Fenofibrate reduces adiposity in pregnant and virgin rats but through different mechanisms

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Fenofibrate has been proven to reduce adiposity. Since gestation produces an increase in white adipose tissue (WAT) mass, we comparatively studied this drug-effect in virgin and pregnant rats. Fenofibrate reduced lumbar WAT weight in both pregnant and virgin rats. Fenofibrate treatment did not modify plasma free fatty acid (FFA) concentration in virgin rats, it greatly increased it in pregnant animals. Remarkable differences between the two groups were obtained for two proteins related to fatty acid oxidation and esterification and storing. Respectively, the mRNA levels of carnitine palmitoyltransferase I (CPT-I) were increased by the fenofibrate only in the virgin rats and a similar finding was observed for the expression of phosphoenolpyruvate carboxykinase (PEPCK). These findings indicate that fenofibrate reduces adiposity in pregnant and virgin rats through different mechanisms: a) in virgin rats, by promoting fatty acid oxidation; and b) in pregnant rats, by enhancing fatty acid output. [BMB reports 2009; 42(10): 679-684]

INTRODUCTION

Fibrates have been effectively used to reduce plasma triacylglycerol levels under conditions of hypertriacylglycerolemia (1). The molecular bases for the action of fibrates on lipid metabolism have been elucidated (2, 3) and involve the activation of transcriptional factors, known as peroxisome proliferator-activated receptors (PPAR), principally the PPARα form. However, there are still aspects of the effects of fibrates on lipid metabolism that remain to be understood. Some fibrates have also been shown to have opposite effects on plasma and liver lipids in rats depending on the degree of hypertriacylglycerolemia (4), and have also been shown to have different regulatory effects on gene expression in rodents versus humans (for a review, 3, 5).

During late pregnancy hypertriacylglycerolemia is consistently developed (6) as a consequence of enhanced white adipose tis-

sue (WAT) lipolytic activity (7), enhanced liver production of VLDL (8), and decreased extrahepatic lipoprotein lipase activity (9). Moreover, hyperinsulinemia and insulin resistance also develop during late pregnancy (10), these being responsible for most of the changes in maternal lipid metabolism (11). Thus, the hypertriacylglycerolemia present during late pregnancy may be comparable to that normally seen in Type 2 diabetic patients, in which the use of fibrates is recommended (12).

In a previous work (13), we showed that fenofibrate, a PPARα agonist, was unable to maintain its hypotriacylglycerolemic effect beyond two days in pregnant rats, whereas in virgin rats it efficiently produced the expected reduction on plasma triacylglycerol throughout its treatment. Such inefficiency of fenofibrate on the triacylglycerolemia of pregnant rats was ascribed to the elevated amount of FFA which reached the liver in treated pregnant rats and which were not sufficiently oxidized and/or stored, and therefore had to be canalized back to the plasma as triacylglycerols (13, 14). This implies that WAT in treated pregnant rats would be releasing fatty acids into the plasma.

It has been reported that fenofibrate can reduce body weight gain in animal models of diabetes (15), obesity (16), and insulin resistance such as seen in obese Zucker rats (17) and high fat fed C57BL/6 mice (18), as well as in normal rats (19). This effect of fenofibrate on body weight gain and on the reduction of WAT mass has been ascribed to increased fat catabolism in liver mainly through the induction of target enzymes involved in hepatic fatty acid oxidation (16, 18). In fact, it has been proposed that PPARα activators reduce insulin resistance and WAT depots secondary to their effects on liver. Curiously, no effects were reported in WAT (18). Although there are authors who only determined the hepatic expression of PPARα target genes (20), in spite of having found reductions in adiposity after fenofibrate treatment, recent works indicate that PPARα is also expressed in WAT, where it is able to regulate genes involved in fatty acid oxidation (21).

Due to the above mentioned different responses to fenofibrate in liver of pregnant and virgin animals (13,14), the aim of the present work was to determine the expression of PPAR and related genes in WAT of pregnant and virgin rats receiving or not such treatment. Moreover, since fenofibrate has been proven to reduce adiposity (16-19) and gestation produces an increase in WAT mass (22), we comparatively studied this drug-effect in virgin and pregnant rats. Thus, we found that fe-

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nofibrate treatment reduces lumbar WAT in both virgin and late pregnant rats, although its effect on the mRNA expression of some involved proteins differs between these conditions, indicating a different mechanism of action.

