Opposite metabolic response to fenofibrate treatment in pregnant and virgin rats

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Abstract **The level of maternal circulating triglycerides during late pregnancy has been correlated to newborns' weight in humans. To investigate the response to fenofibrate, a hypotriglyceridemic agent, in pregnant rats, 0, 100, or 200 mg of fenofibrate/kg body weight as oral doses were given twice a day from day 16 of gestation and studied at day 20. Virgin rats were studied in parallel. Liver weight was higher in pregnant than in virgin rats, and either dose of fenofibrate increased this variable in both groups. The highest dose of fenofibrate decreased fetal weight. Although plasma triglycerides decreased during the first 2 days of fenofibrate treatment in pregnant rats, the effect disappeared on day 3, and plasma triglycerides were even enhanced at day 4. In virgin rats, fenofibrate decreased plasma triglycerides throughout the experiment. Plasma cholesterol levels in pregnant rats decreased during the first 3 days of treatment, and the effect disappeared on day 4, whereas in virgin rats, values remained decreased. Changes in plasma triglycerides paralleled those of VLDL triglycerides. In pregnant rats, VLDL cholesterol levels increased while LDL cholesterol decreased with the treatment, whereas in virgin rats, cholesterol levels decreased in all lipoprotein fractions. Only in virgin rats did liver triglyceride concentration increase with fenofibrate treatment. Lumbar adipose tissue LPL was lower in pregnant than in virgin rats, and fenofibrate treatment decreased this variable in both groups. Maternal fenofibrate treatment increased fetal plasma and liver triglyceride and cholesterol concentrations. It is proposed that the opposite effects of fenofibrate treatment in virgin and pregnant rats are a consequence of both the enhanced liver capability for VLDL triglyceride production and a rebound response to the drug in the latter.**—Soria, A., C. Bocos, and E. Herrera. **Opposite metabolic response to fenofibrate treatment in pregnant and virgin rats.** ***J. Lipid Res.*** **2002.** 43: **74–81.**

Supplementary key words pregnancy • fibrates • triglyceride • VLDL triglyceride • cholesterol • lipoprotein lipase • rat

Elevated plasma triglyceride levels and low HDL cholesterol levels have been shown to be independent risk factors for cerebrovascular and coronary heart disease (1–3), and fibrates have been effectively used to reduce these factors (4). The molecular bases for the action of fibrates on lipid metabolism have been recently elucidated (5), and involve the activation of transcriptional factors known as peroxisome proliferator-activated receptors (PPAR), especially the PPAR-α form that is expressed in liver. Throughout this mechanism, fibrates have been reported to decrease apolipoprotein (apo)C-III (6), to increase acyl-coenzyme A synthetase and fatty acid transport protein (7), to increase apoA-I and apoA-II (8, 9), and to induce the expression and activity of LPL in the liver (10). This latter effect occurs despite that under normal conditions, the adult liver does not express LPL, and it has been proposed that by reverting this situation, fibrates might enhance the liver clearance of triglyceride-rich lipoproteins (10), this effect contributing to their hypotriglyceridemic action.

Although these effects are clearly established, still there are aspects of fibrates' effects on lipid metabolism that remain to be understood, including their effects on liver lipid concentration, triglycerides, and cholesterol, which have been found to be either unchanged (11), decreased (12, 13), or even enhanced (14), although liver weight was consistently enhanced and, therefore, total lipid content may result augmented. Some fibrates also have been shown to have opposite effects on plasma and liver lipids depending on the degree of hypertriglyceridemia in the rats (14). In addition, fibrates have been shown to have different regulatory effects in rodents than in humans. Thus, whereas in rodents fibrates may decrease HDL cholesterol (14) as result of both a decreased expression of the genes for HDL apolipoproteins, apoA-I and apoA-II (15–18), as well as of the enzymes involved in their metabolism such as hepatic lipase and LCAT (19, 20), in humans they have a positive effect on HDL cholesterol as well as on apoA-I and apoA-II concentrations (21, 22). This different response seems to reside in differences between the two species in the apoA-I gene promoter (23). This different composition allows the induction of the apoA-I gene expression by fibrates to be mediated via the interaction

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of PPARα with its peroxisome proliferator response element (PPRE) in humans, whereas in rodents, this PPRE cannot be used by PPARα (24). In addition, whereas rats are responsive to hepatic peroxisome proliferation upon fibrate administration, humans seem to be a nonresponsive species (25), and differences in the amount of hepatic PPARα and/or the composition of PPRE in putative target genes have been proposed to explain interspecies differences in the fibrate response (25).

During late pregnancy, hypertriglyceridemia rather than hypercholesterolemia is consistently developed (26) as a consequence of enhanced adipose tissue lipolytic activity (27–30), enhanced liver production of VLDL (31, 32), and decreased extrahepatic LPL activity (33, 34). Although treatment with hypocholesterolemic drugs in pregnant rats has been shown to impair fetal growth (35, 36), no studies have been carried out to determine the effects of pharmacological reductions of circulating triglycerides, despite the proposed role of maternal hypertriglyceridemia on fetal growth in humans (32, 37).

The present work was therefore undertaken to determine how treatment with fenofibrate, as a hypotriglyceridemic agent, affects maternal lipidic metabolism, and to determine its consequences to fetal growth during late pregnancy in the rat. For comparison, normal virgin rats were studied in parallel, and results show that in addition to impairing fetal growth, fenofibrate responsiveness greatly differs between pregnant and virgin animals.

MATERIAL AND METHODS

Animals, drug administration, and collection of samples

Female Sprague Dawley rats from our animal quarter weighing 180–210 g, 55–60 days old, 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 in Madrid, Spain. One-half of the animals were mated (day 0 of pregnancy was determined when spermatozoids were found in vaginal smears), whereas the other half was kept virgin. From day 16 of gestation, rats were given, by oral gavage, two daily doses of 100 or 200 mg/kg body weight of fenofibrate (from Sigma, St. Louis, MO), one at 8:00 AM and the other at 6:00 PM, suspended in 2% Tween-80. Controls received only the medium by oral gavage. The doses of fenofibrate were chosen under the base of previous studies in the rat (6, 13, 17, 38). Just before the daily morning treatments, blood was collected from the tail into receptacles containing Na_2-EDTA, whereas on the morning of day 20 of pregnancy, 14 h after the last dose, rats were decapitated and blood was collected into tubes containing Na_2-EDTA. Liver and lumbar fat pads were immediately dissected, and aliquots of both tissues were placed into liquid nitrogen and kept at −80°C until analysis. The conceptus was also dissected and, after being weighed, fetuses were counted and weighed. Fetuses were decapitated and blood from all pups of the same mother was collected and pooled into receptacles containing Na_2-EDTA. Fetus livers were also dissected and placed in liquid nitrogen, and those from the same mother were pooled. Virgin rats received the same treatment and were always studied in parallel.

