Lecithin:cholesterol acyltransferase gene expression is regulated in a tissue-selective manner by fibrates

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Abstract Plasma lipoprotein metabolism is influenced by several factors that may act by regulating the expression of proteins involved in lipoprotein metabolism, such as lecithin:cholesterol acyltransferase (LCAT). We determined the influence of several hormones and hypolipidemic drugs on hepatic LCAT gene expression and plasma LCAT activity. Liver LCAT mRNA levels were resistant to regulation by the hormones ethinylestradiol, L-thyroxine, hydrocortisone, or by the hypolipidemic drugs probucol, simvastatin, and nicotinic acid. In contrast, hepatic LCAT mRNA levels decreased to 67%, 64%, and 46% of the control levels after treatment with the fibric acid derivatives clofibrate, gemfibrozil, and fenofibrate, respectively. Fenofibrate lowered liver LCAT mRNA levels in a dose-dependent manner, which was paralleled by a decrease in plasma LCAT activity to 54% of the controls at a dose of 0.5% (w/w) in rat chow. The decrease in liver LCAT mRNA levels was maximal after 1 day, whereas the fall in plasma LCAT activity trailed by 2 days. Cessation of treatment with fenofibrate restored liver LCAT mRNA levels to control levels within 1 week. The transcription rate of the LCAT gene decreased by 25% in nuclei isolated from fenofibrate-treated rat liver, thereby indicating that hepatic LCAT gene expression is, at least partly, regulated at a transcriptional level. In contrast to the liver, brain and testis LCAT mRNA levels remained constant after treatment with fenofibrate, indicating that fibrates regulate LCAT gene expression in a tissue-selective manner.—**Staels, B., A. van Tol, G. Skretting, and J. Auwerx.** Lecithin:cholesterol acyltransferase gene expression is regulated in a tissue-selective manner by fibrates. *J. Lipid Res.* 1992. **33:** 727–735.

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The enzyme lecithin:cholesterol acyltransferase (LCAT, EC 2.3.1.43) is responsible for the formation of cholesteryl esters in plasma, both in humans and laboratory animals (1). Using lecithin as an acyl donor and with apoA-I, and possibly apoA-IV, apoC-I and apoE, as co-factors (2–7), LCAT catalyzes the esterification of free cholesterol contained in HDL or coming from peripheral tissues or cells. In humans and animals that have plasma cholesteryl ester transfer protein (CETP) activity, part of these cholesteryl esters are transferred to lipoproteins of low density and subsequently removed by the liver for degradation (8). In contrast, in animals lacking plasma CETP activity, such as the rat, the cholesteryl esters remain in HDL particles and may be cleared from the circulation by the liver via several mechanisms (9). Consequently, LCAT may play an important role in the process of reverse cholesterol transport. This is illustrated by the observed cholesterol accumulation in peripheral tissues of patients with familial LCAT deficiency (10).

Recently, human, rat, and mouse cDNA and genomic clones have been isolated and the LCAT gene has been shown to be expressed in liver, testis, and brain (11–16). Relatively little, however, is known about the regulation of the expression of the LCAT gene. The aim of these studies was, therefore, to investigate the influence on hepatic LCAT gene expression of several different factors known to affect plasma lipoprotein metabolism, such as hormones and hypolipidemic drugs. Changes observed in LCAT gene expression were consequently correlated with plasma LCAT activity.

MATERIALS AND METHODS

Animals and treatments

Hormones. Female, ovariectomized rats (n = 4) were injected subcutaneously for 7 days with ethinylestradiol

Abbreviations: LCAT, lecithin:cholesterol acyltransferase; apo, apolipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; VLDL, very low density lipoprotein; LPL, lipoprotein lipase; CETP, cholesteryl ester transfer protein.

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(2000 μg/day). Control ovariectomized animals (n = 4) received vehicle only.

Hyperthyroidism was induced by daily injections of L-thyroxine (1 μg/g body weight) for 20 days. Hypothyroidism was induced by continuous administration for 30 days of 0.1% (w/w) n-propylthiouracil dissolved in the drinking water. Hydrocortisone was injected daily for 20 days at a dose of 100 μg/g body weight. Each treatment group consisted of four adult male rats and was matched with a control group (n = 4) receiving vehicle only.

