Circulating Triacylglycerols, Lipoproteins, and Tissue Lipoprotein Lipase Activities in Rat Mothers and Offspring During the Perinatal Period: Effect of Postmaturity

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Mammary gland and adipose tissue lipoprotein lipase activities have been implicated in the changes of circulating triacylglycerol levels which occur in the mother at late gestation. In the newborn the temporal accumulation of triacylglycerols in the liver coincides with the appearance of a lipoprotein lipase peak. The relationships of these changes with the rise in circulating prolactin in the mother before parturition and the extrauterine nutritional status in the offspring were studied in a postmaturity model produced in the rat by subcutaneous injection of 7 mg progesterone/day to pregnant animals from the 20th day of gestation. Pregnant controls received the medium. Parturition occurred at day 21.5 of gestation in pregnant controls while it did not occur before the 23rd day in those receiving progesterone. At the 20th day of gestation, plasma triacylglycerol concentrations and all lipoprotein fractions (especially VLDL) were much higher in mothers not receiving progesterone than in age-matched virgins, and these differences disappeared at the 21st day of gestation. Lipoprotein lipase activity was maintained low in control mothers' adipose tissue until the 23rd postfecundation day while it greatly increased in mammary gland from parturition time. In progesterone treated mothers, both triacylglycerol and lipoprotein fractions (especially VLDL) in plasma were maintained elevated until the 23rd postfecundation day and adipose tissue and mammary gland lipoprotein lipase activities were maintained low until this time. Circulating prolactin levels increased around parturition in control mothers while they did not change in the progesterone treated mothers at any of the times studied. In offspring from control mothers, plasma triacylglycerols were low and their most abundant circulating lipoprotein fraction appeared to be LDL. In contrast to mother's liver, in offspring liver a marked lipase activity with all the inhibitory characteristics of lipoprotein lipase in the presence of both NaCl and protamine sulfate appeared around birth, coinciding with a sharp increase in liver triacylglycerol concentration and plasma ketone body levels. In postmature fetuses, liver lipoprotein lipase activity and triacylglycerol content decreased from the 20th to the 23rd postfecundation day while levels of both acetoacetate and beta-hydroxybutyrate rose in blood. Results indicate that increased mammary gland lipoprotein lipase in the mother may play a role in the decline of hypertriglycerolemia before parturition and that the initial change is driven by the rise in circulating prolactin at that time. On the contrary, the reduction in adipose tissue lipoprotein lipase activity in late gestation is not associated with the increase in circulating prolactin levels. We speculate that in offspring from control mothers, the high fat content of the mother's milk produces a substrate induction mechanism on liver lipoprotein lipase converting the liver of the newborn into a temporary lipid storage organ. Consumption of endogenous resources and maintenance of maternal ketone bodies transfer through the placenta ensure development of postmature fetus.

IN THE LATTER HALF of pregnancy maternal lipids increase[1-7] as result of an increase in their mobilization from adipose tissue,[7-10] and enhanced production of endogenous triacylglycerol,[11] and a reduced removal of triacylglycerol-rich lipoproteins due to decreased activity of adipose tissue lipoprotein lipase (EC 3.1.1.34),[4,12-14] all of which produce a specific hypertriacylglycerolemia and increases in lipoproteins of density <1.006 at late gestation.[4,12,15-19] At parturition, plasma triacylglycerol levels return to normal as result of both a decrease in the rate of entry of triacylglycerol into the circulation[20] and a diversion of blood triacylglycerol fatty acids from storage in adipose tissue to milk production in mammary gland,[4] as suggested by the increase lipoprotein lipase in the mammary gland[12,21,22] and the low activity in adipose tissue[4,12,13] still found at this stage. The mechanism which regulates some of these changes in the mother is probably controlled by hormonal variations occurring around parturition. It has recently been proposed[23] that changes in enzyme activities in rat mammary glands during that phase are indirectly triggered by the well-know decline in plasma progesterone concentration and the subsequent release of prolactin that occurs during the last 2 days of pregnancy.[24-26] It has also been suggested that prolactin mediates the control of lipoprotein lipase activity in both mammary gland and adipose tissue during late pregnancy and lactation in the rat.[17,27] Lipid metabolism in the offspring also changes very dramatically during the perinatal phase although the mechanisms which control these changes are unclear. In the offspring of different species including the rat it has been shown that liver content of triacylglycerol is low in the fetus but increases rapidly around parturition[7,28-30] to decrease later to adult values. We have reported the transitory presence of

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lipoprotein lipase activity in the liver during the perinatal phase in the rat[13] that would convert the newborn liver into a triacylglycerol utilizer instead of an exporter as it occurs in the adult. At birth, the plasma concentration of triacylglycerol in the offspring is low rising immediately thereafter[29,31] and the plasma lipoprotein pattern also differs from that in adults, containing only negligible amounts of very low density lipoproteins (VLDL) at birth both in humans[32,33] and in the rat.[19] As these lipoproteins are the main transport system for triacylglycerols from endogenous origin these findings could fit with the temporal retention of triacylglycerol in the liver of the newborn. The present work was performed to determine whether some of these changes in both rat mother and offspring are directly affected by parturition. For this study, postmaturity was induced by treating the pregnant rat with progesterone to avoid the late rise in circulating prolactin and parturition, without affecting offspring growth. Circulating lipoproteins and lipoprotein lipase activities were measured in both mother and offspring and were compared with animals of the same postconceptual age receiving no treatment and with virgin adults treated or not with progesterone. Results indicate that prolactin rise before parturition modulates changes in circulating triacylglycerol levels and mammary gland lipoprotein lipase activity but not lipoprotein lipase activity in adipose tissue in the mother while extrauterine nutritional environment is the main factor modulating lipidic metabolism in the newborn.

