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Differential effects of fibrates on the acyl composition of microsomal phospholipids in rats

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1 The time-course and comparative effects of treatment with clofibrate (CFB), bezafibrate (BFB), and gemfibrozil (GFB) on the acyl composition of the main microsomal phospholipids, i.e. phosphatidylcholine and phosphatidylethanolamine, have been studied in male Sprague-Dawley rats.

2 The administration of the three fibrates caused a strong peroxisomal induction and a hypolipidaemic effect. Concerning the changes in acyl composition, CFB and BFB behaved in a similar way, with differences which could be attributed to their different potency as peroxisome inducers, whereas GFB showed a somewhat distinct profile.

3 The three drugs increased the relative content of palmitic, palmitoleic and oleic acids, whereas the levels of stearic acid and also those of long chain, highly unsaturated fatty acids docosatetraenoic, docosapentaenoic and docosahexaenoic acids were reduced. In general, these effects appeared from the first day of treatment and were highly correlated with peroxisomal proliferation. In addition, they were more evident in the phosphatidylcholine than in the phosphatidylethanolamine fraction.

4 Fibrates increased total monounsaturated fatty acids, whereas a decrease in total polyunsaturated fatty acids in the phosphatidylcholine fraction was observed in CFB- and BFB-, but not in GFB-treated rats. Clear differences appeared between CFB and BFB on the one hand, and GFB on the other when the influence of fibrate treatment on the molar percentages of linoleic, eicosatrienoic, arachidonic and mead acids was analyzed.

5 GFB increased linoleic acid content in phosphatidylethanolamine, whereas CFB and BFB decreased its level in both phospholipid fractions. In contrast, CFB and BFB enhanced eicosatrienoic and mead acids in both fractions and arachidonic acid in phosphatidylethanolamine, whereas GFB had practically no effect.

6 The different behaviour of GFB may be explained on the basis of two different observations. First, GFB is the weakest peroxisomal inducer of the three fibrates tested. Second, GFB is the strongest inhibitor *in vitro* of some of the enzyme activities related to fatty acid chain modelling, particularly elongases and desaturases.

Keywords: Fibrates; phospholipid acyl chains; rat liver; peroxisomal proliferation

Introduction

Fibric acid derivatives are hypolipidaemic drugs mainly used in hypertriglyceridaemic states (Klosiewicz-Latoszek & Szostak, 1991). They reduce VLDL synthesis and enhance their catabolism by activating lipoprotein lipase (Illingworth 1987; Catapano, 1992). Nevertheless, the biochemical mechanism of action underlying these effects is not well understood (Sirtori *et al.*, 1991). Among the biochemical events following the administration of fibrates we have considered the importance of the modifications in fatty acid synthesis caused by these drugs (Sánchez *et al.*, 1992a,b; 1993; Vázquez *et al.*, 1993; Alegret *et al.*, 1994). These modifications may result in changes in the relative proportion of fatty acid components in membranes, and as a consequence, in changes in their physicochemical properties (such as the fluidity) which are, to a great extent, determined by the chain length and the degree of unsaturation of their constitutive fatty acids (Stubbs & Smith, 1990). As membrane proteins, e.g. receptors, enzymes, are highly sensitive to the lipid environment, it could be hypothesized that changes in some enzyme activites caused by fibrate treatment may be related to changes in the acyl composition of membranes or, further, of plasma lipoproteins. It is known that the type of fatty acids (chain length and number of unsaturations) present in lipoproteins has an important role in the control of their own metabolism, modulating the enzyme activities directly related to their handling in the organism, e.g. lipoprotein lipase (Wang *et al.*, 1992) or lecithin-cholesterol acyltransferase (Hida *et al.*, 1993). Furthermore, fatty acids may modify the binding affinity of receptors, such as the acetylated low density lipoprotein receptor (Gavino *et al.*, 1992).

The present work was undertaken to study the comparative effects of a treatment with clofibrate (CFB), bezafibrate (BFB) and gemfibrozil (GFB) on the acyl composition of the main microsomal phospholipids, i.e. phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in rats. Other authors have already investigated the effect of CFB, the prototype fibrate drug (Kawashima *et al.*, 1984). Nevertheless, CFB is being replaced in clinical practice by other fibrates, like BFB and GFB, which are more efficient hypolipidaemic drugs. There is no evidence that all fibrates modify the acyl composition of microsomal phospholipids in the same way as CFB. Moreover, only one time point has been studied per treatment (i.e. 7 or 15 days), which precludes any discussion about the onset and the maximum of the changes caused by the treatment. Thus, we studied the time-course of these effects and their relationship with the reported changes in enzyme activities produced by treatment with fibrates (Kawashima *et al.*, 1984; Hawkins *et al.*, 1987). Furthermore, the administration of fibrates has the ability to promote peroxisomal proliferation in rats and mice, while this change seems not to occur in guinea-pigs or man (Lake & Gray, 1985; Vázquez *et al.*, 1993). Thus, we were also interested in the relationship between the changes observed following the administration of fibrates to rats and the presence of peroxisome proliferation.

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Methods

Animals

Male Sprague-Dawley rats (150) from Letica (Spain), weighing 110–120 g at the beginning of treatment, were maintained under conditions of constant humidity and temperature (22±2°C) under a constant light-dark cycle and were fed standard diet (Panlab, Barcelona, Spain) for five days before the beginning of the studies. The animals were distributed randomly into four groups of 36. Each group was fed, respectively, control diet or a diet containing CFB, BFB or GFB. The six remaining rats were used as controls on day 0 of treatment to establish basal values. Throughout the study, the weight and daily food intake of the animals were measured. The 36 rats in each group were randomly killed in groups of 6 after 1, 2, 4, 7, 10 and 15 days of treatment. The concentration of CFB administered in the diet was 0.3% w/w, as described by others (Berge & Bakke, 1981; Stahlberg *et al.*, 1989). The concentration of the other two drugs (0.45% for BFB and 0.3% w/w for GFB) was chosen in such a way as to be equimolar with CFB. The diets were prepared by soaking the pellets in an acetone solution of the drug (Alegret *et al.*, 1994). To avoid any possible effect of the solvent, the control diet was also soaked in acetone and dried.

Experimental

The animals were killed on the assigned day by decapitation between 08 h 00 min and 09 h 00 min. Blood samples were collected from the neck in EDTA tubes and plasma was obtained by centrifugation at 3000 *g* for 10 min at 4°C. 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. The subcellular fractions (postnuclear fraction and microsomes) were obtained by differential centrifugation as described previously (Alegret *et al.*, 1994), and the protein content was determined by the method of Bradford (1976), with bovine serum albumin used as standard.

