Fatty acid metabolism in hepatocytes cultured with hypolipidaemic drugs

Role of carnitine

Polymnia GERONDAES, K. George M. M. ALBERTI and Loranne AGIUS*

Department of Medicine, University of Newcastle upon Tyne, Newcastle upon Tyne NE2 4HH, U.K.

The direct effects of clofibrate analogues on carnitine acyltransferase activities and fatty acid metabolism were studied in cultured hepatocytes. Rat hepatocytes cultured with bezafibrate or ciprofibrate (0.1–10 µg/ml) for 48 h had increased activities of carnitine acetyltransferase (CAT; 4–6-fold) and carnitine palmitoyltransferase (CPT; 12–34 %). The increase in CAT was higher in hepatocytes from the periportal zone (440 %) of rat liver compared with cells from the perivenous zone (266 %). In human hepatocytes, in contrast with rat, the fibrates did not cause a marked increase in CAT activity. The effects of fibrates on palmitate metabolism were dependent on the carnitine status. In the presence of exogenous carnitine (1 mM), rat hepatocytes cultured with bezafibrate had higher rates of total palmitate metabolism (29–34 %) without increased partitioning of palmitate towards β -oxidation, relative to control cultures. At low endogenous carnitine concentrations, cells cultured with bezafibrate had a greater increase in palmitate metabolism, esterification and cellular accumulation of triacylglycerol compared with the corresponding increases in the presence of carnitine. The changes in palmitate metabolism at either high or low carnitine concentrations were small in comparison with the changes in CAT activity. It is concluded that the increase in hepatic carnitine that occurs *in vivo* after fibrate feeding probably plays the major role in the changes in partitioning of fatty acid between β -oxidation and esterification.

INTRODUCTION

Clofibrate analogues (fibrates) are used clinically to lower the plasma [triacylglycerol] in patients with hyperlipidaemia. The mechanism of action of these compounds seems to involve both increased clearance of very-low-density lipoproteins and decreased production by the liver [1,2]. The effects of fibrates on the liver have been studied mainly in rodents. In the rat, fibrates cause: marked proliferation of peroxisomes [3], increased peroxisomal [4] and mitochondrial [5] β -oxidation capacity, increased activities of carnitine acetyltransferase (CAT) and acyl-CoA hydrolases [6–8], and increased hepatic carnitine [9,10] and CoA [11,12]. Several mechanisms have been proposed by which fibrates decrease hepatic production of triacylglycerol, including: (i) decreased fatty acid availability [1]; (ii) increased intrahepatic diversion of fatty acids towards peroxisomal and mitochondrial β -oxidation, as opposed to esterification to glycerolipid [13]; (iii) inhibition of glycerolipid formation [14]; and (iv) inhibition of very-low-density-lipoprotein secretion [15]. Studies on perfused liver or hepatocytes from rats treated with fibrates *in vivo* have shown increased rates of fatty acid β -oxidation [5,10,16–19], associated with either decreased [16,19] or unchanged or increased [5,10] rates of fatty acid esterification. Clofibrate has been shown to lower plasma insulin in the rat [20]. Since hepatic fatty acid metabolism is critically dependent on plasma insulin and glucagon concentrations, it is not clear to what extent the observed

changes in fatty acid metabolism in hepatocytes isolated from rats treated with fibrates *in vivo* are due to direct effects of fibrates on the liver or to secondary endocrine changes.

The aims of the present study were: first, to investigate whether fibrates have a direct effect on the partitioning of fatty acids between β -oxidation and esterification in cultured hepatocytes, and to examine whether insulin and glucagon influence the effects of fibrates on lipid metabolism; second, to examine whether changes in fatty acid metabolism correlate with changes in CAT activity; third, to compare some of the effects of these drugs in rat and human hepatocytes.

MATERIALS AND METHODS

Materials

Bezafibrate {2-[4-(chlorobenzamidoethyl)phenoxy]-2-methyl-propanoic acid} was a gift from Dr. M. Hertz, Boehringer, Mannheim, Germany. Ciprofibrate {2-[4-(2,2-dichlorocyclopropyl)phenoxy]-2-methylpropanoic acid} was from Sterling-Winthrop (Alnwick, Northumberland, U.K.). Sources of other reagents were as in [21].

