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Tissue-specific effect of clofibrate on rat lipogenic enzyme gene expression

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Abstract

Fibrate derivatives are commonly used to treat hyperlipidaemia; however, the mechanism of the antilipidaemic action of these drugs is still unknown. The effect of clofibrate (fibrate derivative) administration for 14 days on lipogenesis and on malic enzyme (EC 1.1.1.40) and fatty acid synthase (EC 2.3.1.85) gene expression in brown and white adipose tissues and in the liver was examined in rats. The rate of brown adipose tissue lipogenesis in the clofibrate-treated animals was significantly lower than that of the control rats. The rate of liver and white adipose tissue lipogenesis was not affected significantly by clofibrate. In brown adipose tissue, the drug treatment resulted in a depression of fatty acid synthase and malic enzyme mRNA levels. The fatty acid synthase mRNA level did not change significantly in the liver, whereas the malic enzyme mRNA level increased approximately 6-fold in this organ after clofibrate treatment. The malic enzyme mRNA level in white adipose tissue increased about 2-fold, while the fatty acid synthase mRNA level was unchanged after clofibrate feeding. The results presented in this paper provide further evidence that the hypolipidaemia caused by treatment of rats with clofibrate cannot be related to the inhibition of fatty acid synthesis in the liver and white adipose tissue. These data also indicate that clofibrate exhibits tissue specificity.

Keywords: Clofibrate; Malic enzyme; Fatty acid synthase; mRNA; Lipogenesis; Brown adipose tissue; Liver; White adipose tissue; (Rat)

1. Introduction

Fibrate derivatives are commonly used to treat hyperlipidaemia. Numerous reports indicate that fibrates cause a significant decrease in serum triacylglycerol, and total and low-density lipoprotein–cholesterol concentrations (Miyazawa et al., 1975; Augustyn et al., 1978; Laker and Mayes, 1979; O'Brien et al., 1981; Martini et al., 1982; Heller and Harvengt, 1983; Lewis, 1983; Maxwell et al., 1983; Shirai et al., 1983). A variety of mechanisms has been proposed for the antilipidaemic action of these drugs (Schoonjans et al., 1996a,b). Fibrates have been suggested to be involved in the increase of lipoprotein triacylglycerol hydrolysis, stimulation of the cellular uptake of fatty acid and their conversion to acyl-CoA derivatives, stimulation of mitochondrial and peroxisomal β-oxidation pathways, and reduction of fatty acid and triacylglycerol synthesis and very low density lipoprotein production (Schoonjans et al., 1996a,b). It seems that all these mechanisms of action of fibrates involve peroxisome proliferator-activated (PPA) receptors which act in concert with several genes and lead to a decreased lipid concentration in human serum (Schoonjans et al., 1996a,b). The relative importance of these mechanisms for the antilipidaemic action of fibrates is still unknown.

The ability of fibrates to decrease the synthesis of fatty acids and triacylglycerols is still the most controversial issue, probably because of the variety of experimental models used and parameters measured. For instance, Laker and Mayes (1979) showed that lipogenesis in the liver was depressed upon addition of the drug to the perfusate, but was increased significantly in livers of rats treated with clofibrate for 1 week. Miyazawa et al. (1975) also reported an increase in liver lipogenesis after clofibrate treatment of rats. We have shown previously that clofibrate administration for 7 days to rats significantly decreased, slightly increased and was without effect on the rate of lipogenesis in brown adipose tissue, liver and white adipose tissue, respectively (Kochan et al., 1993). Furthermore, we have shown that the activity of fatty acid synthase and malic enzyme is depressed by clofibrate in brown adipose tissue

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(Kochan et al., 1993). In contrast, clofibrate feeding caused a several-fold increase in malic enzyme activity but was without effect on fatty acid synthase activity in the liver (Kochan et al., 1993).

Thus, an additional study appeared to be necessary to resolve the conflicting findings concerning the effect of fibrates on lipogenesis. In the present study, we compared the effects of clofibrate treatment on lipogenesis and on the relative abundance of mRNAs encoding malic enzyme and fatty acid synthase in the liver, white and brown adipose tissues.

2. Materials and methods

2.1. Chemicals

Digoxigenin DNA labelling kit, nylon membrane positively charged, anti-digoxigenin–AP conjugate, chemiluminescent substrate: CDP-*Star* (disodium 4-chloro3-(4-methoxyspiro{1,2-dioxetane-3,2′-(5′-chloro)tricyclo[3.3.1^{3,7}]decan}-4-yl) phenyl phosphate) and blocking reagent were obtained from Boehringer Mannheim (Mannheim, Germany). Clofibrate and Tween 20 were provided by Bioproducts (Heidelberg, Germany). Tritiated water was obtained from Amersham International (Aylesbury, England). All other chemicals were purchased from Sigma (St. Louis, MO, USA). Kodak XAR film (Eastman Kodak, Rochester, NY, USA) and Polaroid 665 film (Polaroid, Cambridge, USA) were also used.

2.2. Animals

Male Wistar rats weighing approximately 230 g were used for the experiments. The rats were housed in wire mesh cages at 20°C with alternating 12-h light/12-h dark and were fed ad libitum on a commercially available standard laboratory diet and tap water. The experimental group of rats was given clofibrate (as a water suspension prepared as described previously; Zelewski and Swierczynski, 1983) by stomach tube at a daily dose of 250 mg/kg of body weight for 14 successive days.