Plasma cholesterol and triglyceride concentration measurement

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

Peroxisomal β-oxidation activity

Peroxisomal β-oxidation was determined in the postnuclear fraction by the method of Lazarow (1981). The incubation medium contained, in final concentrations: Tris-HCl buffer 50 mM, pH 8.0, KCN 1 mM, dithiothreitol 1 mM, FAD 10 μM, CoA 100 μM, BSA 75 μg ml^{-1}, Triton X-100 0.09 μl ml^{-1}, NAD 200 μM, and of postnuclear protein 400 μg. After 2 min of preincubation at 37°C the reaction was started by adding 20 μM palmitoyl-CoA, and the increase in absorbance at 340 nm with respect to a blank cuvette without palmitoyl-CoA was recorded in a Perkin-Elmer 550 UV-VIS Spectrophotometer.

Fatty acid analysis from microsomal phospholipids

Lipids were extracted from microsomes by the method of Bligh & Dyer (1959). PC and PE fraction were isolated by thin layer chromatography and recovered from silica as described previously (Vázquez *et al.*, 1993). 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 × 0.20 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

Palmitoyl-CoA, fatty-acid-free bovine serum albumin (BSA), CoA, FAD, and Trizma base (tris[hydroxymethyl]aminomethane) were obtained from Sigma Chemical Co. (Madrid, Spain); EDTA and butylhydroxytoluol were from Merck (Barcelona, Spain); sodium methylate was from Supelco (Barcelona, Spain) and Triton X-100 from Scharlau (Barcelona, Spain). Other general chemicals were obtained from commercial sources and were of the highest purity available.

Drugs

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

Statistical evaluation

Results are expressed as means±s.e.mean of 6 experiments. Statistical differences were established by a two-way ANOVA test (treatment × time) by means of the FOUNDS computer programme; when differences were found, multiple comparisons were performed between treatment groups at different time points using Duncan's test. Differences with $P<0.05$ were considered significant. Correlations between two variables were performed by linear regression using the GPIP computer programme.

Results

Serum lipids

Fibrate treatment produced a marked hypolipidaemic effect in rats. Serum triglyceride concentrations were significantly reduced with respect to the control values by the three fibrates from the first day of treatment. This decrease persisted during the rest of the treatment e.g. on day 15 there was a 37, 55, and 59% decrease for CFB, BFB, and GFB, respectively, with respect to control values (Table 1). Serum cholesterol concentrations were also reduced by the treatment with CFB and BFB. Both drugs significantly decreased the levels of plasma cholesterol from the first day, reaching the maximum effect (about 35% reduction) on day 15. In contrast, GFB significantly reduced serum cholesterol only on the first day of the treatment. On the following days, serum cholesterol concentrations approached those of the control group (Table 2). Fibrate treatment also modified some parameters such as food

Table 1 Effect of fibrate treatment on plasma triglyceride levels (mg dl^{-1})

Day of treatment	*Treatment*[a]			
	Control	*CFB*	*BFB*	*GFB*
0	140.7±12.0	–	–	–
1	116.3±8.5	85.4±12.6	78.5±12.8	68.2±6.2
4	94.1±12.0	61.0±4.7	56.0±3.8	55.9±7.6
7	119.9±9.3	80.9±12.2	56.8±5.1	59.2±4.9
15	128.3±6.2	80.9±4.7	58.0±5.4	52.8±7.1

Results are means±s.e.mean of 6 experiments performed in duplicate. [a]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). Data for all groups of treatment on a given day are different from respective controls, $P<0.05$.

intake, and body weight increase. The body weight of treated rats was similar to that of control rats up to the seventh day of treatment. From that time on, treated rats tended to gain weight slower than control rats (data not shown). Daily food intake was also significantly modified by the treatment, as BFB- and GFB-treated animals consumed more than the corresponding controls (e.g., on day 14 the daily food intake was 31.3±2.8 g per day for control animals, and 30.6±2.6, 37.8±4.0, and 37.3±7.8 g per day for CFB-, BFB- and GFB-treated animals, respectively). These results agree with those showed in a previous paper (Alegret *et al.*, 1994), where they are extensively discussed.

Peroxisomal proliferation markers

Figure 1 shows the effect of fibric acid derivatives on peroxisomal β-oxidation activity, which is commonly used as a marker of peroxisomal proliferation (Hawkins *et al.*, 1987). The three drugs studied strongly increased this activity respect to the control values from the second day of the treatment on. The potency order observed was BFB>CFB>GFB and the maximum increase was achieved on day 10, when BFB, CFB and GFB increased peroxisomal β-oxidation activity 8.6, 4.9 and 3.5 fold respectively. These data, as well as the increase in liver/body weight ratio, which was significant from day 2 of treatment on (Figure 2) indicated the proliferation of peroxisomes induced by these agents.

Table 2 Effect of fibrate treatment on plasma cholesterol levels (mg dl^{-1})

Day of treatment	*Treatment*[a]			
	Control	*CFB*	*BFB*	*GFB*
0	96.5±4.2	–	–	–
1	105.8±3.5	82.6±3.2*	84.9±2.9*	81.0±5.5*
4	101.7±2.7	76.5±4.6*	69.6±3.8*	91.3±4.0
7	113.9±7.1	61.9±1.8*	70.3±6.0*	97.3±9.8
15	91.1±4.4	59.4±3.9*	59.4±3.0*	85.4±4.3

Results are means±s.e.mean of 6 experiments performed in duplicate. [a]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 are different from controls, $P<0.05$.

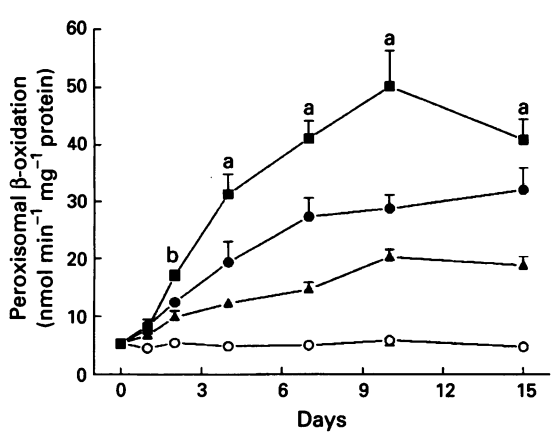

Figure 1 Time-course of the effect of a standard diet (○) or a diet supplemented with 0.3% w/w CFB (●), 0.45% w/w BFB (■), and 0.3% w/w GFB (▲) on cyanide-insensitive peroxisomal β-oxidation determined in the postnuclear fraction of livers from male Sprague-Dawley rats. Results are means±s.e.mean of 6 experiments performed in duplicate. [a] Values for CFB, BFB, and GFB are all different from controls, $P<0.05$; [b] values for CFB and BFB are different from controls, $P<0.05$. For abbreviations in this and subsequent legends, see text.

