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Triglyceridemia and peroxisome proliferator-activated receptor-α expression are not connected in fenofibrate-treated pregnant rats

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Abstract

To investigate the response to fenofibrate in pregnant rats, 0 mg, 100 mg or 200 mg of fenofibrate per kilogram body weight oral doses were given twice a day from day 16 of gestation and studied at day 20. Virgin rats were studied in parallel. Whereas in pregnant rats plasma triglycerides significantly increased, in virgin rats, fenofibrate decreased plasma triglycerides which accumulated in liver. Fenofibrate faithfully modulated the hepatic expression of PPARα responsive genes. Fenofibrate increased mRNA contents corresponding to both acyl-CoA oxidase, carnitine palmitoyltransferase (CPT), and peroxisome proliferator-activated receptor alpha (PPAR), and lowered mRNA amounts of apolipoproteins B and C-III, both in virgin and pregnant rats. However, genes related to hepatic lipogenesis, such as PPARγ and stearoyl-CoA desaturase (SCD), showed an augmented expression by fenofibrate in virgin rats, but not in pregnant animals. We propose that the opposite effects of fenofibrate treatment in virgin and pregnant rats are a consequence of the enhanced capability for VLDL-triglyceride production in the latter, further promoted by the elevated amount of free fatty acids (FFA), which reach the liver in treated pregnant rats and were not sufficiently oxidized and/or stored, and therefore would have to be canalized as triglycerides to the plasma. Thus, the present study shows how fenofibrate, in spite of efficiently exerting its expected molecular effects in the liver (i.e., to induce fatty acid and lipoprotein catabolism, and to reduce TG-rich lipoprotein secretion), was unable to reverse the typical hypertriglyceridaemia of gestation. (Mol Cell Biochem **273:** 97–107, 2005)

Key words: fibrates, mRNA levels, pregnancy, PPARα, rat, triglycerides

Abbreviations: PPAR, peroxisome proliferator-activated receptor; FFA, free fatty acids; ACO, peroxisomal acyl-CoA oxidase; CPT, carnitine palmitoyltransferase; SREBP, sterol regulatory element binding protein; FAS, fatty acid synthase; ACC, acetyl-CoA carboxylase; SCD, stearoyl-CoA desaturase; FAT/CD36, fatty acid translocase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RT-PCR, reverse transcriptase polymerase chain reaction; A.U., arbitrary units

Introduction

Fibrates have been effectively used to reduce plasma triglyceride levels under conditions of hypertriglyceridemia [1]. Endogenous hypertriglyceridemia is associated with insulin resistance and impairment in glucose metabolism [2, 3]; and although fibrates have been shown to have a triglyceride-lowering effect with no significant influence on

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insulin sensitivity [4], there are also reports showing that they reduce insulin resistance conditions in rats [5] and mice [6]. The molecular bases for the action of fibrates on lipid metabolism have recently been elucidated ([7] for a review; [8]) and involve the activation of transcriptional factors, known as peroxisome proliferator-activated receptors (PPAR), principally the PPAR-α form expressed in liver. Throughout this mechanism, fibrates have been reported to decrease apolipoprotein C-III, and increase both acyl-coenzyme A oxidase [9] and fatty acid translocase (FAT/CD36) [10] in rodent liver.

Although these mechanisms are clearly established, there are still aspects of the effects of fibrates on lipid metabolism that remain to be understood, including their effect on liver lipid concentration, triglycerides and cholesterol, which have been found to be either unchanged [11], decreased [12] or even enhanced [13], although liver weight was consistently enhanced and, therefore, total lipid content may result augmented. Some fibrates have also been shown to have opposite effects on plasma and liver lipids, depending on the degree of hypertriglyceridemia in rats [13], and have also been shown to have different regulatory effects on gene expression in rodents versus humans [14]. In fact, as recently reported [15], the degree of effect of fibrates on the expression of hepatic genes of fatty acid metabolism may depend on the animal model employed and the extent of the hypertriglyceridemia.

During late pregnancy, hypertriglyceridemia is consistently developed [16] as a consequence of enhanced adipose tissue lipolytic activity [17, 18], enhanced liver production of VLDL [19], and decreased extrahepatic lipoprotein lipase activity [20]. Moreover, hyperinsulinemia and insulin resistance also develop during late pregnancy [21, 22], which is responsible for most of the changes in maternal lipid metabolism [23]. Thus, the hypertriglyceridemia present during late pregnancy may be comparable to that normally seen in Type 2 diabetic patients, in which the use of fibrates is recommended [24].

In a previous work [25], we showed that fenofibrate was unable to maintain a hypotrigliceridemic effect beyond 2 days in pregnant rats, whereas it efficiently produced the expected reduction on plasma triglyceride in virgin rats. Since it is well known that this drug is a ligand for the PPAR [8], and that the capability of fibrates to reduce triglyceridemia is mediated by PPAR [26], the lack of hypotriglyceridemic effect of fenofibrate on pregnant rats could be due to an impaired fibrate-mediated activation of PPAR and/or a diminished hepatic expression of this receptor. In fact, there are reports showing that hepatic PPARα mRNA content and plasma triglycerides level are inversely related [27, 28].

Furthermore, if the action of fibrates on lipid metabolism is primarily mediated by their role as ligands for PPARα [29] the enhanced arrival of free fatty acids (FFA) (which are also ligands for PPARα) [29] to the liver in the late-pregnant rat [30] could decrease the availability of the former to their corresponding PPAR site. This would substantially reduce the capability of fibrates to activate PPARα and, consequently, its metabolic effects [7].