On day 15, the rats were used for the estimation of in vivo fatty acid synthesis. Fatty acid synthesis in vivo was estimated by measuring the incorporation of tritium from tritiated water into fatty acids (Stansbie et al., 1976; Trayhurn, 1980). Rats were injected intraperitoneally with 5 mCi $^{3}H_2O$. One hour after injection, the animals were killed by decapitation, blood was collected, and the total interscapular brown adipose tissue and approximately 1 g of liver and white adipose tissue (epididymal) were removed. Tissues were weighed, saponified with ethanolic KOH, acidified and fatty acids were extracted with petroleum ether (Stansbie et al., 1976). The incorporation of tritium into fatty acids was assayed using an LS-6000 liquid scintillation spectrometer (Beckman). The rate of fatty acid synthesis was calculated as micromoles ^{3}H incorporated per hour per gram of wet tissue by dividing the radioactivity in the lipid fraction by the specific radioactivity of plasma water measured in the blood sample taken at the same time as the tissue sample.

2.3. Probes designing and labelling

The fatty acid synthase (EC 2.3.1.85) coding sequence was obtained from the EMBL database (Gene Bank accession number M84761). Antisense oligonucleotide (32-mer: 5′-GAT AGA GGT GCT GAG CCA GCG TGC TGA GCG TG-3′) was designed with the aid of the GeneRunner program (Hastings Software). Its uniqueness was checked against the EMBL data library using the Fasta program. The oligonucleotide was synthesized commercially (GENSET, Paris, France) with a single digoxigenin ligand at the 3′ end. The cDNA probe targeted to the malic enzyme (EC 1.1.1.40) coding sequence (Gene Bank accession number M12545) was synthesized and labelled with digoxigenin-11-dUTP using the random-primed method and the digoxigenin DNA labelling kit. As a DNA template, the pME 6 (pUC 13-JM103 cloning vector with a 1278-b insert—a fragment of malic enzyme coding sequence) was used. The pME 6 was a gift from Dr. Vera Nikodem, NIH, Bethesda (Magnuson et al., 1986).

2.4. Isolation of RNA and Northern blot RNA analysis

The animals were killed by decapitation. Liver, white adipose tissue (epididymal), brown adipose tissue (interscapular), kidney cortex (separated from the renal medulla), heart, skeletal muscle and brain were rapidly removed and frozen in liquid nitrogen. Total RNA was extracted from frozen tissues by a guanidinium isothiocyanate–phenol/chloroform method (Chomczynski and Sacchi, 1987) and finally dissolved in dimethyl pyrocarbonate-treated water. The RNA concentration of the extracts was determined from the absorbance at 260 nm and all samples had an 260/280 nm absorbance ratio of about 2.0.

The RNA samples from control rats and from clofibrate-treated rats were applied (10 μg/lane) to a 1% agarose gel containing 0.41 M formaldehyde, and fractionated by horizontal gel electrophoresis. After electrophoretic fractionation, RNA was transferred overnight to a positively charged nylon membrane by capillary blotting and fixed with UV light. Pre-hybridization was performed at 42°C for 45 min in 25 ml of pre-hybridization solution [7% sodium dodecyl sulfate (SDS), 50% formamide, 5× saline–sodium citrate buffer (SSC), 2% blocking reagent, 50 mM sodium phosphate (pH 7.0), 0.1% *N*-lauroylsarcosine]. Hybridization was at 42°C overnight in 25 ml of pre-hybridization solution containing oligonucleotide probe (25 ng/ml). In the case of the probe specific for rat malic enzyme gene, pre-hybridization and hybridization were at 55°C. The following post-hybridization washes were performed: twice for 5 min in 2× SSC/0.1% SDS (at room temperature), twice for 15 min in 0.1× SSC/0.1% SDS at 48°C (68°C for plasmid-derived probe). The membranes were then rinsed briefly with

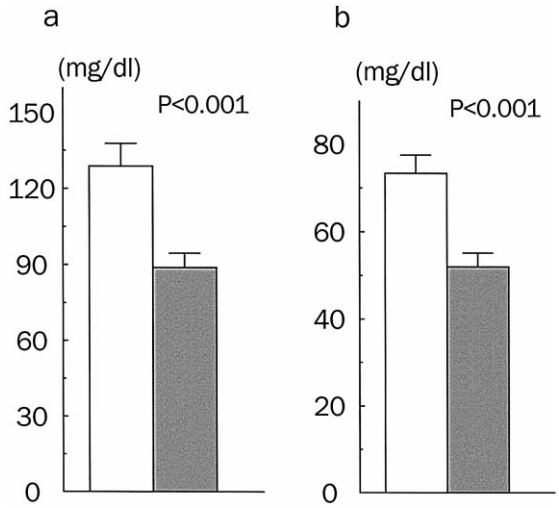

Fig. 1. The effect of clofibrate treatment on rat plasma triacylglycerol (a) and cholesterol (b) concentration. The significance of differences in lipid concentrations between clofibrate-treated (black) and control (white) rats is shown in the figure.

washing buffer containing 0.1 M maleic acid (pH 7.5), 0.15 M NaCl and 0.3% Tween 20. They were blocked by incubation for 30 min at room temperature with blocking buffer [1% blocking reagent, 0.1 M maleic acid (pH 7.5), 0.15 M NaCl] and incubated (at the same conditions as described above) with blocking buffer containing a polyclonal antibody against digoxigenin (Fab fragment conjugated to alkaline phosphatase diluted 1:20,000). After being washed twice for 15 min with washing buffer, the membranes were rinsed for 5 min with detection buffer (0.1 M Tris–Cl, pH 9.5, 0.1 M NaCl) and immersed for 5 min in CDP-*Star* solution (25 mM CDP-*Star* diluted in detection buffer). Membranes were exposed to Kodak XAR film for 15–60 min (at 37°C).