Fatty acid composition of microsomal phospholipids

Saturated fatty acids (SFA) Figure 3a and 3b shows the time-course of the effect of the three drugs on the relative

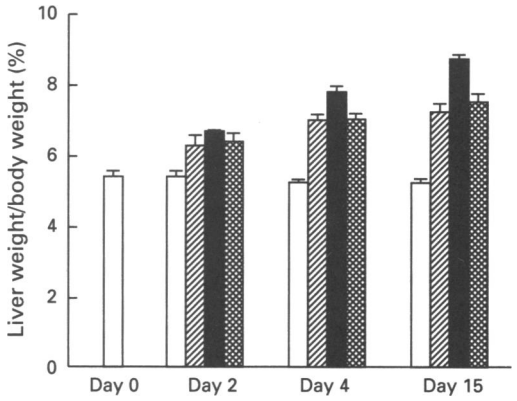

Figure 2 Time-course of the effect of a standard diet (open column) or a diet supplemented with 0.3% w/w CFB (hatched column), 0.45% w/w BFB (solid column), and 0.3% w/w GFB (cross-hatched column) on liver/body weight ratio (%) of male Sprague-Dawley rats. Results are means±s.e.mean of 6 experiments performed in duplicate. All results were statistically significant from day 2 of treatment.

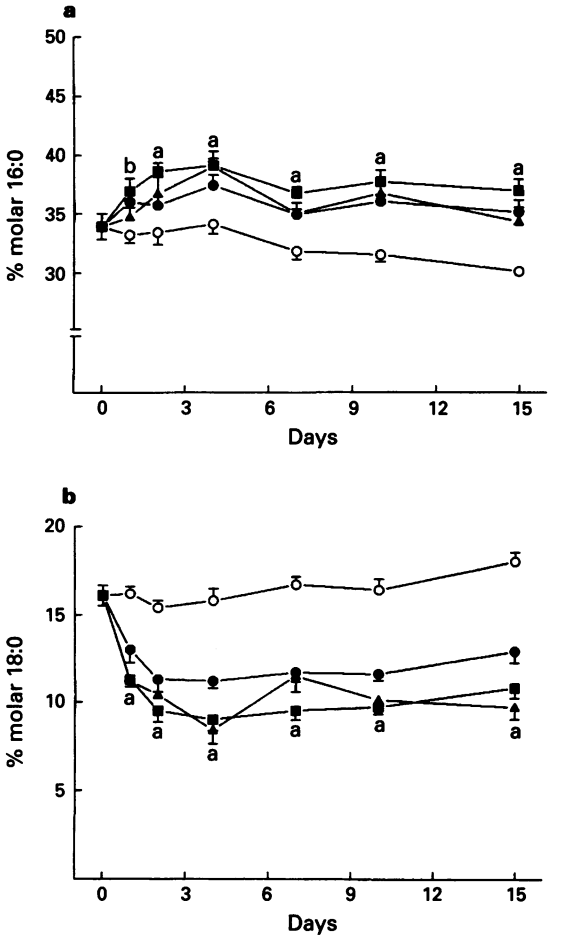

Figure 3 Time-course of the effect of a standard diet (○) or a diet supplemented with 0.3% w/w CFB (●), 0.45% w/w BFB (■), or 0.3% w/w GFB (▲) on the relative content of (**a**) 16:0, and (**b**) 18:0 in the phosphatidylcholine fraction of microsomes from livers of male Sprague-Dawley rats. Results are means±s.e.mean of 6 experiments performed in duplicate. [a] Values for CFB, BFB, and GFB are all different from controls, $P<0.05$; [b] values for CFB and BFB are different from controls, $P<0.05$.

content of palmitic acid (16:0) and stearic acid (18:0) in microsomal PC fraction. The three fibrates significantly increased the relative content of 16:0 in the PC fraction, whereas the relative content of 18:0 was concomitantly reduced. It is remarkable that these changes became evident even on the first day of treatment. The effects on PE fraction were not so clear, as the three drugs slightly decreased 16:0, whereas only GFB modified 18:0 relative content throughout the treatment (Figure 4a and b). Overall, total SFA were not modified by fibrate treatment in PC fraction (data not shown), while GFB decreased total SFA in PE (Figure 4c).

Monounsaturated fatty acids (MUFA) The three fibrates produced a significant increase in monoenoic species, both in PC (Figure 5a–c) and in PE fractions (Figure 6a–c). Particularly relevant due to its quantitative importance was the increase in oleic acid (18:1) relative content in PC, especially in the group of rats treated with BFB, which showed a significant increase from the first day of treatment, reaching a 76% maximum increase on day 10. Moreover, GFB produced an outstanding increase in palmitoleic acid (16:1) content in both phospholipid fractions on the first day of treatment (82 and 119% increase in PC and PE, respectively) and also a marked

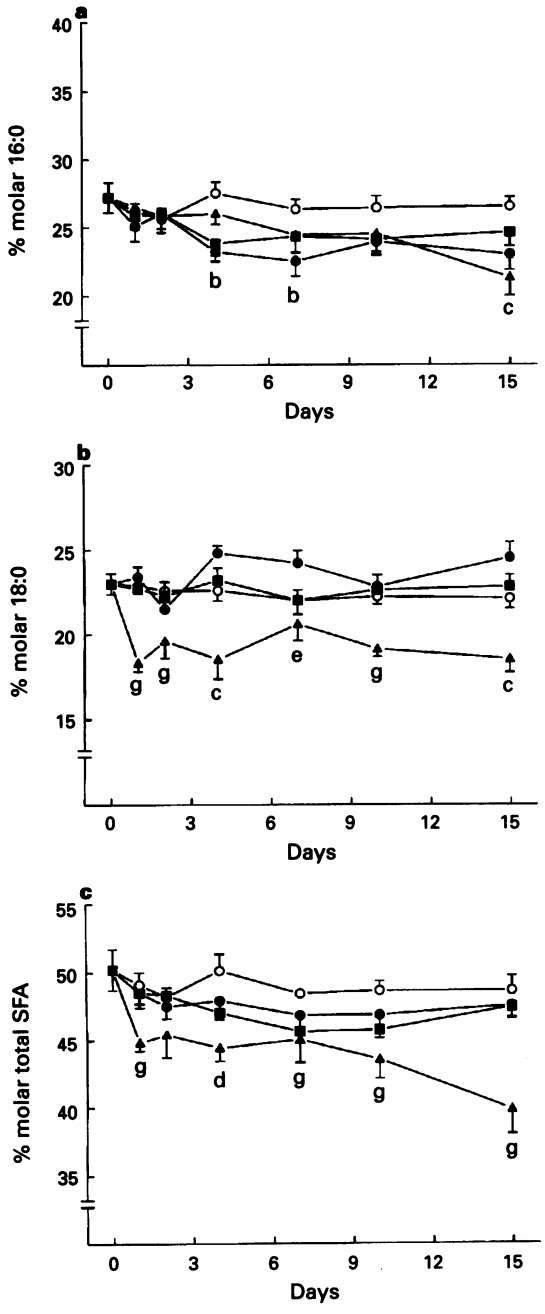

Figure 4 Time-course of the effect of a standard diet (○) or a diet supplemented with 0.3% w/w CFB (●), 0.45% w/w BFB (■), or 0.3% w/w GFB (▲) on the relative content of (**a**) 16:0, (**b**) 18:0, and (**c**) total SFA (16:0+18:0) in the phosphatidylethanolamine fraction of microsomes from livers of male Sprague-Dawley rats. Results are means ± s.e.mean of 6 experiments performed in duplicate. [b] Values for CFB and BFB are different from controls, $P<0.05$; [c] values for CFB and GFB are different from controls, $P<0.05$; [d] values for BFB and GFB are different from controls, $P<0.05$; [e] values for CFB are different from controls, $P<0.05$; [g] values for GFB are different from controls, $P<0.05$.