RESULTS AND DISCUSSION

As shown in Table 1, lumbar WAT weight appeared higher in pregnant than in virgin rats, in agreement with previous findings (22), while it was decreased by the fenofibrate treatment in a dose-dependent manner in the two groups. Although the effect of the 100 × 2 mg dose did not reach statistical significance in either group, a significant decrease was found in rats treated with the 200 × 2 mg dose. Thus, it was found that WAT weight was reduced in virgin and pregnant rats treated for 4 days with fenofibrate. Such an effect has been previously reported for this and other fibrates in non-pregnant rats subjected to different treatment periods and different doses from those used here (15, 16, 18, 19, 23), even after treatment with other peroxisome proliferators, such as perfluorooctanoic acid (24).

In order to obtain an index of the net WAT fatty acids release into the circulation, free fatty acids (FFA) plasma levels were determined (Table 2). Whereas at day 0 of treatment (day 16 of preg-

nancy in the case of pregnant rats) plasma FFA levels did not differ between virgin and pregnant rats, values increased in pregnant rats at day 20 of pregnancy. Moreover, whereas fenofibrate treatment did not modify this variable in virgin rats, it caused a significant increase in pregnant rats, not only when compared to virgin rats but also when compared to the pregnant rats not receiving treatment. These findings would indicate that fenofibrate reduces adiposity in pregnant and virgin rats through different mechanisms.

To test whether specific marker genes of WAT may drive the effect of fenofibrate reducing adiposity, the mRNA levels of PPARγ and leptin were determined. The PPARγ mRNA levels in both virgin and pregnant rats decreased after treatment with fenofibrate (Fig. 1A), the effect being dose-dependent and greater in WAT from pregnant than from virgin rats. In fact, in rats receiving the 100 × 2 mg/kg/day dose, the adipose PPARγ mRNA was significantly lower in pregnant than in virgin rats, whereas the difference in those receiving 200 × 2 mg/kg/day did not reach significance due to the high variability of the groups (Fig. 1A). As was to be expected from previous studies (25), leptin mRNA levels appeared lower in virgin than in pregnant rats not receiving the drug (Fig. 1C), but fenofibrate treatment did not affect this variable in neither virgin nor pregnant rats (Fig. 1C). The fenofibrate dose-dependent reduction of PPARγ mRNA expression found here fits with the diminished adipose tissue LPL activity previously found in virgin and pregnant rats receiving the same treatment (13). Although these two effects agree with the reported decline in PPARγ expression in rat adipocytes after fibrate exposure (26), which would indicate a conversion of triacylglycerol storing cells into cells with a higher capacity to oxidize fatty acids, this view is not supported by our leptin values. In virgin rats, the mRNA expression of this adipocyte marker was practically kept stable after fenofibrate treatment.

To test whether an augmented mitochondrial fatty acid β-oxidation of fatty acids by WAT could also be involved in the decreased WAT size after fenofibrate treatment, the mRNA expression of carnitine palmitoyl transferase type I (CPT-I) was determined. CPT-I is a rate-limiting enzyme for long-chain fatty

Table 1. Effects of fenofibrate on lumbar WAT weight in virgin and pregnant rats

WAT weight (g)	Dose (× 2 mg/kg/day)		
	0	100	200
Virgin	1.16 ± 0.09[a,A]	1.01 ± 0.07[a,A]	0.77 ± 0.08[b,A]
Pregnant	1.68 ± 0.13[a,B]	1.35 ± 0.14[ab,B]	1.15 ± 0.09[b,B]

Values are means ± S.E. of 7-8 rats/group. 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

Table 2. Effects of fenofibrate on plasma FFA in virgin and pregnant rats

	Dose (× 2 mg/kg/day)					
	0		100		200	
	Day of treatment		Day of treatment		Day of treatment	
	0	4	0	4	0	4
FFA (mM)						
Virgin	0.296 ± 0.024[a,A]	0.291 ± 0.033[a,A]	0.263 ± 0.026[a,A]	0.263 ± 0.021[a,A]	0.274 ± 0.026[a,A]	0.259 ± 0.040[a,A]
Pregnant	0.237 ± 0.021[a,A]	0.515 ± 0.058[b,B]	0.259 ± 0.033[a,A]	0.950 ± 0.073[c,B]	0.240 ± 0.029[a,A]	0.914 ± 0.087[c,B]