Signals were scanned and quantified using the Sigma Scan software program (Jandel Scientific) and the level of mRNA for lipogenic enzymes was estimated. The values were normalized to the corresponding amount of 18S RNA. Results expressed in arbitrary units are presented as means ± standard error of the mean (S.E.M.) of samples from 4 to 16 rats. The statistical significance of differences between groups was assessed by one-way analysis of variance (ANOVA) followed by Student's *t*-test or by Mann–Whitney test, using Systat software (Systat). Differences between groups were considered significant when $P < 0.05$.

2.5. Measurements of plasma triacylglycerol and cholesterol concentrations

Plasma triacylglycerol and cholesterol concentrations were analysed by standard enzymatic procedures (Boehringer Mannheim, Mannheim, Germany).

3. Results

The food consumption and the changes in total body weight of control and clofibrate-treated rats were essen-

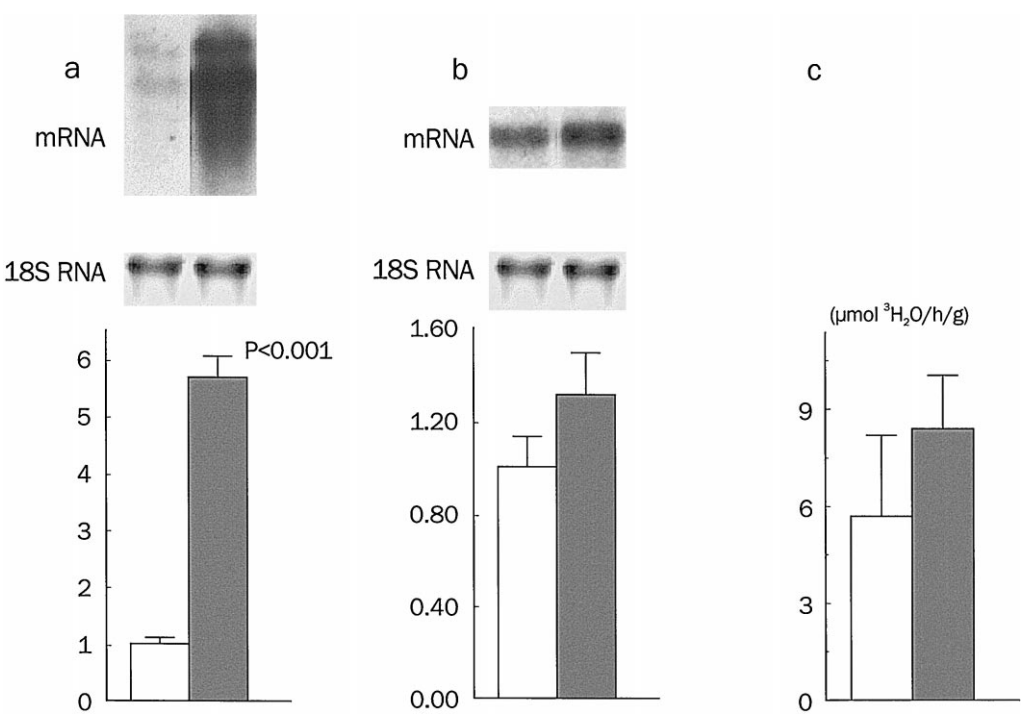

Fig. 2. The effect of clofibrate treatment on malic enzyme (a), fatty acid synthase (b) mRNA levels, and on in vivo liver lipogenesis (c). Malic enzyme and fatty acid synthase mRNA concentrations were measured by Northern blot hybridization (representative Northern blots are presented) and calculated as described in Section 2. The significance of differences in the mRNA concentrations expressed in arbitrary units and in the rate of incorporation of ^3H from ^3H_2O into lipids between clofibrate-treated (black) and control (white) rats is presented in the figure.