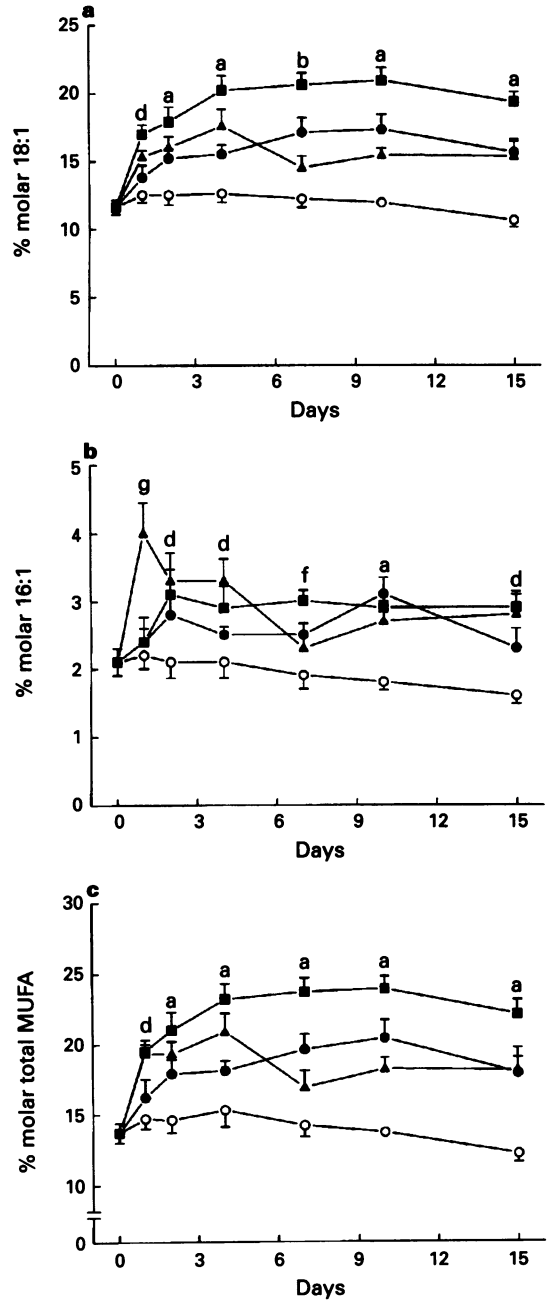

Figure 5 Time-course of the effect of a standard diet (○) or a diet supplemented with 0.3% w/w CFB (●), 0.45% w/w BFB (■), or 0.3% w/w GFB (▲) on the relative content of (**a**) 18:1, (**b**) 16:1, and (**c**) total MUFA (16:1+18:1) in the phosphatidylcholine fraction of microsomes from livers of male Sprague-Dawley rats. Results are means ± s.e.mean of 6 experiments performed in duplicate. [a] Values for CFB, BFB, and GFB are all different from controls, $P<0.05$; [b] values for CFB and BFB are different from controls, $P<0.05$. [d] values for BFB and GFB are different from controls, $P<0.05$; [f] values for BFB are different from controls, $P<0.05$; [g] values for GFB are different from controls, $P<0.05$.

increase (84% with respect to control values) in 18:1 content in the PE fraction on day 15 of treatment.

Polyunsaturated fatty acids (PUFA) n-6 series The changes caused by GFB in the relative content of fatty acids belonging to the n-6 series were different from those caused by BFB or CFB under the same conditions (Figure 7a–d for PC fraction, and Figure 8a–c for PE fraction).Thus, BFB and CFB treatment significantly decreased the content of linoleic acid 18:2(n-6) in PC from the first day while GFB had practically no effect on this fatty acid (Figure 7a). The effect of treatment was different in PE, as GFB increased 18:2(n-6) from the first day, reaching a maximum increase (about 40%) on day 10 (Figure 8a). In contrast, BFB and CFB decreased the molar percentage of 18:2(n-6), from day 4 to the end of the treatment, when the decrease was maximum (about 50% for both drugs).

GFB also produced a different effect from that shown by CFB and BFB on the proportion of eicosatrienoic acid 20:3(n-6) in PC (Figure7b). CFB and BFB markedly increased the relative content of 20:3(n-6) and, in this case CFB had a stonger effect than BFB. In contrast, GFB did not increase the relative content of 20:3(n-6) and even decreased it significantly on day 15. Changes in PE fraction were similar to those in PC but of lesser magnitude (data not shown).

No significant changes were observed in the proportion of arachidonic acid 20:4(n-6) in microsomal PC (data not shown). The molar percentage of this fatty acid in all groups and days of treatment ranged from 13.2 to 19.0%. However, the treatment changed the relative content of 20:4(n-6) in microsomal PE (Figure 8b). CFB and BFB significantly increased the levels of 20:4(n-6) from day 4 and, as for 20:3(n-6), CFB showed a stronger effect than BFB. Again, GFB showed a different effect, causing only a slight decrease in the levels of 20:4(n-6) on day 1.

The three drugs decreased the proportion of docosatetraenoic acid 22:4(n-6) in microsomal PC. Nevertheless, while CFB and BFB produced a significant reduction of the proportion of 22:4(n-6) throughout the treatment (83% and 76% decrease for CFB and BFB on day 15), GFB reduced the proportion only in the first four days of treatment, and the levels returned to control values thereafter (Figure 7c). Similar behaviour was observed for 22:4(n-6) in PE (Figure 8c).

None of the fibrates significantly modified γ-linolenic acid 18:3(n-6) relative content in microsomal phospholipids throughout the treatment (data not shown). The molar percentage of this fatty acid in all groups and days of treatment ranged from 0.6 to 1.4% in PC and from 1.0 to 2.2% in PE fractions.