The aim of the present work was, therefore, to investigate the factors involved in the lack of fenofibrate ability in reducing plasma lipids under certain conditions. Therefore, the hepatic expression of PPAR and related genes was determined in pregnant and virgin rats treated or not with that hypolipidemic drug.

Materials and methods

Animals, drug administration, and collection of the samples

Female Sprague–Dawley rats weighing 180–210 g were fed *ad libitum* standard rat chow (B & K Universal, Barcelona, Spain) and housed under controlled light and temperature conditions (12-h light–dark cycle; 22 ± 1 °C). The experimental protocol was approved by the Animal Research Committee of the University San Pablo-CEU, Madrid, Spain. Half of the animals were mated, and day 0 of pregnancy was determined by the appearance of spermatozoids in vaginal smears, whereas the other half were kept virgin. From day 16 of gestation, rats were given by oral gavage two daily doses of 0 mg, 100 mg or 200 mg of fenofibrate (from Sigma, USA) per kilogram of body weight, one at 8:00 h and the other at 18:00 h, suspended in 2% Tween 80. On the morning of the 20th day of pregnancy (after 4 days of treatment), corresponding to 14 h after receiving the last treatment, rats were decapitated and blood collected into tubes containing Na_2-EDTA. After centrifugation, plasma was kept at -20 °C until processed for triglycerides analysis using a commercial kit (Menarini, Italy). Liver was immediately removed, placed in liquid nitrogen and kept at -80 °C until analysis. Virgin rats received the same treatment and were studied in parallel.

Total RNA preparation and analysis

Total hepatic RNA was isolated by a modification of the guanidium isothiocyanate method, using Ultraspec RNA according to the manufacturer's instructions (Biotecx Labs, Houston, USA). Total RNA concentration was determined by absorbance measurement at 260 nm. The 260/280 absorption ratio of all samples was between 1.8 and 2.0. Twenty micrograms of denatured total RNA was separated on 1.2% agarose gels and transferred to nylon membranes in 20× SSC. The cDNA probes for Northern blot analysis were labeled with [α-^{32}P]dCTP using the random primer DNA labeling kit provided by Amersham. Probes used were: 1.1 kb *PstI* fragment of rat peroxisomal acyl-CoA oxidase (ACO) cDNA (kindly supplied by Dr. H. N. Sorensen, University of Oslo, and with

Table 1. Sequences of oligonucleotide primers and PCR conditions

Gene name	Size (bp)	5′-oligonucleotide	3′-oligonucleotide	Annealing (°C)	Cycles	Reference
Apo B	281	TCCTCAGCAGATTCATGATTATCT	AGCATTTTTAGCTTTTCAATGATT	55	30	[34]
L-CPT-1	629	TATGTGAGGATGCTGCTT	CTCGGAGAGCTAAGCTTG	60	30	[35]
SREBP-1c	185	GGAGCCATGGATTGCACATT	GCTTCCAGAGAGGAGGCCAG	65	35	[36]
FAS	297	TGCTGTGGACCTCATCACTA	TGGATGATGTTGATGATAGAC	55	35	[37]
ACC	242	ACAGTGAAGGCTTACGTCTG	AGGATCCTTACAACCTCTGC	55	35	[37]
SCD-1	521	GCTCATCGCTTGTGGAGCCAC	GGACCCCAGGGAAACCAGGAT	65	25	[38]
GAPDH	452	ACCACAGTCCATGCCATCAC	TCCACCACCCTGTTGCTGTA	57	30	[39]

permission of Dr. T. Osumi, Humeji Institute of Technology, Hyogo, Japan), the rat apo CIII cDNA fragment spanning nucleotides +54 to +356 (a generous gift of Dr. J. Auwerx, Strasbourg, France), and a fragment of rat β-actin cDNA (kindly supplied by Dr. D. Langin, INSERM U317, Toulouse, France). Blots were prehybridized at 42 °C for 2 h and hybridized overnight at the same temperature. Washing conditions were 0.1× SSC, 0.1% SDS at 53 °C for 15 min three times and membranes were exposed to Kodak X-OMAT films at −70 °C from 1 h to 3 days. Filters were stripped of label at 75–80 °C for 1 h with 0.1 × SSC, 0.5% SDS, 0.1% tetrasodium pyrophosphate and then rehybridized. Autoradiograms were analysed by quantitative scanning densitometry (GS-700 Imaging Densitometer, BioRad, California, USA).

An aliquot of hepatic total RNA was also subjected to RT-competitive PCR for determination of PPARα and PPARγ mRNA. Briefly, it consisted of the reverse transcription of the specific mRNA and then the co-amplification of the target cDNA with known amounts of the specific competitor DNA in the same tube [31]. The sequences of the primers, and the protocol and validation of the RT-competitive PCR assays have been reported in detail elsewhere [32, 33].