Hypolipidemic drugs. Male Wistar rats (90 days old) were fed standard rat chow supplemented with one of the following drugs for 14 days: 5% (w/w) nicotinic acid (Bios, Leuven, Belgium); 1% (w/w) probucol (Merrell Dow Pharmaceuticals Inc., Cincinnati, OH); 0.25% (w/w) simvastatin (Merck Sharp & Dohme Research Laboratories, Rahway, NJ); 0.3% (w/w) clofibrate (Sigma Chemical Company, St. Louis, MO); and 0.5% gemfibrozil (Warner-Lambert, Ann Arbor, MI). Fenofibrate (Laboratories Fournier, Daix, France) mixed at the indicated concentrations (w/w) with standard rat chow was administered to 90-day-old male Wistar rats for the indicated periods of time.

At the end of the experiments animals were fasted overnight and killed by exsanguination after ether anesthesia. Blood was collected in EDTA-containing tubes and plasma was used for determination of plasma LCAT activity. Liver, testis, and brains were removed immediately, rinsed with 0.9% NaCl, and frozen in liquid nitrogen.

Measurement of plasma LCAT activity

Plasma levels of LCAT activity were measured using excess exogenous substrate as previously described (17). The activities are expressed in relative units (% of activity in control animals). The intraassay coefficient of variation was 5.1%.

RNA analysis

RNA was prepared by the guanidine isothiocyanate/cesium chloride procedure from livers and testes of individual animals (18). Northern and dot-blot hybridizations of total cellular RNA were performed as described previously (19). LCAT mRNA levels were measured using a human LCAT cDNA probe (12). As control probes a rat C/EBP (20) and a chicken β-actin cDNA clone (21) were used. All probes were labeled by random primed labeling (Boehringer Mannheim). Filters were hybridized to 1.5 × 10^6 cpm/ml of each probe as described (19). They were washed in 500 ml of 0.5 × SSC and 0.1% SDS for 10 min at room temperature and twice for 30 min at 65°C and subsequently exposed to X-ray film (X-OMAT-AR, Kodak). Autoradiograms were analyzed by quantitative scanning densitometry (LKB 2202 Ultrascan Laser Densitometer) as described (19).

Isolation of nuclei and transcriptional rate assay

Nuclei were prepared fresh from livers of untreated rats and from livers of rats treated for 14 days with fenofibrate (0.5%, w/w, in rat chow) exactly as described by Gorski, Carneiro, and Schibler (22). Transcription run-on assays were performed as described by Nevins (23). Equivalent amounts of labeled nuclear RNA were hybridized (36 h at 42°C) to 5 μg of purified cDNAs immobilized on Hybond-C Extra filters (Amersham). The following cDNA probes were spotted: a human LCAT (12) and a rat C/EBP (20) cDNA probe. As a control, 5 μg of the vector DNA was also applied to the filter. After hybridization, filters were washed at room temperature for 10 min in 0.5 × SSC and 0.1% SDS and twice for 30 min at 65°C and subsequently exposed to X-ray film (X-OMAT-AR, Kodak). Autoradiograms were analyzed by quantitative scanning densitometry (LKB 2202 Ultrascan Laser Densitometer).

Statistical methods

Analysis of variance (ANOVA) was used to evaluate the results of the dose-response and time-course experiments. Values observed between different groups were compared by contrast statements. A two-tailed unpaired Student's *t*-test was used to evaluate differences between means in all other experiments.

RESULTS

Regulation of hepatic LCAT gene expression by hormones and hypolipidemic drugs

To investigate the influence of thyroid hormones on hepatic LCAT gene expression, rats were treated with the thyroid hormone agonist L-thyroxine, or with the thyroid hormone antagonist n-propylthiouracil. Neither of these treatments caused significant changes in hepatic LCAT mRNA levels (**Table 1**). In addition, no major changes were observed after administration of pharmacological doses of the corticosteroid hydrocortisone or the estrogen ethinylestradiol (Table 1). Similarly, no significant changes were observed in liver LCAT mRNA levels when rats were treated with the hypolipidemic drugs probucol, simvastatin, or nicotinic acid (Table 1).