Determinations

Plasma aliquots were processed fresh for lipoprotein isolation by ultracentrifuge sequential fractionation following the method previously described (39), whereas other aliquots were kept at −30°C until processing for the analysis of triglycerides, cholesterol, and FFA by enzymatic commercial kits (Menarini, Menarini, and Wako, respectively).

Frozen liver aliquots were used for lipid extraction (40), and aliquots of lipid extracts were quantified after image analysis and separation by one-dimensional TLC (41) using the G5-700 BIOIMAGE TLC scanner of Bio-Rad (Hercules, CA). Spots were quantified as integrated optical densities against an internal standard of cholesteryl formate and calibration curves of triglyceride standards.

LPL activity was measured in acetone powders from frozen adipose tissue aliquots by the method previously described (42).

Expression of results and statistical evaluation

Results were expressed as means ± SEM. When necessary, data were log transformed to achieve equal variance among means. Treatment effects were analyzed by one-way ANOVA using a computer software system (Systat Version 5.03; Wilkinson, Evanston, IL). When treatment effects were significantly different ($P < 0.05$), means were tested by Tukey's test. Differences between two groups were analyzed by Student's *t*-test.

RESULTS

As shown in **Table 1**, treating rats twice daily with either 100 mg or 200 mg fenofibrate/kg body weight/day for 4 days did not modify net body weight in pregnant (free of conceptus) or virgin rats. Liver weight was higher in pregnant than in virgin rats, and either dose of fenofibrate increased this variable in both groups. Fenofibrate treatment did not modify fetal number, but although the 2 × 100 mg/kg body weight/day dose did not modify fetal body weight, this variable was significantly decreased with the 2 × 200 mg dose (Table 1).

The hypolipidemic effect of fenofibrate differed between pregnant and virgin rats. As shown in **Fig. 1**, in control virgin rats, plasma triglycerides remained unchanged throughout the experiment, whereas either dose of fenofibrate significantly decreased plasma triglyceride levels after day 1 of treatment, and the effect remained stable up to the end of the experiment (day 4 of treatment). However, in control pregnant rats, plasma triglycerides increased, although not significantly, at day 4 of the experiment, corresponding to day 20 of gestation. In addition, in pregnant rats, although plasma triglycerides were lower after day 1 and 2 of treatment with either dose of fenofibrate, the effect disappeared after day 3, and was even reverted in a dose-dependent manner after day 4. In fact, values in pregnant rats treated with the highest dose were significantly higher than in the same rats prior to the onset of treatment (day 0) as well as in control rats on the same day of gestation. In a different set of rats receiving the same treatment, blood was collected 2 h after the evening drug administration, and it was found that plasma triglyceride levels were consistently lower in fenofibrate-treated pregnant rats than in controls (data not shown),

TABLE 1. Effects of fenofibrate on body and liver weight in virgin and pregnant rats, and on fetal number and weight

	Dose[a]		
	0	100	200
Initial body weight (g)			
Virgin	192 ± 2	196 ± 2	196 ± 2
Pregnant	196 ± 3	195 ± 3	196 ± 3
Final net body weight (g)			
Virgin	242 ± 2	243 ± 3	244 ± 3
Pregnant	287 ± 4[b]	280 ± 6[b]	276 ± 5[b]
Liver weight (g)			
Virgin	9.66 ± 0.23	11.40 ± 0.22[c]	12.13 ± 0.30[d]
Pregnant	15.13 ± 0.40[b]	17.96 ± 0.32[b,d]	17.14 ± 0.23[b,d]
Fetus			
Number/litter	13.5 ± 0.7	13.9 ± 0.7	13.4 ± 0.4
Average body weight (g)	4.08 ± 0.08	4.00 ± 0.06	3.78 ± 0.05[c]

Results are mean ± SEM, n = 10/group.
[a] Dose given in mg/kg body weight/day. Two oral doses of fenofibrate were given per day.
[b] $P < 0.001$ from virgin rats.
[c] $P < 0.01$ from dose 0.
[d] $P < 0.001$ from dose 0.

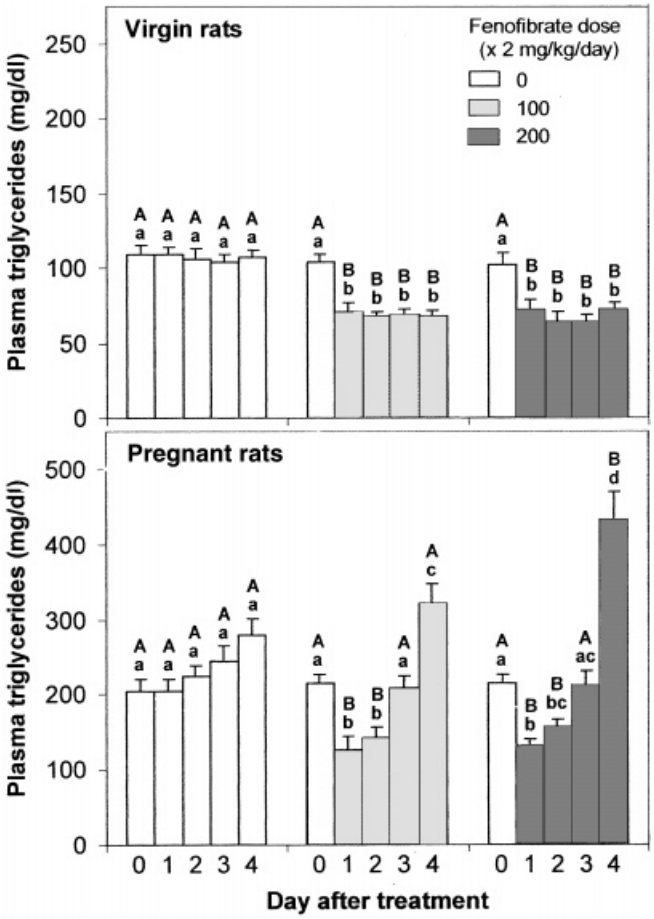

Fig. 1. Plasma triglyceride levels in virgin and pregnant rats at different days of treatment with fenofibrate. Values are means ± SEM, n = 10. Within-group statistical comparisons for rats receiving the same treatment at different days are shown by lowercase letters, whereas comparisons between groups receiving different treatments for virgin and pregnant rats are shown by uppercase letters. Different letters indicate significant differences between the groups ($P < 0.05$).

indicating that the enhanced values found in the morning extractions during the last days of treatment corresponded to a rebound effect. As shown in **Fig. 2**, the change in plasma cholesterol levels paralleled that of triglycerides in virgin rats because either dose of fenofibrate significantly decreased this variable after day 1 of treatment, and values remained stable until day 4. Plasma cholesterol levels in control pregnant rats remained stable throughout the experiment, whereas they decreased in treated rats after day 1, 2, and 3 of treatment, to return to basal values (day 0) on day 4 of treatment, the effect being similar with either dose (Fig. 2).