Hepatocyte isolation

Rat hepatocytes were isolated by collagenase perfusion of the liver [22] of male Wistar rats (180–220 g body wt.) bred in the Animal House of the Medical School, University of Newcastle upon Tyne. After liver perfusion,

Abbreviations used: CPT, carnitine palmitoyltransferase (EC 2.3.1.21); CAT, carnitine acetyltransferase (EC 2.3.1.7).

* To whom correspondence should be addressed.

the hepatocytes were preincubated in Minimum Essential Medium (Eagle) for 20 min at 37 °C. They were then sedimented (50 g, 2 min) and washed twice in the same medium and once in medium with 1% (w/v) bovine serum albumin. Cell viability was > 90%.

Rat periportal and perivenous hepatocytes were isolated from male Wistar rats (150–200 g body wt.) obtained from Bantin and Kingman (Hull, U.K.) by digitonin/collagenase perfusion by the method of Quistorff [23] with the following modifications: (i) perfusion media were buffered with Hepes instead of bicarbonate (10 mM, pH 7.5, for the first two media; 20 mM and 50 mM, pH 7.6, for the collagenase medium and digitonin soln. respectively); (ii) separate peristaltic pumps were used for each medium, and the digitonin soln. (4 mg/ml) was injected with a 10 ml syringe, approx. 5 ml in 1 min for isolation of periportal cells and 3 ml in 3 s for isolation of perivenous cells. After liver perfusion, which lasted about 40–50 min, the hepatocytes were sedimented and washed as above, except that the preincubation was omitted. Cell yield was $(1-2) \times 10^8$ for periportal cells and $(0.5-1) \times 10^8$ for perivenous cells, and viability was > 90%.

Human hepatocytes were isolated from liver sections (100–150 g) from kidney donors (male; 48, 19 and 29 years old) by a two-step perfusion. This involved perfusion without recirculation with 1.5 litres of buffer containing 150 mM-NaCl, 6.7 mM-KCl, 10 mM-Hepes and 0.1 mM-EDTA, pH 7.5, followed by perfusion with 500 ml of buffer containing 120 mM-NaCl, 6.7 mM-KCl, 30 mM-Hepes, 5 mM-CaCl₂ and collagenase (Type IV; 90 mg/500 ml), pH 7.6, which was recirculated for 45 min. After dissociation of the liver, the hepatocytes were allowed to sediment at 1 g for 20 min at room temperature and then washed as described for rat hepatocytes. Cell viability was 60–70%.

Hepatocyte culture

The hepatocytes were suspended in Minimum Essential Medium with 5% (v/v) fetal-bovine serum (4×10^5 cells/ml) and plated (4 ml per flask) in 25 cm² culture flasks. The time allowed for attachment was 4–7 h for rat hepatocytes and 12 h for human hepatocytes. The medium was then replaced by serum-free Minimum

Essential Medium containing 10 nM-dexamethasone, 10 nM-insulin and the test concentrations of bezafibrate or ciprofibrate. Where indicated, insulin was replaced by 100 nM-glucagon. The flasks were equilibrated with CO₂/air (1:19) and incubated at 37 °C. The medium was replaced after 24 h with fresh medium containing the same hormones and drug concentrations. Fatty acid metabolism was studied after 48 h, and enzyme activities were determined after either 2 or 4 days.