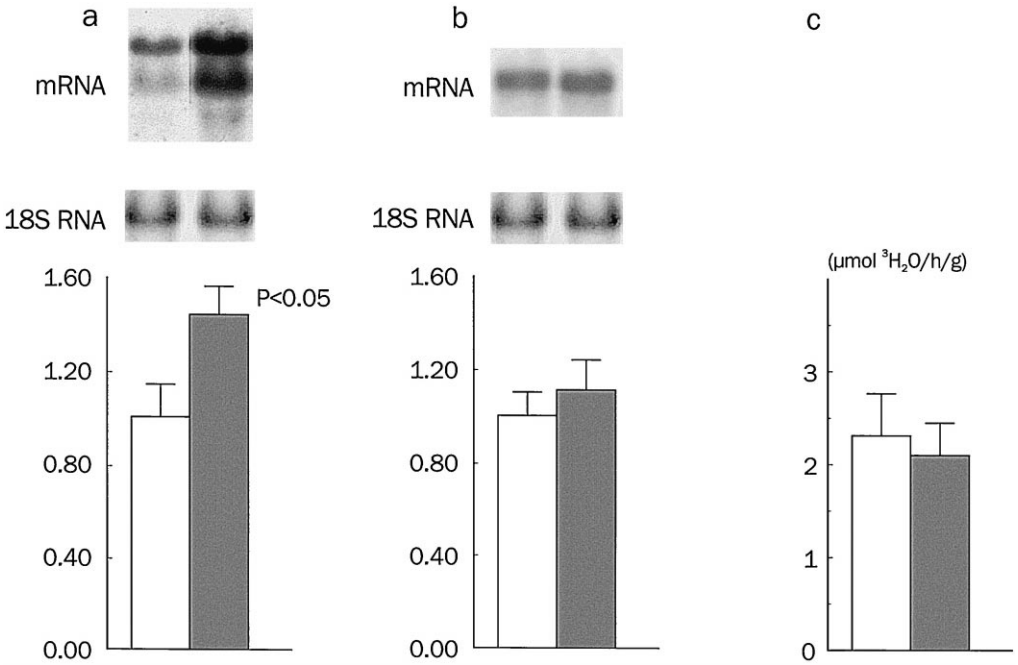

Fig. 3. The effect of clofibrate treatment on malic enzyme (a), fatty acid synthase (b) mRNA levels, and on in vivo white adipose tissue lipogenesis (c). Malic enzyme and fatty acid synthase mRNA concentrations were measured by Northern blot hybridization (representative Northern blots are presented) and calculated as described in Section 2. The significance of differences in the mRNA concentrations expressed in arbitrary units and in the rate of incorporation of ^3H from ^3H_2O into lipids between clofibrate-treated (black) and control (white) rats is presented in the figure.

tially the same. Thus, all the changes presented below may be assumed to be caused by the drug itself and not by dietary factors. Plasma triacylglycerol (Fig. 1a) and cholesterol (Fig. 1b) concentrations were significantly lower in clofibrate-treated rats. Clofibrate treatment is known to induce malic enzyme activity in the liver and hep-

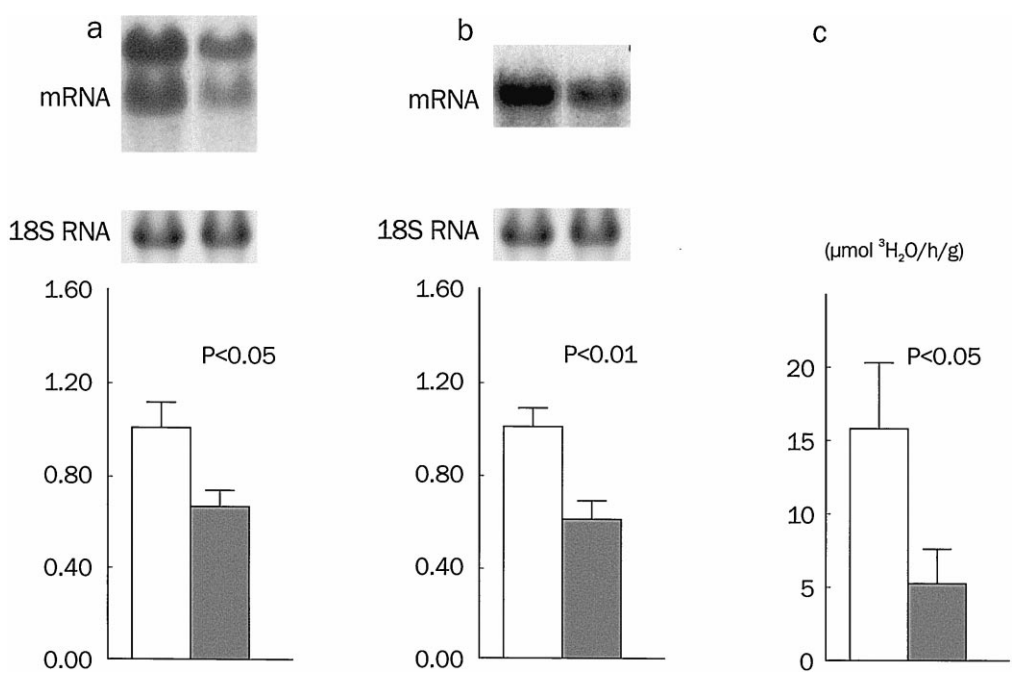

Fig. 4. The effect of clofibrate treatment on malic enzyme (a), fatty acid synthase (b) mRNA levels, and on in vivo brown adipose tissue lipogenesis (c). Malic enzyme and fatty acid synthase mRNA concentrations were measured by Northern blot hybridization (representative Northern blots are presented) and calculated as described in Section 2. The significance of differences in the mRNA concentrations expressed in arbitrary units and in the rate of incorporation of ^3H from ^3H_2O into lipids between clofibrate-treated (black) and control (white) rats is presented in the figure.