MATERIALS AND METHODS

Female Wistar rats from our own colony were mated at 2 mo-of-age (160–180 g) and maintained in a controlled environment (23 ± 2°C, 12 hr light–dark cycles). From day 20 of gestation (estimated by the appearance of spermatozoids in vaginal smears), half of the pregnant rats received a daily subcutaneous injection of progesterone (7 mg dissolved in 0.2 ml of ricine-oil/rat/day) while the remaining half received only the oil. The mothers and their fetuses or pups were sacrificed by decapitation at different postfecundation times (20,21,22 or 23 days). Age- and sex-matched virgin rats were treated or not with either progesterone (7 mg/rat/day) or oil for 3 days and sacrificed by decapitation. Blood was collected from the neck into two different receptacles, one containing Na_2-EDTA, and the other containing heparin. Blood from offspring was always pooled for all pups from the same mother. A piece of liver, lumbar fat pads, and mammary gland from mothers or virgin adult rats were rapidly exised and placed in liquid N_2 immediately after decapitation and one body per litter (without head and liver) was also placed into liquid N_2 for carcass determinations.

Plasma aliquots (25 μl/plate) from the blood collected in Na_2-EDTA were used for electrophoresis in 1% agarose (Sigma Co., St. Louis, USA) and processed as previously described.[34,35] After fixation with ethanol:water: acetic acid (15:4:1 by volume), the slides were stained with Sudan Black B and the lipoprotein fractions were estimated by densitometry. Electrophoretic mobility and floating characteristics in ultracentrifugation under different densities were previously estimated in plasma samples from pregnant and offspring rats[19] to validate the electrophoretic method used here. Other plasma aliquots were used for the estimation of the triacylglycerols after alkaline hydrolysis,[36] using 0.15 M $MgSO_4$ instead $HClO_4$ for precipitation after the hydrolysis, and for prolactin which was measured by the specific RIA developed for the rat by NIAMDD of the NIH, USA. Aliquots of whole blood from the samples collected with heparin were used for protein precipitation[37] and both acetoacetate and beta-hydroxybutyrate were measured[37] in the protein-free supernatants.

Water content was determined in the whole body of fetuses and pups by drying at 100°C up to constant weight, and protein concentration was evaluated after alkali digestion.[39] Liver triacylglycerols were measured by extracting lipids with chloroform-methanol.[40] Phosphoacylglycerides were removed from the lipid extacts by using activated silicic acid and Hyflo-Super-Cell in chloroform medium[41] and aliquots of the neutral lipid extracts were dried at 40°C under N_2 for alkali saponification and triacylglycerol determination as indicated herein for blood. Lipoprotein lipase activity was measured in aliquots of the liquid N_2 frozen tissues following the methods already described.[13,42,43] Protein concentration in aliquots of the enzyme preparations were measured[39] to make the proper enzyme activity calculations.

In order to determine whether enzyme activity in the fetus liver showed inhibitory characteristics similar to those assayed in adult adipose tissue and liver, samples from these preparations were assayed in the presence of 0.5 M and 1.0 M NaCl and 0.5, 1.0 and 2.0 mg/ml of protamine sulfate. While a similar inhibition was found in virgin adult adipose tissue and fetal (21.5 days of gestation) liver (15.5%–30.0% of the basal activity found for adipose tissue preparations and 25.3%–37.7% for the basal liver samples, under the described conditions), no inhibition was observed when using the adult liver preparation (91.4%–97.6% of the basal). To further test the characteristics of the enzyme assayed, another experiment of inhibition was carried out in acetone-ether extracts[13] of liver from fetuses of 21 days and newborns of 0.5 and 1.5 days postpartum and compared with those from both liver and lumbar fat pads of virgin adults and pregnant rats of 21 days' gestation. The tissue extracts were assayed for lipoprotein lipase activity in the presence of either 1.0 M NaCl or 3.5 mg/ml of protamine sulfate. Observed values are shown in Table 1 where it is seen that enzyme activity was inhibited by NaCl and protamine in liver from offspring around birth and in lumbar fat pads from both 21-day pregnant rats and virgin adults. Inhibition, however, was not observed in liver extracts from either the pregnant mothers or virgin adults. As hepatic triacylglycerol lipase is considered resistant to NaCl and protamine inhibition[42,44,45] these results show that assayed activity in offspring liver around birth corresponds to lipoprotein lipase of similar characteristics as in the adult adipose tissue enzyme. Activity assayed in adult liver, however, may be attributed to other lipases.

