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Relationship between lipoprotein lipase and peroxisome proliferator-activated receptor-α expression in rat liver during development

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The present study was addressed to determine whether the high expression of peroxisome proliferator-activated receptor-α (PPAR-α) in rat liver during the perinatal stage plays a role in the induction of liver lipoprotein lipase (LPL) expression and activity. Parallel increases in liver mRNA PPAR-α and LPL activity were found in newborn rats, and after a slight decline, values remained elevated until weaning. Anticipated weaning for 3 days caused a decline in those two variables as well as in the mRNA LPL level, and a similar change was also found in liver triacylglycerol concentration. Force-feeding with Intralipid in 10-day-old rats or animals kept fasted for 5 h showed high mRNA-PPARα and –LPL levels as well as LPL activity with low plasma insulin and high FFA levels, whereas glucose and a combination of glucose and Intralipid produced low mRNA-PPARα and –LPL levels as well as LPL activity. Under these latter conditions, plasma insulin and FFA levels were high in those rats receiving the combination of glucose and Intralipid, whereas plasma FFA levels were low in those force-fed with glucose. It is proposed that the hormonal and nutritional induction of liver PPAR-α expression around birth and its maintained elevated level throughout suckling is responsible for the induction of liver LPL-expression and activity during suckling.

Key words: PPAR-α, LPL, Triacylglycerols, Anticipated weaning, Insulin, FFA, Suckling.

The functional fraction of lipoprotein lipase (EC 3.1.1.34, LPL) is located at the luminal side of the endothelial cells, where it hydrolyzes triacylglycerols of chylomicrons and very low density lipoproteins (VLDL), and the fatty acids released are taken up by the subjacent tissue. The enzyme is widely distributed in the body, expressing its highest activity in adipose

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tissue, cardiac muscle and skeletal muscle, and the mammary gland during lactation (24, 35). Although LPL is considered an extrahepatic enzyme because it is normally absent in adult liver, where there is no mRNA LPL expression, its activity has been reported present in adult rat liver in some situations (8, 31, 37, 38). In fact, under these conditions, the liver enzyme is believed to originate in peripheral tissues and to be transported through plasma to the liver, where it is still active for some time before it is degraded (23). However, fibrate treatment of adult rats has been shown to reinduce the LPL gene in liver, and therefore, the LPL activity restored by fibrate in adult liver is actually due to the enzyme produced in the organ itself, rather than coming from extrahepatic tissues (32, 34).

Different to what occurs in adults (33), the fetal and neonatal liver has high LPL activity, as initially reported in the rat (12) and latterly extended to other species (1, 9, 11, 15, 16, 19). The activity increases after birth (5, 26) but declines progressively after the first day of extrauterine life. Whereas adult rat liver hardly synthesizes LPL, newborn rat liver has an active LPL synthesis rate (2, 36). The expression of LPL progressively decreases during suckling, reaching about the adult situation at weaning, as shown by age-related decreases in LPL activity (5, 20), LPL synthesis (36) and LPL mRNA (22).

The LPL activity found in newborn liver is functional, with properties similar to those found in adult adipose tissue LPL (27): it has been shown to increase with either fasting or undernutrition; it is related to plasma and liver triacylglycerol concentrations (7, 13); it is directly related to birth (26) and is modulated by hormones (10). However, the way how the liver LPL gene is initially expressed and latterly repressed is not known. The reported pattern of liver LPL expression is similar to what we found for the liver mRNA expression of peroxisome proliferator-activated receptor-α (PPAR-α) and of some of its related genes: rapidly increasing after birth, decreasing throughout suckling and attaining the adult liver level at the time of weaning (17). Since the LPL gene has also been reported to be a PPAR-α related gene (14, 32), there exists the possibility that changes in liver LPL expression be modulated by the changes in PPAR-α taking place around birth and during the weaning period. Recently we have found that the high lipid intake and decreased plasma insulin levels are responsible for the high PPAR-α expression detected in rat neonates (18). Thus, the possibility that these same factors modulate the changes in liver LPL expression during suckling also exists.

The present paper is therefore addressed to determine the relationships between liver LPL activity and expression and PPAR-α expression during suckling, both under basal conditions, and after force-feeding with either glucose or Intralipid.