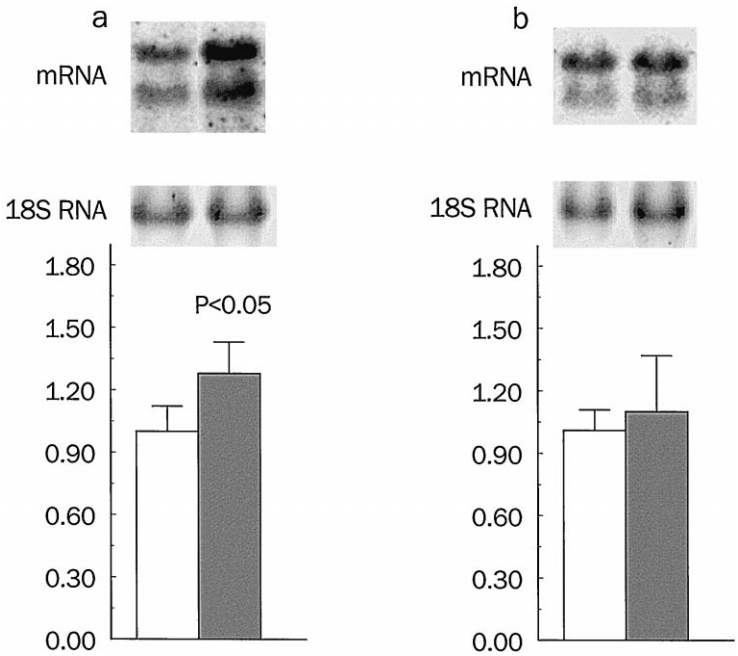

Fig. 5. The effect of clofibrate treatment on malic enzyme mRNA levels in kidney cortex (a) and brain (b). Malic enzyme mRNA concentrations expressed in arbitrary units were measured by Northern blot hybridization (representative Northern blots are presented) and calculated as described in Section 2. The significance of differences in the mRNA concentrations between clofibrate-treated (black) and control (white) rats is presented in the figure.

atomegaly (Zelewski and Swierczynski, 1983). Thus, to validate the effectiveness of clofibrate treatment under the present experimental conditions, we determined the liver weight and the relative abundance of malic enzyme mRNA. Administration of clofibrate to rats for 14 days led to a significant increase in liver weight (not shown) and to a several-fold increase in the level of liver malic enzyme mRNA (Fig. 2a), thus confirming the validity of our experimental model. Under the same conditions, the fatty acid synthase mRNA level (Fig. 2b) and the rate of fatty acid synthesis in the liver (Fig. 2c) were not enhanced significantly in clofibrate-treated rats as compared to control animals. To check whether clofibrate treatment affected the expression of the genes for malic enzyme and fatty acid synthase in white adipose tissue, we measured the relative abundance of the mRNA encoding these enzymes. The malic enzyme mRNA level was increased approximately 2-fold after clofibrate treatment (Fig. 3a), but the fatty acid synthase mRNA level (Fig. 3b) and the rate of lipogenesis (Fig. 3c) were unchanged by clofibrate treatment.

In contrast to liver and white adipose tissue, in brown adipose tissue, the levels of mRNA for the malic enzyme (Fig. 4a) and fatty acid synthase (Fig. 4b), as well as the rate of fatty acid synthesis (Fig. 4c), were significantly depressed in the clofibrate-treated animals.

To gain more information about the tissue specificity of the regulation of malic enzyme gene expression by fibrates, we measured the malic enzyme mRNA level in the kidney cortex, brain, heart and skeletal muscle of control and drug-treated rats. Administration of clofibrate for 14 days caused an increase in the malic enzyme mRNA level in the kidney cortex (Fig. 5a). No effect of clofibrate feeding on the malic enzyme mRNA level was observed in the brain (Fig. 5b), skeletal muscle and heart (not shown). The lack of response of malic enzyme mRNA to clofibrate treatment seen in the brain, heart and skeletal muscle, the increase in the liver, white adipose tissue and kidney cortex, and the decrease in brown adipose tissue indicate that malic enzyme gene expression is tissue-specific.

4. Discussion

For the last three decades, fibrates have been widely prescribed to treat human hyperlipidaemia. However, the primary mechanism by which these drugs lower the plasma lipid concentration is still unknown. The most controversial is the effect of fibrates on lipogenesis. The discovery of PPA receptor (Issemann and Green, 1990) has opened up new opportunities to understand how the fibrate hypolipidaemic drugs act at the molecular level. Clofibrate, a potent peroxisomal proliferator, increases the activity of liver malic enzyme—one of the lipogenic enzymes (Zelewski and Swierczynski, 1983). Hertz et al. (1996) reported that the thyromimetic effect of fibrates with respect to the malic enzyme results from transcriptional activation of the malic enzyme gene. Thus, one would expect that clofibrate would increase rather than decrease liver lipogenesis. Furthermore, the presence of peroxisome proliferator response element (PPRE) in the promoter region of the malic enzyme gene has been reported (Castelein

et al., 1994; Hertz et al., 1996; IJpenberg et al., 1997). The recent suggestion that fatty acid synthase gene expression is inhibited by peroxisome proliferators, even though no functional PPRE has been identified in the promoter region of fatty acid synthase gene (Schoonjans et al., 1996a,b; Staels et al., 1998), led us to analyse whether fatty acid synthase gene expression is subject to regulation by clofibrate.

