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Relationship between plasma lipids and palmitoyl-CoA hydrolase and synthetase activities with peroxisomal proliferation in rats treated with fibrates

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1 The time-course of the effect of clofibrate (CFB), bezafibrate (BFB) and gemfibrozil (GFB) on lipid plasma levels and palmitoyl-CoA hydrolase and synthetase activities, as well as the correlations with the peroxisomal proliferation phenomenon have been studied in male Sprague-Dawley rats.

2 The administration of the three drugs caused a significant reduction in body weight gain, accompanied with a paradoxical increase in food intake in groups treated with BFB and GFB.

3 Drug treatment produced gross hepatomegaly and increase in peroxisomal β-oxidation, and these parameters were strongly correlated. The order of potency was BFB > CFB ≥ GFB.

4 Both plasma cholesterol (BFB ≈ CFB > GFB) and triglyceride (BFB ≈ GFB > CFB) levels were reduced in treated animals. There was an inverse correlation between these parameters and peroxisomal β-oxidation, although the peroxisomal proliferation seemed to explain only a small part of the hypolipidemic effect observed.

5 Cytosolic and microsomal (but not mitochondrial) palmitoyl-CoA hydrolase activities were increased by the three drugs (BFB > CFB > GFB), probably by inducing the hydrolase I isoform, which is insensitive to inhibition by fibrates *in vitro*. The increased hydrolase activities were directly and strongly correlated with peroxisomal β-oxidation.

6 Palmitoyl-CoA synthetase activity was also increased by the treatment with fibrates (BFB > CFB > GFB), probably as a consequence of the enhancement of hydrolase activities.

7 Some of the effects of fibrate treatment can be explained, at least in part, in terms of peroxisomal induction and caution should be exercised in the extrapolation of these results to species, such as man, that are insensitive to peroxisomal proliferation.

Keywords: Fibrates; peroxisomal proliferation; β-oxidation; palmitoyl-CoA hydrolase; palmitoyl-CoA synthetase

Introduction

Fibric acid derivatives are a well known group of hypolipidaemic drugs used mainly in the treatment of hypertriglyceridaemia and mixed hyperlipidaemia (Sirtori *et al.*, 1991; Klosiewicz-Latoszek & Szostak, 1991). Although they have been in therapeutic use for more than twenty years, the mechanism(s) by which they reduce blood lipis is not fully understood.

A considerable body of information has been gathered regarding the effect of fibric acid derivatives on several enzyme activities related to lipid biosynthesis (Bremer *et al.*, 1981; Reddy & Lalwani, 1983; Hawkins *et al.*, 1987). With few exceptions, most of these studies share similar drawbacks: (1) They are based on the effect of only one drug, clofibrate, accepted as a hypolipidaemic drug prototype, but which is gradually being replaced by more efficient drugs, like bezafibrate and gemfibrozil. (2) Usually, only one time point is studied per treatment (i.e. 7 or 15 days), making it difficult to relate the modification of one particular enzyme activity with the hypolipidaemic effect. (3) Fibrates are typical peroxisome inducers in rats (Esbenshade *et al.*, 1990; McGuire *et al.*, 1991); as the rat is usually the model studied, few authors give clear-cut information about the possible involvement of this phenomenon in the modification of the lipogenic enzyme activities reported. (4) There is a lack of information about the effect of fibrates *in vitro* on some of the enzyme activities studied *in vivo*. Given the prevailing role of fatty acids in lipoprotein metabolism (Desreumaux *et al.*, 1979; Vance & Vance, 1990), in the last few years we have studied the effect of fibric acid derivatives on enzyme activities related to fatty acid biosynthesis and its involvement in the hypolipidaemic activity (Alegret *et al.*, 1991; Sánchez *et al.*, 1992a,b; 1993a,b) In this paper, we report the effect *in vivo* of clofibrate, bezafibrate and gemfibrozil on two hepatic enzyme activities involved in fatty acid biosynthesis: palmitoyl-CoA hydrolase (EC 3.2.2.1) and palmitoyl-CoA synthetase (EC 6.2.1.3). Further, we have studied the temporal evolution of these activities and their relationship with the hypolipidaemic effect and peroxisome induction phenomena.

