

Gemfibrozil modifies acyl composition of liver microsomal phospholipids from guinea-pigs without promoting peroxisomal proliferation

(*Received* 19 *February* 1993; *accepted* 24 *June* 1993)

Abstract—Treatment with gemfibrozil modifies acyl composition of hepatic microsomal phosphatidylcholine and phosphatidylethanolamine in guinea-pigs. Palmitic (16:0) and palmitoleic (16:1) fatty acids are increased, and stearic (18:0) and oleic (18:1) are decreased; further, while linoleic acid [18:2 (n-6)] is increased by gemfibrozil treatment, the other constituents of the n-6 fatty acids family, including arachidonic acid [20:4 (n-6)], are decreased. As gemfibrozil is a potent inhibitor of fatty acid elongation *in vitro* (Sánchez *et al.*, *FEBS Lett* **300**: 89–92, 1992), the inhibition of this enzyme system by gemfibrozil treatment could be responsible for the observed results *in vivo*. These changes in fatty acid composition are accompanied by a decrease in serum lipids and, more important, are independent of peroxisomal proliferation

Gemfibrozil (GFB*) is a hypolipidaemic agent generally classified as a fibric acid derivative, but showing some different pharmacological effects [1]. It is particularly effective in states of hypertriglyceridaemia and furthermore increases plasmatic high density lipoprotein-cholesterol. GFB also increases lipoprotein lipase activity and reduces hepatic triglyceride production [2]. GFB, like other fibrates and a wide variety of structurally unrelated compounds, has the ability to promote peroxisome formation in rodent liver, and is classified as a peroxisome proliferator [3]. Moreover, treated animals show a considerable increase in the activity of enzymes related to fatty acid metabolism, accompanied by a characteristic hepatomegaly [4]. Kawashima *et al.* [5] reported that the administration of clofibric acid to rats or mice, and also tiadenol, another peroxisome proliferator unrelated to fibrates, produced a marked increase in the activities of both stearoyl-CoA desaturase and palmitoyl-CoA chain elongation in hepatic microsomes. These enzymes have an important role in the modification of the fatty acids needed for the biosynthesis of glycerolipids and, as a result, acyl composition of liver microsomal phospholipids is modified. In contrast, no change was observed in the activity of hepatic microsomal enzymes involved in fatty acid biosynthesis or in the acyl composition of phospholipids when these drugs were administered to guinea-pigs, in which peroxisome proliferation does not occur. In the present study we demonstrate that GFB treatment can modify acyl composition in both phosphatidylcholine (PC) and phosphatidylethanolamine (PE) fractions of hepatic microsomal phospholipids from guinea-pigs under conditions where peroxisome proliferation is not observed and, further, a clear hypolipidemic effect is attained.

Materials and Methods

The guinea-pigs used in this study were purchased from Letica (Barcelona, Spain). They had free access to a commercial diet (Panlab, Spain) or a diet containing 0.3% (w/w) GFB for 2 weeks. Animals were killed by decapitation at 9:00 a.m. Blood samples were collected for determination of triglyceride and total cholesterol values (Boehringer Mannheim enzymatic tests). Livers were removed and perfused with ice-cold 0.9% NaCl to remove contaminating haemoglobin. Microsomes from livers were prepared as described previously [6]. Protein was determined by the method of Bradford [7], using bovine serum albumin as standard. Lipids were extracted from microsomes by the method of Bligh and Dyer [8]. The organic fraction was applied to Silica gel 60 F_{254} plates (20 × 20 cm, E. Merck, Darmstadt, Germany) for the separation of phospholipids, and the plates were developed with chloroform/methanol/acetic acid/water (25:15:3:2, by vol.). The spots were visualized by comparison with authentic standards under fluorescent light at 254 nm. All solvent systems used contained 0.01% (w/v) butylhydroxytoluol. Silica gel corresponding to each phospholipid was scraped off and extracted twice with 5 mL Bligh and Dyer reagent, and dried under a stream of N_2. Fatty acid methyl esters were formed by incubation of lipids in 1 mL tetrahydrofuran (containing 0.01% butylhydroxytoluol) and 1 mL 0.5 N sodium methylate in methanol for 10 min at 50°. After the addition of 100 μL acetic acid and 3 mL water, the methyl esters were extracted three times with 2 mL *n*-hexane. The combined hexane extracts were dried with anhydrous sodium sulphate. Fatty acid methyl esters were analysed using a Hewlett–Packard gas chromatograph model 5890 equipped with a flame ionization detector and a Supelcowax 10 fused silica capillary column (30 m × 0.20 mm i.d.). Peaks were identified by comparison of retention times with those of authentic fatty acid methyl esters. Peak areas were determined by a Hewlett–Packard model 3390A integrator. For the analysis of microsomal lipid composition, aliquots of the microsomal Bligh and Dyer extracts were dried under N_2 and the lipids were determined by enzymatic assays (Boehringer Mannheim). Peroxisomal β-oxidation, the commonly used marker of peroxisomal proliferation [9], was measured by the method of Lazarow [10]. Data are presented as means ± SD (standard deviation). Statistical comparisons (Student's *t*-test) were performed by the FOUNDS computer program.