When analyzing data for total n-6 fatty acids, a decrease in BFB- and CFB-treated animals was observed in PC fraction, whereas GFB-treated animals were not affected (Figure 7d). Total n-6 fatty acids in PE were not modified by fibrates (data not shown), but there were qualitative differences. Thus, in CFB- and BFB-treated rats, the decrease in 18:2(n-6) was

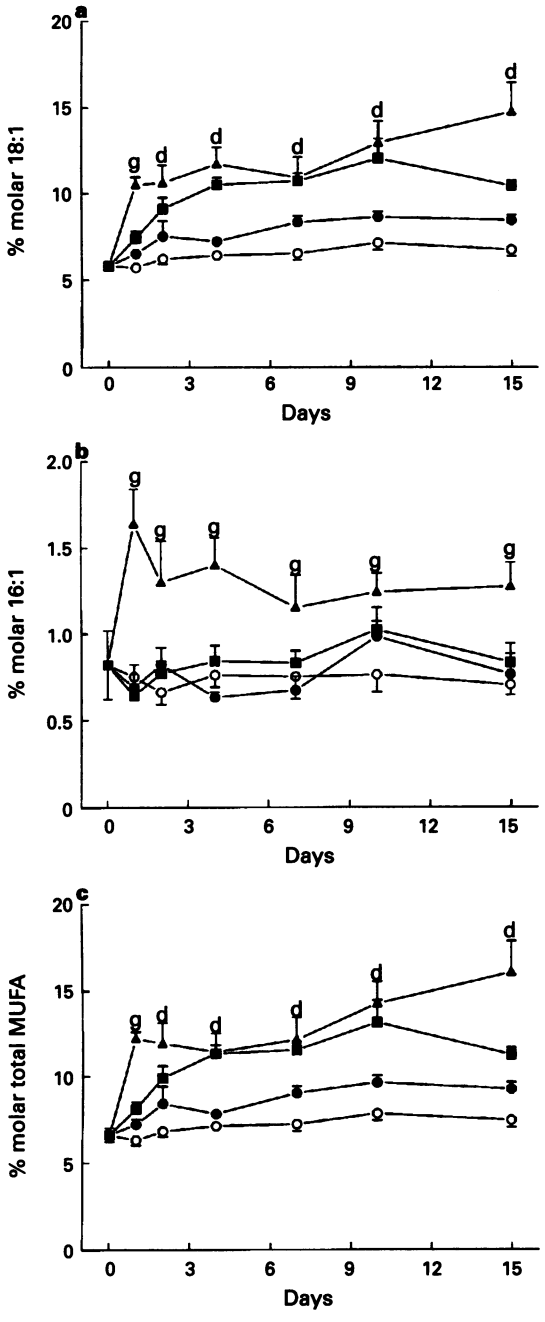

Figure 6 Time-course of the effect of a standard diet (○) or a diet supplemented with 0.3% w/w CFB (●), 0.45% w/w BFB (■), or 0.3% w/w GFB (▲) on the relative content of (a) 18:1, (b) 16:1 and (c) total MUFA (16:1+18:1) in the phosphatidylethanolamine fraction of microsomes from livers of male Sprague-Dawley rats. Results are means±s.e.mean of 6 experiments performed in duplicate. [d] Values for BFB, and GFB are all different from controls, $P<0.05$; [g] values for GFB are different from controls, $P<0.05$.

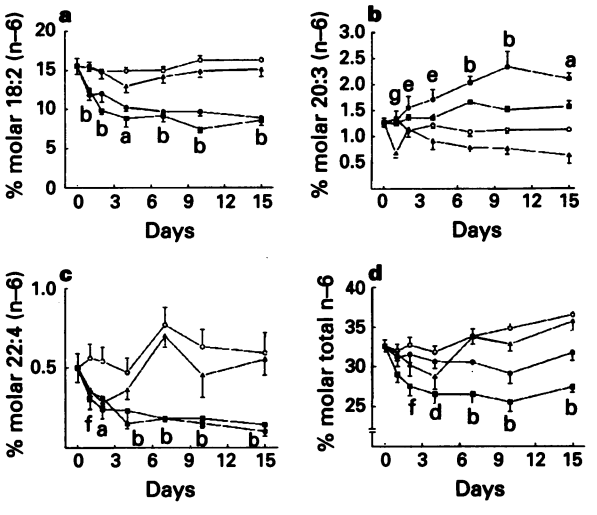

Figure 7 Time-course of the effect of a standard diet (○) or a diet supplemented with 0.3% w/w CFB (●), 0.45% w/w BFB (■), or 0.3% w/w GFB (▲) on the relative content of (a) 18:2(n-6), (b) 20:3(n-6), (c) 22:4(n-6), and (d) total n-6 in the phosphatidylcholine fraction of microsomes from livers of male Sprague-Dawley rats. Results are means±s.e.mean of 6 experiments performed in duplicate. [a] Values for CFB, BFB, and GFB are all different from controls, $P<0.05$; [b] values for CFB and BFB are different from controls, $P<0.05$; [d] values for BFB and GFB are different from controls, $P<0.05$; [e] values for CFB are different from controls, $P<0.05$; [f] values for BFB are different from controls, $P<0.05$; [g] values for GFB are different from controls, $P<0.05$.

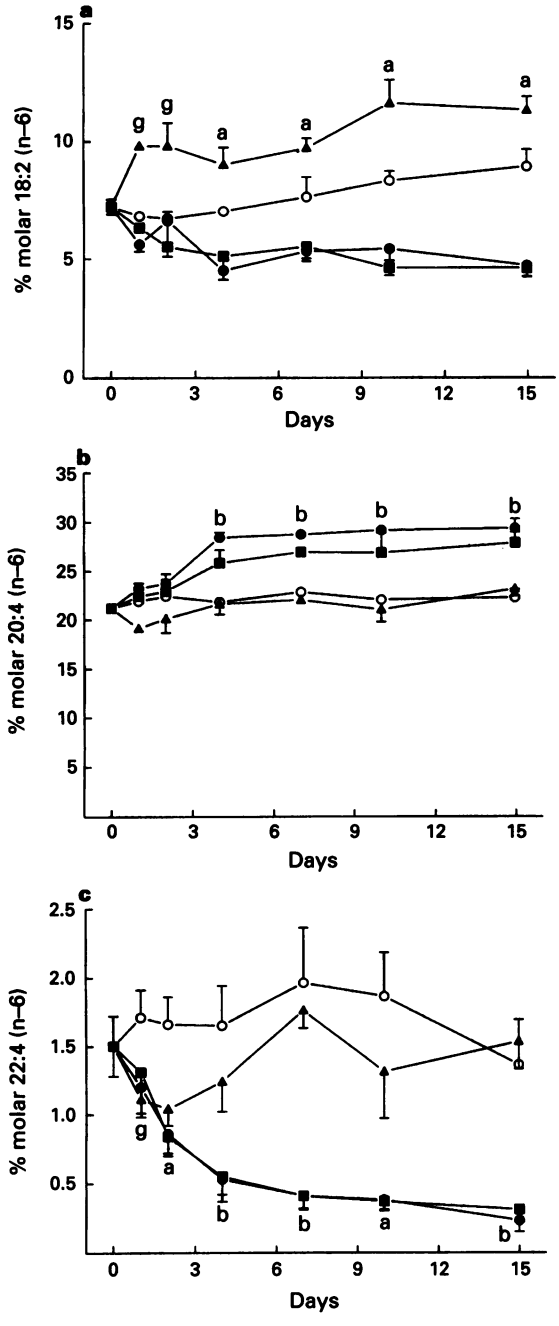

Figure 8 Time-course of the effect of a standard diet (○) or a diet supplemented with 0.3% w/w CFB (●), 0.45% w/w BFB (■), or 0.3% w/w GFB (▲) on the relative content of (a) 18:2(n-6), (b) 20:4(n-6), and (c) 22:4(n-6) in the phosphatidylethanolamine fraction of microsomes from livers of male Sprague-Dawley rats. Results are means ± s.e.mean of 6 experiments performed in duplicate. [a] Values for CFB, BFB, and GFB are all different from controls, $P<0.05$; [b] values for CFB and BFB are different from controls, $P<0.05$; [g] values for GFB are different from controls, $P<0.05$.