Material and Methods

Animals.– Female Sprague-Dawley rats from our animal quarters were fed, ad libitum, standard rat chow (B&K Universal, Barcelona, Spain) and housed under controlled light and temperature conditions (12 hours light/dark cycle; 22 ± 1 °C). The experimental protocol was approved by the Animal Research Committee of the University San Pablo-CEU, Madrid, Spain. Rats were mated when they weighed 160-190 g and, the day that spermatozoids appeared in vaginal smears was considered day 0 of pregnancy. Some mothers and their fetuses were sacrificed

by decapitation on day 21 (d –1) of post-fecundation, whereas other pregnant rats were allow to deliver. On the day of birth, each litter was reduced to nine pups per mother and pups were killed at 0, 1, 2, 5, 10, 20, 25 or 30 days after birth. On the 20th day of life, the lactating mothers were removed from the cage to stop the suckling period. In another set of lactating rats, half of the pups were subjected to anticipated weaning by removing their mothers from the cages on day 17, whereas the other half remained with their mothers until the 20th day of age. In both groups, pups were killed at different ages.

In another experiment, ten-day-old suckling rats were separated from their mothers, maintained at 37 °C, in high humidity, and subjected to oral force-feeding three times (at 10.00 a.m., 11.00 a.m. and 13.00 p.m.) with 10-20 µL/g of body weight of a solution containing either 25% glucose, 10% Intralipid (emulsion of soybean oil), glucose plus Intralipid or the medium alone (bidistillated water). All pups were decapitated two hours after the last administration. The liver was immediately removed, placed in liquid nitrogen and stored at –80 °C until analysis. Blood was collected from the neck into EDTA-Na_2 containing receptacles. After centrifugation at 1,500 g for 15 min at 4 °C, plasma was kept at –20 °C until processed for insulin and free fatty acid (FFA) analysis, using commercial kits (DiaSorin, USA; and Wako, Germany, respectively).

RNA analysis.– RNA analysis was carried out as previously described (17, 18) Total cellular RNA was prepared either from the liver of individual animals or from liver pools of three animals of the same litter in the case of fetuses and 0- to 2-day-old newborns. Total RNA was isolated by a modification of the guanidium isothiocyanate method using Ultraspec RNA according to the manufacturer's instructions (Biotecx Labs, Houston, USA). For Northern blot analysis, around 25 µg of total RNA was denatured, separated on 1.2% agarose gels and transferred to nylon membranes in 20 x SSC. For dot blot analysis, 10, 20 and 30 µg of total RNA was denatured, spotted and fixed to a nylon membrane. The cDNA probes were labeled with [α-^{32}P]dCTP using the random primer DNA labeling kit provided by Amersham. Probes used were: 1.6 kb *Eco RI* fragment of rat PPARα cDNA (a generous gift from Dr. J-Å Gustafsson, Karolinska Institute, Huddinge, Sweden), 1.5 kb *EcoRI* fragment of rat lipoprotein lipase (LPL) cDNA and a fragment of rat β-actin cDNA (both kindly supplied by Dr. D. Langin, INSERM U317, Toulouse, France). Blots were prehybridized at 42 °C for 2 h and hybridized overnight at the same temperature. Membranes were exposed to Kodak X-OMAT films at –70 °C from 1 h to three days. Filters were stripped of label at 75-80 °C for 1 h with 0.1x SSC, 0.5% SDS, 0.1% tetrasodium pyrophosphate and then rehybridized. Linearity of the relationship between signal intensity and RNA concentration was confirmed for each sample and probe.

Hepatic lipid composition analysis.– Frozen liver aliquots were used for lipid extraction (4), and aliquots of lipid extracts were quantified after image analysis and separation by one-dimensional TLC (30), using the G5-700 BIOIMAGE TLC scanner of Bio-Rad (USA). Spots were quantified as integrated optical densities against an internal standard of cholesteryl formate and calibration curves of triacylglycerol standards.

LPL activity.– It was measured in acetone powders from frozen liver aliquots by the method previously described (12).