Fatty acid metabolism

After culture for 48 h with bezafibrate or ciprofibrate, the medium was replaced by Minimum Essential Medium containing 1 mM-[U-¹⁴C]palmitate (0.03 Ci/mol), 1 mM-L-carnitine, 1 mM-pyruvate and 7.5 mg of bovine serum albumin/ml. The flasks were incubated at 37 °C for 4 h, except in time-course experiments, where incubations were terminated at 1 h intervals up to 5 h. The medium was decanted on termination of the incubation, and 0.5 ml samples were deproteinized with 1 M-HClO₄ (100 µl). The supernatant of the deproteinized extract was used for determination of ¹⁴C-labelled acid-soluble metabolites, acetoacetate and 3-hydroxybutyrate [24]. Palmitate was determined in the untreated medium enzymically [25]. The hepatocyte monolayer was rinsed twice with 150 mM-NaCl, and the cells were extracted in buffer containing 100 mM-KCl, 50 mM-Tris, 20 mM-KF, 5 mM-EDTA and 0.05% (w/v) Lubrol PX, pH 7.9. Triacylglycerol was determined in the cell extract [26]. The rate of fatty acid metabolism was determined from the difference in [palmitate] in the medium at the beginning and end of the 4 h incubation. β -Oxidation of palmitate was determined from the conversion of [U-¹⁴C]-palmitate into ¹⁴C-labelled-acid-soluble metabolites, and ketogenesis was determined from the accumulation of acetoacetate and 3-hydroxybutyrate. Rates were linear during 4 h.

Enzyme activities

Enzymes were assayed at 30 °C in a centrifugal analyser (Cobas Bio 8326; Hoffmann-La Roche). CAT and CPT were assayed by monitoring the formation of CoA by using the thiol reagent 5,5'-dithiobis-(2-nitrobenzoate) (DTNB) by modifications of established methods [27,28].

Table 1. Effects of bezafibrate on enzyme activities in rat hepatocytes cultured with either insulin or glucagon

Hepatocytes were cultured with the concentrations of bezafibrate (Bez) indicated and either insulin (10 nM) or glucagon (100 nM) for 48 h. Enzyme activities are expressed as munits/mg of cell protein. Values are means $\pm$ S.E.M. for either duplicate (insulin) or quadruplicate (glucagon) flasks from three cultures. Values that are significantly different from the respective controls are shown: * $P < 0.05$; ** $P < 0.005$.

Additions present during 48 h culture	Activity (munits/mg of cell protein)				Cellular protein (mg/flask)
	CAT	CPT	Acetyl-CoA hydrolase	Palmitoyl-CoA hydrolase	
Insulin (10 nM)	3.0 $\pm$ 0.3	11.9 $\pm$ 0.5	11.1 $\pm$ 1.2	5.5 $\pm$ 0.4	0.84 $\pm$ 0.12
+ Bez (0.1 µg/ml)	3.5 $\pm$ 0.4	12.8 $\pm$ 1.1	11.0 $\pm$ 1.2	5.3 $\pm$ 0.3	0.90 $\pm$ 0.15
+ Bez (1 µg/ml)	8.8 $\pm$ 1.3**	11.9 $\pm$ 0.9	11.7 $\pm$ 1.2	6.2 $\pm$ 0.5	1.00 $\pm$ 0.16
+ Bez (10 µg/ml)	18.0 $\pm$ 2.0**	14.2 $\pm$ 2.4	12.3 $\pm$ 1.1	7.7 $\pm$ 0.4*	0.96 $\pm$ 0.11
Glucagon (100 nM)	3.5 $\pm$ 0.6	11.1 $\pm$ 0.7	7.9 $\pm$ 0.9	6.5 $\pm$ 0.2	0.59 $\pm$ 0.09
+ Bez (0.1 µg/ml)	4.1 $\pm$ 0.7	12.6 $\pm$ 0.6	8.7 $\pm$ 1.0	7.0 $\pm$ 0.4	0.61 $\pm$ 0.11
+ Bez (1 µg/ml)	8.5 $\pm$ 1.3**	13.7 $\pm$ 0.5	10.5 $\pm$ 0.4*	8.1 $\pm$ 0.3*	0.65 $\pm$ 0.10
+ Bez (10 µg/ml)	14.8 $\pm$ 2.2**	14.9 $\pm$ 0.7*	11.9 $\pm$ 0.4**	9.5 $\pm$ 0.4**	0.70 $\pm$ 0.11

Table 2. Effects of bezafibrate on carnitine acyltransferase activities in rat periportal and perivenous hepatocytes

Rat periportal (PP) and perivenous (PV) hepatocytes were isolated as described in the Materials and methods section. The activities of alanine aminotransferase, fructose biphosphatase, pyruvate kinase and glutamate dehydrogenase were determined in culture flasks that were terminated 4 h after inoculation. CAT and CPT were determined in hepatocyte monolayers that were cultured without or with bezafibrate (10 $\mu\text{g}/\text{ml}$) for 2 days. Activities are expressed as munits/mg of cell protein. Values are means $\pm$ S.E.M. for the numbers of cultures indicated in parentheses; NS, not significant.