At the time of sacrifice, corresponding to 14 h after the last dose, plasma lipoprotein fractionation was secured. Lipoprotein distribution in virgin rats showed that the decrease in plasma triglycerides mainly corresponded to those of VLDL, although the effect in rats receiving the 2 × 100 mg dose did not reach statistical significance (**Fig. 3**). In pregnant rats, the increase in plasma triglycerides seen 14 h after the last treatment also corresponded to VLDL triglycerides, and although the difference in the rats receiving the 2 × 100 mg dose did not reach statistical significance, a significant increase in HDL triglycerides was also found in rats treated with the 2 × 200 mg dose (Fig. 3). As shown in **Fig. 4**, in virgin rats, the decrease in plasma cholesterol levels found 14 h after the last administration of fenofibrate (day 4) corresponds to a significant reduction in the cholesterol associated to all lipoprotein fractions, VLDL, LDL, and HDL, the effect being similar with either dose. However, in pregnant rats, fenofibrate treatment enhanced plasma VLDL cholesterol concentration and decreased LDL cholesterol levels without modifying HDL cholesterol, the effects being similar for either dose (Fig. 4).

To understand the changes taking place in plasma VLDL, liver lipids were measured. In virgin rats (**Table 2**), fenofibrate treatment greatly increased liver triglyceride concentration (mg/g) and content (mg/organ), whereas

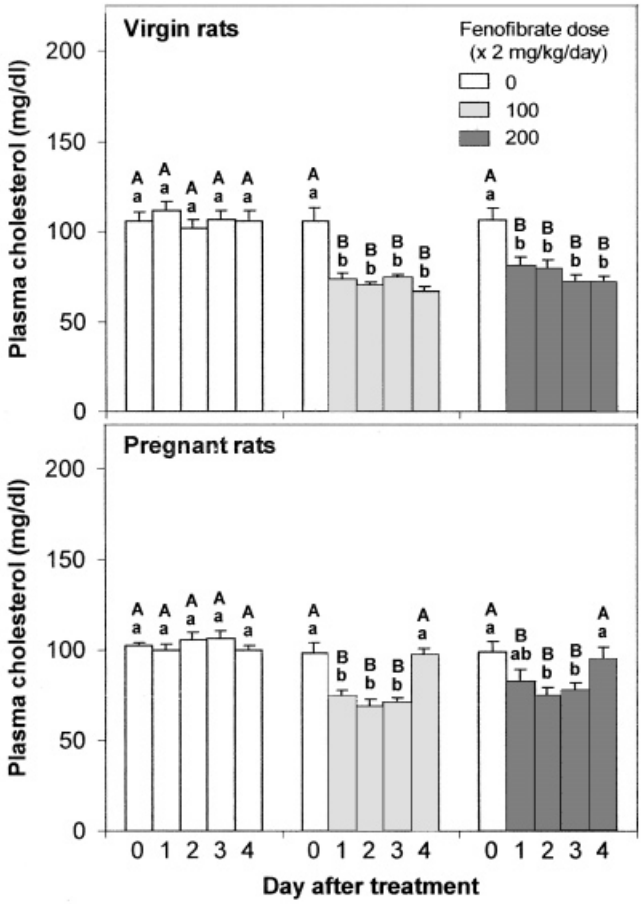

Fig. 2. Plasma cholesterol levels in virgin and pregnant rats at different days of treatment with fenofibrate. Values are means ± SEM, n = 10. Within-group statistical comparisons for rats receiving the same treatment at different days are shown by lowercase letters, whereas comparisons between groups receiving different treatments for virgin and pregnant rats are shown by uppercase letters. Different letters indicate significant differences between the groups ($P < 0.05$).

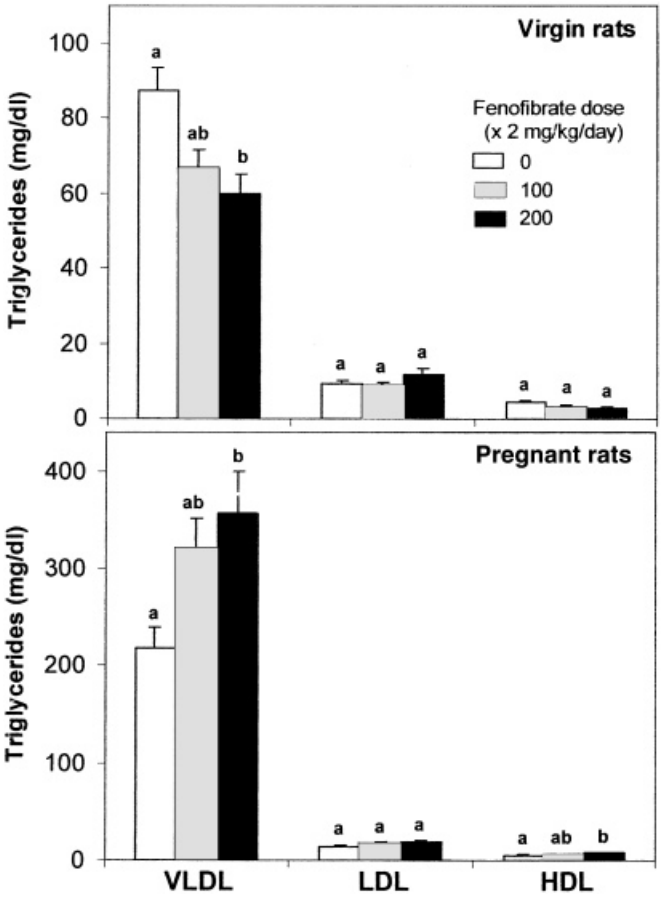

Fig. 3. Plasma triglycerides in VLDL, LDL, and HDL in virgin and 20-day pregnant rats after 4 days of treatment with fenofibrate. Values are means ± SEM, n = 8. Statistical comparison between rats receiving different doses, for the same variable, is shown by lowercase letters. Different letters indicate significant differences between the groups ($P < 0.05$).

liver cholesterol content (mg/organ) increased only under the 2 × 200 mg dose. However in pregnant rats, fenofibrate treatment did not modify either liver triglyceride or cholesterol concentrations, the values being significantly lower than in virgin rats, except for cholesterol values expressed per total organ content (Table 2).