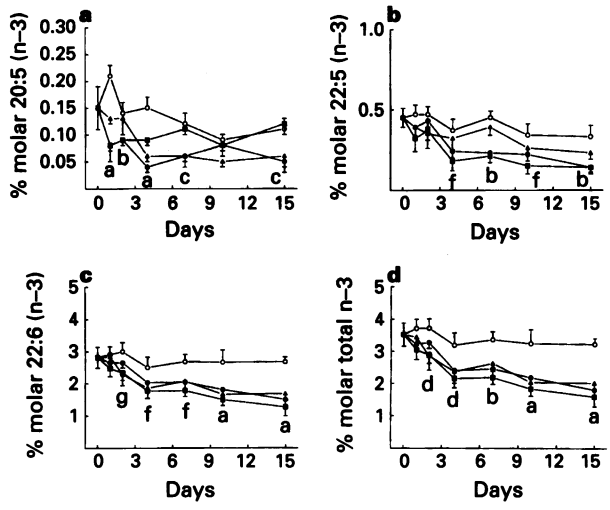

Figure 9 Time-course of the effect of a standard diet (○) or a diet supplemented with 0.3% w/w CFB (●), 0.45% w/w BFB (■), or 0.3% w/w GFB (▲) on the relative content of (a) 20:5(n-3), (b) 22:5(n-3), (c) 22:6(n-3), and (d) total n-3 in the phosphatidylcholine fraction of microsomes from livers of male Sprague-Dawley rats. Results are means ± s.e.mean of 6 experiments performed in duplicate. [a] Values for CFB, BFB, and GFB are all different from controls, $P<0.05$; [b] values for CFB and BFB are different from controls, $P<0.05$; [c] values for CFB and GFB are different from controls, $P<0.05$; [d] values for BFB and GFB are different from controls, $P<0.05$; [f] values for BFB are different from controls, $P<0.05$; [g] values for GFB are different from controls, $P<0.05$.

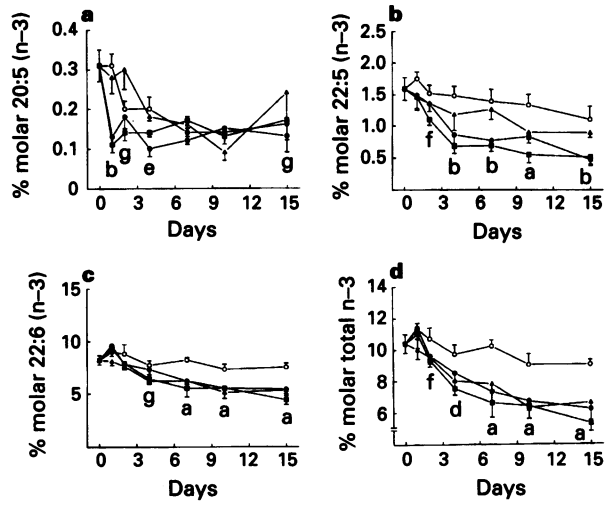

Figure 10 Time-course of the effect of a standard diet (○) or a diet supplemented with 0.3% w/w CFB (●), 0.45% w/w BFB (■), or 0.3% w/w GFB (▲) on the relative content of (a) 20:5(n-3), (b) 22:5(n-3), (c) 22:6(n-3), and (d) total n-3 in the phosphatidylethanolamine fraction of microsomes from livers of male Sprague-Dawley rats. Results are means ± s.e.mean of 6 experiments performed in duplicate. [a] Values for CFB, BFB, and GFB are all different from controls, $P<0.05$; [b] values for CFB and BFB are different from controls, $P<0.05$; [d] values for BFB and GFB are different from controls, $P<0.05$; [e] values for CFB are different from controls, $P<0.05$; [f] values for BFB are different from controls, $P<0.05$; [g] values for GFB are different from controls, $P<0.05$.

compensated for by an increase in 20:4(n-6) whereas in GFB-treated animals the increase in 18:2(n-6) was balanced by the slight decrease in the other n-6 fatty acids.

n-3 series The three fibrates decreased the relative content of highly unsaturated n-3 fatty acids, namely eicosapentaenoic 20:5(n-3), docosapentaenoic 22:5(n-3), and docosahexaenoic 22:6(n-3) acids, both in PC (Figure 9a–d) and in PE fractions (Figure 10a–d). α-Linolenic acid 18:3(n-3) was not significantly modified by any of the treatments (data not shown). The molar percentage of the latter fatty acid in all groups and days of treatment ranged from 0.06 to 0.18% in PC and from 0.13 to 0.34% in PE fractions.

n-9 series An impressive increase in the content of mead acid 20:3(n-9) in microsomal PC was observed after CFB and BFB

treatment (Figure 11). On day 10, the relative content of 20:3(n-9) increased 4.8 and 8.9 fold in CFB- and BFB-treated rats, respectively. Conversely, GFB did not change the proportion of 20:3(n-9) in microsomal PC. The changes in the relative content of 20:3(n-9) in PE were similar, but of a smaller magnitude than those seen in PC (Figure 12).

The content of total PUFA in PC (Figure 13) showed an overall decrease in CFB- and BFB-treated groups, whereas no effects were observed following treatment with GFB. Total PUFA in PE was practically unaffected (data not shown).

Correlation with peroxisomal β-oxidation

The correlation between some of the changes observed with peroxisomal β-oxidation activity, expressed as r squared values, is showed in Table 3. At least for 18:1, 18:2(n-6) and 20:3(n-9) in microsomal PC and 20:5(n-3) in PE, more than 40% of the change may be attributed to peroxisome proliferation.