Total RNA-genomic DNA-free samples were also used to analyse the expression of the following genes: apolipoprotein B (apo B), liver carnitine palmitoyl transferase type I (L-CPT-I), sterol regulatory element binding protein (SREBP-1c), fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), stearoyl-CoA desaturase (SCD-1), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as endogenous control, by means of semi-quantitative RT-PCR, according to the following protocols. Briefly, total RNA (2.5 μg) was digested with 5 U RNase free DNase I (Roche, USA) for 20 min at 37 °C to remove traces of genomic DNA. The DNase was inactivated at 64 °C for 10 min and cDNA was synthesized from total RNA by oligo(dT)-primed reverse transcription with Superscript II (Invitrogen, USA), according to the manufacturer's instructions. PCRs were performed in a 25 μl reaction mix containing 20 pmol of both forward and reverse primer, 10 mmol/l of each deoxyribonucleotide triphosphate, appropriate dilutions of the cDNA stock, 2.5 μl of PCR 10 × buffer, and Accu Taq-polymerase (Sigma, USA). Table 1 shows primers and their references, along with PCR conditions (size of PCR products, number of cycles and primer annealing temperatures) for each gene tested. All reactions were performed in a PTC-100 Thermocycler (MJ Research, USA) in which samples underwent a 3 min initial denaturing step, followed by the number of cycles indicated (Table 1) of 1 min at 94 °C, 45 s to 2 min at the annealing temperature indicated in Table 1, and a primer extension step at 72 °C for 45 s to 2 min. The final extension step was 5–10 min at 72 °C. The PCR products were analysed by agarose gel electrophoresis and DNA was visualized by ethidium bromide staining and using a UV-light box. The intensities of the bands on the images were determined by quantitative scanning densitometry (GS-700 Imaging Densitometer, BioRad, California, USA). To determine the linear range of the PCR, dilutions of the cDNA preparations were previously used for each gene and experimental group of rats. Results for the expression of specific mRNAs were always presented relative to the expression of the control gene (GAPDH).

Statistical analysis

Results were expressed as means ± S.E.M. Treatment effects were analysed by one-way analysis of variance (ANOVA). When treatment effects were significantly different ($p < 0.05$), means were tested by Tukey multiple range test. When necessary, the Mann–Whitney U test was used instead. Differences between the two groups were analysed by using the Student's t-test.

Results

Effect of fenofibrate on serum triglycerides in pregnant and virgin rats

Fenofibrate significantly lowered serum triglycerides in virgin rats treated for 4 days (Fig. 1), independently of the dose used. As expected, in non-treated pregnant rats,

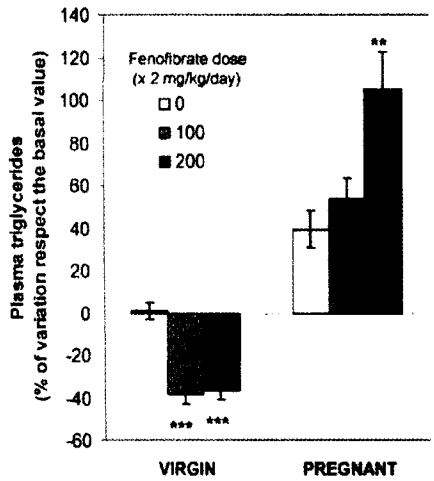

Fig. 1. The effect of 4 days treatment with fenofibrate on serum triglycerides in virgin and pregnant rats. Control rats received vehicle only. Serum triglyceride concentrations were measured at the beginning and the end of the treatment period and changes in serum triglyceride concentrations are expressed as a percentage (mean ± standard error) of the pretreatment values. Statistically significant differences between values versus control group are shown by asterisks (**$p < 0.01$; ***$p < 0.001$).

triglyceridemia increased after 4 days of study due to the gestational state. However, in pregnant rats, fenofibrate did not produce a hypolipidemic effect but, on the contrary, produced a dose-dependent increase on serum triglycerides whose levels became even higher than those caused by the pregnancy itself.

Effect of fenofibrate in β-oxidation gene expression in pregnant and virgin rats

The effect of fenofibrate on hepatic β-oxidation gene expression mediated by PPARα in female non-pregnant rats, such as ACO and mitochondrial carnitine palmitoyl transferase type I (CPT-I) conformed to what has previously been published using male rat and cell culture after exposure to fibrates [40, 41]. Thus, in virgin rats, hepatic ACO mRNA levels were augmented by the treatment, independently of the dose used (Fig. 2A), and hepatic L-CPT-1 mRNA levels were increased by fenofibrate in a dose-dependent manner (Fig. 2B). In pregnant rats treated with fenofibrate, both hepatic ACO mRNA and L-CPT-1 mRNA levels increased (Figs. 2A and 2B, respectively) independently of the dose used, indicating that the lower dose used here was sufficient to stimulate the expression of these PPAR-target genes.

Effect of fenofibrate in hepatic TG-rich lipoprotein metabolism gene expression in pregnant and virgin rats

The effect of fenofibrate on TG-rich lipoprotein metabolism gene expression related to PPARα, such as apolipoprotein B (apo B) [42] and apolipoprotein C-III (apo CIII) [9] was also studied. In virgin rats, hepatic apo B mRNA levels were decreased by the treatment, regardless of the dose used (Fig. 3A), and hepatic apo CIII mRNA levels conformed to what has previously been published using male rats [9], that is, the levels decreased by fenofibrate in a dose-dependent manner (Fig. 3B). In pregnant rats treated with fenofibrate, both hepatic apo B mRNA and apo CIII mRNA levels were reduced (Figs. 3A and 3B, respectively) independently of the dose used. Thus, these findings would again indicate that the lower dose of fenofibrate used here was sufficient to influence the expression of those PPAR-related genes in pregnant rats.

Effect of fenofibrate in PPARα and PPARγ mRNA in pregnant and virgin rats

Hepatic PPARα mRNA levels in both virgin and pregnant rats increased similarly after treatment with fenofibrate (Fig. 4A). Although there were no significant differences between the rats treated with 100 × 2 mg dose and 200 × 2 mg/kg per day of fenofibrate in virgin or pregnant rats, the increase found in the PPARα expression caused by the drug effect was apparently dose dependent in both virgin and pregnant rats.