Statistical analysis.– Data are expressed as mean ± standard error. Results were subjected to a one-way analysis of variance (ANOVA), differences in mean values among groups were tested using the Tukey multiple range test, and were considered statistically different at $p < 0.05$. For results of plasma insulin levels, the Mann-Whitney *U*-test was used. Comparisons between two groups was done by the Student's *t* test. Linear regressions were calculated by the least-squares methods.

Results

As shown in Fig. 1A and 1B, both mRNA for PPARα and LPL activity in liver of rat 21 d fetuses (d –1) were already detectable although low, whereas at birth (d 0), both variables rapidly increased, reaching their highest value around day 1 in case of mRNA and at day 0 in case of LPL activity. Although not so elevated, they remained high during suckling, to decline at the time of weaning (day 20), remaining low from that time on. A similar trend to that seen for liver LPL activity is seen for liver triacylglycerol concentration (Fig. 1C), which was very low in 21 d fetuses, then rapidly increased at birth, attaining in this case its highest value on day 2. It declined on day 5, reaching low values on day 10 and remaining so on days 20 and 30.

As shown in Fig. 2A, plasma insulin levels sharply decreased just after birth, to increase from day 5 up to day 15, decreasing again to attain the lowest level in the 25- day-old pups, whose values did not differ from those of 30-day-old rats. In contrast, plasma FFA levels rapidly increased just after birth (Fig. 2B) followed by a significant decline in 5-day-old pups. However, values of FFA remained elevated throughout suckling to

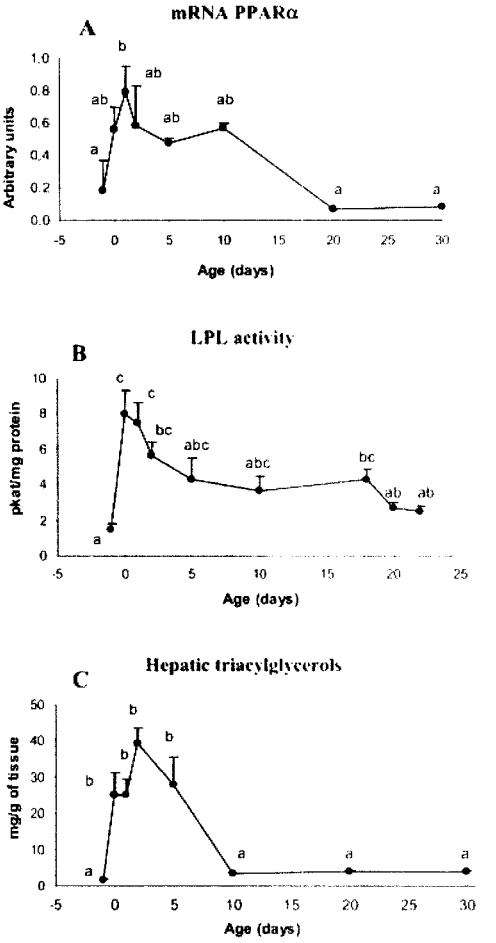

Fig. 1. *PPAR-α mRNA levels (Northern blot), LPL activity and triacylglycerol concentration in liver throughout development in rat fetuses and neonates.* Values are mean ± SEM of 5 rats/group. Letters correspond to the statistical comparisons (one-way analysis of variance, ANOVA) between the time-points throughout the development for each parameter (values not sharing a common letter are significantly different at $p < 0.05$). Day 0 represents the day of birth and day 20 when the neonates were weaned.

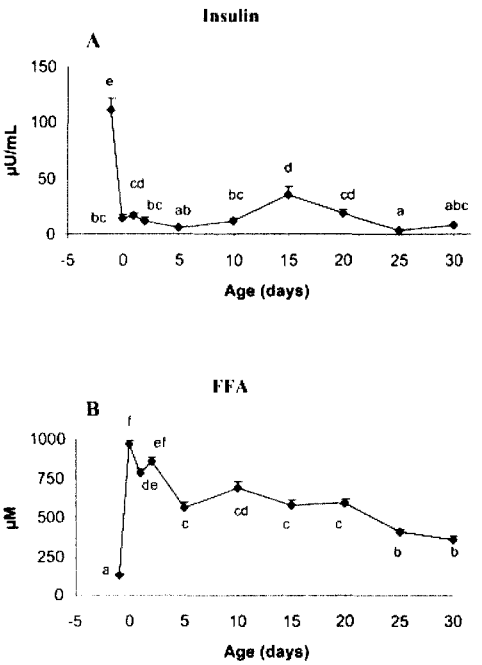

Fig. 2. *Plasma insulin and FFA levels throughout development in rat fetuses and neonates.* Values and letters as in Fig. 1.

further decrease in 25- and 30-day-old rats, although they always remained higher than in fetuses.