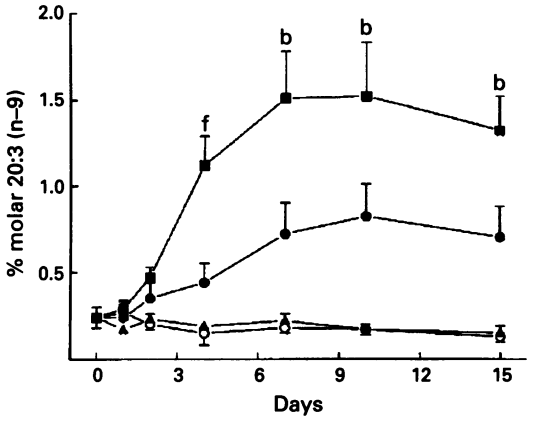

Figure 11 Time-course of the effect of a standard diet (○) or a diet supplemented with 0.3% w/w CFB (●), 0.45% w/w BFB (■), or 0.3% w/w GFB (▲) on the relative content of 20:3(n-9) in the phosphatidylcholine fraction of microsomes from livers of male Sprague-Dawley rats. Results are means ± s.e.mean of 6 experiments performed in duplicate. [b] Values for CFB and BFB are different from controls, $P<0.05$; [f] values for BFB are different from controls, $P<0.05$.

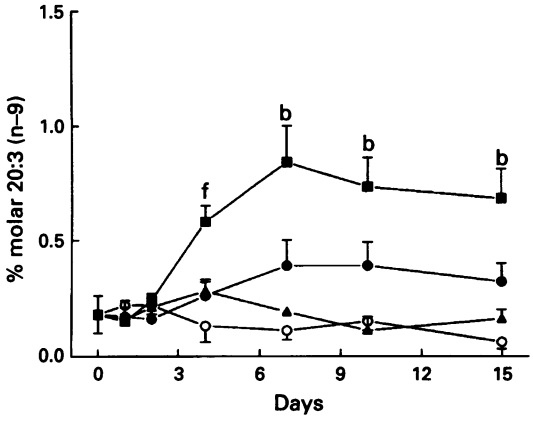

Figure 12 Time-course of the effect of a standard diet (○) or a diet supplemented with 0.3% w/w CFB (●), 0.45% w/w BFB (■), or 0.3% w/w GFB (▲) on the relative content of 20:3(n-9) in the phosphatidylethanolamine fraction of microsomes from livers of male Sprague-Dawley rats. Results are means ± s.e.mean of 6 experiments performed in duplicate. [b] Values for CFB and BFB are different from controls, $P<0.05$; [f] values for BFB are different from controls, $P<0.05$.

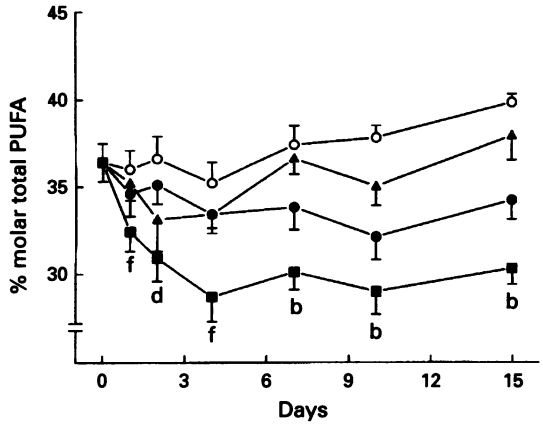

Figure 13 Time-course of the effect of a standard diet (○) or a diet supplemented with 0.3% w/w CFB (●), 0.45% w/w BFB (■), or 0.3% w/w GFB (▲) on the relative content of total PUFA in the phosphatidylcholine fraction of microsomes from livers of male Sprague-Dawley rats. Results are means ± s.e.mean of 6 experiments performed in duplicate. [b] Values for CFB and BFB are different from controls, $P<0.05$; [d] values for BFB and GFB are different from controls, $P<0.05$; [f] values for BFB are different from controls, $P<0.05$.

Table 3 Correlation between fatty acid relative proportions and peroxisomal β-oxidation activity

	Phosphatidylcholine	*Phosphatidylethanolamine*
16:0	0.182*†	0.102*†
16:1	0.055	0.000
18:0	0.273*†	0.019
18:1(n-9)	0.432*	0.141*
18:2(n-6)	0.475*	0.145*†
20:3(n-9)	0.435*†	0.329*
20:3(n-6)	0.151*	0.019
20:4(n-6)	0.017	0.378*
20:5(n-3)	0.088	0.078†
22:4(n-6)	0.249*†	0.360*†
22:5(n-3)	0.305*†	0.413*†
22:6(n-3)	0.310*†	0.310*†

Results are obtained by linear regression from 146 pairs of data. Tabulated values are r^2, determination coefficient. *Significant correlation between the two variables with a P value <0.0001; †negative correlation.

Discussion

Administration of fibric acid derivatives to rats results in the proliferation of peroxisomes in liver. This causes hepatomegaly and the induction of several enzymes such as peroxisomal β-oxidation, as well as hepatic enzymes involved in fatty acid biosynthesis (Kawashima *et al.*, 1984; Hawkins *et al.*, 1987; Alegret *et al.*,1994). In the present work, BFB was the most powerful peroxisomal inducer as measured by the increase in peroxisomal β-oxidation activity (Figure 1), whereas CFB reached intermediate values and GFB was the weakest.

Serum triglyceride and cholesterol concentrations were markedly decreased by the three fibrates. These effects have been related to peroxisomal induction, although an important part of the pharmacological effect could be regarded as independent of such phenomenon (Alegret *et al.*, 1994). It should be borne in mind that a hypolipidaemic response is also found in species which are refractory to peroxisomal induction, such as guinea-pigs (Hawkins *et al.*, 1987; Vázquez *et al.*, 1993) or humans (Lake & Gray, 1985; Hawkins *et al.*, 1987).

The effects of CFB on the fatty acyl composition of microsomal phospholipids in rat liver have already been reported (Kawashima *et al.*, 1984). However, there is little information

about the effects of BFB and GFB. Here, the changes induced by the three drugs were similar in some cases, but striking differences were also observed. Overall, CFB and BFB behaved in a similar way, with differences which could be attributed to their different potency, while GFB showed a distinct profile. Among the similarities, the three drugs increased the relative content of 16:0, 16:1 and 18:1, while the levels of 18:0 were reduced. Previous studies using CFB showed similar trends (Kawashima *et al.*, 1984). These changes have been attributed to the increase in the activities of the elongation-desaturation system (Δ^9-desaturase and palmitoyl-CoA elongase activities) (Kawashima *et al.*, 1984). In the parallel study performed in our laboratory we have confirmed significant increases in the activities of Δ^9-desaturase and palmitoyl-CoA elongase caused by the three drugs in rat liver (Alegret *et al.*, 1995). It has been reported that the proliferation of peroxisomes requires an increase in the production of lipids to form the membrane of these organelles (Kawashima *et al.*, 1990). Moreover, we have found a good correlation between the increase in Δ^9-desaturase activity and peroxisomal proliferation (Alegret *et al.*, 1995). Accordingly, in the present study a good correlation between the increase in 18:1 and peroxisomal β-oxidation was found, at least in the PC fraction (Table 3).