Curiously, hepatic PPARγ mRNA levels were increased in virgin rats after treatment with the higher dose of fenofibrate (Fig. 4B). However, there were no significant differences between the pregnant rats treated or not with fenofibrate.

Effect of fenofibrate in lipogenesis gene expression in pregnant and virgin rats

The effect of fenofibrate on hepatic lipogenic gene expression in virgin and pregnant rats was studied. As shown in Fig. 5A, the expression of sterol regulatory element binding protein (SREBP-1c), a transcription factor implicated in the regulation of expression of multiple lipogenic genes including FAS, ACC and SCD-1 [43], was reduced in non-pregnant rats after treatment, regardless of the dose used. In pregnant rats, although the trend was also shown to be reduced by fenofibrate, it did not become significant. On the other hand, the expression of FAS and ACC was not significantly changed by fenofibrate in neither virgin nor pregnant rats (Figs. 5B and 5C, respectively). However, SCD-1 expression in virgin rats was significantly augmented by fenofibrate with the two doses of the drug used (Fig. 5D). An induction on liver SCD-1 gene expression by fibrates has previously been reported [44]. In pregnant rats, however, the drug did not affect the SCD-1 expression.

Interestingly, the CD36 mRNA levels whose expression has been previously demonstrated to be induced by fibrates treatment [6] were increased in liver of virgin rats by the

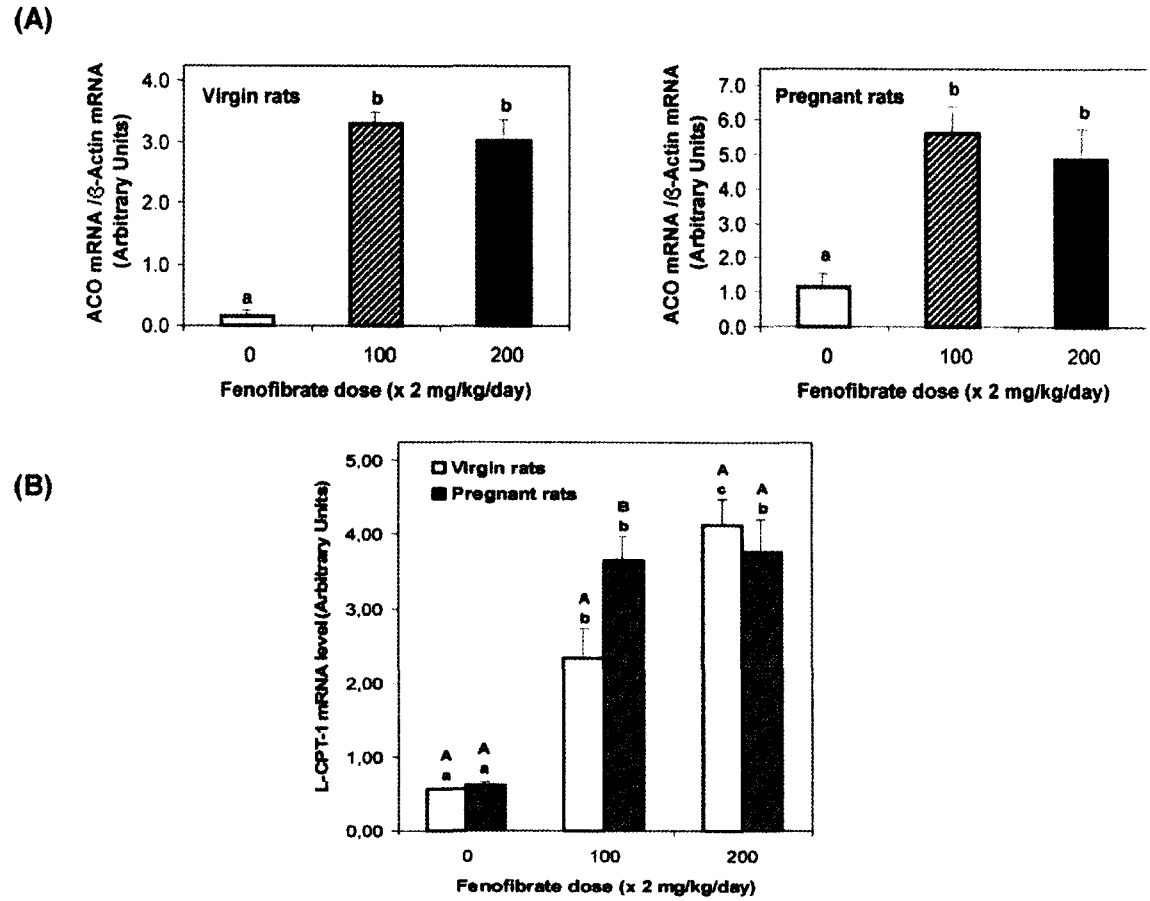

Fig. 2. (A) Relative amount of mRNA of hepatic acyl-CoA oxidase (ACO) from virgin and pregnant rats treated or not with fenofibrate, measured by Northern blot assays. Values obtained by densitometric scanning of the blots and normalized against actin was represented using arbitrary units. Statistically significant differences are represented by letters, and values not sharing a common letter are significantly different at $p < 0.05$. Each value represents the mean ± standard error of four to six animals. (B) Relative amount of mRNA of liver carnitine palmitoyl transferase type I (L-CPT-I) from virgin and pregnant rats treated or not with fenofibrate, measured by semi-quantitative RT-PCR. Values were normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression and were represented using arbitrary units. The capital letters correspond to the statistical comparisons between pregnant and virgin rats receiving the same treatment. The small letters correspond to the statistical comparisons between rats receiving different drug doses. Values not sharing a common letter are significantly different at $p < 0.05$. Each value represents the mean ± standard error of five animals.

higher dose of fenofibrate used here (Fig. 5E). However, in pregnant rats, fenofibrate did not produce any effect on the CD36 expression levels (Fig. 5E).