Hepatocytes ...	Activity (munits/mg of protein)		PP/PV ratio	<i>P</i> value (<i>t</i> test) PP versus PV
	PP (6)	PV (7)		
Enzyme activities determined after 4 h				
Alanine aminotransferase	172 ± 16	120 ± 4	1.43	< 0.02
Fructose bisphosphatase	22.9 ± 1.5	16.5 ± 1.7	1.39	< 0.05
Pyruvate kinase	60 ± 10	73 ± 8	0.82	NS
Glutamate dehydrogenase	1667 ± 32	1901 ± 62	0.88	< 0.03
Enzyme activities determined after 2 days				
CAT				
Control	2.38 ± 0.17	2.54 ± 0.33	0.94	NS
Bezafibrate	12.75 ± 0.59	9.29 ± 0.49	1.37	< 0.001
CPT				
Control	9.8 ± 1.3	8.8 ± 0.6	1.13	NS
Bezafibrate	11.1 ± 0.7	10.2 ± 0.8	1.08	NS

The assay contained 100 mM-KCl, 50 mM-Tris, 2 mM-MgCl₂, 0.1 mM-DTNB, 2.5 mM-L-carnitine, and either 150 μM -acetyl-CoA or 20 μM -palmitoyl-CoA. Carnitine acyltransferase activities were determined from the difference in rates of increase in absorbance with and without L-carnitine, and hydrolase activities were determined from the rates in the absence of carnitine. Alanine aminotransferase (EC 2.6.1.2), glutamate dehydrogenase (EC 1.4.1.2) and fructose-1,6-bisphosphatase (EC 3.1.3.11) were assayed as in ref. [29]. Pyruvate kinase (EC 2.7.1.40) was assayed as in ref. [29], except that 0.5 mM-phosphoenolpyruvate and 50 μM -fructose 1,6-bisphosphate were used. Enzyme activities are expressed as munits/mg of cell protein, where 1 munit is the amount converting 1 nmol of substrate/min at 30 °C. Protein was determined by the Lowry [30] method.

Except for the experiments on periportal and perivenous hepatocytes, all test conditions were in duplicate flasks in each culture. Results are expressed as means $\pm$ S.E.M. for the numbers of hepatocyte preparations indicated. Statistical analysis was by Student's *t* test (paired or unpaired).

RESULTS

Effects of bezafibrate on enzyme activities in rat hepatocyte cultures

In rat hepatocytes cultured for 48 h with either insulin (10 nM) or glucagon (100 nM), bezafibrate caused 6- and 4-fold increases in CAT activity respectively. The activities of CPT, acetyl-CoA hydrolase and palmitoyl-CoA hydrolase were increased by 20–50 % (Table 1). Total cell protein was also increased (12–18 %, *P* < 0.01).

Table 3. Effects of bezafibrate and ciprofibrate on CAT activity in rat and human hepatocytes

Rat and human hepatocytes were isolated as described in the Materials and methods section. They were cultured with 10 nM-insulin and the concentrations of bezafibrate (Bez) and ciprofibrate (Cip) indicated, and CAT activity was determined after 48 h. Enzyme activities are expressed as munits/mg of protein and are means $\pm$ S.E.M. for duplicate flasks from five cultures for the rat cells and three cultures for the human hepatocytes. Values in parentheses are activities expressed as percentages of the controls. Activities of ciprofibrate-treated cultures that are significantly different from the corresponding bezafibrate-treated values are indicated: **P* < 0.05; ***P* < 0.005.