Since we previously found that anticipation of the normal weaning day, which normally occurs at day 20, by 3 days (to day 17), greatly decreases the level of hepatic PPARα mRNA (18), an experiment was carried out to determine whether weaning on day 17 has a similar effect on liver LPL mRNA expression and activity. As shown in Fig. 3, 24 h after anticipated weaning (day 17) the liver mRNA level of both PPARα and LPL, as well as liver LPL activity greatly decreased. As also shown in Fig. 3, anticipated weaning also affects hepatic triacylglycerol concentrations, which appeared lower in 18-, 19- and 20-day-old pups that were weaned on day 17 than in those that were weaned on day 20. In the latter, liver triacylglycerols declined just after weaning (days 21 and 22), and values in 22-day-old rats that were weaned on day 20 were lower than in those weaned on day 17 (Fig. 3). Parallel changes in PPARα and LPL mRNA expressions in this experiment of anticipated weaning prompted us to determine the existence of a linear correlation between these two variables, and as shown in Fig. 4A, it was found that such correlation was highly significant ($p<0.001$).

In order to determine whether nutritional and hormonal factors simultaneously affect mRNA-PPARα and –LPL expressions as well as LPL activity, as previously suggested for other PPARα-target genes (18), 10-day-old suckling pups were subjected to force-feeding for three times during a total of 5 h with either glucose, Intralipid or both. Another group were kept fasted for the 5 h period receiving only the vehicle (bidistilled water), as described in Material and Methods. As shown in Fig. 5, plasma insulin levels had the lowest values in those pups receiving Intralipid, whereas the highest values were in those receiving glucose plus Intralipid, with no significant difference between those pups treated with glucose and those kept fasted (i.e. receiving the medium). However, as also shown in Fig. 5, plasma FFA levels were similarly high in those pups receiving either Intralipid or glucose plus Intralipid and in those kept fasted, as compared to those treated with glucose. As shown in Fig. 6, pups receiving Intralipid alone or kept fasted for the 5 h period showed the highest levels of liver mRNA for both PPARα and LPL, whereas those receiving glucose alone or glucose plus Intralipid showed the lowest levels. A similar intergroup relationship was found for liver LPL activity (Fig. 6),

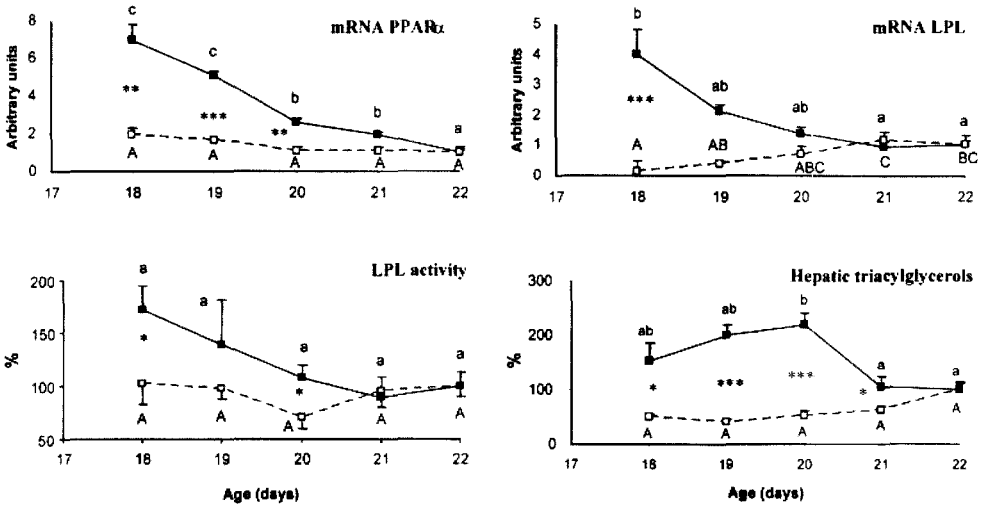

Fig. 3. *mRNA levels of PPARα (dot blot) and LPL (Northern blot), LPL activity and liver triacylglcerol concentrations in rats weaned either at the normal weaning day (day 20, continuous line) or 3 days earlier (day 17, dotted line).*