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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 rats. Each group was fed, respectively, a 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 the basal values. Throughout the study, the weight and daily food intake of the animals was measured. The 36 rats in each group were killed randomly 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 other authors (Berge &

Bakke, 1981; Stahlberg et al., 1989). The concentrations of the other two drugs (0.45% for BFB and 0.3% w/w for GFB) were chosen in such a way as to be equimolar to CFB. The diets were prepared as described by Berge & Bakke (1981), by soaking in an acetone solution of the drug. 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. The 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 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 BSA used as standard. In order to check that the treatment did not affect the sedimentation distribution of cell organelles, fractions obtained from livers of control and CFB-treated rats were assayed for enzyme marker activities, succinate dehydrogenase activity (Earl & Korner, 1965) for heavy mitochondria, KCN insensitive palmitoyl-CoA β-oxidation (Lazarow, 1981) for light mitochondria, NADPH cytochrome c reductase (Alegret et al., 1991) for microsomes and glucose-6-phosphate dehydrogenase (Sánchez et al., 1993a) for cytosol. For the studies *in vitro*, cytosol obtained from control or GFB-treated rats was incubated with 5 mM GFB. The drug was added from a stock solution 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.

Plasma cholesterol and triglyceride concentration measurement

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

Enzyme assays

All enzyme activites were measured by spectrophotometric methods using a Perkin-Elmer 550 UV-Vis spectrophotometer with a recorder accessory. Palmitoyl-CoA hydrolase and palmitoyl-CoA synthetase activities were assayed as described in previous studies (Sánchez et al., 1992a; 1993b). 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 post-nuclear 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.

Chemicals

Palmitic acid, palmitoyl-CoA, fatty-acid-free bovine serum albumin (BSA), HEPES, CoA, FAD and Trizma base (tris[hydroxymethyl]aminomethane) were obtained from Sigma Chemical Co. (Madrid, Spain); NADH, NADPH, DTNB and ATP were from Boehringer Mannheim (Barcelona, Spain); EDTA was from Merck (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.d. of *n* experiments performed in duplicate. By means of the FOUNDS computer programme, statistical differences were established by a two-way ANOVA test (treatment × time); 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

Non enzymatic parameters

Although all the animals showed a steady increase in body weight during the treatment, fibrate-treated animals gained less weight than control group from day 7 on (Figure 1a). Fibrate-treated animals weighed about 10% less than control in the last four days of treatment: on day 15, the average weight was 261.3 ± 15.9 g for control, and 239.5 ± 15.9, 236.2 ± 12.8 and 238.9 ± 21.9 g for the CFB, BFB and GFB treated groups. Surprisingly, this reduction in body weight gain was accompanied, in the case of BFB- and GFB-treated animals, by a significant increase in the daily food intake (Figure 1b). Thus, on day 11 of treatment, while daily food intake for control and CFB-treated animals was 28.9 ± 1.9 and 28.0 ± 2.1 g/day per rat, the animals treated with BFB and GFB consumed 36.6 ± 4.9 and 38.2 ± 7.9 g/day per rat.

The temporal evolution of the typical hepatomegaly produced by fibrates is shown in Figure 1c as the ratio between liver and body weight. As expected, while this ratio remained fairly constant in the control group, it increased from day 2 onwards for CFB, BFB and GFB-treated animals. While CFB and GFB showed similar capacity to produce hepatomegaly, BFB surpassed them at every time point studied; for instance, on day 15, the liver weight/body weight ratios (as percentages) were 4.9 ± 0.2, 7.4 ± 0.6, 8.5 ± 1.0, and 7.2 ± 0.6 for control, CFB, BFB and GFB-treated animals, respectively.