Discussion

Our previous work [25] showed that fenofibrate decreases plasma triglycerides levels in female virgin rats (Fig. 1), which is in agreement with previous works using this fibrate or others, although carried out in male rats [9, 45, 46]. However, surprisingly, fenofibrate was not only unable to produce its expected hypolipidemic action in pregnant rats ([25] and Fig. 1) but, on the contrary, produced a dose-dependent increase in serum triglycerides over the hypertriglyceridemia already caused by pregnancy. This unexpected effect of fenofibrate in pregnant rat triglyceridemia seems to be a rebound effect, since after an initial reduction, plasma triglycerides increased over the values seen in non-treated controls at the fourth day of treatment [25]. In relation to that, it is well known that fenofibrate is a ligand for the PPAR [8], which is a key factor in the regulation of hepatic lipid metabolism, and that the capability of fibrates to reduce triglyceridemia is mediated by PPAR [26]. Thus, the lack of a prolonged hypotriglyceridemic effect of fenofibrate in pregnant rats could be due to an impaired fibrate-mediated activation of PPAR and/or a diminished hepatic expression of this receptor.

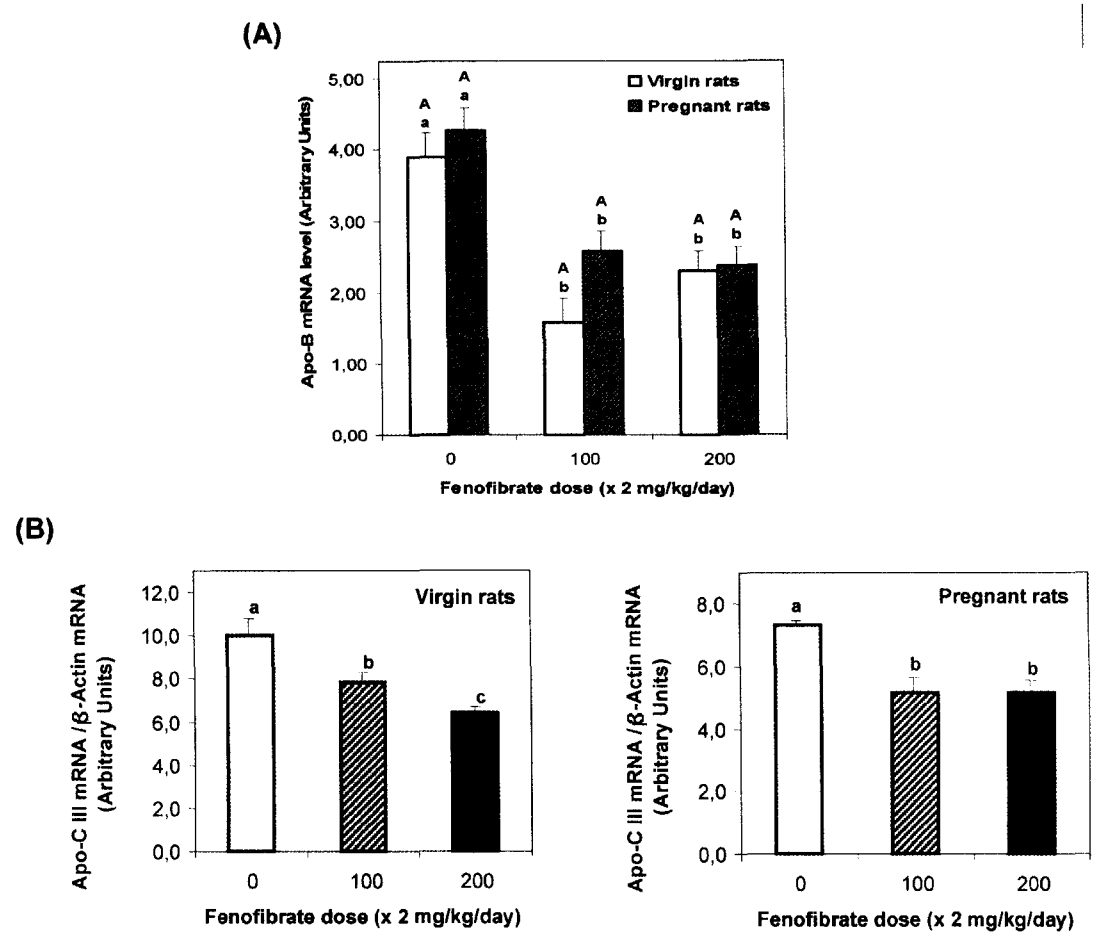

Fig. 3. (A) Relative amount of mRNA of liver apolipoprotein B (apo B) from virgin and pregnant rats treated or not with fenofibrate, measured by semi-quantitative RT-PCR. Values were normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression and were represented using arbitrary units. The capital letters correspond to the statistical comparisons between pregnant and virgin rats receiving the same treatment. The small letters correspond to the statistical comparisons between rats receiving different drug doses. Values not sharing a common letter are significantly different at $p < 0.05$. Each value represents the mean ± standard error of five animals. (B) Relative amount of mRNA of hepatic apolipoprotein C-III (apo CIII) from virgin and pregnant rats treated or not with fenofibrate, measured by Northern blot assays. Values obtained by densitometric scanning of the blots and normalized against actin was represented using arbitrary units. Statistically significant differences are represented by letters, and values not sharing a common letter are significantly different at $p < 0.05$. Each value represents the mean ± standard error of four to six animals.

