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Different effects of fibrates on the microsomal fatty acid chain elongation and the acyl composition of phospholipids in guinea-pigs

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1 The effects *in vitro* and *in vivo* of three fibric acid derivatives, clofibrate (CFB), bezafibrate (BFB) and gemfibrozil (GFB) on some enzyme activities related to fatty acid biosynthesis, namely palmitoyl-CoA synthetase and hydrolases (microsomal and cytosolic), NADH and NADPH cytochrome c reductases and acyl-CoA elongases were investigated in guinea-pigs.

2 The three fibrates inhibited acyl-CoA elongation *in vitro*, irrespective of the substrate of elongation used (saturated, monounsaturated, polyunsaturated) and with an order of potency GFB > BFB > CFB. In the case of GFB, inhibition occured at concentrations that can be reached *in vivo*.

3 Microsomal palmitoyl-CoA hydrolase and synthetase were also inhibited *in vitro* (GFB ≥ BFB > CFB), whereas NADH cytochrome c reductase activity was increased by GFB. Nevertheless, the magnitude of changes were lower than those observed in elongation activities.

4 Treatment with fibrates did not produce peroxisomal proliferation in guinea-pigs, as measured by peroxisomal β-oxidation activity and liver weight/body weight ratio. Nevertheless, fibrates provoked a reduction in plasma cholesterol and triglycerides, at least in GFB- and BFB-treated animals.

5 Fatty acid elongation was significantly modified by GFB treatment *in vivo*. The remaining enzyme activities studied were only slightly changed by fibrate treatment.

6 Treatment with BFB and to a lesser extent with CFB, increased the relative proportion of MUFA (palmitoleic and oleic acids) in microsomal phospholipids, whereas PUFA (mainly linoleic acid) decreased. GFB behaved differently, increasing palmitic and linoleic acids and decreasing stearic and oleic acids. The latter changes are attributable to an inhibition of elongation activity by GFB.

7 The changes observed after fibrate treatment in both rats and guinea-pigs, as they are not directly related to peroxisome proliferation, could be more reliably extrapolated to man than those observed only in rats.

Keywords: Fibrates; elongases; peroxisomal proliferation; microsomal phospholipids; guinea-pigs

Introduction

Fibric acid derivatives (fibrates) constitute a well known group a hypolipidaemic drugs, mainly used in the treatment of mixed hyperlipoproteinaemia and hypertriglyceridaemia (Shepherd *et al.*, 1991). In spite of their wide prescription, the biochemical bases for their hypolipidaemic effect are not fully understood (Catapano, 1992). Besides, fibrate treatment increases the content of monounsaturated fatty acids (palmitoleic and/or oleic acids) and decreases the content of polyunsaturated fatty acids (mainly linoleic) in the cholesteryl ester, triglyceride, or phospholipid plasma fractions from hyperlipidaemic subjects (Vessby *et al.*, 1980; Vessby & Lithell, 1990; Agheli & Jacotot, 1991; Tavella *et al.*, 1993). The biochemical mechanism(s) underlying these latter effects are not established. It is also unknown whether there is a relationship between changes in fatty acid composition and the hypolipidaemic effect (Tavella *et al.*, 1993; Alegret *et al.*, 1995), although there are rational bases to think so. Thus, we are aware that the type of fatty acid, especially the length and number of unsaturations present in their structure, modulates the enzyme activities directly related to the handling of lipoproteins in the organism, e.g. lipoprotein lipase (Wang *et al.*, 1992) or lecithin-cholesterol acyltransferase (Grove & Pownall, 1991).

Fibrates modify some of the enzyme activites related to hepatic fatty acid synthesis in rats, either *in vitro* (Alegret *et al.*, 1991; Sánchez *et al.*, 1992a,b; 1993a,b) or *in vivo* (Kawashima *et al.*, 1984; 1986; Alegret *et al.*, 1994; 1995). Fibrates also behave as potent peroxisome proliferators in mice and rats (Sirtori *et al.*, 1992). We have demonstrated that the activities of some of the enzymes modified by fibrate treatment in rats, such as palmitoyl-CoA hydrolases, palmitoyl-CoA synthetase and Δ^9-desaturase are correlated with the peroxisomal proliferation phenomena, while others, such as the decrease in NADH cytochrome c reductase activity and the increase in palmitoleoyl CoA elongation or Δ^6-desaturation are not (Alegret *et al.*, 1994; 1995). Thus, in order to obtain further insight into the effect of fibrates on enzyme activities related to fatty acid biosynthesis, either *in vitro* or *in vivo*, and their relationship with peroxisome proliferation, we chose the guinea-pig as a species that is insensitive to peroxisome proliferation (Lake & Gray, 1985). Human subjects are assumed to be resistant to fibrate-induced peroxisomal proliferation (Lake & Gray, 1985; Hawkins *et al.*, 1987), thus results from guinea-pigs could be more reliably extrapolated to man than those obtained in rats. Here we compare the effects *in vitro* and *in vivo* of three fibric acid derivatives, clofibrate (CFB), bezafibrate (BFB) and gemfibrozil (GFB) on some enzyme activities related to fatty acid biosynthesis which were modified by fibrate treatment in previous studies in rats, namely palmitoyl-CoA synthetase and hydrolases (microsomal and cytosolic), NADH and NADPH cytochrome c reductases and acyl-CoA