To determine whether changes in liver triglycerides could be related to the amount of incoming FFA, plasma FFA concentrations were also measured (**Table 3**). This variable was always higher in pregnant than in virgin rats, and although fenofibrate treatment further increased plasma FFA in a dose-dependent manner in pregnant rats, no effect was found in virgin rats.

As it would be expected, lumbar adipose tissue LPL activity appeared lower in pregnant than in virgin rats not receiving the drug (**Table 4**). Fenofibrate treatment significantly decreased LPL activity in both groups of rats, and the effect in virgin rats was dose dependent. Because lumbar adipose tissue in rats receiving the fenofibrate treatment weighed less than in control rats, total tissue LPL activity in fenofibrate-treated rats decreased even more than when expressed per unit of fresh tissue (data not shown).

Maternal treatment with fenofibrate affected fetal lipid metabolism, as shown by increments in fetal plasma and liver triglyceride and cholesterol concentrations, the effect being especially marked and significant with the highest dose used (**Table 5**).

DISCUSSION

The present study shows that from the first day, and along 4 days of treatment with high doses of fenofibrate, plasma triglyceride and cholesterol levels decreased in virgin rats, the effect corresponding to specific reductions in plasma VLDL triglycerides and plasma cholesterol associated with all lipoprotein fractions. However, in pregnant rats, after an initial reduction, plasma triglycerides increased over values seen in untreated controls at the fourth day of treatment, and this effect corresponded to an increase in the triglyceride content both in VLDL and HDL, the effect being especially marked in the former.

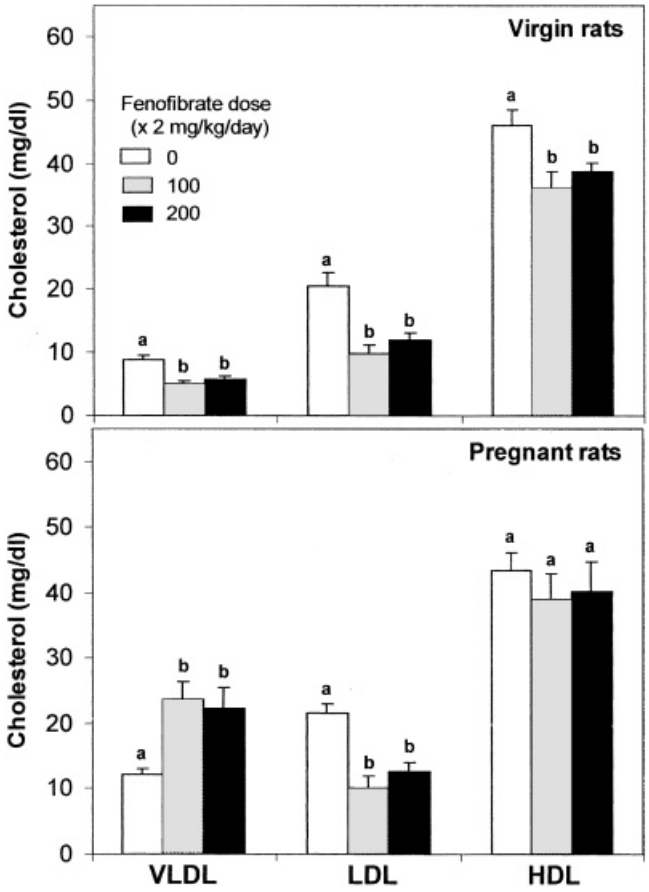

Fig. 4. Plasma cholesterol in VLDL, LDL, and HDL in virgin and 20-day pregnant rats after 4 days of treatment with fenofibrate. Values are means ± SEM, n = 8. Statistical comparison between rats receiving different doses, for the same variable, is shown by lowercase letters. Different letters indicate significant differences between the groups ($P < 0.05$).

Cholesterol levels also declined shortly after fenofibrate treatment in pregnant rats to return later on to basal values, the effect corresponding to an increase in VLDL cholesterol that was compensated by a reduction in LDL cholesterol. These changes are followed by major differences in the liver lipid content: an intense accumulation of triglycerides in the liver of virgin rats receiving the fenofibrate treatment, no changes in the concentration of cholesterol in these same rats, and unchanged liver triglyceride and cholesterol concentration in pregnant animals.

In virgin rats, the decrease in plasma VLDL triglycerides caused by fenofibrate seems to be the result of their decreased liver production, as suggested by the concomitant accumulation of liver triglycerides. This finding could be the result of the decreased liver VLDL triglyceride production (43) and/or an enhanced liver LPL activity (10) reported in rats receiving fibrate treatment. In fact, it has been shown previously that conditions where there is an increase in liver LPL activity such as in the fasted late pregnant rat or under Intralipid administration to fasted virgin rats, contribute to liver triglyceride accumulation (44, 45), switching the liver from a triglyceride exporter organ into an acceptor. An enhanced extrahepatic VLDL catabolism secondary to decreased hepatic apoC-III expression has been proposed to contribute to the fibrate-mediated triglyceride lowering (6). Because previous studies have demonstrated that LPL activity is inhibited by high amounts of apoC-III (46), it was proposed that reductions in plasma apoC-III levels would facilitate LPL-mediated VLDL catabolism (6). However, fibrates have shown lack of regulation of adipose tissue LPL in rats and in humans (10, 11, 47), and we found, even here, that high doses of fenofibrate caused significant reductions both in plasma VLDL triglycerides and adipose tissue LPL activity. Increments in adipose tissue LPL, however, have been reported in fibrate-treated rats (48), even by ourselves (12), but depending on the type of drug used and the dose, fibrates have been also reported to cause opposite effects on different variables (14).

In any case, because reductions of adipose tissue LPL were found here under conditions where fenofibrate had caused significant reductions in plasma VLDL triglycerides in normolipidemic virgin rats, no possibility exists to claim an enhanced removal of triglycerides unless LPL in

TABLE 2. Effects of fenofibrate treatment on liver triglycerides and cholesterol concentrations in virgin and pregnant rats

Dose[a]	Gestation	Liver Triglycerides		Liver Cholesterol	
		mg/g	*mg/organ*	*mg/g*	*mg/organ*
0	−	5.05 ± 0.41	49.9 ± 3.8	2.37 ± 0.07	22.8 ± 0.6
100	−	8.08 ± 1.00[b]	109.2 ± 10.5[d]	2.18 ± 0.08	23.4 ± 1.3
200	−	9.22 ± 1.22[c]	105.3 ± 13.2[d]	2.41 ± 0.09	26.3 ± 1.0[c]
0	+	2.33 ± 0.19[g]	35.9 ± 2.6[f]	1.52 ± 0.17[g]	23.2 ± 2.3
100	+	1.85 ± 0.19[g]	31.5 ± 2.5[g]	1.61 ± 0.15[f]	29.5 ± 2.2[e]
200	+	1.90 ± 0.10[g]	32.5 ± 1.3[g]	1.28 ± 0.13[g]	22.2 ± 2.1

Results are mean ± SEM, n = 10/group.
[a] Dose given in mg/kg body weight/day. Two oral doses of fenofibrate were given per day.
[b] $P < 0.05$ from dose 0.
[c] $P < 0.01$ from dose 0.
[d] $P < 0.001$ from dose 0.
[e] $P < 0.05$ from virgin rats.
[f] $P < 0.01$ from virgin rats.
[g] $P < 0.001$ from virgin rats.