Hepatocytes ...	CAT (munits/mg of protein)	
	Rat	Human
Control	2.7 $\pm$ 0.2 (100)	22.1 $\pm$ 6.1 (100)
+ Bez (0.1 $\mu\text{g}/\text{ml}$)	2.9 $\pm$ 0.3 (107)	
+ Bez (1 $\mu\text{g}/\text{ml}$)	6.8 $\pm$ 0.7 (252)	
+ Bez (10 $\mu\text{g}/\text{ml}$)	13.5 $\pm$ 0.8 (500)	26.8 $\pm$ 6.1 (121)
+ Cip (0.1 $\mu\text{g}/\text{ml}$)	5.5 $\pm$ 0.5 (204)*	
+ Cip (1 $\mu\text{g}/\text{ml}$)	11.8 $\pm$ 0.8 (437)**	
+ Cip (10 $\mu\text{g}/\text{ml}$)	15.9 $\pm$ 1.4 (500)	27.9 $\pm$ 6.7 (126)

in cells cultured with bezafibrate for 2 days relative to controls (Table 1). In experiments where the effects of bezafibrate (0.1–10 $\mu\text{g/ml}$) were examined after either 2 or 4 days, the highest bezafibrate concentration caused a 9-fold increase in CAT activity after 4 days (cf. 6-fold at 2 days) compared with controls. Total cell protein and CAT activity declined between 2 and 4 days by about 40 % in the control cultures, and, although the difference in CAT activity between the bezafibrate-treated cultures and the controls was greater in percentage terms after 4 days, the difference was similar in absolute terms at 2 and 4 days. In the rest of this study experiments were terminated after 2 days.

Studies on the effects of cell inoculation density showed that the effects of bezafibrate (0.1–10 $\mu\text{g/ml}$) on the activities of CAT, CPT and acetyl- and palmitoyl-CoA hydrolases after 48 h were similar in cultures inoculated at densities of 0.63×10^6 , 1.25×10^6 and 2.5×10^6 cells per flask ($n = 3$ cultures; results not shown).

Table 2 shows the effects of bezafibrate on CAT activity in rat hepatocytes isolated from either the periportal or the perivenous zone of rat liver. In agreement with previous studies [23,31], periportal cells had higher alanine aminotransferase activity, lower glutamate dehydrogenase activity (Table 2) and higher rates of gluconeogenesis (D. Tosh & L. Agius, unpublished work) compared with perivenous cells. There was no significant difference between periportal and perivenous cells in CAT activity in the control cultures, but in the bezafibrate-treated cultures CAT activity was significantly higher in periportal cells (436 % increase) than in perivenous cells (266 % increase; Table 2).

Effects of fibrates in rat and human hepatocytes

In rat hepatocyte cultures, bezafibrate and ciprofibrate had similar effects on CAT activity at the highest concentration (10 $\mu\text{g/ml}$), but ciprofibrate was more potent at lower concentrations (Table 3). In human hepatocytes, CAT activity was 8-fold higher than in rat hepatocytes (Table 3), and acetyl-CoA hydrolase activity was not detectable. This contrasts with the lower transferase relative to hydrolase activity in rat liver cells (Table 1). In human hepatocytes, in contrast with rat cells, the fibrates did not cause a marked increase in CAT activity. The small increase (Table 3) was not statistically significant, but the trends were similar in the three experiments.

Effects of bezafibrate on fatty acid metabolism in rat hepatocytes

Table 4 shows the effects of bezafibrate on the rates of palmitate metabolism (expressed per flask) and also the rates expressed per mg of protein. The total cell protein was higher ($P < 0.005$) in the insulin-treated than in glucagon-treated cultures (Table 4), presumably because of a higher rate of proteolysis in the latter cultures. The glucagon-treated cultures had higher rates of palmitate metabolism compared with the insulin-treated cultures when rates were expressed on a protein basis (Table 4).