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elongases (see above for references). The peroxisomal proliferation marker, peroxisomal β-oxidation, as well as plasma lipid levels were also measured *in vivo*. As changes in enzyme activities related to fatty acid biosynthesis induced by fibrate treatment are translated to modifications in the acyl composition of microsomal phospholipids in rats (Vázquez *et al.*, 1995), we attempted to determine whether fibrates also modify the acyl composition of the main microsomal phospholipids, namely phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in guinea-pigs. Our results provide evidence of a differential effect of GFB, which strongly inhibits the chain elongation system both *in vitro* and *in vivo*. These modifications may help to explain the different behaviour of GFB compared to other fibrates in plasma fatty acid composition in man (Tavella *et al.*, 1993).

Methods

Animals

Male Dunkin-Hartley guinea-pigs from Letica (Barcelona, Spain), weighing about 350 g, were maintained in a 12 h light-dark cycle under conditions of constant humidity and temperature (22±2°C) and were fed standard Panlab diet (Barcelona, Spain) for five days prior to the beginning of the studies. In *in vivo* studies, animals were distributed randomly according to their weight in four groups of 6. Each group was fed, respectively, a control diet or a diet containing 0.3% w/w CFB, 0.45% w/w BFB or 0.3% w/w GFB (equimolar doses). The 6 guinea-pigs of each group were killed after 15 days of treatment. The diets were prepared as described previously (Alegret *et al.*, 1994).

Experimental

The animals were killed by decapitation between 08 h 00 min and 09 h 00 min, to avoid circadian variations of the enzyme activities (Dato *et al.*, 1972). Samples of blood were collected from the neck and plasma was obtained by centrifugation at 3000 g for 10 min. Livers were removed, perfused with ice-cold 0.9% NaCl, weighed and homogenized in eight volumes of 0.25 M sucrose, 50 mM Tris-HCl buffer, pH 7.4. Subcellular fractions were obtained by differential centrifugation as described previously (Nagi *et al.*, 1989), and the protein content was determined by the method of Bradford, (1976) with bovine serum albumin used as standard.

Enzyme assays

Palmitoyl-CoA hydrolase and palmitoyl-CoA synthetase activities were assayed as described in previous studies (Sánchez *et al.*, 1992a; 1993b). The enzyme activities related to the microsomal electron transport chains, namely NADH and NADPH cytochrome c reductase and also the peroxisomal β-oxidation activity were determined as described previously (Alegret *et al.*, 1991; 1994). Microsomal fatty acid elongation was assayed by the measurement of [2-^{14}C]-malonyl-CoA incorporation into exogenous acyl-CoAs essentially as described previously (Alegret *et al.*, 1995). 18:3 CoA was synthesized by the mixed anhydride procedure as described elsewhere (Sánchez *et al.*, 1993b). Concentrations of the CoA derivatives were determined by the method of Ellman (1959) after cleavage of the thioester bond with hydroxylamine. For the studies *in vitro* the subcellular fractions were incubated with either 1 or 5 mM fibrate. Drugs were added from stock solutions adjusted to pH between 7.5 and 8 with 0.1 N NaOH. The volume added did not modify the pH of the assay mixture.

Analysis of fatty acids from microsomal phospholipids

Lipids were extracted from microsomes by the method of Bligh & Dyer (1959). PC and PE fractions were isolated by thin layer chromatography and recovered from silica as described previously (Vázquez *et al.*, 1995). The fatty acids in these phospholipids were transmethylated with 0.5 N sodium methylate. Analysis of fatty acids was performed on a Hewlett-Packard gas chromatograph model 5890 equipped with a flame ionization detector and a Supelcowax 10 fused silica capillary column (30 m × 0.2 mm internal diameter). Peaks were identified by comparison of retention times with those of authentic fatty acid methyl esters (Sigma). Peak areas were determined by a Hewlett-Packard 3390A integrator.