Results and Discussion

GFB treatment affected neither body weight increase nor the liver weight/body weight ratio (Table 1). In the same conditions, a parallel treatment applied to rats (not shown) produced an increase of up to 43% in the liver weight/body weight ratio. Peroxisomal β-oxidation activity was not affected by the treatment. This finding, together with the absence of hepatomegaly, indicates a lack of peroxisome proliferation. Under similar conditions, an about 5-fold increase in β-oxidation can be observed in rats (personal data). Serum triglyceride and cholesterol concentrations were reduced in 22 and 47%, respectively.

* Abbreviations: GFB, gemfibrozil; PC, phosphatidylcholine; PE, phosphatidylethanolamine; 16:0, palmitic acid; 18:0, stearic acid; PUFAs, polyunsaturated fatty acids; 20:4 (n-6), arachidonic acid; 16:1, palmitoleic acid; 18:2, (n-6), linoleic acid; 22:4 (n-6), docosatetraenoic acid; 20:3 (n-6), eicosatrienoic acid; 22:6 (n-3), docosahexaenoic acid.

Table 1. Effect of a treatment with GFB on liver weight, serum and microsomal lipids, and peroxisomal β-oxidation in guinea-pigs

	Control	GFB-treated
Body weight increase (g)	102.5 ± 23.0	107.5 ± 18.8
Liver weight/body weight	0.065 ± 0.009	0.071 ± 0.011
Serum cholesterol (mg/dL)	54.3 ± 10.8	28.9 ± 7.2*
Serum triglycerides (mg/dL)	54.0 ± 16.6	42.3 ± 13.4
Microsomal cholesterol (μg/mg microsomal protein)	14.6- ± 3.9	10.7 ± 2.1
Microsomal triglycerides (μg/mg microsomal protein)	33.5 ± 6.7	31.2 ± 5.6
Microsomal phospholipids (μg/mg microsomal protein)	87.7 ± 25.4	93.5 ± 24.6
Peroxisomal β-oxidation (nmol/min/mg protein)	2.59 ± 0.55	3.00 ± 0.79

Guinea-pigs were fed on a standard diet or the same diet containing 0.3% (w/w) GFB for 2 weeks. Substrate concentrations and peroxisomal β-oxidation activity are measured as described in the text.

Values are means ± SD from six animals.

* $P < 0.001$ relative to control, Student's *t*-test.

Table 2. Effects of GFB on acyl composition of PC and PE in hepatic microsomes of guinea-pigs

	Composition (mol %)			
	PC		PE	
	Control	GFB	Control	GFB
16:0	17.6 ± 1.6	22.9 ± 1.5‡	10.4 ± 1.6	16.1 ± 2.4‡
16:1	0.71 ± 0.24	1.13 ± 0.13†	0.57 ± 0.20	0.75 ± 0.16
18:0	30.7 ± 1.2	25.8 ± 1.3‡	35.6 ± 2.2	31.1 ± 1.8†
18:1	11.2 ± 2.2	8.0 ± 0.5†	4.22 ± 0.97	3.98 ± 0.30
18:2 (n-6)	35.0 ± 1.7	38.4 ± 1.0†	36.4 ± 0.4	37.1 ± 1.0
18:3 (n-6)	1.09 ± 0.26	0.85 ± 0.31	1.99 ± 0.92	1.29 ± 0.41
18:3 (n-3)	0.04 ± 0.00	0.05 ± 0.00	0.08 ± 0.04	0.08 ± 0.02
20:3 (n-9)	0.02 ± 0.04	0.02 ± 0.03	0.05 ± 0.07	0.07 ± 0.06
20:3 (n-6)	0.69 ± 0.09	0.43 ± 0.04‡	0.46 ± 0.08	0.32 ± 0.07†
20:4 (n-6)	2.16 ± 0.38	1.66 ± 0.30*	7.0 ± 1.2	6.6 ± 0.9
20:5 (n-3)	0.24 ± 0.10	0.21 ± 0.14	0.54 ± 0.16	0.52 ± 0.12
22:4 (n-6)	0.12 ± 0.03	0.04 ± 0.04†	0.45 ± 0.19	0.35 ± 0.10
22:5 (n-3)	0.13 ± 0.05	0.06 ± 0.04	0.76 ± 0.26	0.73 ± 0.11
22:6 (n-3)	0.32 ± 0.17	0.50 ± 0.20*	1.52 ± 0.33	1.55 ± 0.25
Total saturated	48.4 ± 0.7	48.7 ± 0.4	46.0 ± 1.4	47.1 ± 1.0
Total monounsaturated	11.9 ± 2.4	9.1 ± 0.9*	4.8 ± 0.9	4.7 ± 0.3
Total PUFAs	39.8 ± 2.0	42.2 ± 0.6*	49.2 ± 1.6	48.1 ± 0.9
Total n-6 fatty acids	39.0 ± 2.0	41.3 ± 0.7*	46.4 ± 1.1	45.6 ± 0.6
Total n-3 fatty acids	0.73 ± 0.20	0.83 ± 0.22	2.90 ± 0.54	2.50 ± 0.24