TABLE 3. Effects of fenofibrate on plasma free fatty acids in virgin and pregnant rats

	Dose[a]		
Free Fatty Acids	0	100	200
	mM		
Virgin	0.181 ± 0.018	0.185 ± 0.017	0.171 ± 0.020
Pregnant	0.306 ± 0.021[b]	0.449 ± 0.034[b,c]	0.481 ± 0.035[b,d]

Results are mean ± SEM, n = 10/group.
[a] Dose given in mg/kg body weight/day. Two oral doses of fenofibrate were given per day.
[b] $P < 0.001$ from virgin rats.
[c] $P < 0.01$ from dose 0.
[d] $P < 0.001$ from dose 0.

other extrahepatic tissues such as skeletal muscle was enhanced. If this was the case, increments in plasma HDL cholesterol would have been predicted under the base of studies in humans (49, 50) and in rats (51); however, to the contrary, a reduction was clearly found here in agreement with similar results found by others after bezafibrate treatment (14), which also causes liver triglyceride accumulation. The mechanism for such effect is not yet known, but fenofibrate has been shown to decrease VLDL apolipoprotein synthesis (43), and it has been shown by us and others that fibric acid derivatives in the nonpregnant rat decrease liver VLDL triglycerides production (11, 12).

This is the first time that the effect of fenofibrate has been studied in late pregnant rats where a hypertriglyceridemic condition is normally present. This condition is caused by enhanced liver VLDL triglycerides production (31), partially supported by an enhanced arrival of FFA and glycerol to the liver as result of enhanced adipose tissue lipolytic activity (30, 52), and decreased circulating clearance of these lipoproteins due to decreased adipose tissue LPL (33, 53). The increase in VLDL triglycerides seen here after 4 days of treatment with fenofibrate in pregnant rats instead of the decrease seen in virgin rats may result from the enhanced VLDL triglycerides production capability of the mother (54, 55). Such increased VLDL triglycerides production in the liver of the fenofibrate-treated pregnant rats allows these animals to avoid the liver triglyceride accumulation seen in virgin rats, and must be supported by an enhanced arrival of FFA to the liver. This possibility is supported by the fact that significant linear correlations are found when individual values of plasma FFA (Table 3) are plotted against plasma triglycerides in pregnant rats receiving 2 × 100 mg fenofibrate/kg body weight/day (FFA = 0.0011 TG + 0.01, n = 13, R^2 = 0.6729, $P < 0.01$) or 2 × 200 mg fenofibrate/kg body weight/day (FFA = 0.0008 TG + 0.0661, n = 14, R^2 = 0.6722, $P < 0.01$), whereas this relationship was not significant in the case of pregnant controls or in virgin rats being treated or not (data not shown). Increments in plasma VLDL triglycerides in fenofibrate-treated pregnant rats are followed by parallel changes in VLDL cholesterol, which together with decreased LDL cholesterol levels, fit with the decreased adipose tissue LPL activity seen in these animals, suggesting that the conversion of VLDL to LDL is impaired in these animals.

If the action of fibrates on lipid metabolism is primarily mediated by its role as ligands for PPARα (56), the enhanced arrival of FFA (which are also ligands for PPARα) (56) to the liver in the late pregnant rat (52) 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. Furthermore, a displacement of fibrates for binding to fatty acid-binding protein by FFA, which would be influenced by their intracellular concentration, cannot be ruled out (57). On the other hand, recent evidence suggests that alteration of gene transcription by FFA and fibrates is often disconnected (58). Thus, the enhanced arrival of FFA to liver in pregnant rats, along with fibrate, might be affecting an additional battery of genes in which transcription would be altered, in comparison to the situation in virgin rats.

Increments in plasma triglycerides seen in pregnant rats after 4 days of treatment with fenofibrate were preceded by maintained hypotriglyceridemia during the first 2 days of treatment, and occurred 14 h after the last fenofibrate administration when the drug still caused a transitory hypotriglyceridemic effect. Thus, such hypertriglyceridemia appears to be the result of a rebound effect that takes place during late pregnancy when the mother is known to be in a catabolic condition (26) and when adipose tissue lipolytic activity is enhanced (30, 59, 60). Such

TABLE 4. Effects of fenofibrate on lumbar adipose tissue LPL activity in virgin and pregnant rats

	Dose[a]		
LPL	0	100	200
	pkatal/g of fresh tissue		
Virgin	1,074 ± 300	595 ± 146[b]	276 ± 45[c]
Pregnant	854 ± 135[d]	368 ± 38[b]	340 ± 86[b]

Results are mean ± SEM, n = 5/group.
[a] Dose given in mg/kg body weight/day. Two oral doses of fenofibrate were given per day.
[b] $P < 0.05$ from dose 0.
[c] $P < 0.01$ from dose 0.
[d] $P < 0.05$ from virgin rats.

TABLE 5. Effects of maternal treatment with fenofibrate on fetal plasma and liver lipids

	Dose[a]		
	0	100	200
Plasma triglycerides (mg/dl)	83.5 ± 2.8	85.1 ± 2.9	94.3 ± 3.1[b]
Plasma cholesterol (mg/dl)	83.1 ± 4.2	83.3 ± 3.3	98.6 ± 5.2[b]
Liver triglycerides (mg/g)	1.52 ± 0.09	2.48 ± 0.13[d]	2.51 ± 0.10[d]
Liver cholesterol (mg/g)	0.74 ± 0.01	0.92 ± 0.04[c]	1.17 ± 0.04[d]

Results are mean ± SEM, n = 10/group for plasma lipids; n = 6/group for liver lipids.
[a] Dose given in mg/kg body weight/day. Two oral doses of fenofibrate were given per day.
[b] $P < 0.05$ from dose 0.
[c] $P < 0.01$ from dose 0.
[d] $P < 0.001$ from dose 0.

