High Liver Lipoprotein Lipase Activity in Hyperlipemic Developing Rats from Undernourished Pregnant Mothers

D. López-Tejero,[1] M. Llobera[1] and E. Herrera[2]

To study the potential relationship between circulating triacylglycerol (TAG) levels and lipoprotein lipase (LPL) activity in the newborn rat liver, pups from undernourished or normal control mothers were nursed by normal dams, and studied at 0, 1, 15 or 30 days of age. Plasma TAG levels and liver TAG concentration increased more in pups from undernourished mothers than they did in controls. At birth, liver LPL activity was similarly high in both groups but, whereas in controls it decreased progressively after birth, in pups from undernourished mothers it remained stable until 15 days of age. Results suggest that the hypertriglyceridemia present in pups from undernourished mothers may be responsible for the sustained high LPL activity in their liver which may enhance the hepatic uptake of circulating TAG.

INTRODUCTION

Lipoprotein lipase (LPL, E.C.3.1.1.34) is an enzyme bound to capillary endothelium. It hydrolyzes the triacylglycerols (TAG) present in circulating very low density lipoproteins and chylomicra, allowing the utilization of the TAG hydrolytic products by the tissues (1). Lipoprotein lipase is found mainly in some extrahepatic tissues such as adipose tissue, heart, skeletal muscle and lactating mammary gland (2). In agreement with its TAG-exporter role, the liver has been traditionally considered lacking in LPL activity. It contains another heparin-releasable lipase, called hepatic lipase (HL). The physiological role of this HL is clearly different to that of LPL (3). However, the existence of high LPL activities in the liver of some species, as well as in some experimental conditions, has been well documented (4–19). We reported that the appearance of LPL activity in the newborn rat liver (5, 18) is parturition-dependent (9) and increases under

---

[1] Departamento de Bioquímica y Fisiología, Facultad de Biologia, Universidad de Barcelona, Avda Diagonal 645, 08028 Barcelona, Spain.
[2] Departamento de Investigación, Centro Ramón y Cajal, Ctra. de Colmenar Km 9, 28031 Madrid, Spain.

starvation (13). We suggested that this LPL activity might well convert the liver into a net importer rather than a net exporter of TAG, and may be responsible for the accumulation of TAG observed in neonatal livers (9, 13).

Although the mechanism of hepatic LPL appearance is unknown, this appearance has been repeatedly observed in adult rats under hyperlipidemic conditions, such as after Intralipid administration (17) or during starvation in late pregnancy (16).

In the present work we study liver LPL activity, liver TAG concentration, and plasma TAG levels during postnatal development of rats from undernourished dams, since undernourishment of the mother is known to alter the lipidic metabolism in the offspring (20–22).

MATERIALS AND METHODS

Pups from both undernourished or control primiparous Wistar rats were used. The experimental undernourishment was achieved by maintaining the animals on an otherwise standard diet diluted 50% with pure cellulose (Panlab, UAR) for two months, this total period extending from before fecundation to the end of pregnancy. The total caloric intake of these animals was a mean 75% of that of the age-matched controls (23).

After parturition, newborn rats from both undernourished and control mothers were immediately cross-fostered with normal dams under standard feeding conditions (these had delivered their litters less than 6 hours before the experimental dams). At birth the number of pups per dam was adjusted to 8–10 to obtain optimize litter size in relation to milk supply (24, 25). Pups were weighed and sacrificed by decapitation either at birth or at 1, 4, 15 or 30 days of age.

Blood samples from each litter were pooled and collected into chilled heparinized beakers. Plasma was separated for triacylglycerol (26) and free fatty acid (27) determination. Immediately after sacrifice livers were excised and frozen in liquid nitrogen. They were used for triacylglycerol quantification (26) and lipoprotein lipase (LPL) activity measurement in acetone-ether extracts (28). Inhibition of LPL activity by NaCl, protamine sulphate and specific antibodies, and behaviour in heparin-Sepharose affinity chromatography was previously studied with rat adipose tissue, adult liver and newborn liver, and had shown that the activity measured with this method (28) actually corresponds to LPL but not to HL (17, 18).