Results are expressed as mean ± S E. After analysis of variance, the intergroup comparisons were made according to Tuckey's method.[46]

RESULTS

Litter Size and Maternal Plasma Lipoprotein Profile

Parturition in rat mothers treated with 7 mg of progesterone per day from the 20th day of gestation was delayed until the 23rd day while in controls treated with the oil carrier, parturition always occurred at the end of the 21st day. As shown in Table 2, litter size, offspring body weight, and size and carcass water and protein concentrations were similar in control rats and in those treated with progesterone at the same postfecundation time. Plasma triacylglycerol concentrations

Table 1. Inhibition of Lipoprotein Lipase Activity Assayed in Acetone-Ether Extracts from Liver of Normal Offspring Around Birth and Liver and Lumbar Fat Pads from Pregnant and Virgin Rats

	Percentage of Activity Versus Basal Assay Conditions			
Additions to the Assay Medium:	NaCl (1M)	*p*	Protamine Sulfate (3.5 mg/ml)	*p*
Offspring				
Liver from 21-day fetuses	19.98 ± 5.51	<0.001	46.59 ± 13.59	<0.05
Liver from 0.5-day newborns	15.69 ± 1.00	<0.01	51.89 ± 4.36	<0.05
Liver from 1.5-day newborns	14.68 ± 2.14	<0.01	25.04 ± 15.26	<0.05
21-Day Pregnant Mother				
Liver	86.93 ± 4.11	NS	123.35 ± 11.83	NS
Lumbar fat pad	6.30 ± 2.50	<0.001	30.90 ± 2.20	<0.05
Virgin Adults				
Liver	94.67 ± 1.64	NS	99.97 ± 2.90	NS
Lumbar fat pads	3.90 ± 0.41	<0.01	26.70 ± 2.25	<0.05

Values are expressed as mean ± SEM of four samples per group.

p Corresponds to the paired t-comparisons versus basal assay conditions (100%).

NS = Not Significant.

Table 2. Effects of Progesterone Treatment in Pregnant Rat on Litter Size and Maternal Plasma Lipoprotein Pattern

	Offspring					Maternal Plasma			
Days Postfecundation	Number per Litter	Body Weight (g)	Body Size (cm)	Carcass Water (g/100g)	Carcass Protein (mg/g)	Triacyl-Glycerols (mM)	VLDL	LDL	HDL
							(Absolute Areas)		
Controls									
0 (Virgins)						1.88 ± 0.24	6.17 ± 1.56	4.10 ± 0.96	11.41 ± 3.21
20	11.6 ± 0.4	3.54 ± 0.12	4.4 ± 0.1	87.3 ± 0.1**	50.1 ± 6.4	4.02 ± 0.16*	92.85 ± 18.31**	24.19 ± 4.21**	25.97 ± 1.33**
21	10.5 ± 0.9	5.18 ± 0.15	5.1 ± 0.1	86.7 ± 0.1	44.2 ± 2.6	2.59 ± 0.52	9.02 ± 2.98	6.44 ± 1.08	11.29 ± 1.95
22 (0.5 days postpartum)	9.0 ± 0.9	6.23 ± 0.42	5.3 ± 0.1	84.1 ± 0.4	58.4 ± 3.7	2.09 ± 0.31	3.80 ± 0.96	4.08 ± 0.20	10.47 ± 1.40
23 (1.5 days postpartum)	9.6 ± 0.7	6.58 ± 0.27	5.4 ± 0.1	82.4 ± 0.2	55.5 ± 1.4	0.66 ± 0.25**	1.02 ± 0.16**	2.19 ± 0.33	7.11 ± 0.83
Progesterone									
0 (Virgins)						1.51 ± 0.07	7.02 ± 2.42	4.43 ± 1.71	10.80 ± 4.07
21	11.5 ± 0.5	5.11 ± 0.19	4.9 ± 0.1	86.5 ± 0.1	49.7 ± 4.0	4.86 ± 0.83**	53.61 ± 11.61°*	27.47 ± 6.84°*	28.24 ± 5.97
22	10.3 ± 0.6	6.22 ± 0.21	5.3 ± 0.1	86.2 ± 0.2°°	53.3 ± 2.4	4.51 ± 0.76°*	66.29 ± 19.67°*	27.95 ± 2.99°*	22.80 ± 6.15
23	8.8 ± 0.9	6.77 ± 0.75	5.6 ± 0.1	85.0 ± 0.6°°	53.8 ± 0.9	5.32 ± 1.02°*	91.81 ± 17.64°*	32.46 ± 7.22°*	31 93 ± 2.52°*

At the 20th day of gestation, half of the pregnant rats were subcutaneously treated with 7 mg progesterone/day until sacrifice while controls received the medium. Sex- and age-mached virgin animals given the same treatments were also studied. Plasma triacylglycerols were measured by enzymatic glycerol determination after ethanol-alkali digestion and lipoprotein fractions were separated by agarose electrophoresis and quantified by densitometry after staining with Sudan black B, their values being expressed as absolute area units of the densitometer.

Means ± SEM of 6 animals per group.

° – Statistical comparisons versus controls.

* – Pregnant versus virgins.

° or * – $p < 0.05$.

°° or ** – $p < 0.01$.