Effect of treatment on plasma cholesterol and triglyceride concentrations

Figures 2a and 2b show the temporal evolution of cholesterol and triglyceride levels in control and fibrate-treated animals. The hypocholesterolemic activity of fibrates was already evident on day 2 of treatment, but while CFB and BFB decreased cholesterol levels throughout the treatment (mean decrease of 31% and 37% versus control values for CFB and BFB), cholesterol concentration in GFB-treated animals reverted to control values on day 10 of treatment.

Triglyceride levels were significantly lower in the three treated groups than in the control one from day 1 of treatment. BFB and GFB showed similar hypotriglyceridemic potency, with mean reductions of triglyceride levels of 49 and 44%, respectively, while CFB was somewhat less effective (mean reduction of 31%).

Enzyme activities

As reported previously (Lazarow et al., 1982; Bodnar & Rachubinski, 1991), the sedimentation patterns of the various organelles were similar for both untreated and fibrate-treated

rats (data not shown). Liver protein content, measured in the post-nuclear homogenate was not significantly modifed by drug administration (data not shown).

As shown in Figure 3, the three fibric acid derivatives studied behaved as typical peroxisomal proliferators, significantly increasing the cyanide-insensitive palmitoyl-CoA oxidation from day 2 of treatment on, in the case of CFB and BFB, and from day 7 on in the case of GFB. Maximal increases were achieved on day 10 of treatment (6.4, 10 and 5.7 fold increase for CFB, BFB and GFB versus control values). Throughout the treatment, the order of potency as β-oxidation inducers was BFB > CFB > GFB.

The palmitoyl-CoA hydrolase activity present in the mitochondrial fraction (heavy and light) was practically unchanged by fibrate treatment (data not shown). Microsomal palmitoyl-CoA hydrolase was slightly increased by GFB-treatment, showing an average 30% increase in activity from day 2 to the end of treatment (Figure 4). Although CFB and BFB also increased this activity from day 2 to 7, the progressive increase in control values over time blunted this effect in the second week of treatment.

CFB, BFB and GFB strongly increased cytosolic palmitoyl-CoA hydrolase activity (Figure 5). Maximal induction was achieved on day 7 of treatment, with approximately 4, 7 and 2 fold increase over control values for CFB, BFB and GFB-treated animals, respectively. GFB was by far the least effective inducer; thus, while BFB-treated animals showed significant increases from the first day of treatment, in GFB-