After clofibrate treatment, liver LCAT mRNA levels dropped to two-thirds of the levels in control rats (Table 1). In order to investigate whether the changes in liver LCAT mRNA levels observed after treatment with clofibrate were general effects of fibric acid derivatives, rats were treated for 14 days with two other fibrates, fenofibrate and gemfibrozil, given at comparable doses. All three fibrates provoked a lowering of liver LCAT mRNA levels (**Fig. 1**). Gemfibrozil and fenofibrate treatment lowered LCAT mRNA levels to less than two-thirds of the levels in control livers (Fig. 1). Hybridization of the same blots with a probe for the liver-specific transcription fac-

TABLE 1. Influence of hormones and hypolipidemic drugs on hepatic LCAT mRNA levels in rats

Treatment	Treated	Control
	relative activity units (% of control)	
Thyroid hormones		
L-Thyroxine	113 ± 2	100 ± 15
n-Propylthiouracil	95 ± 22	100 ± 12
Corticosteroids		
Hydrocortisone	101 ± 11	100 ± 9
Estrogens		
Ethinylestradiol	89 ± 13	100 ± 7
Hypolipidemic drugs		
Probucol	90 ± 11	100 ± 27
Simvastatin	106 ± 12	100 ± 30
Nicotinic acid	106 ± 19	100 ± 22
Clofibrate	67 ± 7*	100 ± 12

Animals, treatments, and hepatic LCAT mRNA determinations were exactly as described in Materials and Methods. Values represent the mean ± SD.

*Statistically significant differences from controls (*t*-test, $P < 0.005$).

tor, C/EBP, showed no effects of the different fibrates on liver C/EBP mRNA levels (Fig. 1), thereby indicating that fibrates act specifically on LCAT gene expression without having general toxic effects.

Influence of different doses of fenofibrate

Since treatment with fenofibrate caused the largest decrease in liver LCAT mRNA levels (Fig. 1), the effects of this drug were investigated in more detail. Male rats were treated for 14 days with different doses of fenofibrate mixed in rat chow. Administration of fenofibrate caused a dose-dependent decrease in liver LCAT mRNA levels, which became significantly different from the controls at the intermediate dose of 0.05% (**Fig. 2A**). In addition it can be seen that the decrease after administration of a dose of 0.5% is similar to the lowering observed in the first experiment (compare Fig. 1 and Fig. 2A). In contrast, no significant changes were observed in liver C/EBP mRNA levels at any dose of fenofibrate (Fig. 2A).

The changes in liver LCAT mRNA levels as measured by dot-blot hybridization were confirmed by Northern blot hybridization (**Fig. 3**). Hybridization of the same Northern blot with a probe for C/EBP demonstrated no change in liver C/EBP mRNA levels (Fig. 3). In addition it can be seen that both probes specifically hybridize to a single band of the corresponding size under the conditions used.

Parallel to the changes in hepatic LCAT mRNA levels, plasma LCAT activity decreased in a dose-dependent fashion to nearly 50% of the control values at the highest dose tested (Fig. 2B).

Time-dependent regulation of liver LCAT mRNA and plasma LCAT activity by fenofibrate

To investigate whether the decrease in hepatic LCAT gene expression preceded the changes in plasma LCAT activity, a time-course experiment was performed. Treatment of rats with fenofibrate mixed at 0.5% (w/w) in rat chow maximally decreased hepatic LCAT mRNA levels after 1 day of treatment, whereas C/EBP mRNA levels did not change significantly (**Fig. 4A**). Plasma LCAT activity trailed the decrease in liver LCAT mRNA levels, since a nearly maximal decrease was observed only from day 3 on (Fig. 4B).