Table 3. Lipoprotein Lipase Activity in Different Tissues from Pregnant Rats Treated or Not with Progesterone

	Activities (*n* Kat/g protein)			
Days Postfecundation	Mammary Gland	Adipose Tissue	Liver	Placenta
Controls				
0 (Virgins)	5.69 ± 1.35	111.88 ± 19.58	1.69 ± 0.08	
20	10.94 ± 2.62	62.43 ± 8.23*	1.55 ± 0.24	2.94 ± 0.20
21	13.15 ± 2.28	39.20 ± 8.63*	1.15 ± 0.10	4.13 ± 0.47
22 (0.5 days postpartum)	37.06 ± 12.47**	21.77 ± 2.55**	1.58 ± 0.20	
23 (1.5 days postpartum)	51.03 ± 13.44**	21.76 ± 3.16**	1.25 ± 0.17	
Progesterone				
0 (Virgins)	4.49 ± 0.26	121.91 ± 21.33	2.07 ± 0.24	
21	10.73 ± 2.04	61.86 ± 21.49*	1.12 ± 0.16*	2.54 ± 0.32
22	5.32 ± 1.25°°	34.73 ± 11.92**	1.71 ± 0.27	3.21 ± 0.41
23	4.43 ± 0.70°°	23.51 ± 8.05**	1.53 ± 0.14	2.37 ± 0.33

Experimental conditions of the animals and statistical comparisons are as indicated for Table 2. Lipoprotein lipase activity was measured in acetone-ether powders following a method previously described (Nilsson-Ehle & Schotz, 1976; Corey & Zilversmit, 1977) with minor modifications (Llobera, Montes & Herrera, 1979).

were higher in the 20-day pregnant rats than in age-matched virgin females, and values decreased progressively in the 21st day of pregnancy and on days 0.5 and 1.5 postpartum in the control mothers (Table 2). Progesterone treatment did not affect plasma triacylglycerol levels in virgin animals, but in the pregnant rats values remained high until the 23rd postfecundate day, the difference from the postfecundate time matched mother controls being statistically significant on day 22 and 23 (Table 2). These changes were parallel with those determined in the plasma lipoprotein profile by agarose electrophoresis (Table 2). All lipoprotein fractions appeared augmented at the 20th day of gestation in the control mothers as compared with virgins, and this change was maximal for the VLDL fraction (Table 2). At day 21 of gestation and days 0.5 and 1.5 postpartum, all the lipoprotein fractions decreased progressively in control mothers while in those treated with progesterone, values remained elevated until the 23rd postfecundate day. This rise was most marked for VLDL after the 21st postfecundate day compared with values in their respective control mothers. No differences in either of these parameters were found in virgin animals treated or not with progesterone.

Lipoprotein Lipase Activity in Maternal Tissues

As differences in circulating triacylglycerol and VLDL are influenced by lipoprotein lipase activities, this enzyme was determined in different tissues (Table 3). Lipoprotein lipase activity increased sharply in the mammary glands after parturition but in progesterone treated mothers it remained as low as in virgins (Table 3), the difference with postfecundate matched control mothers being highly significant from the 22nd day (Table 3). These changes differed substantially from those found for the enzyme activity in adipose tissue in which values decreased progressively with postfecundate time in both control and progesterone treated mothers as compared with the virgin groups. No differences were found in this parameter in either group with progesterone treatment (Table 3). Lipoprotein lipase activity assayed in the liver was much lower than in either mammary gland or adipose tissue and was slightly lower in mothers than in virgin rats; progesterone treatment did not affect this parameter in either group. Lipoprotein lipase activity was also low in the placenta (Table 3) and progesterone treatment did not affect it. In view of the known relationships between changes in circulating triacylglycerol levels and adipose tissue and mammary gland lipoprotein lipase activities around parturition[4,12-14,21,22] values of these parameters in the mothers treated or not with progesterone were graphically represented as shown in Fig. 1. A sharp decrease in plasma triacylglycerol concentration around parturition occurs in the mother with a progressive decrease in adipose tissue lipoprotein lipase activity, coinciding with initiation of a marked increase in enzyme activity in mammary gland. Prolongation of gestational period by progesterone treatment maintained elevated plasma triacylglycerol levels and did not modify the progressive decrease in adipose tissue lipoprotein lipase while preventing any change in the enzyme activity in mammary gland as compared with untreated mothers of the same postfecundation age (Fig. 1).

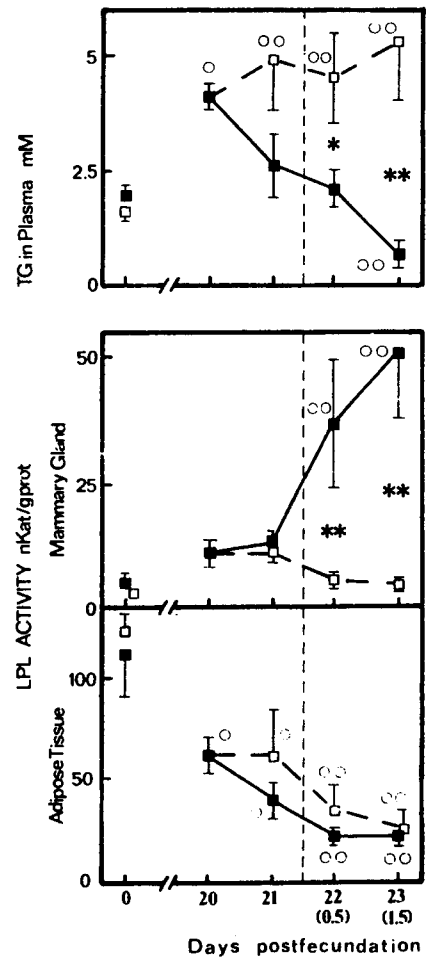

Fig. 1. Circulating triacylglycerol (TG) levels and mammary gland and lumbar fat pads lipoprotein lipase activity in pregnant rats treated (□— —□) or not (■—■) with progesterone (7 mg/day) from the 20th day of gestation and from virgin controls (day 0) receiving (□) or not (■) the treatment. Parturition in the nontreated mothers occurred at day 21.5, indicated in the figure by the vertical line, while in the treated animals it did not occur during the entire period studied. Number in brackets correspond to days after parturition in untreated mothers. O – Statistical comparisons versus virgins. • – Statistical comparisons between treated and nontreated animals of the same postfecundation time; O or • – $p < 0.05$, OO or •• – $p < 0.01$.