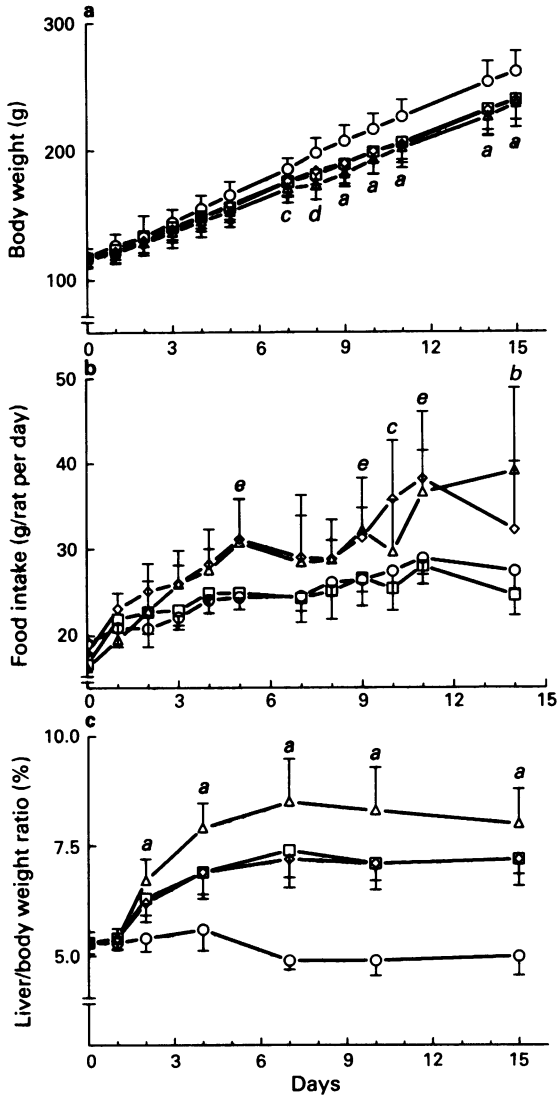

Figure 1 Time-course of the effect of a standard diet (○) or a diet supplemented with 0.3% w/w clofibrate (CFB) (□), 0.45% w/w bezafibrate (BFB) (△), and 0.3% w/w gemfibrozil (GFB) (◇) on (**a**) body weight, (**b**) daily food intake, and (**c**) liver/body weight ratio (%) of male Sprague-Dawley rats. Results are means ± s.d. of 6 experiments performed in duplicate. [a]means that values for CFB, BFB and GFB are all different from controls, $P<0.05$; [b]means that values for BFB are different from controls, $P<0.05$; [c]means that values for GFB are different from controls, $P<0.05$; [d]means that values for CFB and BFB are different from controls, $P<0.05$; [e]means that values for BFB and GFB are different from controls, $P<0.05$.

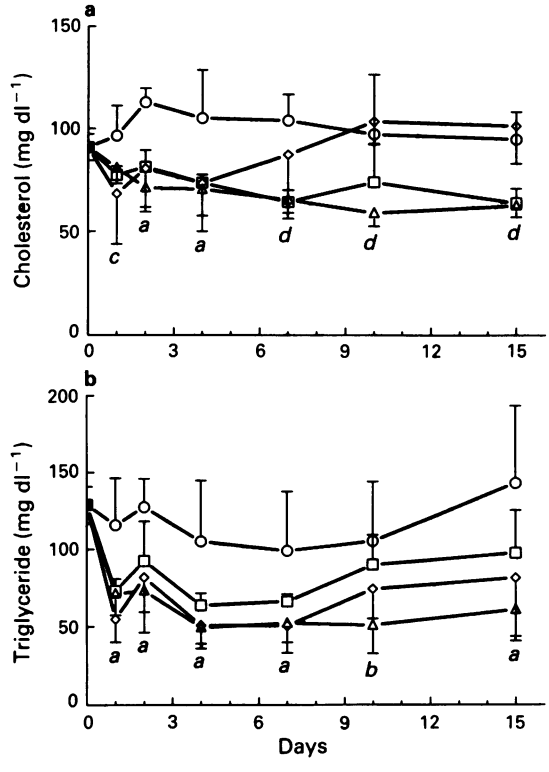

Figure 2 Time-course of the effect of a standard diet (○) or a diet supplemented with 0.3% w/w clofibrate (CFB) (□), 0.45% w/w bezafibrate (BFB) (△), and 0.3% w/w gemfibrozil (GFB) (◇) on (**a**) plasma cholesterol levels, and (**b**) plasma triglyceride levels of male Sprague-Dawley rats. Results are means ± s.d. of 6 experiments performed in duplicate. Legends for significance are the same as in Figure 1.