Therefore, to gain more insight, we studied liver mitochondrial and peroxisomal β-oxidation gene expressions and particularly ACO and L-CPT-I mRNA levels, which were previously shown to increase after fenofibrate treatment [9, 41]. Although female rats have been described as less responsive to peroxisome proliferators than male rats [47], we found that the drug was equally effective on potentiating the mitochondrial and peroxisomal β-oxidation gene expression in virgin as well as in pregnant rats, and it would therefore be expected that the fatty acid catabolism was activated by fenofibrate in both cases.

In relation to the lipoprotein metabolism, liver production of VLDL was estimated by measuring apo B mRNA content and VLDL catabolism by apo CIII mRNA levels. Since it has been shown that PPARα-deficient hepatocytes have an increased capacity for apo B and VLDL secretion [43], it is expected that activators of PPARα would produce a decrease on apo B mRNA liver content, which corresponds with the effect observed here for virgin and pregnant rats treated with fenofibrate. Furthermore, Roglans *et al.* [27] have observed that apoB plasma levels were opposite to PPARα mRNA hepatic levels, and accordingly we found that liver apoB and PPARα mRNA contents were inversely related (Fig. 3A versus Fig. 4A). Furthermore, the decrease of apoB levels mediated by fibrate treatment has been previously reported, although affecting other steps on the synthesis

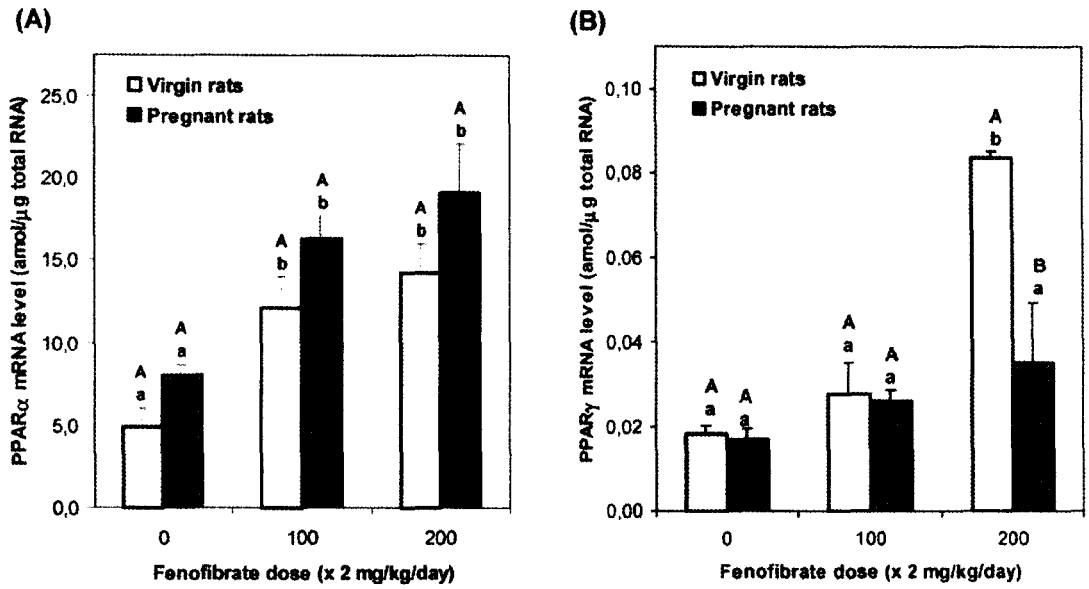

Fig. 4. Amount of mRNA of liver (A) peroxisome proliferator-activated receptor alpha (PPARα) and (B) peroxisome proliferator-activated receptor gamma (PPARγ) from virgin and pregnant rats treated or not with fenofibrate, measured by competitive RT-PCR. Values were represented using amol of PPAR mRNA per microgram of total RNA. The capital letters correspond to the statistical comparisons between pregnant and virgin rats receiving the same treatment. The small letters correspond to the statistical comparisons between rats receiving different drug doses. Values not sharing a common letter are significantly different at $p < 0.05$. Each value represents the mean ± standard error of five animals.

of apolipoprotein B, such as its translation, the intracellular degradation of apolipoprotein B, or both [48]. However, the hepatic apoB mRNA content and the serum apoB levels not always show a good correlation, as previously found by others [49]. Nevertheless, most authors agree that VLDL secretion is not only regulated by the apoB production rate, but is also controlled by the availability of fatty acids and the biosynthesis of triglycerides [48, 50]. Moreover, apo CIII is known to inhibit lipoprotein lipase (LPL) activity and, therefore, is often used to explain the hypotriglyceridemic action of fibrates [8]. Thus, the decrease in apo CIII gene expression found after fenofibrate treatment in both pregnant and virgin rats, would point to an upregulation of extrahepatic LPL. However, we previously showed that adipose tissue LPL activity, measured in rats with the same treatment, was diminished in a dose-dependent manner by fenofibrate in both groups [25], suggesting that VLDL catabolism was similarly impaired by the treatment in virgin as well as in pregnant rats.