Chemicals

Malonyl-CoA, palmitoyl-CoA (16:0 Co-A), palmitoleoyl-CoA (9−16:1 CoA), γ-linolenic acid (6,9,12−18:3), NAD(P)H, fatty acid free bovine serum albumin (BSA), GSH, ATP, CoA and Trizma were obtained from Sigma (St. Louis, MO, U.S.A.). [2-^{14}C]-malonyl-CoA (50 mCi mmol^{-1}) was purchased from New England Nuclear (Boston, MA, U.S.A.). Scintillation fluids (Co 136 and Co 36) were from Scharlau Co. (Barcelona, Spain). Butylhydroxytoluol was from Merck (Barcelona, Spain) and sodium methylate from Supelco (Barcelona, Spain). General chemicals were obtained from commercial sources and were of analytical grade.

Drugs

Clofibrate (CFB) was a generous gift from ICI-Farma (Pontevedra, Spain), bezafibrate (BFB) from Boehringer Mannheim (Barcelona, Spain), and gemfibrozil (GFB) from Parke-Davis (Barcelona, Spain).

Plasma cholesterol and triglyceride concentration

Cholesterol was determined using the colorimetric test Monotest Cholesterol CHODPAP No. 290319, and triglyceride concentration was assayed by means of a Peridochrom Triglyceride GPO-PAP No. 701882 test, both from Boehringer Mannheim (Barcelona, Spain).

Statistical evaluation

Results are expressed as means±s.e.mean of six experiments performed in duplicate. Statistical differences were established by a two way ANOVA test and the *a posteriori* multiple comparison Duncan test, using the FOUNDS computer programme. Differences with $P<0.05$ were considered significant.

The abbreviations used are: CFB, colifibrate; BFB, bezafibrate; GFB, gemfibrozil; phosphatidylcholine, PC; phosphatidylthanolamine, PE; 16:0, palmitic acid; 16:1, palmitoleic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2(n-6), linoleic acid; 18:3(n-6), γ-linolenic acid; 20:3(n-9), mead acid 20:3(n-6), dihomo-γ-linoleic acid; 20:4(n-6), arachidonic acid; MPCH, microsomal palmitoyl-CoA hydrolase; CPCH, cytosolic palmitoyl-CoA hydrolase; PCS, palmitoyl-CoA synthetase; NADH CR, NADH cytochrome c reductase; NADPH CR, NADPH cytochrome c reductase; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Results

Enzyme activities in vitro

Table 1 shows the percentage variation with respect to control values of several enzyme activities in the presence of two different concentrations (1 and 5 mM) of CFB, BFB and GFB. Five mM of either BFB or GFB significantly reduced microsomal palmitoyl-CoA hydrolase (MCPH) (34 and 51% inhibition, respectively) and palmitoyl-CoA synthetase activities (PCS) (32 and 34% inhibition). Five mM GFB also increased NADH cytochrome c reductase activity (NADHCR) by 43%.

Effects of 1 mM fibrate on these enzyme activities showed the same trends as 5 mM, but variations were always small. The effect of the three fibrates was more marked on the chain elongation system (Figure 1). Elongation activity was strongly inhibited even at 1 mM, which can be attained in plasma during the therapeutic use of fibrates (Brandes *et al.*, 1990). GFB was the strongest inhibitor and after the addition of 1 mM of this drug the elongation system activity of saturated, monounsaturated and polyunsaturated fatty acids was reduced by 90, 89 and 77%, respectively.

Studies in vivo

Peroxisomal proliferation markers Guinea-pigs fed with a diet supplemented with fibrates for 15 days showed no significant differences in body weight gain with the control group (data not shown). Fibrate treatment did not modify either the liver weight/body weight ratio or the peroxisomal β-oxidation activity in guinea-pigs (Table 2), both markers of peroxisome proliferation.

Plasma lipid levels BFB and GFB treatment produced moderate hypolipidaemic effects, reducing both plasma cholesterol (17 and 43% reduction for BFB- and GFB-treated animals, respectively), and triglycerides (25 and 23%) (Table 2). Due to variability, only cholesterol reduction in GFB-treated group was statistically significant at the level of 0.05. CFB did not produce lipid reduction in the same conditions.