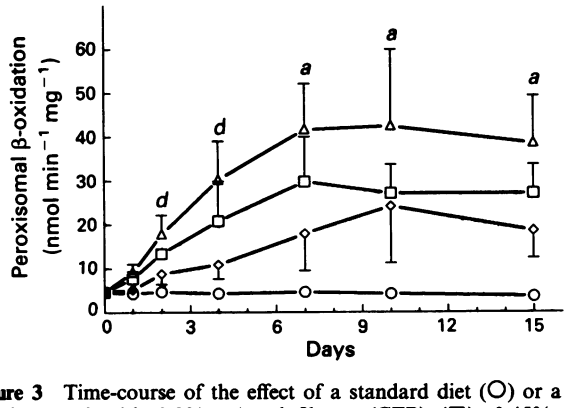

Figure 3 Time-course of the effect of a standard diet (○) or a diet supplemented with 0.3% w/w clofibrate (CFB) (□), 0.45% w/w bezafibrate (BFB) (△), and 0.3% w/w gemfibrozil (GFB) (◇) on cyanide-insensitive peroxisomal β-oxidation determined in the post-nuclear fraction of livers from male Sprague-Dawley rats. Results are means ± s.d. of 6 experiments performed in duplicate. Legends for significance are the same as in Figure 1.

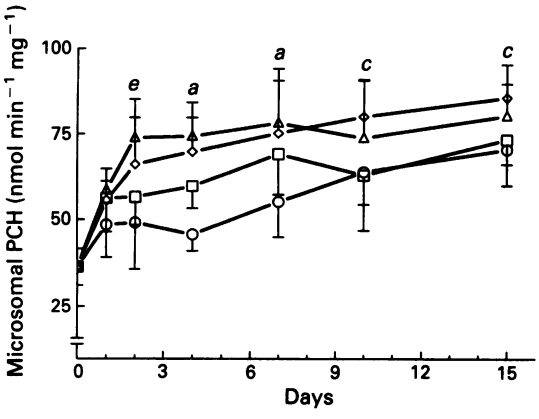

Figure 4 Time-course of the effect of a standard diet (○) or a diet supplemented with 0.3% w/w clofibrate (CFB) (□), 0.45% w/w bezafibrate (BFB) (△), and 0.3% w/w gemfibrozil (GFB) (◇) on palmitoyl-CoA hydrolase (PCH) activity in the microsomal fraction of livers from male Sprague-Dawley rats. Results are means ± s.d. of 6 experiments performed in duplicate. Legends for significance are the same as in Figure 1.

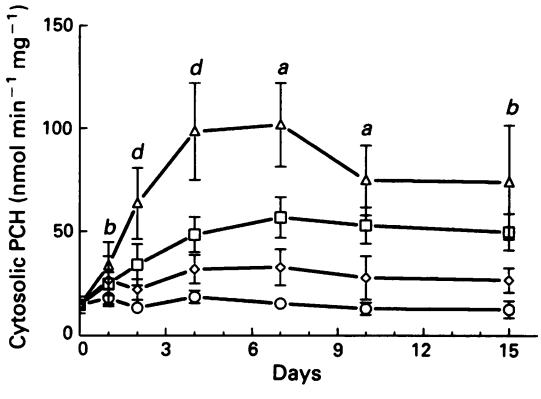

Figure 5 Time-course of the effect of a standard diet (○) or a diet supplemented with 0.3% w/w clofibrate (CFB) (□), 0.45% w/w bezafibrate (BFB) (△), and 0.3% w/w gemfibrozil (GFB) (◇) on palmitoyl-CoA hydrolase (PCH) activity in the cytosolic fraction of livers from male Sprague-Dawley rats. Results are means ± s.d. of 6 experiments performed in duplicate. Legends for significance are the same as in Figure 1.

treated animals this was accomplished after 7 days of drug consumption.

Palmitoyl-CoA hydrolase activity of cytosol from control animals was almost completely inhibited by 5 mM GFB *in vitro*, while only a partial inhibition of cytosol activity from GFB-treated animals was observed (Table 1).

As shown in Figure 6, the three drugs significantly increased microsomal palmitoyl-CoA synthetase activity from day 4 for the CFB- and BFB-treated groups and from day 7 for the GFB group. The order of potency was BFB > CFB- > GFB.