Values are means ± S.E. of 7-8 rats/group. 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

(B)

(A)

(C)
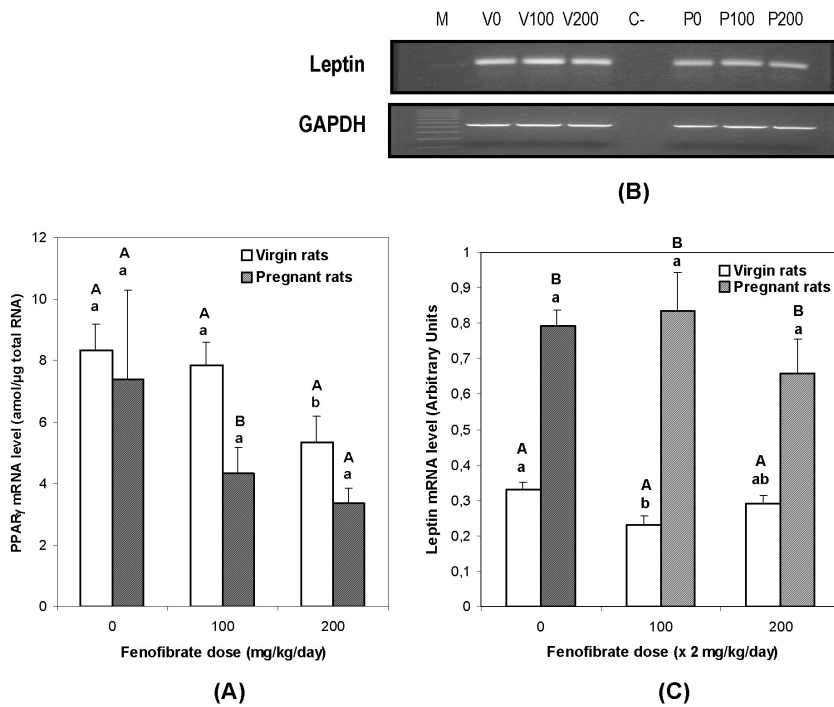
Fig. 1. Effects of fenofibrate on the expression of adipocyte markers. (A) Amount of mRNA of peroxisome proliferator-activated receptor gamma (PPARγ) in lumbar WAT from virgin and pregnant rats treated or not with fenofibrate. Values are represented using amol of PPAR mRNA per mg of total RNA. (B) Blots and (C) relative amounts of mRNA of leptin in WAT from virgin and pregnant rats treated or not with fenofibrate. Values were normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression and are 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.

(A)

(B)

(C)
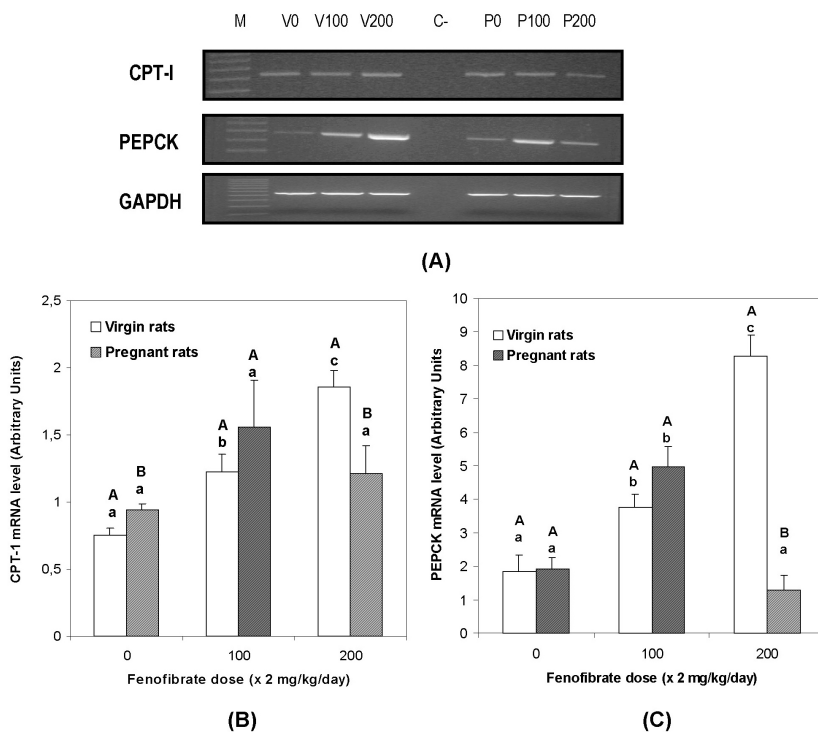
Fig. 2. Effects of fenofibrate on fatty acid metabolism gene expression. (A) Blots and relative amounts of mRNA of: (B) carnitine palmitoyl transferase type I (CPT-I) and (C) phosphoenolpyruvate carboxykinase (PEPCK) in WAT from virgin and pregnant rats treated or not with fenofibrate. Values were normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression and are 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.