RESULTS AND DISCUSSION

Liver LPL in Control Newborns

Figure 1 summarizes the circulating levels of TAG and hepatic concentration of TAG and LPL activity in pups both from undernourished and normally fed dams, at birth, and at different times after birth, being nursed by foster dams.
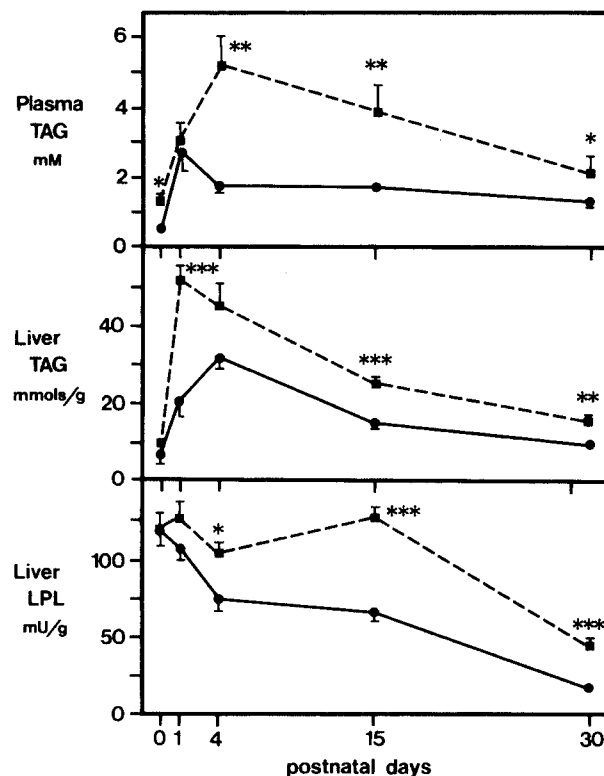
Fig. 1. Plasma and liver triacyglycerols and liver lipoprotein lipase activity in pups from undernourished (■-----■) or control (●———●) mothers. Values are means ± standard error of 8–9 data, each of which is the mean of values from one litter. Statistical differences between groups by Student's t test: $* = p < 0.05$, $** = p < 0.01$, $*** = p < 0.001$.

Control pups showed a peak of circulating TAG the first day after birth, which was followed by a wider peak in liver TAG concentration. These findings agree with those previously reported (9, 29), and may be related to the role of the liver as a temporary fat storage organ in the suckling rat due to the delayed development of white adipose tissue in the rat and to the high fat content of the milk ingested. During this phase of early life, hepatic lipogenesis is inhibited (30) and fatty acid utilization is greatly enhanced, and mainly used for ketone body production (9). There is, thus, an enhanced liver uptake of circulating fatty acids, fuelled by milk. On the basis of these considerations we previously postulated that the appearance of LPL activity in the newborn liver enables this organ to take up circulating TAG from VLDL and chylomicra (9, 13) in the same way as it does in extrahepatic tissues. This hypothesis is supported by the progressive decrease with age of both liver LPL activity and liver TAG content found in control pups (Fig. 1), and by our previous findings showing that exogenous LPL infused to livers of adult rats maintains its lipolytic activity (31).

Hyperlipidemia in Newborns from Undernourished Mothers

As shown in Fig. 1, pups from undernourished mothers had a greater and more prolonged increase in circulating TAG than those of controls, reaching a peak level at 4 days of age rather than on day 1, and maintaining their higher circulating TAG levels up to 30 days of age. During pregnancy, hyperlipidemia (32), diabetes (33) and betamethasone-treatment (34) are known to cause hyperlipidemia in the newborns, but the present findings are the first report of postnatal hyperlipidemia in the pups produced by maternal undernourishment. As yet we do not know the mechanism behind it but the potential effects of maternal undernourishment on milk production and/or composition need not be taken into account as the pups were suckled from birth by control dams. Pups from iron-deficient mothers develop hyperlipidemia (35), and a possible deficit in this and/or other trace elements as the main cause of the elevated pup-circulating-TAG levels could not be ruled out.