Effects of fenofibrate on liver LCAT mRNA levels are reversible

To determine whether hepatic LCAT mRNA levels could be restored to the levels in untreated control rats, treatment with fenofibrate (0.5% w/w) was stopped after 14 days and liver LCAT mRNA levels were determined on days 1, 3, 7, 14, and 28 after cessation of fenofibrate treatment. Fenofibrate decreased liver LCAT mRNA levels to

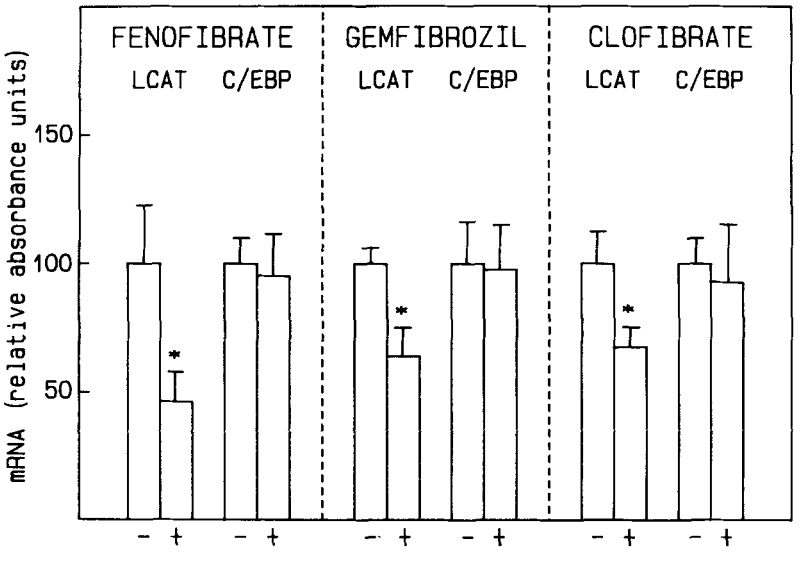

Fig. 1. Influence of different fibrates on hepatic LCAT and C/EBP mRNA levels. Adult male Wistar rats (n = 4) were treated (+) or not (−) with fenofibrate (0.5%, w/w, in rat chow), gemfibrozil (0.3%, w/w, in rat chow) or clofibrate (0.3%, w/w, in rat chow) for 14 days. Liver LCAT and C/EBP mRNA levels were measured as described in Materials and Methods. Values represent the mean ± SD. Statistically significant differences from controls (*t*-test, $P < 0.05$) are indicated by an asterisk.

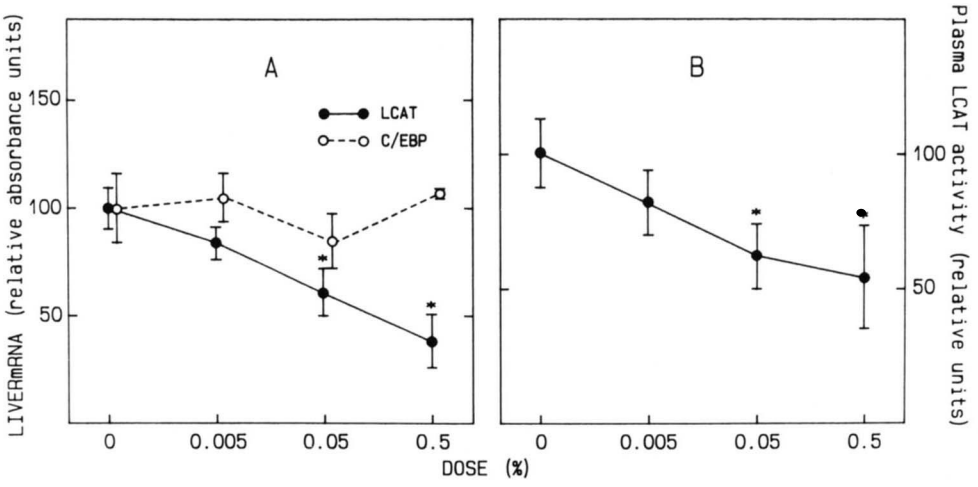

Fig. 2. Influence of treatment with different doses of fenofibrate on liver LCAT mRNA levels (panel A) and plasma LCAT activity levels (panel B). Adult male rats were treated for 14 days with the indicated doses of fenofibrate (w/w, mixed in rat chow). Plasma LCAT activity and liver LCAT and C/EBP mRNA levels were measured and expressed as described in Materials and Methods. Statistically (ANOVA; $P < 0.05$) significant differences from the untreated control animals are indicated by an asterisk. Each value represents the mean ± SD of three animals.

less than 50% of the controls (**Fig. 5**), thereby confirming the previous observations (Figs. 1, 2, and 4). Hepatic LCAT mRNA levels increased 3 days after cessation of fenofibrate administration and reached levels comparable to untreated controls 7 days after cessation of treatment (Fig. 5). Liver C/EBP mRNA levels remained unchanged in all treatment groups (not shown).