The effect of clofibrate on hepatic lipogenesis has been extensively studied, but contradictory results have been reported, namely, inhibition (Maragoudakis, 1969; Adams et al., 1971; Lamb and Fallon, 1972; Laker and Mayes, 1979), stimulation (Duncan and Best, 1969; Giocoli et al., 1971; Capuzzi et al., 1974; Miyazawa et al., 1975; Laker and Mayes, 1979) and no effect (Mishkel and Webb, 1967). The different results from various laboratories may be due to diverse assay systems and different experimental conditions. Our results suggest that clofibrate treatment did not affect significantly ^{3}H incorporation into liver fatty acids (Fig. 2c). In the liver, there was no correlation between the rate of fatty acid synthesis and the level of malic enzyme mRNA. The liver malic enzyme mRNA level was increased approximately 6-fold after feeding of the drug (Fig. 2a), as could have been anticipated from previous work (Platt and Cockrill, 1966; Richert and Westerfeld, 1970; Zakim et al., 1970; Zelewski and Swierczynski, 1983, 1986, 1989). While clofibrate induced hepatic malic enzyme mRNA, it had no effect on fatty acid synthase mRNA. Similarly, Ren et al. (1997) showed that the peroxisome proliferator, gemfibrozil, did not significantly suppress liver levels of fatty acid synthase mRNA. Thus, one can assume that the regulation of liver fatty acid synthase gene expression does not involve PPA receptor and PPRE. The unchanged rate of lipogenesis in epididymal fat pad after clofibrate feeding (Fig. 3c) correlated well with the unchanged fatty acid synthase mRNA level, but not with the malic enzyme mRNA level (Fig. 3a). Taken together, these results indicate that clofibrate regulates malic enzyme gene expression both in the liver and in white adipose tissue (possibly via the activation of the PPA receptor signalling pathway), but is without effect on fatty acid synthase and consequently on de novo lipogenesis in the liver and in white adipose tissue. Thus, one can assume that the induction by clofibrate of mitochondrial and peroxisomal fatty acid β-oxidation (Schoonjans et al., 1996a,b), but not the decrease in fatty acid synthesis, is the cause of the lower availability of fatty acids for triacylglycerol synthesis in the liver. The present study confirms the previous suggestion that the hypolipidaemic effect of this drug is not linked directly to the inhibition of fatty acid biosynthesis in rat liver and white adipose tissue (Giocoli et al., 1971; Miyazawa et al., 1975; Laker and Mayes, 1979).

There were distinct differences between brown adipose tissue and liver as far as the action of clofibrate on malic enzyme and fatty acid synthase mRNAs is concerned. The data presented in this paper provide further evidence that total fatty acid biosynthesis, estimated by the incorporation of tritium from $^{3}H_{2}O$ into lipid, is substantially decreased in brown adipose tissue after clofibrate treatment. The parallel decrease in the levels of mRNA for fatty acid synthase and malic enzyme suggests that the lower brown adipose tissue lipogenesis is a consequence of the diminished transcription rate. However, the pharmacological implication of the effect of clofibrate on malic enzyme and fatty acid synthase gene expression and on lipogenesis in brown adipose tissue is not clear. Considering that brown adipose tissue lipogenesis contributes substantially to the overall fatty acid synthesis in the rat (Mercer and Williamson, 1988; Kochan and Swierczynski, 1992), one can assume that the hypolipidaemic effect of clofibrate is due in part to the inhibition of brown adipose tissue lipogenesis. This cannot be true for humans because the brown adipose tissue content is limited or absent in human adults in whom fibrates are effective as hypolipidaemic drugs.

A striking finding of the experiments presented in this paper is that clofibrate feeding had different effects on malic enzyme mRNA in different tissues. In liver, a large increase in the level of mRNA for malic enzyme was observed after clofibrate administration. The adipose tissue malic enzyme mRNA level was also increased by clofibrate treatment but to a lesser extent than that in the liver. Kidney malic enzyme mRNA was only weakly induced by clofibrate. In contrast to these tissues, the level of mRNA for malic enzyme in brain, skeletal muscle and heart remained unchanged, but in brown adipose tissue, the malic enzyme RNA level was significantly reduced after clofibrate treatment. It should be noted that Schoonjans et al. (1993) also observed a different effect of fenofibrate on the level of mRNA for long-chain acyl-CoA synthetase in different tissues. In this respect, fibrates resemble thyroid hormone (T_3), which also regulates lipogenic enzyme activity in the rat in a tissue-specific manner (Blennemann et al., 1992, 1995). It was also shown that, in brown adipose tissue, lipogenesis was inversely related to the thyroid hormone status (Blennemann et al., 1995). It is well-established that rat tissues express various forms of PPA receptor (Schoonjans et al., 1996a). For instance, the PPA receptor α is predominantly expressed in the liver, kidney, heart and brown adipose tissue (Schoonjans et al., 1996a). There is no correlation between PPA receptor α expression and the change in malic enzyme mRNA level caused by clofibrate (a PPA receptor α activator) treatment. The effect of clofibrate feeding on the level of mRNA for malic enzyme is not correlated with the level of PPA receptor δ and PPA receptor γ expression in the different tissues (Schoonjans et al., 1996a). The lack of effect of clofibrate on malic enzyme gene expression in skeletal muscle may be related to the concentration of the drug in this tissue. It has been shown that, in rats receiving clofibrate, the concentration of the drug is much higher in

the liver than in skeletal muscle (Baldwin et al., 1980). The same could be true for the brain and heart.