acids (LCFAs) β-oxidation in mitochondria (27). As shown in Fig. 2B, fenofibrate treatment increases CPT-I mRNA levels in WAT of virgin rats in a dose-dependent manner. However, whereas in untreated pregnant rats the CPT-I mRNA levels were higher than in virgins, values in this group did not increase significantly with fenofibrate treatment (Fig. 2B). In fact, CPT-I mRNA levels in non-pregnant rats treated with the highest drug dose used here, were signicantly higher than those found in pregnant rats receiving the same dose (Fig. 2B). In accordance with the results previously observed in liver (14) and in adipocytes (26), the CPT-I expression in WAT from non-pregnant rats was augmented by fenofibrate in a dose-dependent manner. However, in pregnant rats we did not observe such a fenofibrate-induced increase, which may be due to the known contrarregulatory action of estrogens (3, 28), whose levels are quite high in late gestation (9). In a recent study with ovariectomized mice, the concomitant treatment with fenofibrate and estradiol showed that the hormone reversed the effects of fibrate on plasma lipids and hepatic PPARα target gene expression (20), although nothing was said about the WAT expression. Furthermore, the possible implication of insulin should not be discarded. Hyperinsulinemia develops during late pregnancy (10), and we (29) and others (30) have described that insulin can counteract the induction effected by PPARα activators.

As shown in Fig. 2C, the expression of phosphoenolpyruvate carboxykinase (PEPCK) in WAT does not differ between virgin and pregnant rats but significantly increases in a dose-dependent manner in non-pregnant rats after fenofibrate treatment. However, in tissues from pregnant rats, an increase in PEPCK mRNA occurred only in those treated with 100×2 mg/kg of fenofibrate whereas no-differences were found in pregnant rats receiving the highest dose of fenofibrate studied here in comparison to basal values (Fig. 2C). In fact, PEPCK mRNA levels in virgin rats treated with this high dose were signicantly higher than those in pregnant rats receiving the same dose (Fig. 2C).The main components of WAT are triacylglycerols, whose synthesis depends on the esterification of fatty acids. This pathway requires glycerol-3-phosphate, which due to the low glycerol kinase activity in WAT (31), must come from either glycolitic glucose utilization or from non-carbohydrate precursors such as pyruvate, lactate or even amino acids, throughout glyceroneogenesis (32). The enzyme PEPCK catalyzes the key step in WAT glyceroneogenesis (32) and in the present study it was observed that adipose tissue PEPCK expression was dose-dependent induced by fenofibrate in non-pregnant rats. This finding agrees with a similar effect seen in adipocytes cultured in the presence of clofibrate (33). In fact, we have previously shown that fibrates are able to promote fatty acid reesterification in adipose tissue of male rats both *in vitro* and *in vivo* (34, 35). However, such a dose-dependent effect of fenofibrate on mRNA PEPCK was not observed in pregnant rats, suggesting the presence of some factor which might be counteracting the induction effect. Thus, glucocorticoids, whose circulating levels are augmented during pregnancy (36), have been described as repressors of PEPCK induction by fibrates in adipocytes (37). Moreover, a possible insulin implication should not be discarded. In fact, varia-

tions in insulinemia are oppositely correlated to PEPCK mRNA levels (33). In agreement with this, we also found that the PEPCK expression increase after a lipid overload was attenuated when insulinemia was elevated (29, 38).