Discussion

Fibric acid derivatives are known to induce peroxisomal proliferation in rats. The typical manifestations of this process are hepatomegaly (Hawkins *et al.*, 1987) and increase in the specific activity of peroxisomal enzymes, such as the β-oxidation system (Reddy & Lalwani, 1983). In our study, both parameters increase quickly from the first day of administration (Figures 1c and 3). Moreover, there is a strong direct correlation between hepatomegaly expressed as

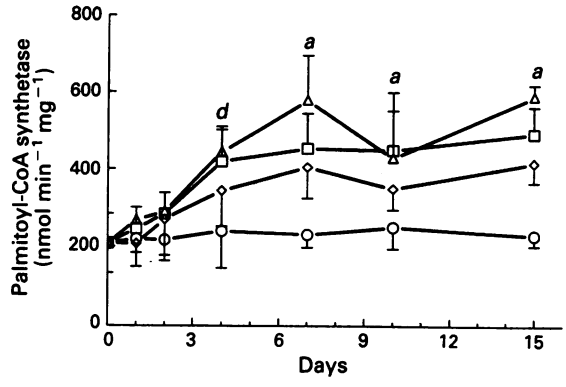

Figure 6 Time-course of the effect of a standard diet (○) or a diet supplemented with 0.3% w/w clofibrate (CFB) (□), 0.45% w/w bezafibrate (BFB) (△), and 0.3% w/w gemfibrozil (GFB) (◇) on palmitoyl-CoA synthetase activity in the microsomal fraction of livers from male Sprague-Dawley rats. Results are means ± s.d. of 6 experiments performed in duplicate. Legends for significance are the same as in Figure 1.

Table 1 Effect of gemfibrozil (GFB) on cytosolic palmitoyl-CoA hydrolase (PCH) activity *in vitro*, cytosol from control and GFB-treated rats

	Cytosol	
	Control	*GFB-treated*
No drug addition	11.5 ± 1.8	25.5 ± 5.1
+ 5 mM GFB	0.6 ± 0.7	14.3 ± 3.3

Activities are nmol min^{-1} mg^{-1} protein. Results are mean ± s.d. of three experiments performed in duplicate. Enzyme activities were assayed as described in Methods section.

Table 2 Correlation among enzymatic and non enzymatic parameters and peroxisomal proliferation (measured as peroxisomal β-oxidation activity)

	Peroxisomal β-oxidation	
	n	r^2
Liver/body weight	149	0.659
Plasma triglycerides	113	0.190*
Plasma cholesterol	115	0.248*
Palmitoyl-CoA synthetase	147	0.535
Cytosolic palmitoy-CoA hydrolase	149	0.473
Microsomal palmitoyl-CoA hydrolase	148	0.293

n are the number of pairs of data analysed. All r^2 values are statistically significant ($P < 0.001$).
*Means negative correlation.

the liver/body weight ratio and the increase in peroxisomal β-oxidation activity (Table 2), although β-oxidation increase could occur even without gross hepatomegaly (Lazarow *et al.*, 1982). Despite the correlation between these parameters, maximal enlargement of the liver is achieved earlier than maximal β-oxidation activities. This delay may be due to the time needed for the transcription and translation of the corresponding β-oxidation genes (Reddy *et al.*, 1986). BFB behaves as the most powerful peroxisomal inducer, whilst GFB is the least potent. As the presence of halogen atoms is a structural requirement for peroxisomal inducers (Esbenshade *et al.*, 1990; McGuire *et al.*, 1991), the lack of halogenation could account for the smaller effect of GFB.

A significant reduction in weight gain was observed in the fibrate-treated groups (Figure 1a), a phenomenon not des-