Values are mean ± SEM of 5 rats/group. For mRNA of both PPARα and LPL, values have been corrected by considering 1.0 the corresponding to 22 days of age, whereas values of both LPL activity and hepatic triacylglycerols correspond to percentages of those at 22 days of age, which in case of LPL activity were 2.48 ± 0.32 pkatals/mg of protein in pups weaned at day 20 and 3.06 ± 0.30 pkatals/mg protein in those weaned at day 17, NS, and in case of hepatic triacylglycerols 3.28 ± 0.39 mg/g in pups weaned at 20 days and 6.86 ± 1.06 mg/g in those weaned at 17 days, $p < 0.02$. Letters correspond to the statistical comparisons (one-way analysis of variance, ANOVA) between the time-points throughout the different days for each parameter (values not sharing a common letter are significantly different at $p < 0.05$). Statistical (*t* test) significant differences between values of the same age are indicated by asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

whose values were higher in those pups receiving Intralipid alone or in those kept fasted (receiving just the medium), as compared to those receiving either glucose alone or glucose plus Intralipid.

Parallel changes in mRNA expression of liver PPARα and LPL in this experiment also led us to calculate their linear correlation, which as shown in Fig. 4B, it was found to be highly significant ($p<0.001$).

Discussion

The present results show a parallel pattern of change for liver PPARα expression and both liver LPL mRNA and LPL activity in rat offspring around birth and during suckling. In the same way, a similar response of those three variables to anticipated weaning as well as to a force-feeding with either glucose, Intralipid or Intralipid plus glucose or just kept fasted was also found in suckling rats. These findings therefore would indicate that a close relationship exists between all those variables, and fit in the known role of LPL as target gene of PPARα (32). Besides, since conditions of induced hyperinsulinemia are known to decrease liver PPARα expression (18), the effect of Intralipid or fasting seen here in 10-day-old rats, enhancing liver mRNA levels for PPARα and LPL, as well as liver LPL activity and lowering the plasma insulin

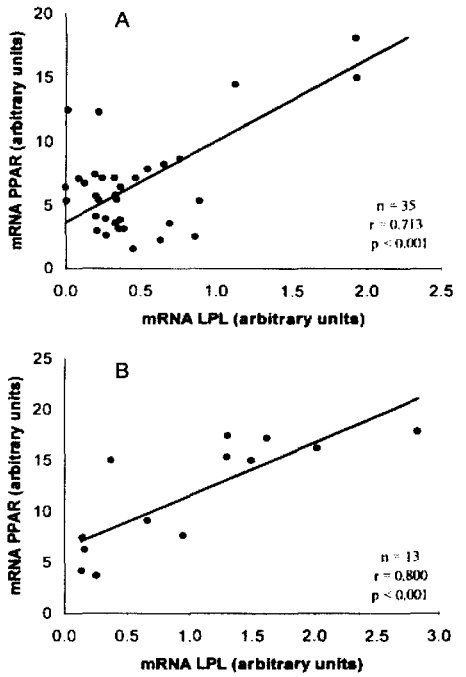

Fig. 4. *Linear correlation between liver mRNA levels of PPARα and LPL in individual animals subjected to normal (day 20) or anticipated weaning (day 17) (A) or in 10-day-old suckling pups subjected to force feeding with either glucose, Intralipid, glucose plus Intralipid or medium (bidistillated water) (B).*