Hepatic LPL Activity in Newborns from Undernourished Mothers

After birth there were no differences in LPL activity between the pups from undernourished and control mothers (Fig. 1), but the decline in activity with age was longer and less marked in the first group, the differences between the two groups being statistically significant at 4, 15 and 30 days of age. Neither of these differences could be attributed to changes in either liver or animal body weight, as there was no difference between either group at any of the studied times (Table 1).

The origin of these high hepatic LPL amounts in the hyperlipidemic newborn rats from undernourished mothers has not yet been established. In hyperlipidemic adult rats, it has been suggested that the appearance of LPL in the liver can be a consequence of the transport of the enzyme from extrahepatic tissues bound onto remnants of TAG-rich lipoproteins (31). In newborns, it also seems that there is a direct relationship between increases in circulating TAG and the appearance of LPL in the liver: postmature pups from progesterone-treated rats neither develop hyperlipidemia nor show increased liver LPL activity (9) and in the present study we have seen the increase in hepatic LPL in a hyperlipidemic situation. However, the problem is not yet fully solved, as we have also found previously that

Table 1. Body and liver weights of rats born from undernourished or control mothers. Values are means ± standard errors of 9–14 data, each of which is the mean of values from one litter. No significant differences between groups by Student's t test

Postnatal	Body weight (g)		Liver weight (g)	
days	Control	Undernourished	Control	Undernourished
0	5.60 ± 0.09	5.58 ± 0.12	0.26 ± 0.01	0.29 ± 0.02
1	6.38 ± 0.22	6.80 ± 0.28	0.26 ± 0.07	0.27 ± 0.01
4	10.47 ± 0.32	9.89 ± 0.59	0.32 ± 0.02	0.32 ± 0.02
15	27.22 ± 1.12	29.45 ± 0.97	0.74 ± 0.04	0.71 ± 0.02
30	91.66 ± 2.23	98.14 ± 2.19	3.43 ± 0.42	3.36 ± 0.46

starvation elicits a decrease in circulating TAG along with an increase in liver LPL activity in the newborn rat (13). The existence of both types of relationship between circulating TAG and liver LPL activity suggests that probably another factor, not directly related to circulating TAG levels, can play a significant role in the control of the liver LPL expression. This is in agreement with the fact that livers from newborn rats can synthesize LPL (19), although the factors that control its expression are unknown.

During the studied period, offspring from undernourished pregnant rats also presented higher TAG levels in the liver than controls (Fig. 1). This increase does not seem due to an increased uptake of circulating free fatty acids since the levels of these metabolites in plasma are not increased until the 30th day of life compared to controls (data not shown). As lipogenesis is inhibited during this early life (30), these high hepatic TAG levels in hyperlipidemic newborns could be explained by an enhancement of the direct uptake of TAG by the liver, due in part to the high LPL activity in this organ.

These results therefore strongly support the previous hypothesis (18, 19) that LPL detected in the liver is fully functional, thus allowing this organ to directly utilize the circulating lipoprotein TAG.

We are currently focusing our efforts on clarifying these aspects since it is evident that the temporal appearance of LPL activity in the liver modifies the role of this organ in the overall lipoprotein metabolism, and may be of pivotal physiological importance in certain conditions, as for instance during suckling, when milk lipids are the major physiological substrates.