a rebound effect has not been previously reported for fenofibrate, but there are reports of rebound effects of other hypolipidemic antilypolitic drugs such as Acipimox after semichronic treatments in humans having stimulated adipose tissue lipolysis such as non-insulin-dependent diabetes mellitus patients (61). Although we do not know whether the hypotriglyceridemia followed by hypertriglyceridemia caused by fenofibrate treatment in the late pregnant rat would affect fetal growth, a direct effect of the drug to the fetus and/or a response of the fetus to maternal alterations in lipoprotein metabolism cannot be discarded. In fact, although neither VLDL triglycerides nor cholesterol directly seem to cross the placental barrier in the rat (62–64), the presence of lipase activities in the placenta allows conditions of maternal-exaggerated hypertriglyceridemia to increase fetal triglycerides (53). In addition, maternal cholestyramine feeding, a nonabsorbable bile acid binding resin, is known to induce 3-hydroxy-3-methylglutaryl coenzyme A reductase and fatty acid synthetase activities and the consequent cholesterol and fatty acid synthesis in the fetus (65, 66). Therefore, the increments of plasma and liver cholesterol and triglyceride concentrations seen here in fetus of dams treated with fenofibrate could be a consequence of the drug-induced perturbation of maternal lipoprotein metabolism. Although a similar explanation could be given to justify the negative effect of the highest dose of fenofibrate on fetal body weight, a direct effect of the drug crossing the placenta on fetal development cannot be discarded, as has been already shown for clofibrate (67).

Thus, present results show opposite effects of fenofibrate treatment in virgin and pregnant rats, the main responsible factor for the difference being both the known enhanced liver capability for VLDL triglyceride production and a rebound response to the drug in the latter.

This work was supported by grants from the Fondo de Investigación Sanitaria, Instituto de Salud Carlos III (98/0158 and 99/0205), Universidad San Pablo-CEU (10/99-00) of Spain, and the European Community (FATLINK, FAIR-CT-98-4141). Ana Soria was recipient of a postdoctoral fellowship from the Universidad Nacional del Litoral, Santa Fe, Argentina. The excellent technical assistance of Milagros Morante and the editorial help of Dr. Beatriz Ramos are greatly appreciated.

Manuscript received 10 April 2001, in revised form 20 July 2001, and in re-revised form 10 October 2001.

REFERENCES

1. Havel, R. 1994. McCollum Award Lecture, 1993: Triglyceride-rich lipoproteins and atherosclerosis—new perspectives. *Am. J. Clin. Nutr.* **59:** 795–799.
2. Lindenstrom, E., G. Boysen, and J. Nyboe. 1994. Influence of total cholesterol, high density lipoprotein cholesterol, and triglycerides on risk of cerebrovascular disease: the Copenhagen City Heart study. *Br. Med. J.* **309:** 11–15.
3. Zilversmit, D. B. 1995. Atherogenic nature of triglycerides, postprandial lipidemia, and triglyceride-rich remnant lipoproteins. *Clin. Chem.* **41:** 153–158.
4. Watts, G. F., and S. B. Dimmitt. 1999. Fibrates, dyslipoproteinaemia and cardiovascular disease. *Curr. Opin. Lipidol.* **10:** 561–574.
5. Staels, B., J. Dallongeville, J. Auwerx, K. Schoonjans, E. Leitersdorf, and J. G. Fruchart. 1998. Mechanism of action of fibrates on lipid and lipoprotein metabolism. *Circulation.* **98:** 2088–2093.
6. Haubenwallner, S., A. D. Essenburg, B. C. Barnett, M. E. Pape, R. B. DeMattos, B. R. Krause, L. L. Minton, B. J. Auerbach, R. S. Newton, T. Leff, and C. L. Bisgaier. 1995. Hypolipidemic activity of select fibrates correlates to changes in hepatic apolipoprotein C–III expression: a potential physiologic basis for their mode of action. *J. Lipid Res.* **36:** 2541–2551.
7. Martin, G., K. Schoonjans, and A. Lefebvre. 1997. Coordinate regulation of the expression of the fatty acid transporter protein (FATP) and acyl CoA synthetase (ACS) genes by PPARα and PPARγ activators. *J. Biol. Chem.* **272:** 28210–28217.
8. Vu-Dac, N., S. Chopin-Delannoy, P. Gervois, E. Bonnelye, G. Martin, J. C. Fruchart, V. Laudet, and B. Staels. 1998. The nuclear receptors peroxisome proliferator-activated receptor α and Rev-erbα mediate the species-specific regulation of apolipoprotein A-I expression by fibrates. *J. Biol. Chem.* **273:** 25713–25720.
9. Vu-Dac, N., K. Schoonjans, V. Kosykh, J. Dallongeville, J-C. Fruchart, B. Staels, and J. Auwerx. 1995. Fibrates increase human apolipoprotein A-II expression through activation of the peroxisome proliferator-activated receptor. *J. Clin. Invest.* **96:** 741–750.
10. Schoonjans, K., J. Peinado-Onsurbe, A. M. Lefebvre, R. A. Heyman, M. Briggs, S. Deeb, B. Staels, and J. Auwerx. 1996. PPARα and PPARγ activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. *EMBO J.* **15:** 5336–5348.
11. Nagao, K., M. Sakono, M. Nakayama, T. Hirakawa, and K. Imaizumi. 1999. Effect of gemfibrozil on triacylglycerol synthesis and secretion by liver and lipoprotein lipase activity in adipose tissue of rats. *Comp. Biochem. Physiol. [B].* **124:** 289–294.
12. Herrera, E., M. A. Lasunción, M. Castro, D. Gomez Coronado, A. Martín, and G. Quack. 1988. Studies with etofibrate in the rat. Part I: effects on glycerol, free fatty acid and triacylglycerol metabolism. *Biochim. Biophys. Acta.* **963:** 42–52.
13. Tsoko, M., F. Beauseigneur, J. Gresti, J. Demarquoy, and P. Clouet. 1998. Hypolipidaemic effects of fenofibrate are not altered by mildronate-mediated normalization of carnitine concentration in rat liver. *Biochimie.* **80:** 943–948.
14. Krause, B. R., B. C. Barnett, A. D. Essenburg, K. A. Kieft, B. J. Auerbach, R. Bousley, R. Stanfield, R. S. Newton, and C. L. Bisgaier. 1996. Opposite effects of bezafibrate and gemfibrozil in both normal and hypertriglyceridemic rats. *Atherosclerosis.* **127:** 91–101.
15. Staels, B., A. Van Tol, T. Andreu, and J. Auwerx. 1992. Fibrates influence the expression of genes involved in lipoprotein metabolism in a tissue-selective manner in the rat. *Arterioscler. Thromb. Vasc. Biol.* **12:** 286–294.
16. Staels, B., A. Van Tol, G. Verhoeven, and J. Auwerx. 1990. Apolipoprotein A-IV messenger ribonucleic acid abundance is regulated in a tissue-selective manner by fibrates. *Endocrinology.* **126:** 2153–2163.
17. Lefebvre, A. M., J. Peinado-Osborne, I. Leitersdorf, M. R. Briggs, J. R. Paterniti, J. Ch. Fruchart, C. Fievet, J. Auwerx, and B. Staels. 1997. Regulation of lipoprotein metabolism by thiazolidinediones occurs through a distinct but complementary mechanism relative to fibrates. *Arterioscler. Thromb. Vasc. Biol.* **17:** 1756–1764.
18. Berthou, L., R. Saladin, P. Yaqoob, D. Branellec, P. Calder, J. C. Fluchart, P. Denèfle, J. Auwerx, and B. Staels. 1995. Regulation of rat liver apolipoprotein A-I, apolipoprotein A-II and acyl-coenzyme A oxidase gene expression by fibrates and dietary fatty acids. *Eur. J. Biochem.* **232:** 179–187.
19. Staels, B., J. Peinado-Onsurbe, and J. Auwerx. 1992. Down-regulation of hepatic lipase gene expression and activity by fenofibrate. *Biochim. Biophys. Acta.* **1123:** 227–230.
20. Staels, B., A. Van Tol, G. Skretting, and J. Auwerx. 1992. Lecithin:cholesterol acyltransferase gene expression is regulated in a tissue-selective manner by fibrates. *J. Lipid Res.* **33:** 727–735.
21. Lussier-Cacan, S., J. M. Bard, L. Boulet, A. C. Nestruck, A. M. Grothé, J. C. Fruchart, and J. Davignon. 1989. Lipoprotein composition changes induced by fenofibrate in dysbetalipoproteinemia type III. *Atherosclerosis.* **78:** 167–182.
22. Berthou, L., N. Duverger, F. Emmanuel, S. Langouët, J. Auwerx, A. Guillouzo, J. C. Fruchart, E. Rubin, P. Denèfle, B. Staels, and D. Branellec. 1996. Opposite regulation of human versus mouse apolipoprotein A-I by fibrates in human apolipoprotein A-I transgenic mice. *J. Clin. Invest.* **97:** 2408–2416.
23. Vu-Dac, N., K. Schoonjans, B. Laine, J-C. Fruchart, J. Auwerx, and