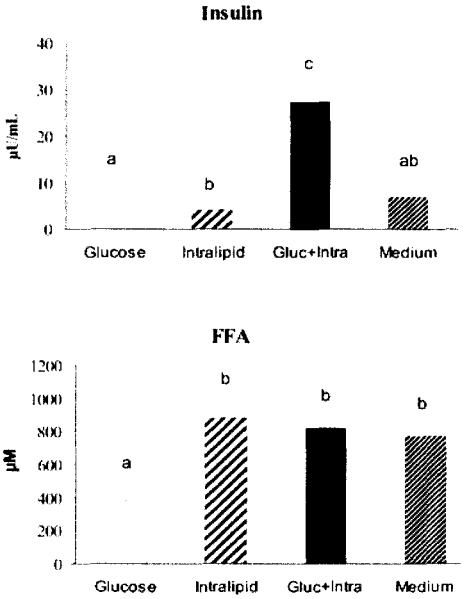

Fig. 5. *Effect of force feeding with either glucose, Intralipid, glucose plus Intralipid, or medium (bidistillated water) on plasma insulin and FFA levels in 10-day-old rats.*
Each value represents the mean ± SEM of 4 animals. Comparison between groups was performed with one-way analysis of variance (ANOVA), and a different letter means differences statistically significant at $p < 0.05$.

levels, would indicate that the rapid and striking decline in plasma insulin levels at birth may play an important role in the above-mentioned changes. The rapid increase in plasma FFA levels found just after birth could also contribute to the induction of liver PPARα mRNA expression and its subsequent induction of LPL expression. However, the fact that conditions of enhanced FFA levels in the presence of hyperinsulinemia show decreased PPARα- and LPL-mRNA expression as well as decreased LPL activity, such as that seen after acute combined administration of glucose and Intralipid in 10-day-old pups, would indicate that maintained elevation of plasma FFA during suckling induces PPARα only if plasma insulin levels are kept low. In fact, the slight but significant increase of plasma insulin levels seen after its initial drop after birth, together with the small but significant decline of plasma FFA, may be responsible for the diminution in LPL activity observed during the first 2-3 days of birth. Values are, however, higher than those seen just after weaning, and this picture is very similar to that previously reported (25).

The rapid increase in LPL activity in liver occurring just after birth allows this organ to become an importer organ for plasma triacylglycerols, which justifies the increase in liver triacylglycerol concentra-

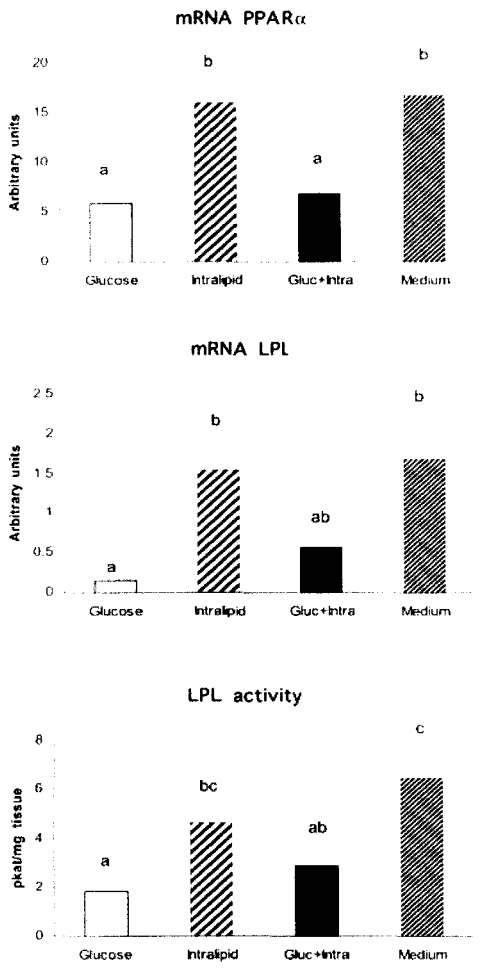

Fig. 6. *Effect of force feeding with either glucose, Intralipid, glucose plus Intralipid, or medium (bidistillated water) on hepatic mRNA levels of PPARα (dot blot) and LPL (Northen blot) and LPL activity in 10-day-old rats.*
Values and symbols as in Fig. 5.

tion that takes place during the first days of suckling. This parallel relationship between hepatic LPL activity and triacylglycerol concentration agrees with previous findings carried out in pups of just 0.5 or 1.5 days of age (26). Since the capacity of newborn liver LPL to hydrolyze triacylglycerols has been well documented (6), throughout this mechanism, the liver may use plasma triacylglycerols as substrates for fatty acid β-oxidation, sustaining the hyperketonemia normally present in the suckling rat (7, 13, 26).