Influence of fenofibrate on hepatic LCAT gene transcription rates

To determine whether the decrease in liver LCAT mRNA levels was caused by a decreased transcription of the LCAT gene, nuclear run-on experiments were performed on nuclei prepared from livers of untreated control rats and rats treated for 14 days with fenofibrate (0.5%, w/w). The transcription of the LCAT gene decreased by approximately 25% after treatment with fenofibrate (**Fig. 6**). In contrast, C/EBP gene transcription did not change markedly, and was even slightly higher in livers from fenofibrate-treated animals (Fig. 6).

Influence of fenofibrate on extrahepatic LCAT gene expression

As LCAT has been reported to be expressed not only in the liver but also in testis and brain (11), the time- and dose-dependent influence of fenofibrate on testis LCAT mRNA levels was investigated. Although testis LCAT mRNA levels showed a tendency to decrease after the highest dose tested, given for 14 days, none of the effects were statistically significant (**Fig. 7A and B**). In contrast, testis β-actin mRNA levels tended to increase slightly after 14 days treatment with fenofibrate mixed in rat chow at 0.5%, but again these effects were not statistically significant (Fig. 7A and B).

Similarly to the testis, no significant changes in brain LCAT mRNA levels were observed after treatment with fenofibrate (data not shown).

DISCUSSION

The results from these studies show that hepatic LCAT gene expression is down-regulated in rat liver by various fibrates. To our knowledge this is the first report on LCAT regulation at the level of gene expression. The decrease in liver LCAT mRNA levels was found to be dose-dependent

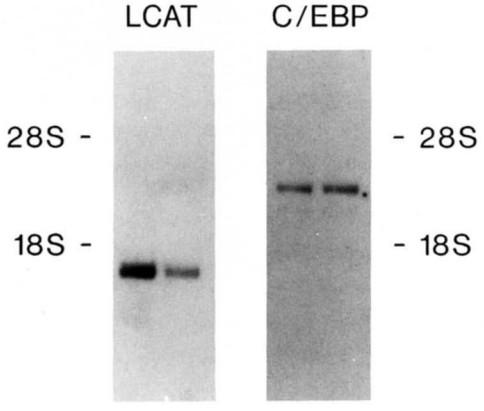

Fig. 3. Northern blot analysis of the influence of fenofibrate on liver LCAT and C/EBP mRNA levels. RNA was prepared from livers of rats treated for 14 days with 0.5% fenofibrate (+) or not (−). Agarose gel electrophoresis of representative RNA samples and hybridizations was performed as described (19). The localization of the 18S and 28S rRNAs are indicated on the autoradiogram.