Enzyme activities None of the three fibrates has a significant effect *in vivo* on any of the enzyme activities that were slightly or not modified *in vitro*, i.e. microsomal and cytosolic palmitoyl-CoA hydrolases, palmitoyl-CoA synthetase and NADH and NADPH cytochrome c reductases (Table 3). Conversely, fibrates affected fatty acid chain elongation, although each drug showed different behaviour (Figure 2). GFB caused a significant increase in fatty acid elongation activities of 77, 60 and 59% for saturated, monounsaturated and polyunsaturated fatty acids, respectively. On the other hand, BFB tended to reduce elongation activity, whereas CFB produced no modification.

Acyl composition of microsomal phospholipids Clear differences appeared among the drugs studied when the molar percentage of fatty acids in phosphatidylcholine (PC) and phosphatidylethanolamine (PE) fractions from guinea-pig he-

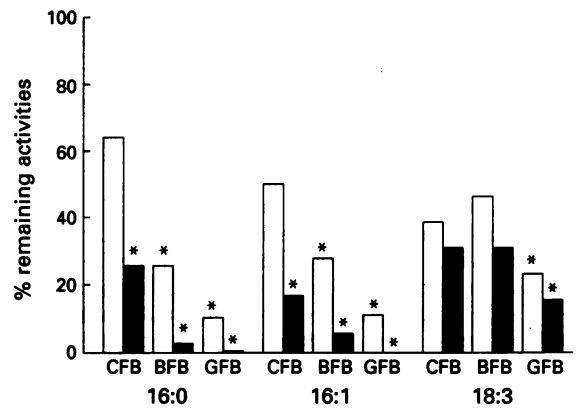

Figure 1 Microsomal elongases activities *in vitro* in the presence of 1 mM (open columns) or 5 mM (solid columns) of fibrates. Results are expressed as percentages with respect to control activities (100%). Control activities (in nmol min^{-1} mg^{-1} protein) were: palmitoyl-CoA elongase (16:0) 0.39±0.07 ($n=7$); palmitoleoyl-CoA elongase (16:1) 0.18±0.02 ($n=6$); gamma-linoleoyl-CoA elongase (18:3) 0.13±0.03 ($n=6$). Values are the mean of n-experiments performed in duplicate. *Values for treatment significantly different from controls, $P<0.05$.

Table 1 Enzyme activities *in vitro* in the presence of two concentrations of fibrates

	MPCH	*CPCH*	*PCS*	*NADHCR*	*NADPHCR*
CFB 1 mM	87	75	83	112	112
5 mM	71	95	85	116	117
BFB 1 mM	75	93	86	110	106
5 mM	66*	86	68	114	108
GFB 1 mM	70	82	75	106	105
5 mM	49*	86	66	143*	107

Results are expressed as percentages with respect to control activities (100%). Control activities (in nmol min^{-1} mg^{-1} protein) were: microsomal palmitoyl-CoA hydrolase (MPCH) 3.26±0.24 ($n=6$); cytosolic palmitoyl-CoA hydrolase (CPCH) 4.40±0.92 ($n=3$); palmitoyl-CoA synthetase (PCS) 65±10 ($n=7$); NADH cytochrome c reductase (NADHCR) 724±36 ($n=6$); NADPH cytochrome c reductase (NADPHCR) 233±29 ($n=6$). Values are the mean of n experiments performed in duplicate. *Values significantly different from control activites, $P<0.05$.

Table 2 Fibrate effects on liver weight, plasma lipids and peroxisomal β-oxidation in guinea-pigs

	Control	*CFB*	*BFB*	*GFB*
Liver weight/body weight (%)	6.4±0.4	5.9±0.6	7.3±0.5	7.1±0.4
Peroxisomal β-oxidation (nmol min^{-1} mg^{-1} protein)	2.20±0.40	1.25±0.24	2.70±0.53	3.00±0.32
Plasma triglycerides (mg dl^{-1})	54.8±6.2	57.3±9.3	40.9±5.0	42.3±5.0
Plasma cholesterol (mg dl^{-1})	46.9±5.8	45.3±5.8	38.9±5.3	26.9±1.6*

Results are means±s.e.mean of 6 experiments performed in duplicate. Animals were fed with a standard diet (control) or a diet containing 0.3% w/w CFB, 0.45% w/w BFB or 0.3% w/w GFB (equimolar doses). *Values for treatment significantly different from controls, $P<0.05$.

patic microsomes was analyzed (Tables 4 and 5). On the one hand, BFB and CFB produced similar changes, even though they were more marked after BFB treatment. BFB and CFB increased the proportion of total MUFA in PC due to an increase in the content of both palmitoleic acid (16:1)(81 and 23% increase for BFB and CFB, respectively) and oleic acid (18:1(n-9))(60 and 25% increase). The same trends were observed in PE, although changes were smaller. The increase in the content of total MUFA in PC was accompanied by a decrease in the total content of PUFA, mainly due to the decrease in the relative content of linoleic acid (18:2(n-6)). BFB also decreased the relative content of dihomo-γ-linolenic acid (20:3(n-6)) and both CFB and BFB increased that of mead acid (20:3(n-9)). On the other hand, GFB showed a different profile, increasing the relative content of 16:0 and 18:2(n-6) in both PC and PE and decreasing 18:1 and 18:0 in PC. Mead acid molar percentage was not modified by GFB treatment.