Guinea-pigs were fed on a standard diet or the same diet containing 0.3% (w/w) GFB for 2 weeks.

Values are means ± SD from six animals.

* $P < 0.05$ relative to control, † $P < 0.01$ relative to control, ‡ $P < 0.001$ relative to control, Student's *t*-test.

This hypolipidaemic effect is particularly relevant if we consider that we were using normolipaemic animals. In contrast, no appreciable change was observed in microsomal lipid composition of livers after treatment with GFB.

Table 2 shows the effect of GFB on the acyl composition of PC and PE from guinea-pig hepatic microsomes. Our results for control values of PC composition were very close to those reported by Kawashima *et al.* [5]. There was a marked difference in the fatty acid profiles in PC and PE, the former showing a higher proportion of palmitic acid (16:0) and monounsaturated fatty acids, while PE was enriched in stearic acid (18:0) and in polyunsaturated fatty acids (PUFAs), especially arachidonic acid [20:4 (n-6)]. These trends have also been reported in rats and may be due, at least in part, to a preferential utilization of molecular species of diacylglycerols containing monoenoic acids for

the formation of PC [11]. When guinea-pigs were treated with GFB, the proportion of 16:0 in PC increased considerably. The increase was compensated by the reduction in the proportion of 18:0. Moreover, among monounsaturated fatty acids, an increment in palmitoleic acid (16:1) and a decrease in oleic acid (18:1) were observed. The proportion of linoleic acid [18:2 (n-6)] also increased significantly. In addition, GFB caused a decrease in the proportion of arachidonic acid [20:4 (n-6)], docosatetraenoic acid [22:4 (n-6)] and especially eicosatrienoic acid [20.3 (n-6)]. Levels of docosahexaenoic acid [22:6 (n-3)] were increased by the treatment. The total amount of monounsaturated fatty acids decreased in the treated group, and an increase in the proportion of PUFAs and total n-6 fatty acids was also observed. The acyl composition of PE was also modified, but to a lesser extent than that of PC. A marked increase in the proportion of 16:0 was observed and this change was also compensated by a decrease in the levels of 18:0. Besides, 20:3 (n-6) was also significantly decreased by the treatment

Many authors report biochemical changes in the hepatic microsomal elongation–desaturation system of rodents, caused by fibric acid derivatives, mainly induction of the activities of both stearoyl-CoA desaturase and palmitoyl-CoA chain elongation and, consequently, alterations in the acyl composition of microsomal phospholipids [5, 12]. For example, when we treated male Sprague–Dawley rats under the same conditions as those described for guinea-pigs, we found that the change in fatty acid composition of PC showed a completely different profile with an increase in the molar percentage of monounsaturated fatty acids (18% GFB versus 12% control) and a decrease in saturated (44% GFB versus 48% control) and polyunsaturated (38% GFB versus 40% control) fatty acids. This change is mainly due to an increase in the level of 18:1 and 16:1 fatty acids, in concordance with the stimulation of stearoyl-CoA desaturase already cited.