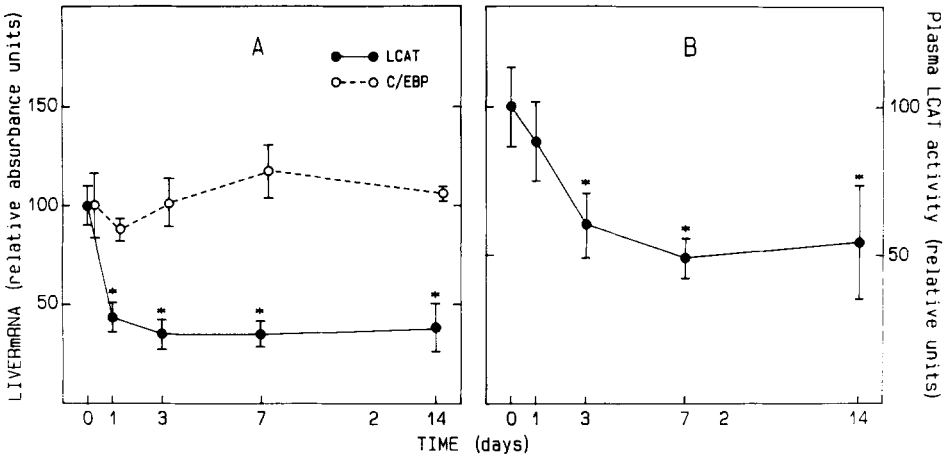

Fig. 4. Influence of duration of treatment with fenofibrate on liver mRNA levels (panel A) and plasma activity (panel B) of LCAT. Adult male rats were treated during the indicated number of days with fenofibrate (0.5%, w/w, mixed in rat chow). Plasma LCAT activity and liver LCAT and C/EBP mRNA levels were measured and expressed as described in Materials and Methods. Statistically (ANOVA; $P < 0.05$) significant differences from day 0 are indicated by an asterisk. Each value represents the mean ± SD of three animals.

after treatment with fenofibrate and was accompanied by a lowered plasma LCAT activity level. Since the drop in plasma LCAT activity trailed the decreased liver LCAT mRNA levels, it is suggested that the decreased LCAT gene expression is responsible for the fall in plasma LCAT activity. This effect of fenofibrate on LCAT activity may contribute to the hypocholesterolemic action of the drug (24–29). Indeed, Dashti and Ontko (26) showed that treatment with clofibrate reduces plasma HDL cholesterol concentrations in rats. In addition, these authors showed that the oleate content of plasma cholesteryl esters increased, whereas the cholesteryl linoleate content decreased after clofibrate (26). As LCAT hydrolyzes the fatty acyl group in the *sn*-2 position of phosphatidylcholine and as LCAT exhibits a significant preference for linoleic acid over oleic acid in this position (30), the changes in fatty acid composition after clofibrate may well reflect a decrease in plasma LCAT activity. In humans treated with different fibrates, similar effects on the fatty acid composition of plasma cholesteryl esters have been described (31–35). In contrast, the effects of fibrates on plasma LCAT activity in humans are less clear: treatment with clofibrate provoked a decrease in the molar esterification rate of cholesterol in plasma of hypercholesterolemic (36) or hypertriglyceridemic (37) subjects, whereas other reports showed no change or an increase in plasma LCAT activity (38–42). In these studies, however, fractional and/or molar cholesterol esterification rates, which are dependent not only on plasma LCAT levels but also on the concentration and composition of plasma lipoproteins, were measured. In contrast, the LCAT assay in this study was performed using excess exogenous substrate (17). This assay is independent of endogenous plasma lipoproteins, and therefore reflects plasma LCAT mass more accurately. Nevertheless, it cannot be excluded that the differences observed between humans and rats are the result of differences in hepatic metabolism after administration of these drugs. Indeed, fibrates are potent hepatic peroxisomal proliferators in rodents. This induction results in an extreme hepatomegaly and may ultimately