Discussion

In previous studies performed in rats we observed that fibrates affected the activities of some of the enzymes related with fatty acid biosynthesis, namely palmitoyl-CoA synthetase and hydrolases, NADH and NADPH cytochrome c reductases and acyl-CoA elongases, either *in vitro* (Alegret *et al.*, 1991; Sánchez *et al.*, 1992a,b; 1993a,b) or *in vivo* (Alegret *et al.*, 1994; 1995). Some of the changes produced by fibrate treatment were correlated with peroxisomal proliferation. Here, we investigated whether these activities were also modified in guinea-pigs, which are insensitive to peroxisome proliferation (Lake & Gray, 1985). A 15 day-fibrate treatment did not cause peroxisome proliferation as no significant changes were observed in the liver weight/body weight ratio or in the peroxisomal β-oxidation activity (Table 2). In contrast, we have

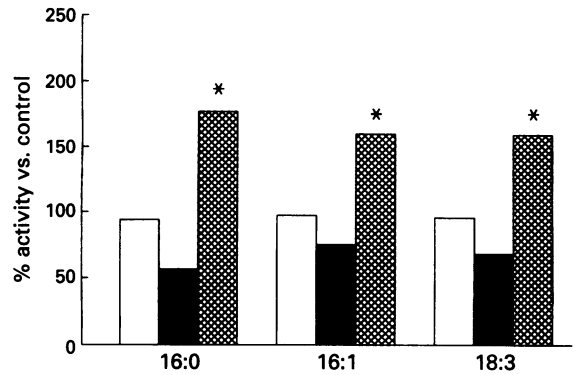

Figure 2 Clofibrate (open columns), bezafibrate (solid columns) and gemfibrozil (cross hatched columns) effect on microsomal elongases *in vivo*. Results are expressed as percentages with respect to control activities (100%). Control activities (in nmol min^{-1} mg^{-1} protein) were: palmitoyl-CoA elongase (16:0) 0.64±0.11; palmitoleoyl-CoA elongase (16:1) 0.72±0.08; gamma-linoleoyl-CoA elongase (18:3) 0.44±0.06. Values are the mean of six experiments performed in duplicate. *Values for treatment significantly different from controls, $P<0.05$.

Table 3 Fibrate effects on enzyme activities *in vivo*

	MPCH	*CPCH*	*PCS*	*NADHCR*	*NADPHCR*
CFB	111	73	84	62	67
BFB	92	127	142	60	73
GFB	108	136	90	87	111

Results are expressed as percentages with respect to control activities (100%). Control activities (in nmol min^{-1} mg^{-1} protein) were: microsomal palmitoyl-CoA hydrolase (MPCH) 3.8±0.4; cytosolic palmitoyl-CoA hydrolase (CPCH) 2.2±0.5; palmitoyl-CoA synthetase (PCS) 72±11; NADH cytochrome c reductase (NADHCR) 850±123; NADPH cytochrome c reductase (NADPHCR) 186±20. Values are the mean of six experiments performed in duplicate.

Table 4 Fibrate effects on acyl composition of PC in hepatic microsomes from guinea pigs