According to the augmented mRNA of CTP-I found in WAT of non-pregnant rats receiving the fenofibrate treatment, it is proposed that the drug is enhancing the mitochondrial β-oxidation, thus contributing in this way to the diminished size of WAT pads. However, this explanation is not valid to account for the decreased adiposity observed in treated-pregnant rats. Pregnancy displays an enhanced lipolytic activity in WAT (7), which is counteracted by an accelerated reesterification of fatty acids, therefore avoiding the depletion of fat stores in the fed state (39). Thus, the lack of effect of fenofibrate in inducing the expression of mRNA PECPK found in WAT of pregnant rats indicates an unmodified glyceroneogenesis which together with an active lipolysis, would produce a net increase in the output of fatty acids into the bloodstream. This explanation fits with the increased plasma FFA levels in pregnant-treated rats and with their lower size of WAT found here.

It is therefore proposed that fenofibrate reduces adiposity in virgin and pregnant rats using different mechanisms. This probably explains the opposite effects found on lipidemia in virgin versus pregnant rats (14). As we (14) and others (20) have already proposed, our present study suggests that fibrates normally act as efficient hypolipidemic agents but, under certain conditions (e.g. patients displaying high estrogen levels; patients with a PPAR receptor deficiency; obese postmenopausal women receiving a combined hormone replacement treatment, etc.) (3, 28), they should be used with care since their effect on such patients may not be as expected (40).

MATERIALS AND METHODS

Animals, drug administration and samples

The experimental design has been reported in detail elsewhere (13). Briefly, female Sprague-Dawley rats weighing 180-210 g were used. Half of the animals were mated, and day 0 of pregnancy was determined by the appearance of spermatozoids in vaginal smears, while the other half were kept virgin. From day 16 of gestation, rats were given two daily doses of 0, 100 or 200 mg of fenofibrate (from Sigma, USA)/kg of body weight suspended in 2% Tween-80, by oral gavage, one at 8.00 h and the other at 18.00 h. The doses of fenofibrate were chosen under the base of previous studies in the rat (13, 14, 34, 35, 41, 42). On the morning of the 20 th day of pregnancy (4 th day after the onset of treatment) rats were decapitated and blood collected. Plasma was kept at -30°C until processing for the analysis of FFA by enzymatic commercial kits (Wako). Lumbar WAT 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. The experimental protocol was approved by the Animal Research Committee of the Faculty of Pharmacy, University CEU San Pablo (Madrid, Spain).

Total RNA preparation and analysis

Total RNA was isolated from WAT by using Ultraspec according to the manufacturer's instructions (Biotecx Labs, Houston, USA). An aliquot of total RNA was subjected to RT-competitive PCR for determination of PPARγ mRNA. Primer sequences and protocol of the RT-competitive PCR assays have been reported in detail elsewhere (43). On the other hand, cDNA was synthesized from 2.5 µg of total RNA-genomic DNA free using Superscript II (Invitrogen, USA). Appropriate dilutions of the cDNA stock were used for PCR. The sense and antisense primer sequences were 5′-TATGTGAGGATGCTGCTT-3′ and 5′-CTCGGAGAGCTAAGCTTG-3′ for CPT-I (629 bp product); 5′-GTGCTGGAGACCCCTGTGCCG-3′ and 5′-AGAATGGGGTGAAGCCCGGGA-3′ for leptin (*ob*) (206 bp product); 5′-AGCCTCGACAGCCTGCCCCAGG-3′ and 5′-CCAGTTGTTGACCAAAGGCTTTT-3′ for PEPCK (575 bp product); and 5′-ACCACAGTCCATGCCATCAC-3′ and 5′-TCCACCACCCTGTTGCTGTA-3′ for GAPDH (452 bp product). The amplification products were separated by agarose gel electrophoresis containing ethidium bromide. UV-stimulated fluorescence was captured using a digital videocamera and quantitated with the GS-700 Imaging Densitometer (BioRad, California, USA). Linearity of the PCR was tested by amplifying dilutions of the cDNA preparations for each gene and experimental group of rats. All experimental values were normalized to GAPDH.

Statistical analysis

Results are expressed as means ± S.E. Treatment effects were analyzed 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 analyzed by using the Student t test.

Acknowledgements

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