B. Staels. 1994. Negative regulation of the human apolipoprotein A-I promoter by fibrates can be attenuated by the interaction of the peroxisome proliferator-activated receptor with its response element. *J. Biol. Chem.* **269:** 31012–31018.
24. Staels, B., and J. Auwerx. 1998. Regulation of apo A-I gene expression by fibrates. *Atherosclerosis.* **137:** S19–S23.
25. Holden, P. R., and J. D. Tugwood. 1999. Peroxisome proliferator-activated receptor alpha: role in rodent liver cancer and species differences. *J. Mol. Endocrinol.* **22:** 1–8.
26. Herrera, E. 2000. Metabolic adaptations in pregnancy and their implications for the availability of substrates to the fetus. *Eur. J. Clin. Nutr.* **54 (Suppl. 1):** S47–S51.
27. Elliott, J. A. 1975. The effect of pregnancy on the control of lipolysis in fat cells isolated from human adipose tissue. *Eur. J. Clin. Invest.* **5:** 159–163.
28. Williams, C., and T. M. Coltart. 1978. Adipose tissue metabolism in pregnancy: the lipolytic effect of human placental lactogen. *Br. J. Obstet. Gynaecol.* **85:** 43–46.
29. Chaves, J. M., and E. Herrera. 1978. In vitro glycerol metabolism in adipose tissue from fasted pregnant rats. *Biochem. Biophys. Res. Commun.* **85:** 1299–1306.
30. Knopp, R. H., E. Herrera, and N. Freinkel. 1970. Carbohydrate metabolism in pregnancy. VIII. Metabolism of adipose tissue isolated from fed and fasted pregnant rats during late gestation. *J. Clin. Invest.* **49:** 1438–1446.
31. Wasfi, I., I. Weinstein, and M. Heimberg. 1980. Increased formation of triglyceride from oleate in perfused livers from pregnant rats. *Endocrinology.* **107:** 584–596.
32. Skryten, A., P. Johnson, G. Samsioe, and A. Gustafson. 1976. Studies in diabetic pregnancy. I. Serum lipids. *Acta Obst. Gynecol. Scand.* **55:** 211–215.
33. Otway, S., and D. S. Robinson. 1968. The significance of changes in tissue clearing-factor lipase activity in relation to the lipaemia of pregnancy. *Biochem. J.* **106:** 677–682.
34. Martin-Hidalgo, A., C. Holm, P. Belfrage, M. C. Schotz, and E. Herrera. 1994. Lipoprotein lipase and hormone-sensitive lipase activity and mRNA in rat adipose tissue during pregnancy. *Am. J. Physiol.* **266:** E930–E935.
35. Hrab, R. V., H. A. Hartman, and R. H. Cox, Jr. 1994. Prevention of fluvastatin-induced toxicity, mortality, and cardiac myopathy in pregnant rats by mevalonic acid supplementation. *Teratology.* **50:** 19–26.
36. Henck, J. W., W. R. Craft, A. Black, J. Colgin, and J. A. Anderson. 1998. Pre- and postnatal toxicity of the HMG-CoA reductase inhibitor atorvastatin in rats. *Fundam. Appl. Toxicol.* **41:** 88–99.
37. Knopp, R. H., R. O. Bergelin, P. W. Wahl, and C. E. Walden. 1985. Relationships of infant birth size to maternal lipoproteins, apoproteins, fuels, hormones, clinical chemistries, and body weight at 36 weeks gestation. *Diabetes.* **34 (Suppl. 2):** 71–77.
38. Dvornik, D., and M. N. Cayen. 1980. Drugs affecting lipoprotein disposition in laboratory animals. *In* Drugs Affecting Lipid Metabolism. R. Fumagalli, D. Kritchevsky, and R. Paoletti, editors. Elsevier/North-Holland Biomedical Press, Amsterdam. 263–272.
39. Munilla, M. A., and E. Herrera. 1997. A cholesterol-rich diet causes a greater hypercholesterolemic response in pregnant than in nonpregnant rats and does not modify fetal lipoprotein profile. *J. Nutr.* **127:** 2239–2245.
40. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **22:** 24–36.
41. Ruiz, J. I., and B. Ochoa. 1997. Quantification in the subnanomolar range of phospholipids and neutral lipids by monodimension thin-layer chromatography and image analysis. *J. Lipid Res.* **38:** 1482–1489.
42. Llobera, M., A. Montes, and E. Herrera. 1979. Lipoprotein lipase activity in liver of the rat fetus. *Biochem. Biophys. Res. Commun.* **91:** 272–277.
43. Petit, D., M. T. Bonnefis, C. Rey, and R. Infante. 1988. Effects of ciprofibrate and fenofibrate on liver lipids and lipoprotein synthesis in normo- and hyperlipidemic rats. *Atherosclerosis.* **74:** 215–225.
44. Herrera, E., M. A. Lasunción, D. Gomez Coronado, P. Aranda, P. Lopez Luna, and I. Maier. 1988. Role of lipoprotein lipase activity on lipoprotein metabolism and the fate of circulating triglycerides in pregnancy. *Am. J. Obstet. Gynecol.* **158:** 1575–1583.
45. Vilaró, S., M. Reina, I. Ramírez, and M. Llobera. 1986. Intralipid administration induces a lipoprotein lipase-like activity in the livers of starved adult rats. *Biochem. J.* **236:** 273–278.
46. Wang, C. S., W. J. McConathy, H. U. Kloer, and P. Alaupovic. 1985. Modulation of lipoprotein lipase activity by apolipoproteins. Effect of apolipoprotein C-III. *J. Clin. Invest.* **75:** 384–390.