	Control	*CFB*	*BFB*	*GFB*
16:0	17.5±0.5	17.5±0.5	17.9±0.4	22.9±0.6*
16:1	0.83±0.12	1.1±0.08	1.5±0.2*	1.1±0.04
18:0	29.4±0.9	28.8±0.4	27.6±0.6	25.8±0.5*
18:1	12.2±1.1	15.3±2.0	19.5±2.7*	8.0±0.2*
18:2(n-6)	33.1±1.8	31.9±2.0	26.2±3.0*	38.4±0.4*
18:3(n-6)	1.1±0.3	0.99±0.16	1.2±0.3	0.85±0.13
18:3(n-3)	0.55±0.39	0.51±0.04	1.4±0.2*	0.05±0.00
20:3(n-9)	0.04±0.02	0.09±0.01*	0.13±0.02*	0.02±0.01
20:3(n-6)	0.66±0.04	0.61±0.05	0.53±0.05*	0.43±0.02*
20:4(n-6)	2.3±0.2	2.3±0.1	2.2±0.2	1.7±0.1*
20:5(n-3)	0.16±0.05	0.05±0.01	0.10±0.03	0.21±0.06
22:4(n-6)	0.12±0.02	0.13±0.09	0.12±0.03	0.04±0.02*
22:5(n-3)	0.15±0.02	0.14±0.01	0.11±0.04	0.07±0.02*
22:6(n-3)	0.47±0.13	0.53±0.05	0.59±0.13	0.50±0.08
Total SFAs	46.9±1.0	46.3±0.09	45.5±0.5	48.7±0.2
Total MUFAs	13.0±1.3	16.4±2.1	21.1±2.9*	9.1±0.4
Total PUFAs	40.1±1.3	37.3±2.1	33.4±3.1*	42.2±0.3
Total n-6	38.7±1.4	36.0±2.1	31.2±2.9	41.4±0.3
Total n-3	1.3±0.4	1.2±0.04	2.1±0.3	0.83±0.09

Results are means±s.e.mean of 6 experiments. Guinea-pigs were fed on a standard diet (control) or the same diet containing 0.3% w/w CFB, 0.45% w/w BFB or 0.3% w/w GFB (equimolar doses). *Values for treatment significantly different from controls, $P<0.05$.

Table 5 Fibrate effects on acyl composition of PE in hepatic microsomes from guinea-pigs

	Control	*CFB*	*BFB*	*GFB*
16:0	10.2±0.5	9.7±0.3	11.0±0.2	16.1±1.0*
16:1	0.58±0.06	0.66±0.08	0.70±0.08	0.75±0.07
18:0	34.4±1.0	33.7±0.5	31.2±0.7*	31.1±0.7*
18:1	4.5±0.4	4.9±0.4	5.8±0.6*	4.0±0.1
18:2(n-6)	34.6±1.1	32.4±0.7	36.3±0.5	37.0±0.4*
18:3(n-6)	1.8±0.4	1.8±0.6	2.3±0.8	1.3±0.2
18:3(n-3)	0.66±0.40	1.0±0.1	1.56±0.08*	0.08±0.01
20:3(n-9)	0.10±0.04	0.24±0.02*	0.23±0.02*	0.07±0.02
20:3(n-6)	0.46±0.04	0.42±0.03	0.36±0.02*	0.32±0.03*
20:4(n-6)	7.7±0.7	9.5±0.5*	5.9±0.4	6.6±0.4
20:5(n-3)	0.45±0.07	0.26±0.02*	0.39±0.03	0.52±0.05
22:4(n-6)	0.58±0.11	0.83±0.11*	0.39±0.04	0.35±0.04
22:5(n-3)	0.99±0.16	1.3±0.1*	0.78±0.07	0.73±0.04
22:6(n-3)	1.8±0.2	3.3±0.4*	1.9±0.4	1.2±0.1
SFA	44.6±1.0	43.4±0.3	42.2±0.5*	47.1±0.4*
MUFA	5.1±0.4	5.5±0.5	6.5±0.7*	4.7±0.1
PUFA	50.4±0.9	51.1±0.7	51.3±0.9	48.2±0.4*
n-6	46.1±0.7	45.0±0.5	46.4±0.8	45.6±0.2
n-3	3.9±0.7	5.9±0.4*	4.6±0.4	2.5±0.1

Results are means ± s.e.mean of 6 experiments. Guinea-pigs were fed on a standard diet (control) or the same diet containing 0.3% w/w CFB, 0.45% w/w BFB or 0.3% w/w GFB (equimolar doses). *Values for treatment significantly different from controls, $P<0.05$.

previously observed that a similar fibrate treatment (same duration, same doses) markedly increases both parameters in rats (Alegret *et al.*, 1994; 1995). For instance, BFB, which was the strongest peroxisomal inducer, produced a 1.7 fold increase in the liver weight/body weight ratio and a 9 fold increase in the peroxisomal β-oxidation activity in rats (Alegret *et al.*, 1994), whereas these parameters are not significantly modified in guinea-pigs. BFB and GFB tend to decrease cholesterol and triglyceride plasma levels in guinea-pigs, although, as confirmed here, these species are refractive to fibrate-induced peroxisomal induction. These data further reinforce the hypothesis that peroxisomal proliferation and hypolipidaemic effects induced by fibrates are mainly independent phenomena (Alegret *et al.*, 1994). Furthermore, the hypolipidaemic effect shown in guinea-pigs is particularly relevant if we consider that, on the one hand, the animals studied were normolipidaemic and on the other, the low values of plasma cholesterol and triglycerides in this species (about 50 mg dl^{-1}), which a further reduction in plasma lipid levels makes difficult.