The decrease in the relative content of long-chain, highly unsaturated fatty acids 22:4(n-6), 22:5(n-3) and 22:6(n-3) is also a common trend for the three fibrates (Figures 7–8c, 9–10b y 9–10c). Again, this reduction may be related to the increase in peroxisomal β-oxidation activity.

Peroxisomal β-oxidation produces degradation of fatty acids, especially those of long chain and high number of unsaturations (Osmundsen *et al.*, 1991). Accordingly, the reduction of the proportion of these fatty acids is more remarkable in the rats treated with CFB and BFB, which are the most potent peroxisomal inducers. This dependence on peroxisomal proliferation is reflected in the very significant correlation among the relative proportions of these fatty acids and peroxisomal β-oxidation activity (Table 3). Furthermore, the relative content of these fatty acids is much less affected in the microsomal phospholipids in guinea-pigs, in which peroxisomal β-oxidation is not enhanced by fibrate treatment (Vázquez *et al.*, 1993).

When the influence of fibrate treatment on the molar percentage of 18:2(n-6), 20:3(n-6), 20:4(n-6) and 20:3(n-9) is analyzed, clear differences appear between CFB and BFB on the one hand, and GFB on the other. The different behaviour of GFB may be explained on the basis of two different observations. First, GFB is the weakest peroxisomal inducer of the three fibrates tested, thus the changes derived from this phenomenon would be less marked than for CFB or BFB. Second, GFB is the strongest inhibitor *in vitro* of some of the activities related to fatty acid modifications, particularly fatty acid elongation (Sánchez *et al.*, 1993). It should be pointed out that inhibition caused by GFB occurs at concentrations well within those reported in plasma in the clinical use of this fibrate (Todd & Ward, 1988). Moreover, given that this drug accumulates in the liver (Cayen, 1985), this inhibition may be reflected *in vivo*. Thus, inhibition of fatty acid elongation may be responsible for the increase in the substrates (18:2(n-6)), at least in PE, and for the decrease in the products (20:3(n-6), 20:4(n-6)) of the chain elongation system in GFB treated rats. Previous results indicate that GFB may inhibit microsomal fatty acid elongation system of guinea-pigs *in vivo* (Vázquez *et al.*, 1993). Thus, in guinea-pigs, GFB increased the content of the substrates (16:0, 16:1, 18:2(n-6)) and decreased the products (18:0, 18:1, 20:3(n-6), 20:4(n-6), 22:4(n-6)) of the elongation system in microsomal phospholipids However, we should also consider the modifications derived from the proliferation of peroxisomes in rats, especially for those fatty acids, such as 18:2(n-6), the relative proportion of which shows a good correlation with this process (see Table 3). This phenomenon may be the cause of the decrease in 18:2(n-6) in CFB- and BFB-treated rats. Further, effects derived from peroxisomal proliferation may counteract the expected increase in 18:2(n-6) due to elongation inhibition in PC of GFB-treated rats. In this respect, it is known that peroxisomal proliferation caused by fibrates is accompanied by an increase of phospholipase and acyltransferase activities, which may be responsible for postsynthetic remodelling of phospholipid species (Kawashima *et al.*, 1989).

Apart from the effects of fibrates on fatty acid elongation, modifications in desaturase systems may also affect fatty acid composition in microsomal phospholipids. We have observed increases in Δ^9, Δ^6, and Δ^5-desaturase activities in rat liver by treatment with the three drugs (Alegret *et al.*, 1995). Particularly interesting was the induction of Δ^6-desaturase, with a potency order (GFB > BFB > CFB) identical to that shown in studies of these drugs *in vitro*, in which they behaved as weak inhibitors (Sánchez *et al.*,1993). This dual behaviour, inhibition *in vitro* and compensatory induction *in vivo*, is common among inhibitors of key lipogenic enzymes, such as statins for hydroxymethylglutaryl-CoA reductase, which is inhibited *in vitro* and induced *in vivo* (Endo, 1986). The effects on desaturase systems may overlap those produced by modifications on elongation *in vivo* and help to explain the differences showed by GFB.

There is a marked discrepancy between the effect of CFB and BFB on the one hand, and GFB on the other, in the content of mead acid (20:3(n-9)) (Figures 11 and 12). The relative content of this fatty acid was greatly increased after CFB and BFB treatment in microsomal phospholipids of rats. In contrast, the content of 20:3(n-9) in GFB-treated rats was unchanged. An increase in the content of 20:3(n-9) in microsomal PC has been reported after CFB treatment (Kawashima *et al.*, 1984). 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, the lack of increase in the content of this fatty acid may also be an indirect effect of the higher levels of 18:2(n-6) in the GFB group than in the CFB or BFB groups. Δ^6-desaturase has greater affinity for C_{18} substrates with the greater number of unsaturations, and in the presence of normal contents of 18:2(n-6) little desaturation of 18:1 occurs (Mead & Slaton, 1956). Therefore, it seems reasonable to suppose that the conversion of 18:1, by the action of the Δ^6-desaturase, in 20:3(n-9) requires a strong decrease in the contents of 18:2(n-6), as occurs in CFB- and BFB- but not in control or GFB-treated rats. The increase in 20:3(n-9) may be considered a compensatory response to the decrease in polyunsaturated fatty acids (PUFA) caused by the increase in peroxisomal β-oxidation (Sardesai, 1992). Moreover, 20:3(n-9) is not increased in guinea-pigs after treatment with GFB (Vázquez *et al.*, 1993).

In summary, treatment with fibrates may affect the acyl composition of rat liver microsomal PC and PE in a different manner, GFB showing different behaviour from CFB and BFB. The results presented here suggest that differences are due to an inhibitory effect of GFB on the enzyme activities responsible for the elongation and desaturation of fatty acids. Further, given that some of the changes are correlated with peroxisomal proliferation, they would be less marked in GFB-treated rats than in CFB- or BFB-treated animals. Changes in the acyl composition of microsomal phospholipids may affect the activity of many enzymes related to lipid metabolism which could be involved in the genesis of the hypolipidaemic effect shown by these drugs. These changes in the acyl composition of microsomal phospholipids may be reflected in the composition of lipoproteins. The initial step in the assembly and secretion of lipoproteins in the liver occurs in the endoplasmic reticulum, which is where microsomal glycerolipids, the intracellular pool of glycerolipids used for lipoprotein assembly, are formed. Furthermore, there appears to be a preference for the secretion of newly made microsomal glycerolipids, rather than pre-existing ones (Vace & Vance, 1990). Therefore, changes in the fatty acid composition of microsomal glycerolipids involves the secretion of hepatic lipoproteins with a modified acyl composition. This may be the cause of the re-

ported enrichment of lipoproteins in oleic acid after treatment with fenofibrate in human subjects (Agheli & Jacotot, 1991). Studies that attempt to determine whether the changes observed in fatty acid composition in microsomal glycerolipids are reflected in plasma lipoprotein composition after treatment with CFB, BFB, and GFB in rats are now in progress.

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