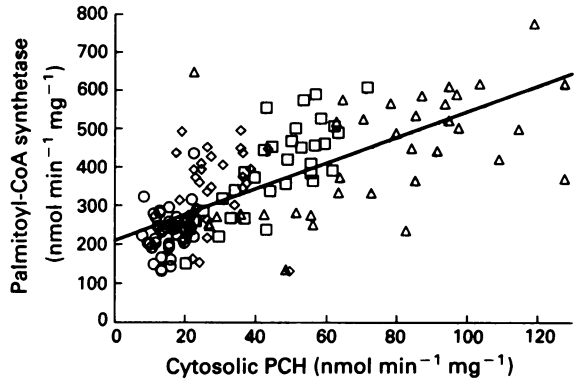

Figure 7 Relationship between palmitoyl-CoA synthetase and cytosolic palmitoyl-CoA hydrolase (PCH) activities in rats treated with a standard diet (○) or a diet supplemented with 0.3% w/w clofibrate (CFB) (□), 0.45% w/w bezafibrate (BFB) (△), and 0.3% w/w gemfibrozil (GFB) (◇). $n = 148$; $r^2 = 0.488$; $P < 0.0001$.

cribed by others (Stahlberg *et al.*, 1989; McGuire *et al.*, 1991). However, in the study by McGuire *et al.* (1991) a different strain of rats was used (CDS albino) while in the study by Stahlberg *et al.* (1989) although the rats were Sprague-Dawley, their higher initial weight (180 vs 120 g in our study) and the lower dose of BFB (0.1 vs 0.45%) make comparison difficult. Nonetheless, a significant increase was observed in the daily food intake of rats treated with BFB and GFB. It is noteworthy that the treatment with dehydroepiandrosterone (DHEA), a steroid hormone that induces peroxisomal proliferation, also causes a marked reduction in body weight of rats (Cleary, 1990; Rao *et al.*, 1992) and mice (Frenkel *et al.*, 1990). The anti-obesity effect of DHEA was thought to be due to the inhibition of glucose-6-phosphate dehydrogenase (Cleary, 1990), an enzyme which is inhibited by GFB *in vitro* (Sánchez *et al.*, 1993a). Nevertheless, the results concerning the action of DHEA are contradictory, and some authors report no effect on this enzymatic activity (Yamada *et al.*, 1991). Another hypothesis to explain the effect of DHEA could be the induction of a futile cycle of deacylation/reacylation of fatty acids in the liver of treated rats (Cleary, 1990). This argument could also be used to explain the effect of fibrates, as these drugs coordinately increase palmitoyl-CoA hydrolase and synthetase activities, as discussed below.

Administration of fibrates reduced plasma cholesterol and triglyceride levels in normolipidaemic rats (Figure 2a and b). The hypolipidaemic effect of these drugs showed a different profile in the study by McGuire *et al.* (1991), in which GFB reduced both cholesterol and triglyceride levels, while BFB showed only hypotriglyceridaemic activity and CFB even increased total cholesterol. However, in that study, experimental conditions were rather different, as animals were fed with a high-fat diet and they were fasted before they were killed. A slight, but significant, inverse correlation between β-oxidation and plasma cholesterol and triglyceride levels was found, suggesting that peroxisomal induction somehow contributes to the hypolipaemic effect of these drugs (Table 2). To our knowlege, only Pourbaix *et al.* (1984) have described a similar association between peroxisomal β-oxidation and plasma cholesterol levels in hamsters. In contrast, in a recent study by Pill *et al.* (1992) using Sprague-Dawley and Lewis rats, even though greater hypolipidaemic effect was achieved in the Lewis rats, which are more sensitive to peroxisomal proliferation, no linear relationship was found between the increase in the β-oxidation activity and the decrease in serum lipids. Moreover, in guinea-pigs (Vázquez *et al.*, 1993) and presumably in man (Hawkins *et al.*, 1987), the hypolipidaemic response to treatment with fibrates is not accompanied by peroxisomal proliferation.