These biochemical responses have been reported to depend on species [5, 13], and thus, after treatment of guinea-pigs with clofibric acid and tiadenol, no change was observed by Kawashima *et al.* [5] in stearoyl-CoA desaturase and palmitoyl-CoA chain elongation. These facts suggested some relationship between the modifications and peroxisome proliferation. Kawashima *et al* [14] reported that peroxisome proliferation requires an increase in the production of lipids to form the membrane of these organellae, especially PUFA, which confers an appropriate fluidity to the membranes Our results show that, in contrast to clofibric acid and other peroxisome proliferators, GFB significantly modifies the acyl composition of hepatic microsomal phospholipids of guinea-pigs *in vivo* without promoting peroxisomal proliferation, suggesting that these phenomena are independent, at least in guinea-pigs. These changes in composition are particularly marked in the 16:0 and 18:0 proportions in PC and PE. The increase in the former is compensated by a decrease in the latter The same situation occurred between 16.1 and 18·1. As 18:0 and 18:1 are known to be formed by chain elongation from 16:0 and 16:1, respectively [15], these results may indicate an inhibitory effect of GFB on the microsomal fatty acid elongation system of guinea-pigs *in vivo*. Inhibition of this enzyme system may be responsible for the increase in its substrates (16·0, 16:1) and for the decrease in the products (18:0, 18·1). This effect is in good agreement with a previous study, in which we found that GFB acted on elongation by inhibiting the condensing step *in vitro* [16], at concentrations well within those reached in therapeutic use [4]. The increase in the proportion of 18:2 (n-6) and the decrease in 20:3 (n-6), 20:4 (n-6) and 22.4 (n-6) can also be explained by the inhibitory effect of GFB on the elongation system. Linoleic acid [18.2 (n-6)] is the substrate for the formation of 20·3 (n-6) by the concerted action of chain elongation and desaturation [17]. Moreover, 20.4 (n-6) and 22·4 (n-6) are the products of successive desaturations and elongations of 20:3 (n-6). In PE the modification of the acyl composition was lower than in PC. The proportion of 16:0 was increased while the proportion of both 18:0 and 20:3 (n-6) decreased significantly. The relative refractoriness of PE to changes in acyl composition has already been described by others [11, 12] but there is no satisfactory explanation to date. As in PC, an inhibitory effect of GFB on chain elongation may explain these changes in the fatty acid profile of PE. Although Δ^5- and Δ^6-desaturase activities can be inhibited by GFB *in vitro*, this occurs at concentrations higher than those at which elongation is completely suppressed (unpublished data). However, concomitant changes in desaturation activity *in vivo* cannot be ruled out, as they could be masked by the inhibition of elongation activity.

Many attempts have been made to ascertain the hypolipidaemic mechanism of fibric acid derivatives [2, 18, 19], but the relative contribution of the proposed mechanisms to the overall pharmacological effect is still controversial Lately, we have tested the effect of three of these drugs *in vitro* (clofibric acid, bezafibrate and gemfibrozil) on several enzymes involved in fatty acid biosynthesis [16, 20–22]. The quality of these fatty acids (chain length and number of unsaturations), either in the free form or incorporated into glycerolipids, has an important role in the control of lipoprotein metabolism [23]. It is known, for example, that lecithin:cholesterol acyltransferase, which is one of the key enzymes involved in reverse cholesterol transport and in the metabolism of high density lipoprotein, shows a greater specificity for some fatty acids of PC [24]. Also, lipoprotein lipase activity is highly influenced by fatty acid composition [25]. Moreover, the acyl composition of phospholipids is known to affect many membrane-associated functions [17, 26] In consequence, the influence of fatty acid composition on glycerolipids on the regulation of lipid metabolism and its contribution to the hypolipidaemic action of fibrates deserves further study.

Acknowledgements—This research was partly supported by grant 91/0147 from the Fondo de Investigaciones Sanitarias de la Seguridad Social and by grant SM91-0020 from DGICYT. M. Vázquez and M. Alegret were recipients of F.P I grants from the Generalitat de Catalunya. We also thank Mr Rycroft (Language Advice Service of the University of Barcelona) for his helpful assistance. Further, we greatly appreciate the technical support provided by Ms T Iglesias.

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REFERENCES

1 Todd PA and Ward A, Gemfibrozil. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic use in dyslipidemia. *Drugs* **36**: 314–339, 1988
2 Schwandt P, Fibrates and triglyceride metabolism. *Eur J Clin Pharmacol* **40** (Suppl 1)· S41–S43, 1991.
3 Brandes R, Kaikus RM, Lysenko N, Ockner RK and Bass NM, Induction of fatty acid binding proteins by