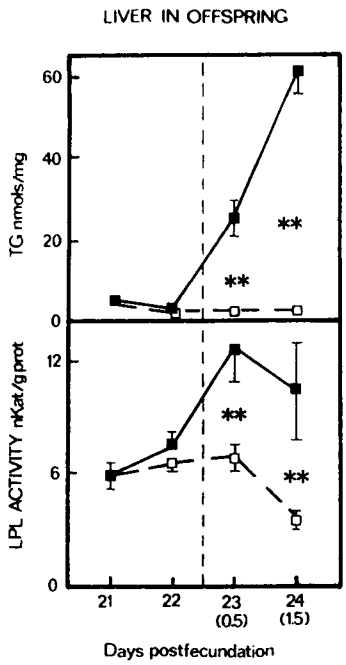

Fig. 2. Triacylglycerol concentration (TG) and lipoprotein lipase (LPL) activity in liver of offspring from mothers treated (□— —□) or not (■—■) with progesterone (7 mg/day) as indicated in Fig. 1. Birth of offspring from untreated mothers occurred at day 21.5 postfecundation, indicated by the vertical line in the figure, while offspring from treated mothers were not born during the time studied. Number in brackets correspond to days after birth in offspring from untreated mothers. Statistical comparisons between both groups at the same postfecundation time: * = $p < 0.05$, ** = $p < 0.01$.

in fetuses from progesterone treated mothers to almost negligible values at the 23rd postfecundation day (Fig. 3).

Plasma RIA-Prolactin Concentration

To determine whether some of the observed changes in both mothers and offspring could be related with variations in the circulating prolactin levels, plasma RIA-prolactin concentration was determined and these values are summarized in Table 6. In the control mothers, plasma prolactin levels were very high on the 21st day of gestation compared with values in virgins, and they decreased after parturition although they remained significantly high until postpartum day 1.5 when the study was terminated. Progesterone treatment did not affect prolactin levels in virgins but it completely abolished the increase found in pregnant rats on the 21st day of gestation, and their levels remained low in progesterone treated mothers until the 23rd gestation day (Table 6). In offspring from both control and progesterone treated mothers, plasma prolactin concentrations were lower than those found in virgins and their respective mothers, and values found in 23-day-old fetuses of progesterone mothers were

Table 5. Blood Ketone Bodies in Offspring from Rat Mothers Treated or Not with Progesterone

Days Postfecundation	Blood Ketone Bodies: Acetoacetate (μM)	Blood Ketone Bodies: β-hydroxy-butyrate (μM)
Virgin Adults	5.32 ± 2.35	10.65 ± 0.63
Offspring (Controls)		
20	3.24 ± 1.27	20.58 ± 2.99
21	4.00 ± 0.71	10.18 ± 1.07
22 (0.5 days postpartum)	12.42 ± 1.91**	62.60 ± 11.73**
23 (1.5 days postpartum)	9.07 ± 2.03*	97.31 ± 14.81**
Offspring (Progesterone)		
21	5.07 ± 0.71	10.96 ± 2.06
22	12.11 ± 1.01**	40.37 ± 9.83§
23	15.98 ± 3.14**	27.81 ± 6.79§°

Experimental conditions and statistical comparisons are as indicated for Table 4. Aliquots of blood samples collected in heparine after decapitation were deproteinized (Somogyi, 1945) and both acetoacetate and beta-hydroxybutyrate were measured enzymatically (Williamson et al., 1962).

slightly but significantly higher than those in postfecundate age-matched controls (Table 6).

DISCUSSION

These findings indicate that the low lipoprotein lipase activity in adipose tissue which occurs in the mother at late pregnancy and maintained after parturition is unaffected by prolongation of the gestational period by progesterone treatment. At the same time, this treatment blocks the rise in plasma prolactin levels

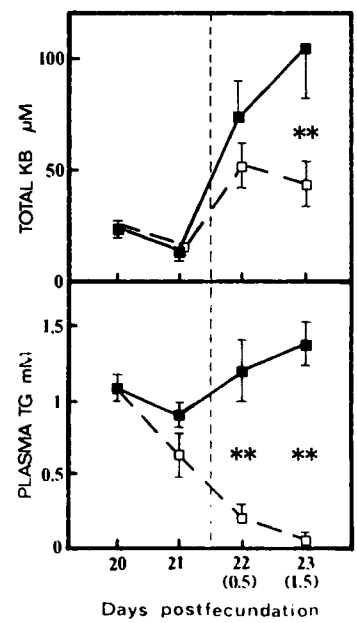

Fig. 3. Plasma concentration of total ketone bodies (KB) and triacylglycerol (TG) in offspring from mothers treated (□— —□) or not (■—■) with progesterone (7 mg/day). Specifications of this figure are the same as those indicated in Fig. 2.