From the enzyme activities assayed *in vitro*, only the fatty acid elongation system was strongly modified by fibrate treatment (Figure 1). The inhibition caused by GFB was marked even at 1 mM and it was independent of the number of unsaturates present in the substrate (90, 89 and 77% inhibition of 16:0, 16:1 and 18:3 elongation, respectively). These data suggest a probable effect of GFB *in vivo*, as a concentration of 1 mM can practically be achieved in the therapeutic use of fibrates (Brandes *et al.*, 1990). Furthermore, higher concentrations may be attained in tissues if we take into account the selective accumulation of GFB in certain organs, such as the liver and the kidney, where fibrate concentration is higher than in plasma (Cayen, 1985). Fibrate effects on fatty acid elongation *in vitro* in guinea-pigs are in agreement with those reported in previous studies in rats where fibrates (Sánchez *et al.*, 1993b), especially GFB (Sánchez *et al.*, 1993a), inhibited the rat-limiting step of the elongation process, the condensing reaction. Furthermore, the potency order observed in guinea-pigs (GFB > BFB > CFB) matched that observed in rats. Other enzyme activities tested *in vitro* showed the same trends already observed in rats, although the effects produced by fibrate addition to a medium containing cellular subfractions from guinea-pigs were generally smaller. The most clear-cut example was microsomal palmitoyl-CoA hydrolase, which was inhibited by fibrates at submillimolar concentrations in rats (the IC_{50} value for GFB is 0.15 mM, Sánchez *et al.*, 1992), whereas concentrations up to 5 mM were needed to produce inhibition in guinea-pigs. Nevertheless, the same trends were shared by both species *in vitro*, i.e., palmitoyl-CoA hydrolases and synthetase activities were inhibited by fibrates, NADH cytochrome c reductase was enhanced and NADPH cytochrome c reductase was not affected.

Fibrate treatment also modified the fatty acid elongation system *in vivo*, which was induced after GFB treatment irrespective of the degree of unsaturation of the substrate used (Figure 2). CFB did not significantly modify the fatty acid elongation system, in agreement with previous studies performed in guinea-pigs (Kawashima *et al.*, 1986). The induction elicited by GFB *in vivo*, in contrast to the inhibitory effect detected *in vitro*, should be regarded as a compensatory response in order to override the drug inhibition. Thus, the organism produces higher amounts of the enzyme (assayed in enzymology studies measuring total activity in optimal conditions), but the overall result is a reduction in product formation due to the presence of the inhibitor. This behaviour has been reported for other drugs acting as inhibitors of key lipogenic enzymes, such as statins for hydroxymethylglutaryl-CoA reductase (Endo, 1986) and it was also described in our previous studies in rats (Alegret *et al.*, 1994; 1995).

Changes in the enzyme activities related to fatty acid biosynthesis caused by fibrate treatment produced qualitative modifications in fatty acid composition of hepatic microsomal phospholipids in rats (Vázquez *et al.*, 1995). In the present study, fibrates also modified acyl composition of the two main microsomal phospholipids, i.e. phosphatidylcholine (PC) and phosphatidylethanolamine (PE), in guinea-pigs. BFB treatment significantly increased the relative content of palmitoleic acid (16:1) and oleic acid (18:1) in hepatic microsomal PC (Table 4). These changes have already been reported in rats after BFB treatment (Vázquez *et al.*, 1995) and they have been explained as a consequence of the inductions of both Δ^9-desaturase and palmitoyl-CoA elongase activities. In a recent study we reported that the induction of the Δ^9-desaturase activity in rats after BFB treatment is mainly dependent on peroxisome proliferation (Alegret *et al.*, 1995). An induction of Δ^9-desaturase activity by BFB in guinea-pigs could be explained by interspecific differences in the mechanisms triggering Δ^9-desaturase induction between guinea-pigs and rats, or it could be the consequence of an initial stage of peroxisomal induction, not detectable by the common markers. We should bear in mind that, of the fibrates studied, BFB is the most potent peroxisomal inducer in rats (Alegret *et al.*, 1994; Vázquez *et al.*, 1995). Moreover, although it is clear that guinea-