Regarding the PPARα mRNA content, fenofibrate produced an increase in liver of virgin rats in accordance with a previous report using male rats treated with that drug [51]. However, this is the first time that the effect of fenofibrate has been studied on PPARα expression and activity in late pregnant rats, and despite the opposite behaviour detected in triglyceridemia in virgin compared to pregnant rats, in both groups there was a similar increase in the expression of that receptor by the treatment. Therefore, in virgin rats, hepatic PPARα mRNA content and plasma triglycerides levels were inversely related, as observed in previous works using different conditions [27, 28, 42]. However, in pregnant rats, both parameters showed a dose-dependent increase. Since plasma FFA levels reported in our previous work [25] did not parallel to the PPARα mRNA content found in liver of virgin rats, the increase observed in the PPARα expression in both virgin and pregnant rats could be mainly attributed to fenofibrate. Thus, since this drug is a ligand for that receptor [29], the augmented expression of PPARα found here would represent a clear example of *in vivo* autoregulation of the receptor [33, 52].

Interestingly, hepatic PPARγ expression augmented with the higher dose of fenofibrate in virgin rats, but not in pregnant rats. This finding may be related to the hepatic triglycerides content, which was not affected by the drug in liver of pregnant rats (35.9 ± 2.6, and 32.5 ± 1.3 mg of triglycerides/organ for control and 2 × 200 mg/kg per day, respectively), but augmented by the drug in liver of virgin rats (49.9 ± 3.8, and 105.3 ± 13.2 mg of triglycerides/organ for control and 2 × 200 mg/kg per day of drug, respectively, $p < 0.001$) [25]. These findings would agree with the observation that many peroxisome-proliferating agents cause transient lipid accumulation in liver [53]. Accordingly, CD36 mRNA levels, a fatty acid membrane transporter whose expression has been related to PPARγ [6, 54], were also increased in liver of virgin rats by the higher dose of fenofibrate used here (Fig. 5E).

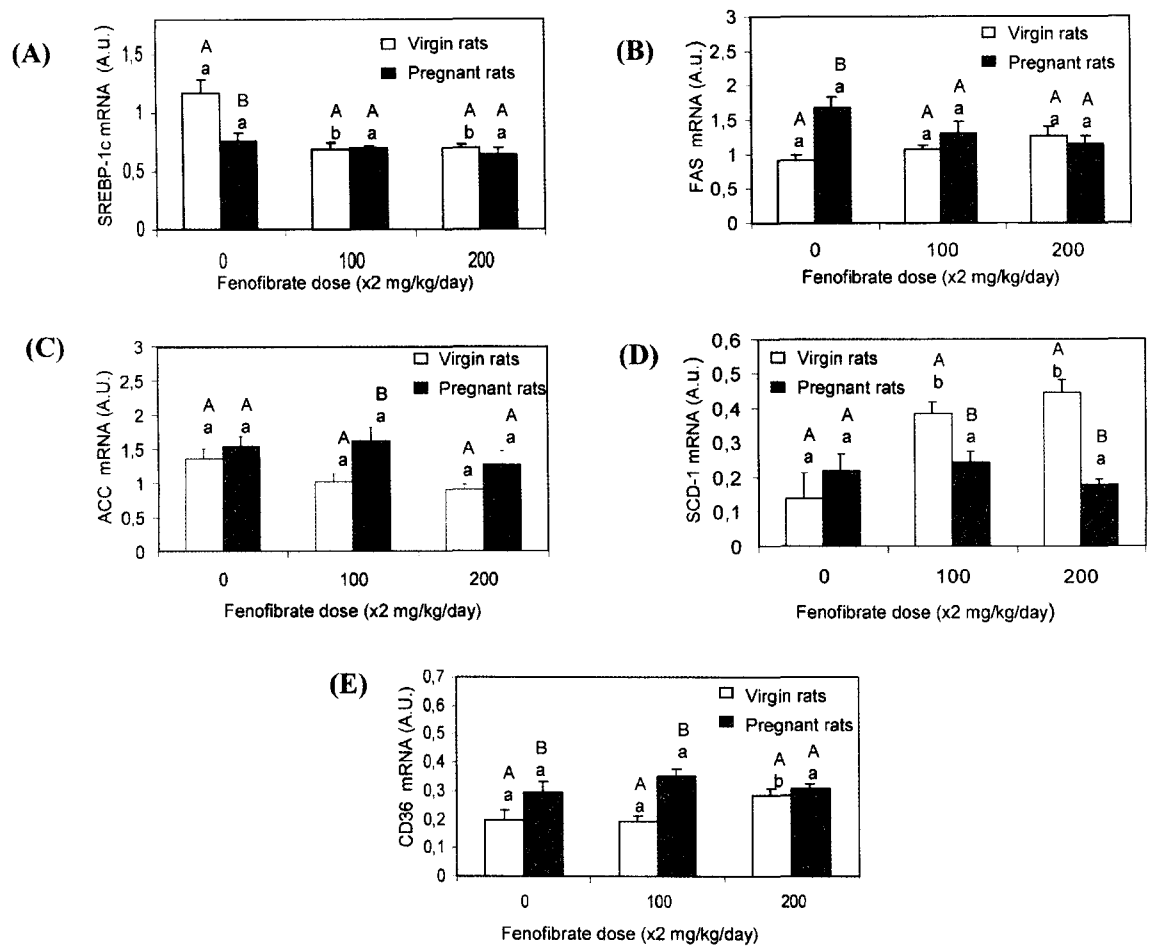

Fig. 5. Relative amount of mRNA of (A) liver sterol regulatory element binding protein (SREBP-1c), (B) fatty acid synthase (FAS), (C) acetyl-CoA carboxylase (ACC), (D) stearoyl-CoA desaturase (SCD-1) and (E) fatty acid translocase (FAT/CD36) from virgin and pregnant rats treated or not with fenofibrate, measured by semi-quantitative RT-PCR. Values were normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression and were represented using arbitrary units. The capital letters correspond to the statistical comparisons between pregnant and virgin rats receiving the same treatment. The small letters correspond to the statistical comparisons between rats receiving different drug doses. Values not sharing a common letter are significantly different at $p < 0.05$. Each value represents the mean ± standard error of five animals.