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peroxisome proliferators in primary hepatocyte cultures and its relationship to the induction of peroxisomal β-oxidation. *Biochim Biophys Acta* **1034**: 53–61, 1990.
4. Hawkins JM, Jones WE, Bonner FW and Gibson GG, The effect of peroxisome proliferators on microsomal, peroxisomal and mitochondrial enzyme activities in the liver and kidney. *Drug Metab Rev* **18**: 441–515, 1987.
5. Kawashima Y, Hirose A and Kozuka H, Alterations by peroxisome proliferators of acyl composition of hepatic phosphatidylcholine in rats, mice and guinea-pigs. Role of stearoyl-CoA desaturase. *Biochem J* **235**: 251–255, 1986.
6. Nagi MM, Laguna JC, Cook L and Cinti DL, Disruption of rat hepatic microsomal electron transport chains by the selenium containing antiinflammatory agent Ebselen. *Arch Biochem Biophys* **269**: 264–271, 1989.
7. Bradford M, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding. *Anal Biochem* **72**: 248–254, 1976.
8. Bligh EG and Dyer WJ, A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* **37**: 911–917, 1959.
9. Reddy JK and Rao MS, Peroxisome proliferators and cancer: mechanisms and implications. *Trends Pharmacol Sci* **7**: 438–443, 1986.
10. Lazarow P, Assay of peroxisomal β-oxidation of fatty acids. *Methods Enzymol* **72**: 315–319, 1981.
11. Kawashima Y, Uy-Yu N and Kozuka H, Sex-related differences in the enhancing effects of perfluoro-octanoic acid on stearoyl-CoA desaturase and its influence on the acyl composition of phospholipid in rat liver. Comparison with clofibric acid and tiadenol. *Biochem J* **263**: 897–904, 1989.
12. Kawashima Y, Hirose A and Kozuka H, Modification by clofibric acid of acyl composition of glycerolipids in rat liver. Possible involvement of fatty acid chain elongation and desaturation. *Biochim Biophys Acta* **795**: 543–551, 1984.
13. Lake BG and Gray TJB, Species differences in hepatic peroxisome proliferation. *Biochem Soc Trans* **13**: 859–861, 1985.
14. Kawashima Y, Musoh K and Kozuka H, Peroxisome proliferators enhance linoleic acid metabolism in rat liver. Increased biosynthesis of omega-6 polyunsaturated fatty acids. *J Biol Chem* **256**: 9170–9175, 1990.
15. Nakagawa M, Kawashima Y and Uchiyama M, Relationship between desaturation and chain elongation of palmitoyl-CoA in rat liver microsomes. *Lipids* **11**: 241–243, 1975.
16. Sánchez RM, Viñals M, Alegret M, Vázquez M, Adzet T, Merlos M and Laguna JC, Inhibition of rat liver microsomal fatty acid chain elongation by gemfibrozil *in vitro*. *FEBS Lett* **300**: 89–92, 1992.
17. Sardesi VM, Nutritional role of polyunsaturated fatty acids. *J Nutr Biochem* **3**: 154–166, 1992.
18. Illingworth DR, Lipid-lowering drugs. An overview of indications and optimum therapeutic use. *Drugs* **33**: 259–279, 1987.
19. Shepherd J and Packard CF, Pharmacological approaches to the modulation of plasma cholesterol. *Trends Pharmacol Sci* **9**: 326–329, 1988.
20. Alegret M, Sánchez R, Adzet T, Merlos M and Laguna JC, *In vitro* effect of clofibric acid derivatives on rat hepatic microsomal electron transport chains. *Biochem Pharmacol* **42**: 2057–2060, 1991.
21. Sánchez R, Alegret M, Adzet T, Merlos M and Laguna JC, Differential inhibition of long-chain acyl-CoA hydrolases by hypolipidemic drugs *in vitro*. *Biochem Pharmacol* **43**: 639–644, 1992.
22. Sánchez R, Vázquez M, Alegret M, Viñals M, Adzet T, Merlos M and Laguna JC, Cytosolic lipogenic enzymes: effect of fibric acid derivatives *in vitro*. *Life Sci* **52**: 213–222, 1993.
23. Bihain BE, Deckelbaum RJ, Yen FT, Gleeson AM, Carpentier YA and Witte LD, Unesterified fatty acids inhibit the binding of low density lipoproteins to the human fibroblast low density liprotein receptor. *J Biol Chem* **264**: 17316–17321, 1989.
24. Grove D and Pownall HJ, Comparative specificity of plasma lecithin:cholesterol acyltransferase from ten animal species. *Lipids* **26**: 416–420, 1991.
25. Desreumaux V, Dedonder E, Dewailly P, Sezille G and Fruchart JC, Effects of unsaturated fatty acids in phospholipids on the *in vitro* activation of the lipoprotein lipase and triglyceride lipase. *Arzneimittel Forsch* **29**: 1581–1583, 1979.
26. Carruthers A and Melchior DL, How bilayer lipids affect membrane protein activity. *Trends Biochem Sci* **11**: 331–335, 1986.