At present, it is difficult to explain the inhibitory effect of clofibrate on malic enzyme and fatty acid synthase gene expression, and consequently on lipogenesis in brown adipose tissue. We can only speculate that the inhibition of these genes in brown adipose tissue by clofibrate does not involve PPA receptor as a common mediator.

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References

Adams, L.L., Webb, W.W., Fallon, H.J., 1971. Inhibition of hepatic triglyceride formation by clofibrate. J. Clin. Invest. 50, 2339–2346.

Augustyn, J.M., Fritz, K.E., Daoud, A.S., Jarmolych, J., Lee, K.T., 1978. Biochemical effects of moderate diet and clofibrate on swine atherosclerosis. Arch. Pathol. Lab. Med. 102, 294–297.

Baldwin, J.R., Witiak, D.T., Feller, D.R., 1980. Disposition of clofibrate in the rat. Acute and chronic administration. Biochem. Pharmacol. 29, 3143–3154.

Blennemann, B., Moon, Y.K., Freake, H.C., 1992. Tissue-specific regulation of fatty acid synthesis by thyroid hormone. Endocrinology 130, 637–643.

Blennemann, B., Leahy, P., Kim, T.S., Freake, H.C., 1995. Tissue-specific regulation of lipogenic mRNAs by thyroid hormone. Mol. Cell Endocrinol. 110, 1–8.

Capuzzi, D.H., Lackman, R.D., Uberti, M.O., Reed, M.A., 1974. Stimulation of hepatic triglyceride synthesis and secretion by clofibrate. Biochem. Biophys. Res. Commun. 60, 1499–1508.

Castelein, H., Gulick, T., Declercq, P.E., Mannaerts, G.P., Moore, D.D., Baes, M.I., 1994. The peroxisome proliferator-activated receptor regulates malic enzyme gene expression. J. Biol. Chem. 269, 26754–26758.

Chomczynski, P., Sacchi, N., 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. Anal. Biochem. 162, 156–159.

Duncan, C.H., Best, M.M., 1969. Stimulation of hepatic synthesis of fatty acids by clofibrate and by a tetralin phenoxy isobutyric acid. Circulation 39–40 (III), 6, Suppl.

Giocoli, G., Tepperman, H.M., Tepperman, J., 1971. Effects of *p*-chlorophenoxyisobutyric acid (CPIB) on adipose tissue triglycerides synthesis, lipogenesis, and some related enzymes. Proc. Soc. Biol. Med. 138, 512–516.

Heller, F., Harvengt, C., 1983. Effects of clofibrate, bezafibrate, fenofibrate and probucol on plasma lipolytic enzymes in normolipidaemic subjects. Eur. J. Pharmacol. 25, 57–63.

Hertz, R., Nikodem, V., Ben-Ishai, A., Berman, I., Bar-Tana, J., 1996. Thyromimetic mode of action of peroxisome proliferators: activation of 'malic' enzyme gene transcription. Biochem. J. 319, 241–248.

IJpenberg, A., Jeannin, E., Wahli, W., Desvergne, B., 1997. Polarity and specific sequence requirements of peroxisome proliferator-activated receptor (PPAR)/retinoid X receptor heterodimer binding to DNA. J. Biol. Chem. 272, 20108–20117.

Issemann, I., Green, S., 1990. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature 347, 645–650.

Kochan, Z., Swierczynski, J., 1992. Development changes of lipogenic enzyme activities and lipogenesis in brown adipose tissue and liver of the rat. Comp. Biochem. Physiol. 101B, 283–288.

Kochan, Z., Bukato, G., Swierczynski, J., 1993. Inhibition of lipogenesis in rat brown adipose tissue by clofibrate. Biochem. Pharmacol. 46, 1501–1506.

Laker, M.E., Mayes, P.A., 1979. The immediate and long-term effects of clofibrate on the metabolism of the perfused rat liver. Biochem. Pharmacol. 28, 2813–2827.

Lamb, R.G., Fallon, H.J., 1972. Inhibition of monoacylglycerolphosphate formation by chlorophenoxyisobutyrate and β-benzalbutyrate. J. Biol. Chem. 247, 1281–1287.

Lewis, B., 1983. Disorders of lipid transport. In: Weatheral, D.J., Ledingham, J.G.G., Warrel, D.A. (Eds.), Oxford Textbook of Medicine. Oxford Univ. Press, Oxford, pp. 985–970.

Magnuson, M.A., Morioka, H., Tecce, M.F., Nikodem, V.M., 1986. Coding nucleotide sequence of rat liver malic enzyme mRNA. J. Biol. Chem. 261, 1183–1186.

Maragoudakis, M.E., 1969. Inhibition of hepatic acetyl-CoA carboxylase by hypolipidaemic agents. J. Biol. Chem. 244, 5005–5013.