47. Simsolo, R. B., J. M. Ong, and P. A. Kern. 1993. Effect of gemfibrozil on adipose tissue and muscle lipoprotein lipase. *Metabolism.* **42:** 1486–1491.
48. Tolman, E. L., H. M. Tepperman, and J. Tepperman. 1970. Effect of ethyl *p*-chlorophenoxyisobutyrate on rat adipose lipoprotein lipase activity. *Am. J. Physiol.* **218:** 1313–1318.
49. Arnon, R., E. Sehayek, and S. Eisenberg. 1993. Disparate effects of a triglyceride lowering diet and of bezafibrate on the HDL system: a study in patients with hypertriglyceridaemia and low HDL-cholesterol levels. *Eur. J. Clin. Invest.* **23:** 492–498.
50. Murdoch, S. J., and W. C. Breckenridge. 1995. Influence of lipoprotein lipase and hepatic lipase on the transformation of VLDL and HDL during lipolysis of VLDL. *Atherosclerosis.* **118:** 193–212.
51. Tsutsumi, K., Y. Inoue, A. Shima, K. Iwasaki, M. Kawamura, and T. Murase. 1993. The novel compound NO-1886 increases lipoprotein lipase activity with resulting elevation of high density lipoprotein cholesterol, and long-term administration inhibits atherogenesis in the coronary arteries of rats with experimental atherosclerosis. *J. Clin. Invest.* **92:** 411–417.
52. Mampel, T., F. Villarroya, and E. Herrera. 1985. Hepatectomy-nephrectomy effects in the pregnant rat and fetus. *Biochem. Biophys. Res. Commun.* **13:** 1219–1225.
53. Soria, A., A. Chicco, N. Mocchiutti, R. A. Gutman, Y. B. Lombardo, A. Martin-Hidalgo, and E. Herrera. 1996. A sucrose-rich diet affects triglyceride metabolism differently in pregnant and nonpregnant rats and has negative effects on fetal growth. *J. Nutr.* **126:** 2481–2486.
54. Gastaldelli, A., S. Baldi, M. Pettiti, E. Toschi, S. Camastra, A. Natali, B. R. Landau, and E. Ferrannini. 2000. Influence of obesity and type 2 diabetes on gluconeogenesis and glucose output in humans—a quantitative study. *Diabetes.* **49:** 1367–1373.
55. Knopp, R. H., B. Bonet, M. A. Lasunción, A. Montelongo, and E. Herrera. 1992. Lipoprotein metabolism in pregnancy. *In* Perinatal Biochemistry. E. Herrera and R.H. Knopp, editors. CRC Press, Boca Raton, FL. 19–51.
56. Forman, B. M., J. Chen, and R. M. Evans. 1997. Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors α and δ. *Proc. Natl. Acad. Sci. USA.* **94:** 4312–4317.
57. Bocos, C., M. Göttlicher, K. Gearing, C. Banner, E. Enmark, M. Teboul, A. Crickmore, and J. A. Gustafsson. 1995. Fatty acid activation of peroxisome proliferator-activated receptor (PPAR). *J. Steroid Biochem. Mol. Biol.* **53:** 467–473.
58. Duplus, E., M. Glorian, and C. Forest. 2000. Fatty acid regulation of gene transcription. *J. Biol. Chem.* **275:** 30749–30752.
59. Aitchison, R. E., R. A. Clegg, and R. G. Vernon. 1982. Lipolysis in rat adipocytes during pregnancy and lactation. The response to noradrenaline. *Biochem. J.* **202:** 243–247.
60. Jarnfelt Samsioe, A., B. Eriksson, L. A. Mattsson, and G. Samsioe. 1987. Serum lipids and lipoproteins in pregnancies associated with emesis gravidarum. *Gynecol. Endocrinol.* **1:** 51–60.
61. Worm, D., J. E. Henriksen, A. Vaag, P. Thye-Ronn, A. Melander, and H. Beck-Nielsen. 1994. Pronounced blood glucose-lowering effect of the antilipolytic drug acipimox in non-insulin-dependent diabetes mellitus patients during a 3-day intensified treatment period. *J. Clin. Endocrinol. Metab.* **78:** 717–721.
62. Belknap, W. M., and J. M. Dietschy. 1988. Sterol synthesis and low density lipoprotein clearance in vivo in the pregnant rat, placenta, and fetus. Sources for tissue cholesterol during fetal development. *J. Clin. Invest.* **82:** 2077–2085.
63. Herrera, E., B. Bonet, and M. A. Lasunción. 1998. Maternal-fetal transfer of lipid metabolites. *In* Fetal and Neonatal Physiology. R. A. Polin and W. W. Fox, editors. W. B. Saunders, Philadelphia. 447–458.
64. Jurevics, H. A., F. Z. Kidwai, and P. Morell. 1997. Sources of cholesterol during development of the rat fetus and fetal organs. *J. Lipid Res.* **38:** 723–733.
65. Haave, N. C., and S. M. Innis. 1991. Hepatic cholesterol and fatty acid synthesis in pregnant and fetal rats: effect of maternal dietary fat and cholestyramine. *J. Nutr.* **121:** 1529–1535.
66. Haave, N. C., and S. M. Innis. 1988. Induction of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in foetal rats by maternal cholestyramine feeding. *J. Dev. Physiol.* **10:** 247–255.
67. Simpson, A. E. C. M., W. J. Brammar, M. K. Pratten, N. Cockcroft, and C. R. Elcombe. 1996. Placental transfer of the hypolipidemic drug, clofibrate, induces *CYP4A* expression in 18.5-day fetal rats. *Drug Metab. Dispos.* **24:** 547–554.