Table 4. Lipid Related Parameters in Offspring from Rat Mothers Treated or Not with Progesterone

Days Postfecundation	Plasma VLDL	Plasma LDL (Absolute Areas)	Plasma HDL	Liver Lipoprotein Lipase (*n* Kat/g protein)	Carcass Lipoprotein Lipase (*n* Kat/g protein)
Virgin Adults	6.54 ± 1.27	2.55 ± 0.54	8.12 ± 1.14	2.17 ± 0.23	
Offspring (Controls)					
20	1.75 ± 0.45*	24.32 ± 4.07**	3.63 ± 0.25**	5.84 ± 0.64**	6.83 ± 0.43
21	4.05 ± 1.57	40.50 ± 7.50**	17.05 ± 8.26	7.45 ± 0.75**	11.89 ± 0.49
22 (0.5 days postpartum)	2.58 ± 0.56	15.32 ± 2.62**	6.74 ± 1.09	12.70 ± 1.81**	13.53 ± 1.83
23 (1.5 days postpartum)	5.17 ± 1.01	21.53 ± 3.56**	8.66 ± 2.32	10.36 ± 2.54**	14.09 ± 0.82
Offspring (Progesterone)					
21	4.37 ± 0.83	42.42 ± 8.55**	16.59 ± 4.20**	6.42 ± 0.31**	11.24 ± 1.37
22	0.61 ± 0.08°*	11.12 ± 1.26**	6.35 ± 0.03	6.66 ± 0.62°*	12.12 ± 0.64
23	0.45 ± 0.02°*	3.39 ± 0.53°°	5.39 ± 1.02	3.55 ± 0.33°°	9.87 ± 1.52°°

Offspring correspond to those mothers whose values are shown in Tables 2 and 3. Determinations were performed as indicated for their mothers. Virgin adults correspond to female rats of 160–180 g body weight not receiving any treatment. Mean ± SEM of 6 samples per group (each sample corresponds to the pool of the samples coming from offspring of each litter).

° = Statistical comparisons versus controls.

* = statistical comparisons of offspring versus virgin adults.

° or * = $p < 0.05$.

°° or ** = $p < 0.01$.

Lipid Related Parameters in Offspring

In 20-day-old fetuses, the main lipoprotein fraction in plasma corresponded to LDL (Table 4). In offspring of control mothers, this LDL fraction remained high in comparison with the other lipoprotein fractions at the 21st day of intrauterine life, becoming less manifest after birth. During the perinatal phase, both VLDL and HDL became more manifest in the offspring of control mothers compared with values in 20-day-old fetuses. In the fetuses of progesterone treated mothers, both VLDL and LDL fractions were reduced at the 22nd postfecundate day and were significantly reduced at day 23 when compared with postfecundate age-matched offspring of control mothers (Table 4). As shown in Table 4, lipoprotein lipase activity in livers of 22-day-old fetuses was higher than in the livers of their mothers (Table 3). This activity was more marked in livers of offspring of control mothers of 1st day of postnatal life and remained elevated on the 2nd day (Table 4). This increased liver lipoprotein lipase activity was not seen in postmature fetuses and their values were significantly lower than those of their controls at days 22 and 23.

To determine whether these differences in liver lipoprotein lipase activity between offspring of the two experimental groups were compensated for by its increase in other structures, carcass lipoprotein lipase activity was also measured (Table 4). After birth, carcass lipoprotein lipase activity remained unchanged in the control offspring, while in the postmature fetuses it was reduced between days 22 and 23 of gestation, being significantly lower than in their controls (Table 4). Triacylglycerol concentrations in offspring liver followed a trend similar to that of lipoprotein lipase activity as shown in Fig. 2, where both parameters are presented for comparison. Liver triacylglycerol concentration did not differ between fetuses of control and progesterone treated mothers at the 21st day of gestation, while it increased rapidly after birth in controls and decreased progressively in postmature fetuses (Fig. 2). Blood ketone bodies were also measured in both mothers and offspring. No differences were found between values in the two mother groups (data not shown). In offspring from both control and progesterone treated mothers, blood acetoacetate and beta-hydroxybutyrate concentrations were similar at the 21st day of gestation (Table 5) and they rose on the 22nd and 23rd postfecundate days in both groups, although beta-hydroxybutyrate values reached a higher level in control offspring (Table 5). Compiled blood total ketone bodies and plasma triacylglycerol concentrations in offspring are shown in Fig. 3. Both of these parameters are lower in postmature fetuses than in control offspring after birth. Total ketone bodies are maintained at a similar level in both groups until the 21st day of postfecundation and increase later on being more pronounced in the control newborns than in the postmature fetuses from progesterone treated mothers. Plasma triacylglycerol concentration was already lower in fetuses from progesterone treated mothers than in controls at the 21st day of intrauterine life and while values increased slightly after birth in the offspring of control mothers, they decreased progressively

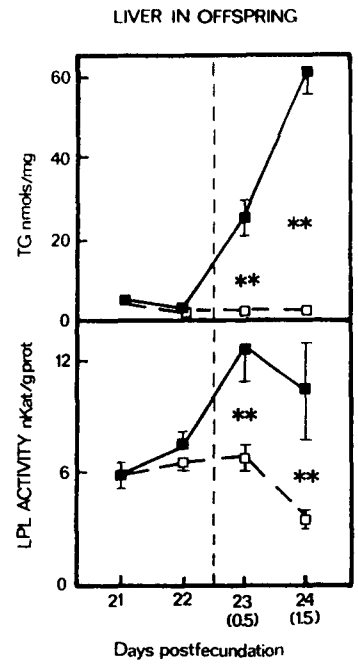

Fig. 2. Triacylglycerol concentration (TG) and lipoprotein lipase (LPL) activity in liver of offspring from mothers treated (□— —□) or not (■—■) with progesterone (7 mg/day) as indicated in Fig. 1. Birth of offspring from untreated mothers occurred at day 21.5 postfecundation, indicated by the vertical line in the figure, while offspring from treated mothers were not born during the time studied. Number in brackets correspond to days after birth in offspring from untreated mothers. Statistical comparisons between both groups at the same postfecundation time: * = $p < 0.05$, ** = $p < 0.01$.

in fetuses from progesterone treated mothers to almost negligible values at the 23rd postfecundation day (Fig. 3).