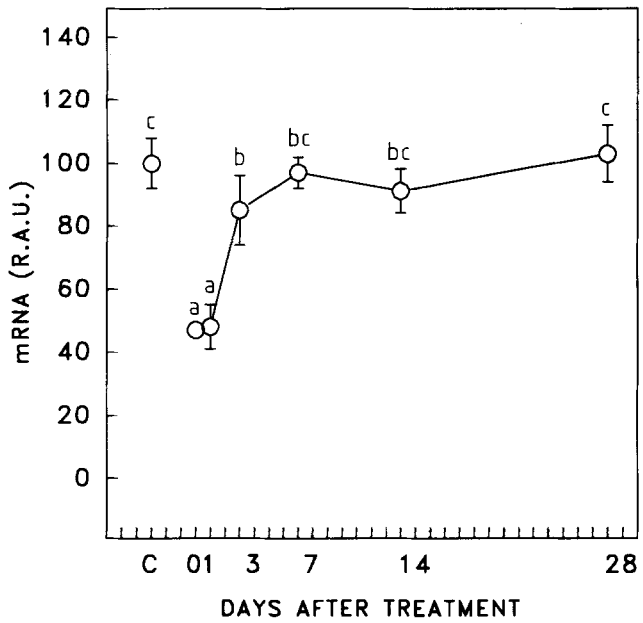

Fig. 5. Influence of cessation of treatment with fenofibrate on liver LCAT mRNA levels. Adult male rats were treated for 14 days with fenofibrate (0.5%, w/w, mixed in rat chow). Administration of fenofibrate was stopped on day 0. Liver LCAT mRNA levels were measured as described in Materials and Methods in livers of untreated control rats (C) and in livers of rats 0, 1, 3, 7, 14, and 28 days after cessation of fenofibrate treatment. Each group consisted of three animals. Statistically (ANOVA, $P < 0.05$) significant differences are observed between values followed by different letters.

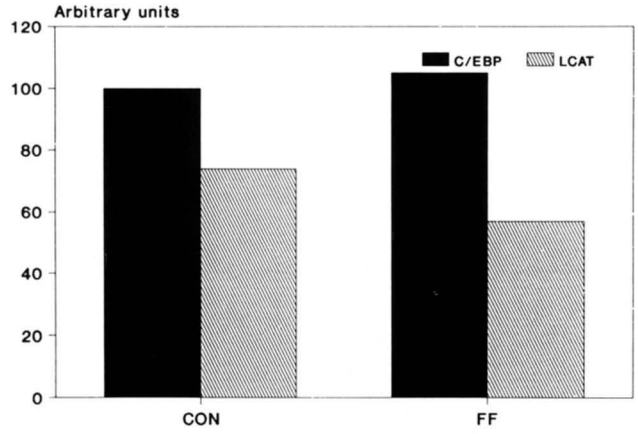

Fig. 6. Nuclear run-on transcription rate assay for LCAT and C/EBP. Relative transcription rates were determined in nuclei from livers of untreated control rats (CON) and rats treated with fenofibrate (FF; 0.5%, w/w) for 14 days. Nuclei were isolated and nuclear run-on assays were performed as described in Materials and Methods. Values, determined by laser densitometric scanning of autoradiograms of filters, are expressed relative to the transcription rate of the C/EBP gene in control nuclei and represent the mean of two independent run-on assays.

lead to the development of hepatocarcinoma in these animals (43–48). However, the decrease in liver LCAT mRNA levels is already evident after only 1 day of treatment. Therefore, it seems unlikely that the changes in hepatic LCAT gene expression are completely mediated by the effects of fibrates on peroxisomal proliferation. Furthermore, it is likely that fibrates exert their effects on peroxisomal proliferation as well as on lipoprotein metabolism through a common mediator. Two alternative mechanisms could be invoked. First, a transcription factor, such as the recently described peroxisomal proliferator-activated receptor (PPAR), a receptor belonging to the steroid hormone receptor supergene family, which is activated upon binding of fibrates in a way similar to the ligand-induced activation of transcription factors belonging to this supergene family, may mediate transcriptional effects (49). Alternatively, fibrates might alter the activity of other as yet unknown transcription factors by displacement of cofactors, such as fatty acids, from the active transcription factor (50).

The down-regulation of liver LCAT mRNA levels after fenofibrate is most likely the result of both transcriptional and post-transcriptional regulation of LCAT gene expression by fibrates. Indeed, the transcription rate of the LCAT gene is lowered in hepatic nuclei isolated from fenofibrate-treated rats. It is, however, unlikely that this relatively small decrease accounts completely for the large decrease in liver LCAT mRNA levels. Our results suggest, therefore, that fenofibrate, in addition to its effects on LCAT gene transcription, influences hepatic LCAT gene expression at a post-transcriptional level.

In rodents, LCAT has been reported to be produced not only in the liver but also in testis and brain (11). In contrast to the liver, testis and brain LCAT mRNA levels were not affected by fenofibrate, indicating that fibrates act primarily on the liver but not on other tissues. A similar tissue-selective regulation of apoA-IV gene expression has been observed after treatment with clofibrate. Indeed, liver apoA-IV mRNA levels decreased more than 10-fold, whereas intestinal apoA-IV mRNA levels remained constant after clofibrate (51).