In the presence of carnitine (1 mM) the rate of palmitate metabolism, determined from the decrease in [palmitate] in the medium, was twice the rate of conversion of palmitate into acid-soluble metabolites. Since the conversion of $[\text{U-}^{14}\text{C}]$ palmitate into $^{14}\text{CO}_2$ represents less

than 5 % of the conversion into acid-soluble metabolites, the latter approximates to the rate of β -oxidation [21]. The rate of esterification was estimated from the difference between rates of palmitate metabolism and β -oxidation. There was little secretion of triacylglycerol into the medium during 4 h (< 15 nmol of triacylglycerol/flask), compared with the rate of palmitate uptake (400–500 nmol of palmitate/flask; Table 4). The triacylglycerol content of the cells before the 4 h incubation with palmitate was about 20–50 % of the triacylglycerol content after 4 h incubation with palmitate, suggesting that 50–80 % of the cellular triacylglycerol at the end of the experiment (Table 4) was formed during incubation with palmitate.

The bezafibrate-cultured cells had higher rates ($P < 0.05$) of palmitate metabolism (30 % and 34 % in the insulin- and glucagon-treated cultures, respectively, expressed per flask), but the percentage of palmitate metabolized by β -oxidation was not increased by bezafibrate, in either the insulin- or glucagon-treated cultures (Table 4). The bezafibrate-treated cultures had a higher protein content than the respective controls (18 % in the insulin-treated cultures and 16–23 % in the glucagon-treated cultures), which probably represents, in part, the induction of specific proteins and proliferation of organelles, and may be analogous to liver hypertrophy induced by fibrates *in vivo*. The effects of bezafibrate on palmitate metabolism were similar in insulin- and glucagon-treated cultures and were small (< 50 %) compared with the changes in CAT activity (400–600 %; Table 1). Ciprofibrate had similar effects to bezafibrate on fatty acid metabolism at concentrations of 0.1, 1 and 10 $\mu\text{g/ml}$ (results not shown).

In incubations without exogenous carnitine, rates of conversion of $[\text{U-}^{14}\text{C}]$ palmitate into acid-soluble metabolites (which presumably represents β -oxidation in mitochondria and peroxisomes) and rates of ketogenesis were lower (61 % and 68 % respectively; $P < 0.0001$) than in the presence of 1 mM-carnitine (Table 4), indicating that endogenous carnitine limits fatty acid oxidation in cultured hepatocytes [21]. Rates of fatty acid esterification were higher ($P < 0.01$) in incubations without carnitine (Table 4), probably because of decreased diversion of fatty acids towards oxidation. The lower rate of β -oxidation was associated with a lower rate of palmitate uptake (22 %, $P < 0.05$), indicating that carnitine deficiency influenced not only the partitioning of palmitate between different pathways but also total palmitate metabolism.

In incubations without carnitine, with increasing [bezafibrate] the formation of ketone bodies decreased (28 %), whereas the formation of acid-soluble metabolites increased (23 %, $P < 0.05$; Table 4); consequently, ketone bodies accounted for a lower proportion of total β -oxidation in bezafibrate-treated cultures (29 % at 10 $\mu\text{g/ml}$) than in controls (47 %). Bezafibrate caused a greater increase in palmitate esterification ($P < 0.01$), which was paralleled by higher cellular triacylglycerol ($P < 0.01$), in the carnitine-free cultures than with 1 mM-carnitine (Table 4).

DISCUSSION

Clofibrate analogues induce proliferation of peroxisomes in rat liver, but not in human liver [32–35]. It is not known which of the changes observed in rat liver are

Table 4. Effects of bezafibrate on fatty acid metabolism in cells cultured with either insulin or glucagon

Hepatocytes were cultured with the concentrations of bezafibrate (Bez) shown and either insulin (10 nM) or glucagon (100 nM) for 48 h. Palmitate metabolism and ketogenesis were determined during a 4 h incubation with 1 mM-palmitate without or with 1 mM-carnitine. The effects of bezafibrate on palmitate metabolism and ketogenesis are expressed as the change in rate per flask, expressed as nmol of substrate or product metabolized/4 h per flask relative to the control cultures shown in parentheses. The amount of protein per flask is expressed in mg, and the rates of palmitate metabolism and ketogenesis are expressed as nmol/4 h per mg of protein. The cellular triacylglycerol content is expressed as nmol/mg of protein. Values that are significantly different from the respective controls without bezafibrate are indicated: * $P < 0.05$; ** $P < 0.005$. Abbreviation: ASM, acid-soluble metabolites. Values are means $\pm$ S.E.M. of duplicate flasks from three cultures.