ACKNOWLEDGEMENTS

REFERENCES

1. Cryer, A. (1981). *Int. J. Biochem.* **13**:525–541.
2. Bensadoun, A. and Koh, T. L. (1977). *J. Lipid. Res.* **18**:768–773.
3. Cryer, A. (1981). *Int. J. Biochem.* **13**:525–541.
4. Kuusi, T., Schröder, T., Bång, B., Lempinen, M., Ehnholm, C. (1979). *Biochim. Biophys. Acta* **573**:443–450.
5. Llobera, M., Montes, A. and Herrera, E. (1979). *Biochem. Biophys. Res. Commun.* **91**:272–277.
6. Jensen, G. L., Bagby, D. L., Brannon, P. M. and Bensadoun, A. (1980). *J. Biol. Chem.* **255**:11141–11148.
7. Ogata, F. and Hirasawa, Y. (1982) *Biochem. Biophys. Res. Commun.* **106**:397–399.
8. Black, D., Kirkpatrick, S. A. and Skinner, E. R. (1983). *Biochem. Soc. Trans.* **11**:708.
9. Ramírez, I., Llobera, M. and Herrera, E. (1983). *Metabolism* **32**:333–341.
10. Paterniti, J. R., Brown, W. V., Ginsberg, H. N. and Artz, K. (1983). *Science* **221**:167–169.
11. Knobler, H., Chajeck-Shaul, T., Stein, O., Etienne, J. and Stein, Y. (1984). *Biochim. Biophys. Acta* **782**:363–371.
12. Masuno, H., Tsujita, T., Nakanishi, H., Yoshida, A., Fukunishi, R. and Okuda, H. (1984). *J. Lipid. Res.* **25**:419–427.

13. Grinberg, D. R., Ramírez, I., Vilaró, S., Reina, M., Llobera, M. and Herrera, E. (1985). *Biochim. Biophys. Acta* **833**:217–222.
14. Reina, M., Vilaró, S. and Llobera, M. (1985). *Biochem. Soc. Trans.* **13**:868–869.
15. Olivecrona, T., Chernick, S. S., Bengtsson-Olivecrona, G., Paterniti, J. R., Brown, W. V. and Scow, R. (1985) *J. Biol. Chem.* **260**:2552–2557.
16. Testar, X., Llobera, M. and Herrera, E. (1985) *Biochem. Soc. Trans.* **13**:134.
17. Vilaró, S., Reina, M., Ramírez, I. and Llobera, M. (1986) *Biochem. J.* **236**:273–278.
18. Reina, M., Vilaró, S. and Llobera, M. (1987) *Biol. Neonate* **51**:45–52.
19. Vilaró, S., Llobera, M., Bengtsson-Olivecrona, G. and Olivecrona, T. *Biochem. J.* (in press).
20. Tulp, O. R., Tyzbir, R. S., Morse, S. G. and Horton, E. S. (1980) *Nutr. Rep. Internat.* **22**:135–146.
21. Jones, A. P. and Friedman, M. I. (1982) *Science* **215**:1518–1519.
22. Stammers, J. P., Elphick, M. C. and Hull, D. (1983) *J. Develop. Physiol.* **5**:395–404.
23. Testar, X., López, D., Llobera, M. and Herrera, E. (1986) *Pharmacol. Biochem. Behav.* **24**:625–630.
24. Yagil, R., Etzion, Z. and Berlyne, G. M. (1976) *Lab. Animal Sci.* **26**:33–37.
25. Babicky, A., Parirek, J., Ostadalova, I. and Kolar, J. (1983) *Physiol. Bohémoslovaca* **22**:557–566.
26. Ramírez, J. I., Llobera, M. and Herrera, E. (1983) *Rev. Esp. Fisiol.* **39**:327–332.
27. Falholt, K., Lund, B. and Falholt, W. (1973) *Clin. Chim. Acta* **46**:105–111.
28. Corey, J. E. and Zilversmit, D. B. (1977) *J. Lab. Clin. Med.* **87**:666–674.
29. Jamdar, S. C., Moon, M., Bow, S. and Fallon, H. J. (1978) *J. Lipid Res.* **19**:763–770.
30. Gandemer, G., Pascal, G. and Durand, G. (1982) *Int. J. Biochem.* **14**:797–804.
31. Vilaró, S., Llobera, M., Bengtsson-Olivecrona, G. and Olivecrona, T. *Amer. J. Physiol.* (in press).
32. Andersen, G. E. and Friis-Hansen, B. (1976) *Pediatrics* **57**:214–218.
33. Mortimer, J. G. (1964) *Arch. Dis. Child* **39**:342–345.
34. Andersen, G. E. and Friis-Hansen, B. (1976) *Pediatrics* **62**:8–11.
35. Sherman, A. R., Bartholomey, S. J. and Perkins, E. G. (1982). *Lipids* **17**:639–643.