Plasma RIA-Prolactin Concentration

To determine whether some of the observed changes in both mothers and offspring could be related with variations in the circulating prolactin levels, plasma RIA-prolactin concentration was determined and these values are summarized in Table 6. In the control mothers, plasma prolactin levels were very high on the 21st day of gestation compared with values in virgins, and they decreased after parturition although they remained significantly high until postpartum day 1.5 when the study was terminated. Progesterone treatment did not affect prolactin levels in virgins but it completely abolished the increase found in pregnant rats on the 21st day of gestation, and their levels remained low in progesterone treated mothers until the 23rd gestation day (Table 6). In offspring from both control and progesterone treated mothers, plasma prolactin concentrations were lower than those found in virgins and their respective mothers, and values found in 23-day-old fetuses of progesterone mothers were

Table 5. Blood Ketone Bodies in Offspring from Rat Mothers Treated or Not with Progesterone

Days Postfecundation	Blood Ketone Bodies: Acetoacetate (μM)	Blood Ketone Bodies: β-hydroxy-butyrate (μM)
Virgin Adults	5.32 ± 2.35	10.65 ± 0.63
Offspring (Controls)		
20	3.24 ± 1.27	20.58 ± 2.99
21	4.00 ± 0.71	10.18 ± 1.07
22 (0.5 days postpartum)	12.42 ± 1.91**	62.60 ± 11.73**
23 (1.5 days postpartum)	9.07 ± 2.03*	97.31 ± 14.81**
Offspring (Progesterone)		
21	5.07 ± 0.71	10.96 ± 2.06
22	12.11 ± 1.01**	40.37 ± 9.83§
23	15.98 ± 3.14**	27.81 ± 6.79§°

Experimental conditions and statistical comparisons are as indicated for Table 4. Aliquots of blood samples collected in heparine after decapitation were deproteinized (Somogyi, 1945) and both acetoacetate and beta-hydroxybutyrate were measured enzymatically (Williamson et al., 1962).

slightly but significantly higher than those in postfecundate age-matched controls (Table 6).

DISCUSSION

These findings indicate that the low lipoprotein lipase activity in adipose tissue which occurs in the mother at late pregnancy and maintained after parturition is unaffected by prolongation of the gestational period by progesterone treatment. At the same time, this treatment blocks the rise in plasma prolactin levels

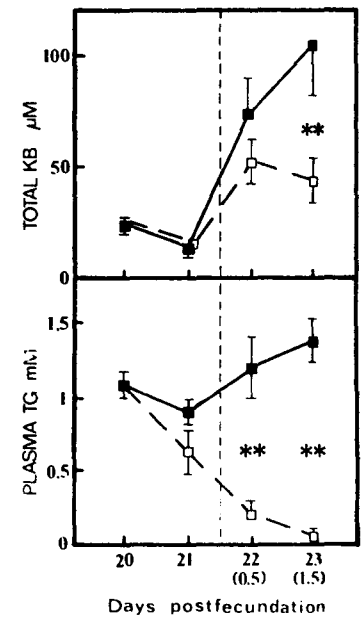

Fig. 3. Plasma concentration of total ketone bodies (KB) and triacylglycerol (TG) in offspring from mothers treated (□— —□) or not (■—■) with progesterone (7 mg/day). Specifications of this figure are the same as those indicated in Fig. 2.

Table 6. Plasma Radioimmunoassayable Prolactin Levels in Mothers and Offspring

Days Postfecundation	Plasma Prolactin (ng/ml) Mothers	Offspring
Controls		
0 (Virgins)	4.72 ± 1.40	
20	5.94 ± 0.55	1.52 ± 0.31**
21	32.51 ± 13.10**	1.71 ± 0.15**
22 (0.5 days postpartum)	12.12 ± 1.94**	0.98 ± 0.21**
23 (1.5 days postpartum)	16.76 ± 3.36**	0.91 ± 0.13**
Progesterone		
0 (Virgins)	5.07 ± 1.40	
21	8.85 ± 1.20°°	1.98 ± 0.16**
22	6.78 ± 0.87	1.88 ± 0.20**
23	6.12 ± 1.12°	3.42 ± 0.49°*

Experimental conditions and statistical comparisons as indicated for Table 2. Radioimmunoassayable prolactin was measured by using the method developed for the rat by NIAMDD of the NIH.

as well as the increase in mammary gland lipoprotein lipase activity and also the decline in hypertriacylglycerolemia which normally takes place in the mothers at late gestation. In offspring the accumulation of triacylglycerol in the liver due to consumption of maternal milk after birth may be associated with the appearance of a lipase which has the inhibitory characteristics of lipoprotein lipase and these manifestations were completely abolished in the postmature fetus of the same postconceptual age.