Additions present during 48 h culture	Carnitine (1 mM)	Change in rate caused by bezafibrate (nmol/4 h per flask)					Total protein (mg/flask)	Rates of palmitate metabolism (nmol/4 h per mg of protein)					Palmitate oxidation (% of uptake)	Cellular triacylglycerol (nmol/mg of protein)
		Palmitate uptake	Palmitate oxidation to ASM	Palmitate esterification	Ketone-body formation	Palmitate uptake		Palmitate oxidation to ASM	Palmitate esterification	Ketone-body formation				
Insulin (10 nM)	+	(433 ± 21)	(220 ± 21)	(214 ± 14)	(447 ± 34)	0.68 ± 0.04	653 ± 52	332 ± 28	321 ± 24	642 ± 82	51 ± 1	171 ± 13		
+ Bez (0.1 µg/ml)	+	38 ± 7	3 ± 10	21 ± 4	19 ± 7	0.68 ± 0.05	690 ± 45	336 ± 29	354 ± 21	704 ± 77	49 ± 2	156 ± 11		
+ Bez (1 µg/ml)	+	89 ± 14*	36 ± 10	52 ± 8*	70 ± 22	0.77 ± 0.06	703 ± 70*	344 ± 35	359 ± 33	699 ± 97	49 ± 1	148 ± 17		
+ Bez (10 µg/ml)	+	128 ± 16**	52 ± 12*	75 ± 4**	93 ± 25*	0.80 ± 0.07	733 ± 77*	358 ± 47	375 ± 31	713 ± 109	48 ± 1	139 ± 14*		
Glucagon (100 nM)	+	(374 ± 23)	(203 ± 5)	(170 ± 22)	(474 ± 24)	0.44 ± 0.05	894 ± 89	489 ± 40	405 ± 67	1135 ± 99	56 ± 3	150 ± 8		
+ Bez (0.1 µg/ml)	+	38 ± 3	7 ± 4	14 ± 12	24 ± 3	0.44 ± 0.02	894 ± 27	493 ± 29	418 ± 21	1165 ± 98	53 ± 2	155 ± 7		
+ Bez (1 µg/ml)	+	64 ± 14	26 ± 9	38 ± 13	55 ± 13	0.48 ± 0.04	929 ± 40	487 ± 22	443 ± 31	1239 ± 59	53 ± 2	155 ± 10		
+ Bez (10 µg/ml)	+	128 ± 18**	43 ± 8**	86 ± 20**	69 ± 6**	0.54 ± 0.05	969 ± 85**	475 ± 39	550 ± 67	1048 ± 95	50 ± 3	161 ± 16		
Glucagon (100 nM)	-	(290 ± 20)	(79 ± 11)	(211 ± 11)	(154 ± 30)	0.44 ± 0.04	679 ± 38	180 ± 11	499 ± 31	343 ± 38	27 ± 2	188 ± 4		
+ Bez (0.1 µg/ml)	-	11 ± 5	-6 ± 4	10 ± 15	-23 ± 12	0.45 ± 0.02	663 ± 24	162 ± 26	492 ± 34	290 ± 36	25 ± 2	222 ± 11		
+ Bez (1 µg/ml)	-	102 ± 7*	5 ± 1	98 ± 9**	-29 ± 12	0.49 ± 0.05	820 ± 58*	172 ± 10	648 ± 46**	260 ± 35	21 ± 1	230 ± 2**		
+ Bez (10 µg/ml)	-	146 ± 10**	19 ± 3*	129 ± 7**	-43 ± 19**	0.51 ± 0.05	888 ± 70**	192 ± 10	696 ± 60**	244 ± 24*	22 ± 2	240 ± 4**		

important for the triacylglycerol-lowering effect in man. Although studies on rats given fibrates have suggested increased hepatic partitioning of fatty acids towards β -oxidation, as opposed to esterification [16,19], this may be due to secondary endocrine or metabolic changes rather than a direct effect of fibrates on enzymes involved in fatty acid metabolism. There is good evidence that hepatocyte cultures are a good model in which to study changes in enzyme activities induced by fibrates [36–38]. Since hepatocyte cultures lose their endogenous carnitine when maintained in the absence of carnitine precursors [21], they can be used to study changes in lipid metabolism induced by fibrates that are independent of carnitine changes. In the present study, the effects of fibrates on fatty acid metabolism in cultured hepatocytes were examined in the absence or presence of exogenous carnitine to establish conditions in which carnitine is either limiting or in excess.