The marked enhancement of liver cytosolic long-chain acyl-CoA hydrolase caused by the three fibrates had already been reported by other authors in CFB-treated rats (Berge *et al.*, 1984; Katoh *et al.*, 1987). Moreover, the increase observed by Katoh *et al.* (1987) correlated well with the induction of peroxisomal β-oxidation. The strong, direct correlation between cytosolic and microsomal acyl-CoA hydrolases and peroxisomal β-oxidation found in this study (Table 2) is in agreement with these reports. Furthermore, the potency order for the induction of palmitoyl-CoA hydrolase is identical to that of peroxisomal proliferating potency: BFB > CFB > GFB. These results also agree with those of Kawashima *et al.* (1983), who demonstrate that treatment with CFB increases hydrolase activity in rats and mice but not in guinea-pigs. Of the three fibrates, GFB is the most potent inhibitor of palmitoyl-CoA hydrolase *in vitro* (Sánchez *et al.*, 1992a). Nevertheless, the lower potency of GFB *in vivo* could not be attributed to the presence of the drug in the cytosolic fraction of treated animals, as activity is the same before and after dialysis of cytosol from GFB-treated rats (data not shown), a procedure which is known to eliminate the drug from the medium (Alegret *et al.*, 1991).

Kawashima *et al.* (1982) proposed the existence of two different inducible palmitoyl-CoA hydrolases in rat liver cytosol, other than the constitutive enzyme. The inducible enzymes were termed hydrolase I, which showed most of the inducible activity after the administration of peroxisomal proliferators, and hydrolase II. Our results *in vitro* confirm the hypothesis that fibrates inhibit the activity of the constitutive enzyme, while they do not affect the induced activity. Thus, if we consider the induced activity as the difference between GFB-treated and control activities, i.e. 25.5 minus 11.5 nmol min^{-1} mg^{-1}, this value closely reflects the activity remaining when cytosol obtained from GFB-treated rats is incubated with 5 mM GFB (about 14 nmol min^{-1} mg^{-1}), whereas this GFB concentration almost completely inhibited the enzyme activity of cytosol obtained from control animals. These results agree with those of previous studies *in vitro* in which the three fibrates were unable to inhibit cytosolic palmitoyl-CoA hydrolase from rat brain (Sánchez *et al.*, 1992a); this enzyme has similar properties to that of the inducible hepatic hydrolase I (Katoh *et al.*, 1987).

The three fibrates greatly increased microsomal palmitoyl-CoA synthetase activity, consistent with the results obtained by Yoshida & Singh (1990) in cell homogenates after treatment of rats with CFB for 14 days. These results cannot be explained by a direct effect of fibrates on the enzyme, as studies *in vitro* demonstrated no effect (CFB, BFB) or inhibition at supraphysiological concentrations (GFB) on palmitoyl-CoA synthetase activity (Sánchez *et al.*, 1993b). The order of potency *in vivo* is similar to that of peroxisomal induction, and in fact the two parameters are strongly correlated (Table 2). Nevertheless, as we found a direct correlation between palmitoyl-CoA synthetase and cytosolic palmitoyl-CoA hydrolase activities (Figure 7), and taking into account that maximal values are achieved earlier for the later enzyme, it seems likely that the induction of palmitoyl-CoA synthetase is an adaptive response to the increase in free CoA caused by the high hydrolase activites, rather than a direct consequence of the peroxisomal induction.

In summary, the time-course of the effect of CFB, BFB and GFB administration to male Sprague-Dawley rats on lipid plasma levels and palmitoyl-CoA hydrolase and synthetase activities, as well as the correlations with the peroxisomal proliferation phenomenon have been studied. From the results reported here, we can assume that: (a) peroxisomal proliferation explains only a relatively small percentage (reflected in the r^2 values) of the observed lipid-lowering effect and (b) given the strong interdependence between the proliferation of peroxisomes and the increase in palmitoyl-CoA synthetase and hydrolase activities, we could conclude that this phenomenon is not directly related to the hypolipidaemic effect of fibrates. Considering that fibrate

therapy is unlikely to cause peroxisomal induction in humans, the increase in these enzyme activities is not expected in humans after fibrate therapy. Thus, in interpreting results obtained from experiments designed to test the fibrate effect on rats, caution should be exercised in their extrapolation to species insensitive to the induction of peroxisomes.

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