The lipidic profile in the pregnant rat at late gestation confirms previous reports of hypertriacylglycerolidemia primarily associated with increase circulating VLDL concentration.[15,18,19] This effect is produced by the juxtaposed effects of an increase entry of triacylglycerols into the circulation[11] and reduced adipose tissue lipoprotein lipase activity[4,12,13] which is known to diminish the uptake of VLDL-triacylglycerides by the tissue.[14] The reduction of circulating triacylglycerols in the mother shortly before parturition is also associated with a decrease in circulating VLDL and occurs when adipose tissue lipoprotein lipase activity is still low and the rate of entry of triacylglycerols into the circulation is known to be still high.[4] Although more direct studies are required to establish definitive conclusions, the increase in mammary gland lipoprotein lipase activity must play an important role in the reduction of circulating triacylglycerols, facilitating their entry into the gland[47] even before its lipogenetic activity is enhanced.[48] Neither the reduction in circulating triacylglycerols and VLDL nor the rise in mammary gland lipoprotein lipase activity at late gestation appeared when mothers were given progesterone to postpone parturition. This treatment did not affect any of the parameters studied in virgin control rats, suggesting that the observed effects in the pregnant rats were secondary to changes other than altered progesterone levels. Actually the absence of increase circulating prolactin level that normally occurs at late gestation and which was produced by progesterone treatment in the pregnant rats, may have impeded the increase in mammary gland lipoprotein lipase activity which is known to be controlled by prolactin.[17,27] The observed changes in circulating triacylglycerols may consequently be affected by the same mechanism.

Adipose tissue lipoprotein lipase activity was kept low in the mothers whose parturition was postponed by progesterone treatment. As no effect was observed on this enzyme by progesterone in virgin animals and the treatment completely abolished the prolactin rise which normally occurs prior parturition in the mother, it may be concluded contrary to previous proposals[17,27] that decreases in adipose tissue lipoprotein lipase in the mother at late gestation are not primarily controlled through the secretion of prolactin. This lack of relationship between adipose tissue lipoprotein lipase activity and circulating prolactin changes is also supported by the fact that the former was already decreased in 20-day pregnant rat when plasma prolactin levels did not yet differ from those in virgin controls.

The increase in liver lipoprotein lipase activity in the newborn coincides with the accumulation of triacylglycerols in the organ, indicating a possible cause-and-effect relationship. The mechanism through which these changes occur is not yet established but some possibilities may be considered. Postmaturity in the fetus completely inhibits the two changes (liver lipoprotein lipase increase and triacylglycerol accumulation) and this is not due to damage of the metabolic conditions in the postmature fetus which maintains a normal growth rate and whole body water and protein content. As a prolactin effect may not be cited to explain the observed changes in liver triacylglycerol content and lipoprotein lipase activity in the newborn, due to their low circulating levels, a nutritional factor may be proposed. It is known that deprivation of milk in the newborn rat completely abolished accumulation of hepatic triacylglycerols.[29] Thus it is possible that the increased availability of triacylglycerols to the newborn coming from the mother's milk may produce a substrate induction mechanism[49] responsible for the increase in liver lipoprotein lipase activity found just after birth which does not occur in the age-matched postmature fetus. The abundance of triacylglycerols in the liver of the newborn provides the substrate for its rapid activation of ketogenesis[50] producing the observed increase in circulating ketone bodies to compensate for the neonatal hypoglycemia.[51,52] The appearance of LDL prior to VLDL in the rat fetus, as

previously described,[19] is in agreement with our present results and contrasts with the well-established metabolism of VLDL in adults which are known to be precursors of LDL at extrahepatic tissues.[53,54] Electrophoretic mobility and ultracentrifugal behavior under different densities were tested for lipoprotein particles in the rat fetus,[19] and although they may have physicochemical characteristics different than those in adults,[55,56] just after birth there was a definite decrease in the plasma LDL fraction which was not followed by an opposite change in VLDL or by a change in plasma triacylglycerol. This finding together with the rapid accumulation of triacylglycerol in the newborn liver, suggest both an impairment in the release of liver triacylglycerol into circulation and an increased flux of circulating triacylglycerol-rich lipoproteins (coming from the absorption of the mother's milk) into the liver, favored by the increase in its lipoprotein lipase activity. The fatty liver is known to last only for the first 5 days of extrauterine life[29] and this coincides with the reduction of liver lipoprotein lipase activity to adult levels[13] and the progressive increase in adipose tissue lipoprotein lipase activity,[57,58] allowing this tissue to take over the role of main fat depository. The proposed relationship between circulating lipoproteins, fatty liver and hepatic lipoprotein lipase activity in the newborn rat are still speculative and must await a better understanding of the actual role of lipases on lipoprotein catabolism, not only in the developing but also in adult liver.

None of these changes occur in the postmature fetus where there is a marked decrease in circulating LDL and VLDL, as well as triacylglycerol from the 22nd day of gestation. This may be caused by a reduced function of the placenta[59] resulting in a rapid decrease in the transport of nutrients from the mother to the fetus[60] which would then need to consume its own resources, as shown by the progressive reduction in liver and plasma triacylglycerol concentration. Ketone bodies are known to cross the placenta easily[61] and as ketogenesis does not seem to operate until after birth,[50] enhanced circulating ketone bodies in the postmature fetus suggest that maternal ketones are still crossing the placenta to be used as the main alternative fuel. This, together with the degradation of liver glycogen,[62] preserves the glycemia in the postmature fetus,[63] allowing it not only to survive but to maintain its normal growth rate for a certain period.

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