In contrast to the effects of fibric acid derivatives, hepatic LCAT gene expression appears to be relatively resistant to challenges by other hypolipidemic drugs, hormones, or diet. Indeed, no changes in liver LCAT mRNA levels were observed after administration of the hypolipi-

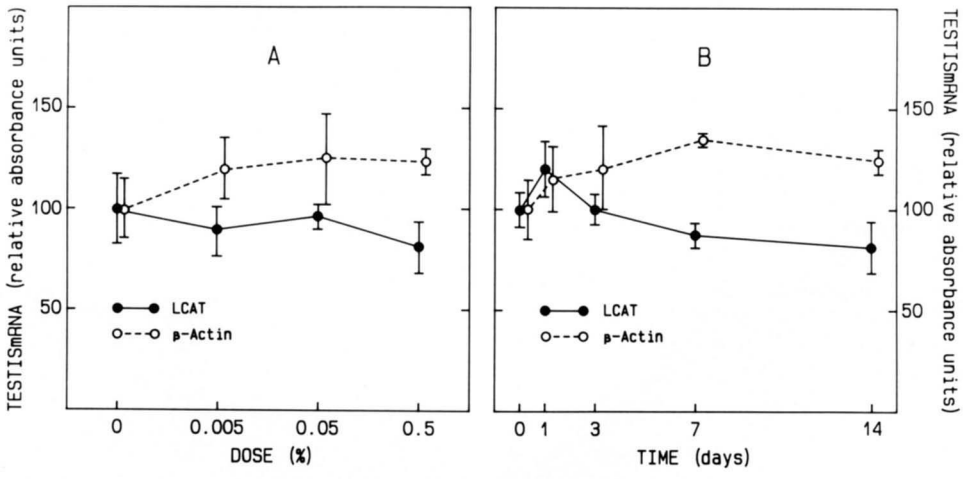

Fig. 7. Dose-dependent (panel A) and time-dependent (panel B) influence of fenofibrate on testis LCAT mRNA levels in rats. Animals and treatments were as described in Figs. 2 and 4. Testis RNA was prepared and LCAT and β-actin mRNA levels were measured as described in Materials and Methods. Values represent the mean ± SD. No statistically (by analysis of variance; $P < 0.05$) significant differences between treated animals and controls were observed.

demic drugs probucol, nicotinic acid, and the HMG-CoA reductase inhibitor, simvastatin. These results agree with previous observations in which mice showed no major changes in liver LCAT gene expression when the hypolipidemic drugs cholestyramine and mevinolin were given simultaneously (11). In addition, administration of diets containing high amounts of cocoa butter–cholesterol–cholic acid did not influence liver LCAT mRNA levels (11).

Administration of different steroid and thyroid hormones at pharmacological doses did not influence hepatic LCAT gene expression. However, rat plasma LCAT activity levels, as well as hepatic LCAT secretion, are reported to be decreased in rats made hypothyroid after administration of n-propylthiouracil (52), whereas LCAT activity is increased in L-thyroxine-treated rats (53). In view of the absence of effects of thyroid hormones on LCAT mRNA levels, it appears that LCAT production may be regulated at a post-transcriptional level by these hormones. In addition, changes in plasma lipoprotein composition due to the hormonal treatment may influence plasma LCAT activity in these studies (53). Finally, it has been shown that changes in plasma LCAT activity may result from changes in LCAT clearance (52). Since plasma LCAT has been shown to be heavily glycosylated, it is possible that the catabolism of plasma LCAT is controlled by desialation reactions in the plasma compartment (54).

In conclusion, the results from these studies demonstrate that LCAT gene expression is regulated by fibrates in rat liver. The effects of fibrates on hepatic LCAT mRNA levels in rats, described in the present paper, do not necessarily occur to the same extent in other mammals and may not always result in decreased levels of plasma LCAT. However, our data clearly show that LCAT mRNA levels in liver can be regulated by fibrates. The absence of LCAT mRNA levels in brain and testis shows that this regulation is tissue-specific.

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