The following conclusions are drawn from this study.

(1) In the presence of exogenous carnitine, bezafibrate caused small increases in rates of palmitate metabolism (29 and 34 % in insulin- and glucagon-treated cultures respectively) and esterification (35 and 51 %), but it did not increase the proportion of palmitate metabolized by β -oxidation. It is therefore inferred that bezafibrate does not inhibit esterification, and for a constant [carnitine] it does not increase the partitioning of palmitate towards β -oxidation.

(2) The effects of bezafibrate on palmitate metabolism were dependent on the carnitine status, such that, at low carnitine concentrations, bezafibrate caused greater increases in palmitate esterification and accumulation of triacylglycerol than at high carnitine concentrations, and it decreased rather than increased ketogenesis. The finding that bezafibrate can have opposite effects on ketogenesis in the absence or presence of exogenous carnitine may explain why, in a recent study, bezafibrate decreased palmitate β -oxidation [39]. The results suggest that bezafibrate increases the capacity for β -oxidation and esterification, but the partitioning between both pathways depends on the carnitine status.

(3) The changes in palmitate metabolism caused by bezafibrate were much smaller than the changes in CAT activity, suggesting that the induction of this enzyme may not be important in the changes in lipid metabolism.

(4) The finding that bezafibrate caused a several-fold increase in CAT activity and a smaller increase in hydrolase activity in cultured rat hepatocytes agrees with the changes *in vivo* [12], and is further evidence that fibrates induce similar changes in enzyme activities in rat hepatocyte cultures to those in rat liver *in vivo*.

(5) The increase in CAT activity was more prominent in rat hepatocytes derived from the periportal zone than from the perivenous zone, suggesting that there may be a heterogeneous response to at least some of the effects of fibrates in different zones of the liver acinus. Fibrates may induce their multiple effects in hepatocytes either by direct interaction with specific recognition sites or receptors [40] or by conversion into CoA esters or other conjugates [41]. The more marked effect of fibrates in periportal cells may be due either to differences in 'recognition sites' in different cells or to more general mechanisms related to conjugation or degradation.

(6) Bezafibrate and ciprofibrate had different effects on CAT activity in rat and human hepatocytes. Since

human hepatocytes have a much higher CAT activity than do rat hepatocytes, the failure to observe a several-fold increase in activity in human hepatocytes is not surprising. Morphometric studies have shown that fibrates have different effects on proliferation of organelles in human and rat liver [32–35]. The present findings suggest that cultures of rat and human hepatocytes *in vitro* can be used to identify and characterize biochemical differences between rat and human liver.

It is well established that fibrates increase hepatic carnitine in the rat *in vivo* [9,10]. Since bezafibrate did not increase the proportion of palmitate that was metabolized by β -oxidation in cultures incubated with 1 mM-carnitine, and it also did not inhibit esterification, the increased partitioning of palmitate towards β -oxidation in hepatocytes or perfused liver isolated from rats treated with fibrates [16,19] is unlikely to be due to either peroxisomal proliferation or inhibition of esterification, but more likely to be due to the increase in hepatic carnitine. Studies on the relation between the rate of β -oxidation and the hepatocyte carnitine content have suggested that, whereas in cells from fasted rats a maximum rate of β -oxidation occurs at a [carnitine] within the physiological range, in cells from re-fed rats a higher than physiological concentration of endogenous carnitine is required for maximum β -oxidation [42]. This suggests that endogenous carnitine may limit β -oxidation in the fed state. This supports the hypothesis that the increase in hepatic carnitine in rats treated with fibrates may be the major factor responsible for increased partitioning of fatty acids towards β -oxidation.

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