Martini, S., Valerio, G., Fellin, R., Baggio, G., Gasparotto, A., Baiocchi, M.R., Baldo, G., Crepaldi, G., 1982. Bezafibrate and clofibrate: effects on plasma lipids, lipoproteins and apoproteins compared in familial hypercholesterolemia. Curr. Ther. Res. 31, 354–361.

Maxwell, R.E., Nawrocki, J.W., Uhlendorf, P.D., 1983. Some comparative effects of gemfibrozil, clofibrate, bezafibrate, cholestyramine and compactin on sterol metabolism in rats. Atherosclerosis 48, 195–203.

Mercer, S.W., Williamson, D.H., 1988. The influence of starvation and natural refeeding on the rate of triacylglycerol/fatty acid substrate cycling in brown adipose tissue and different white adipose site of the rat in vivo. The role of insulin and the sympathetic nervous system. Biosci. Rep. 8, 147–153.

Mishkel, M.A., Webb, W.F., 1967. The mechanism underlying the hypolipidaemic effects of Atromid S, nicotinic acid and benzmolecene-I. Biochem. Pharmacol. 16, 897–905.

Miyazawa, S., Sakurai, T., Imura, M., Hashimoto, T., 1975. Effects of ethyl *p*-chlorophenoxyisobutyrate on carbohydrate and fatty acid metabolism in rat liver. J. Biochem. 78, 1171–1176.

O'Brien, M., Patel, S.T., Mukhopadhyay, A., Newman, H.A.I., Feller, D.R., Kokrady, S.S., Witiak, D.T., Lanese, R.R., Rice, J.C., 1981. Hypolipidaemic effects of clofibrate and selected chroman analogs in fasted rats: I. Chow-fed animals. Lipids 16, 903–911.

Platt, D.S., Cockrill, B.L., 1966. Changes in the liver concentrations of the nicotinamide adenine dinucleotide coenzymes and in the activities of oxidoreductase enzymes following treatment of the rat with ethyl chlorophenoxyisobutyrate (Atromid S). Biochem. Pharmacol. 15, 927–935.

Ren, B., Thelen, A.P., Peters, J.M., Gonzales, F.J., Jump, D.B., 1997. Polyunsaturated fatty acid suppression of hepatic fatty acid synthase and S14 gene expression does not require peroxisome proliferator-activated receptor α. J. Biol. Chem. 272, 26827–26832.

Richert, D.A., Westerfeld, W.W., 1970. Growth and liver glycerophosphate dehydrogenase responses to thyroxine and *p*-chlorophenoxyisobutyrate. Endocrinology 87, 1274–1281.

Schoonjans, K., Staels, B., Grimaldi, P., Auwerx, J., 1993. Acyl-CoA synthetase mRNA expression is controlled by fibric-acid derivatives, feeding and liver proliferation. Eur. J. Biochem. 216, 615–622.

Schoonjans, K., Staels, B., Auwerx, J., 1996a. The peroxisome proliferator-activated receptors (PPARs) and their effects on lipid metabolism and adipocyte differentiation. Biochim. Biophys. Acta 1302, 93–109.

Schoonjans, K., Staels, B., Auwerx, J., 1996b. Role of the peroxisome proliferator-activated receptors (PPARs) in mediating the effects of fibrates and fatty acids on gene expression. J. Lipid Res. 37, 907–925.

Shirai, K., Ishikawa, Y., Nishide, T., Sasaki, N., Murano, S., Matsuoka, N., Saito, Y., Yoshida, S., 1983. Effects of clinofibrate on lipid metabolism of aorta in atherosclerotic rats. Artery 12, 145–155.

Staels, B., Dallongeville, J., Auwerx, J., Schoonjans, K., Leitersdorf, E., Fruchart, J.-C., 1998. Mechanism of action of fibrates on lipid and lipoprotein metabolism. Circulation 98, 2088–2093.

Stansbie, D., Brownsey, R.W., Crettaz, M., Denton, R.M., 1976. Acute effect in vivo of anti-insulin serum on rates of fatty acid synthesis activities of acetyl-Coenzyme A carboxylase and pyruvate dehydrogenase in liver and epididymal adipose of fed rats. Biochem. J. 160, 413–416.

Trayhurn, P., 1980. Fatty acid synthesis in brown adipose tissue in relation to whole body synthesis in the cold-acclimated golden hamster (*Mesocricetus auratus*). Biochim. Biophys. Acta 620, 10–17.

Zakim, D., Paradini, R.S., Herman, R.H., 1970. Effect of clofibrate (ethyl-chlorophenoxyisobutyrate) feeding on glycolytic and lipogenic enzymes and hepatic glycogen synthesis in the rat. Biochem. Pharmacol. 19, 305–310.

Zelewski, M., Swierczynski, J., 1983. The effect of clofibrate feeding on the NADP-linked dehydrogenases activity in the rat tissue. Biochim. Biophys. Acta 785, 152–157.

Zelewski, M., Swierczynski, J., 1986. The influence of prolonged clofibrate treatment on plasma drug concentration and liver NADP-linked enzymes activity. Gen. Pharmacol. 17, 117–119.

Zelewski, M., Swierczynski, J., 1989. Clofibrate feeding increases cytoplasmic but not mitochondrial malic enzyme activity in rat kidney cortex. Cell Biochem. Funct. 7, 97–103.