Fatty acid availability to triglyceride synthesis in liver not only depends on the supply of plasma FFA, but also on *de novo* fatty acid synthesis and oxidation. Regarding lipogenesis, since we observed a decrease on SREBP-1 expression by fenofibrate in treated virgin rats, a diminution in the expression of target genes related to fatty acid synthesis (i.e., FAS, ACC) would be expected, as has been reported under other conditions [43]. However, the only significant change produced by fenofibrate was an induction on SCD-1 expression in non-pregnant rats, which agrees with that previously reported in mice treated with clofibrate or gemfibrozil [44]. This effect could be related to the triglyceride accumulation found in the liver of these animals.

Definitively, in pregnant rats, plasma triglycerides increased after fenofibrate treatment without changing the liver triglycerides content. This lack of effect of fenofibrate-reducing triglyceridemia in pregnant rats could be related to the pharmacokinetics of the drug in the pregnancy state, which would produce an increase in the rate of fenofibrate elimination [55], as the samples were taken 14 h after the rats received the last drug dose [25]. However, in a recent work using peroxisome proliferators in mice for 1 week, the later withdrawal period produced a preservation of drug-induced changes in serum triglycerides and hepatic carnitine palmitoyltransferase (CPT) activity, which lasted 10 days [56]. Accordingly, in our study, the liver of treated pregnant rats at the end of the experiment still showed effects clearly produced by fenofibrate, such as induction of ACO and CPT-I expression. Nevertheless, the most plausible reason would be related to the induced overproduction of VLDL-triglycerides by

liver in pregnancy [16] which, supported by a dose-dependent drug-induced arrival of FFA to the liver [25], favoured by the high expression of CD36, would possibly exceed the known fibrate-induced effects: decrease in apoB mRNA (Fig. 3A), and lipoprotein secretion [12, 45] and increase in fatty acid β-oxidation (Figs. 2A and 2B) [9, 41]. In fact, the effect of fenofibrate-enhancing triglyceridemia in pregnant rats shown here may become evident only in the context of an altered and enhanced flux of fatty acids, such as those seen in pregnant rats. In that sense, it has already been demonstrated that the enhanced lipolytic activity of adipose tissue enhances liver VLDL-triglycerides production and contributes to the augmented plasma level of VLDL-triglycerides during gestation [16]. Furthermore, the diminished adipose tissue LPL activity observed in the treated pregnant rats, which is further decreased by fenofibrate treatment [25], would also contribute to the poor efficiency in removing the elevated concentration of triglycerides from the circulation.

On the contrary, in virgin rats, the drug-induced β-oxidation was probably able to interfere with the assembly and secretion of VLDL from the liver. Moreover, the accumulation of triglycerides observed here in treated virgin rat liver would be also related to the reduction found in triglyceridemia, as observed in previous studies using fibrates [13, 53]. However, such increase in liver triglyceride content found in the treated non-pregnant rats, would not be related to either an impaired expression and/or function of PPARα, as reported elsewhere [27], since ACO, CPT-I, and PPARα expressions were effectively augmented by fenofibrate in these rats. Thus, it could be due to an augmented entry of plasma fatty acids to the liver through CD36 transporter mediated by fenofibrate [10], and/or to a diminished triglyceride-rich lipoprotein production since apoB mRNA levels were reduced by the treatment.

Present results show that fenofibrate produces in virgin rats the expected effects, that is, induction of genes related to fatty acid oxidation, diminution of genes related to lipoprotein secretion and catabolism, stimulation of SCD-1 gene expression, and promotion of triglycerides accumulation in liver, altogether causing the expected hypotriglyceridemia. However, in pregnant rats, fenofibrate produced the same effects like in virgin rats, except on SCD-1 gene expression and hepatic accumulation of triglycerides which were unmodified and the triglyceridemia that, unexpectedly, was augmented. In our previous work, significant linear correlations between plasma FFA and plasma triglycerides were found in fenofibrate-treated pregnant rats but not in pregnant controls neither in virgin rats, treated or untreated [25]. That situation supports an enhanced FFA arrival to the liver which would be overpassing the effects of fenofibrate, that is, stimulation of genes related to fatty acid oxidation and reduction of genes related to lipoprotein production, and therefore the excess of fatty acids has to be exported to the plasma as triglycerides.

Although in the present study plasma estrogens levels and the cross-talk between these hormones and fibrates have not been determined, competition between fenofibrate and estradiol, which levels during late pregnancy are extremely high [57], leading to lipid metabolism should not be discarded [58]. In fact, fibrates have been described as failing, or not sufficiently correcting hypertriglyceridemia under certain conditions, i.e., severe combined hyperlipidaemia [59], overweight associated with diabetes [60], and in combined treatment with hormone replacement therapy in obese postmenopausal women [61]. In conclusion, the study shows that fenofibrate, in spite of efficiently exerting its expected molecular effects in liver (i.e., inducing fatty acids and lipoprotein catabolism, and reducing TG-rich lipoprotein secretion), was unable to reverse the typical hypertriglyceridaemia of gestation.

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