pigs are far less sensitive to peroxisomal induction than rats, it does not mean that such an effect is ruled out. In fact, manifestations of peroxisomal proliferation have been described in species considered refractory, such as primates and humans (Hanefeld *et al.*, 1983; Reddy *et al.*, 1984). Unfortunately, we were not able to measure Δ^9-desaturase activity in guinea-pigs, as its specific activity is at least 10 fold lower than that found in rats. Overall, changes observed in CFB-treated guinea-pigs followed the same trends shown by BFB treatment, but they were rarely significant. Kawashima *et al.* (1986) did not find modifications in acyl composition in PC after treatment with CFB (0.5% for 7 days). GFB showed different behaviour from CFB and BFB, in accordance with our previous studies in rats (Vázquez *et al.*, 1995). GFB increased the molar percentage of both palmitic (16:0) and palmitoleic acids (16:1) and decreased the levels of both stearic (18:0) and oleic acids (18:1). As 18:0 and 18:1 are known to be formed by chain elongation from 16:0 and 16:1, respectively (Nakagawa *et al.*, 1975), these results confirm the inhibitory effect of GFB on the microsomal fatty acid elongation of guinea-pigs observed in the enzymology studies.

BFB-treated guinea-pigs showed a reduction in the percentage of linoleic acid (18:2(n-6)) in PC. A reduction in 18:2(n-6) levels is observed after CFB (Kawashima *et al.*, 1984; 1989) or BFB (Vázquez *et al.*, 1995) administration to rats. Again, changes in BFB-treated guinea-pigs could be attributed to the first signs of peroxisomal induction and/or to the induction of Δ^6-desaturase activity. On the other hand, GFB significantly increased 18:2(n-6) levels, accompanied by decreases in the molar percentage of eicosatrienoic acid (20:3(n-6)), arachidonic acid (20:4(n-6)) and docosatetraenoic acid (22:4(n-6)). These changes may be explained by the inhibitory effect of GFB on the elongation system, as linoleic acid is the substrate for the formation of 20:3(n-6) by the concerted action of chain elongation and desaturation (Sardesai, 1992). Besides, 20:4(n-6) and 22:4(n-6) are the products of successive desaturations and elongations of 20:3(n-6).

There are considerable differences in the content of 20:3(n-9) of PC and PE of CFB- and BFB-treated animals on the one hand, and of GFB-treated animals on the other. 20:3(n-9) content was greatly increased after CFB and BFB treatment, whereas no change was observed after GFB treatment. Mead acid is formed from 18:1 by the concerted action of desaturation and chain elongation. Apart from an inhibitory effect of GFB on the chain elongation system, the lack of increase in the content of this fatty acid may also be an indirect effect of the higher levels of the 18:2(n-6) in the GFB group than in the CFB or BFB groups. Δ^6-desaturase shows a greater affinity for C_{18} substrates with the greater number of unsaturates, and in the presence of normal contents of 18:2(n-6) little desaturation of 18:1 occurs (Sardesai, 1992). However, after CFB and BFB treatment the decrease in the contents of 18:2(n-6) led to the conversion of 18:1 in 20:3(n-9). The increase in 20:3(n-9) in guinea-pigs was less impressive than that observed in rats (even an 800% increase in BFB-treated rats) (Vázquez *et al.*, 1995). In the latter species 20:3(n-9) synthesis is greatly increased in order to compensate for the decrease in long-chain polyunsaturated fatty acids, which are preferentially degraded by the enhanced peroxisomal β-oxidation activity (Sardesai, 1992).

In summary, in the present study we observed that fibrate treatment produced modifications of some enzyme activities related to fatty acid biosynthesis both *in vitro* and *in vivo* in guinea-pigs. These changes occured in the absence of noticeable peroxisomal proliferation and they were reflected in the acyl composition of hepatic microsomal phospholipids. Overall, changes observed in guinea-pigs were less marked than those observed in previous studies in rats, as in the latter species the intrinsic effect of fibrates on enzyme activities overlap with those effects derived from peroxisomal proliferation. Thus, the use of guinea-pigs as experimental models constitutes a more reliable approach to what may occur in human subjects after fibrate treatment. The most outstanding result shown in this study is the clear inhibitory effect of GFB on the chain elongation system. Elongation inhibition modifies the acyl composition of microsomal phospholipids in such a way that an in-crease is observed in the substrates and a decrease in the products of the chain elongation system. These changes could be reflected in the nature of acyl chains in triglycerides, cholesterol esters and phospholipids in plasma lipoproteins, and they may explain the increase in 16:1 and the lack of increase in the content of 18:1 in plasma lipoproteins after GFB treatment in human subjects (Tavella *et al.*, 1993), in contrast to CFB, BFB or fenofibrate treatment, which all increase the relative content of 18:1 (Vessby *et al.*, 1980; Vessby & Lithell, 1990; Agheli & Jacotot, 1991).

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