A relationship between liver PPARα- and LPL-mRNA as well as LPL activity and liver triacylglycerol concentration was found in the experiment of anticipated weaning reported here, where these four variables rapidly decrease when suckling pups are forced to eat solid diet before their normal weaning time. Since the anticipated switch to the solid diet means an important decline in the lipid intake for the weaning pups, that finding further emphasize the role of the high lipid diet during suckling in the induction of liver PPARα expression and its subsequent change in LPL expression and activity, as well as the role of the latter in the hydrolysis and liver uptake of circulating triacylglycerols. Fasting has previously been found to enhance liver LPL activity in suckling rats (7, 20). This was observed in 10-day-old fasted pups receiving just the vehicle (bidistilled water) causing an increase in plasma FFA and a decline in insulin levels, thus producing the appropriate condition for enhanced liver PPARα-mRNA expression and the subsequent changes in liver LPL mRNA and activity. Other hormonal factors have been proposed to modulate liver LPL activity in suckling rats (10), but their contribution as direct or indirect regulators for the enzyme remains to be established.

There is no possibility of extrapolating these findings to the human species because the existence of LPL in human fetal or neonatal liver has not been documented, although a high postheparin plasma LPL which was independent of birth weight and insulin but correlated negatively with cord-serum triacylglycerol

concentration has been reported in newborn infants (28, 29). Rat liver LPL is heparin-releasable (6, 21). Thus, if one assumes that plasma postheparin LPL activity reflects overall body LPL activity, there exists the possibility that newborn infant postheparin plasma LPL includes the enzyme of liver origin, which must be independent of birth weight, as this is mainly determined by adipose tissue mass (3).

In conclusion, besides confirming the presence of liver LPL in rat newborns and during suckling, the present results under different conditions (i.e., developmental age, anticipated weaning, over-feeding and fasting) show the close relationship of LPL expression with the liver PPAR-α expression, its enhancement by FFA and its decline by insulin. It is therefore proposed that although other factors may contribute to the observed findings, the high levels of PPARα mRNA (and also the corresponding protein (17) in neonates, which are induced by hormonal and nutritional factors, play a major role in their induction of liver LPL expression and activity.

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M. PANADERO, C. BOCOS and E. HERRERA. *Relación entre expresión de PPAR-α y de LPL en hígado de rata en desarrollo.* J. Physiol. Biochem., **62** (3), 189-198, 2006.

Se estudia si la alta expresión del receptor activado por proliferadores peroxisomales -α (PPAR-α) que se observa en el hígado de la rata durante la etapa perinatal, contribuye a la inducción de la expresión y actividad de la lipoproteína lipasa (LPL) que se presenta en este órgano. Se observó un incremento paralelo en el mRNA del PPAR-α y en la actividad LPL del hígado en ratas recién nacidas, y tras un ligero descenso, los valores se mantuvieron elevados hasta el destete. Un destete anticipado de 3 días causó un descenso en esas dos variables y en el nivel de mRNA LPL, así como un cambio semejante en la concentración de triacilgliceroles en el hígado. Animales de 10 días de edad sometidos a alimentación forzada con Intralipid o mantenidos en ayunas durante 5 h mostraron valores altos de mRNA-PPARα y –LPL así como de la actividad de la LPL en hígado, y niveles bajos de insulina y altos de FFA en plasma, mientras que la administración de glucosa o la combinación de glucosa e Intralipid produjeron valores bajos de mRNA-PPARα y –LPL así como de actividad LPL en hígado. Los niveles plasmáticos de insulina y FFA fueron altos en las ratas que recibieron la combinación de glucosa e Intralipid, mientras que los niveles de FFA plasmáticos fueron bajos en los sometidos a alimentación forzada con glucosa. En consecuencia, se propone que la inducción hormonal y nutricional de la expresión del PPAR-α en hígado alrededor del nacimiento y su mantenimiento en valores elevados a lo largo de la lactancia es responsable de la inducción de la expresión y la actividad de la LPL durante esta etapa del desarrollo.

Palabras clave: PPAR-α, LPL, Triacilgliceroles, Destete anticipado, Insulina, Ácidos grasos libres, Lactancia

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