

LIPOPROTEIN LIPASE ACTIVITY IN LIVER OF THE RAT FETUS

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SUMMARY

Lipoprotein lipase activity was determined in tissue from pregnant and post-partum rats and virgin adult controls and in liver from fetuses and pups. A glycerol-based emulsion of tri-(1-¹⁴C)-oleoyl-glycerol was used as substrate. According to the inhibitory characteristics in the presence of protamine and NaCl, the measured activity corresponded to the extrahepatic lipoprotein lipase of the adults. Compared to control values, the lipoprotein lipase activity was reduced in the mother's adipose tissue in late gestation and during the first days after parturition while it did not change in heart. Liver activity was negligible in mothers and controls while in the fetus it increased until the time of birth. The presence of this enzyme may allow the fetus liver to remove circulating triglycerides and to store them in preparation for early extrauterine life.

INTRODUCTION

During late gestation, the fetuses of all mammalian species accumulate lipids. There are wide interspecies variations not only in percentile accumulation, ranging from 16% of birth weight in man to 1.1% in the pig and rat (1) but also in the tissues that participate in storage. Although adipose tissue develops during gestation in some mammals including man, it does not develop in the rat until several days after birth (2-4) and the liver may act as a temporary lipid store (5,6). Late fetal and early newborn rat liver may be able to remove circulating triglycerides by the action of lipoprotein lipase (clearing factor lipase, EC 3.1.1.3.), a function later reserved to extrahepatic tissues, mainly adipose tissue and heart (3,4,7,8). No information is presently available on the level of lipoprotein lipase activity in fetal liver. The recently described glycerol-based emulsion technique (9,10) permits rapid measurement of this enzymatic activity with appropriate sensitivity and reproducibility. With this methodology, we investigated lipoprotein lipase activity in rat liver during the perinatal period.

MATERIALS AND METHODS

Female Wistar rats were mated at two months of age and maintained in a controlled environment (23°C; 12 h light-dark cycles). The mothers and their fetuses or pups were sacrificed by decapitation at different times of gestation or post-partum, estimated by the appearance of spermatozooids in vaginal smears. Age and sex matched virgin adult rats were used as controls. Heart, liver and lumbar fat pads from mothers and control animals and liver from fetuses and pups were rapidly excised and placed in ice-cold saline. The tissues (approximately 200 mg) were minced and homogenized in 3 ml of 0.2M Tris-HCl, pH 8.2 and 1 ml aliquots were used to prepare an acetone-ether powder in the presence of 0.1 ml of heated rat plasma (60°C for 10 min) (11). The defatted preparations were dried under N₂ and dissolved in 2 ml of cold 0.05M NH₄OH-NH₄Cl, pH 8.1. The lipoprotein lipase substrate emulsion (9,10) was prepared by taking 69 mg of tri-oleoyl-glycerol, 3.3 mg lecithin and 25 μ Ci of tri-(1-¹⁴C)-oleoyl-glycerol to dryness under N₂. The dried lipids were emulsified in 5 ml of glycerol by sonication in an MSE sonifier (set at 12 microns, 1 minx5) with the glass test tube immersed in an ice bath. The emulsion was stored at room temperature.

The enzyme assay was always performed on the day of sacrifice. Two hundred microliters of the sample, suspended in the NH₄OH-NH₄Cl buffer, were incubated at 37°C for 30 min with mild agitation in the presence of 100 μ l of reaction mixture (6% BSA in 0.2M Tris-HCl, pH 8.2 made up in 0.15 M NaCl/ substrate emulsion/heated rat plasma: 1/1/0.5 by vol.). Incubations were terminated by the addition of 3.5 ml of methanol/chloroform/heptane (1.41/1.25/1, by vol.) and free fatty acids were extracted for counting in the upper phase after the addition of 1 ml of 0.1M potassium carbonate-potassium borate, pH 10.5 (12,13). Blank assay tubes without enzyme were always processed in parallel to make the proper corrections. Protein concentration was determined (14) and enzyme activity was calculated as the amount of tri-glycerides hydrolyzed per unit of protein.

RESULTS

As shown in Table I, the activity of lipoprotein lipase in the fed adult virgin rat was very high in adipose tissue, being lower in heart and negligible in liver. Pregnancy produced a decrease in the enzyme activity in adipose tissue and values became minimal around parturition and were not restored even 5 days after delivery. On the contrary, pregnancy produced no significant changes in enzyme activity in the heart nor did it alter the very low activity found in liver. Lipoprotein lipase activity in the liver of 17 day fetuses was as low as in their mothers, but later on there was a significant enhancement of this parameter which was maximal just before birth and rapidly returned to low levels 24 h after delivery.

TABLE I
Lipoprotein lipase activity in heart, lumbar fat pads and liver in pregnant and virgin adult control rats and liver of fetuses and newborns of different ages.

Group	Heart	Lumbar fat pads	Liver
Virgin controls	31.8±3.0 (12)	98.5±12.7 (12)	2.1±0.2 (12)
Days of gestation		Mother	Fetus or newborn
17			
P	25.0±2.9 (5)	66.8±9.2 (5)	1.3±0.3 (5) N.S. 1.7±0.4 (5)
19			
P	33.7±6.0 (5)	50.0±5.2 (5) *	2.2±0.3 (5) N.S. 6.2±2.0 (5) **
21			
P	35.1±2.7 (5)	35.5±6.7 (5) **	1.8±0.3 (5) 0.01 12.1±1.5 (16) ***
Days postpartum			
0			
P	35.5±4.4 (5)	23.8±8.4 (5) **	1.4±0.3 (5) 0.01 18.0±2.2 (30) ***
1			
P	35.0±3.6 (5)	21.5±3.0 (5) **	1.7±0.3 (5) N.S. 4.7±1.2 (9) *
5			
P	35.5±4.5 (5)	24.9±1.4 (5) **	1.7±0.1 (5) N.S. 2.9±0.4 (6) **

Enzyme activity was expressed per 100 mg of protein and calculated as μ moles of triglyceride hydrolyzed per 30 min. Means \pm S.E.M. Asterisks correspond to the significance between each group and the virgin controls: * = p 0.05, ** = p 0.01 and *** = p 0.001. p values correspond to the significance between the values in mother and fetus or newborn livers.

TABLE II

Characteristics of lipoprotein lipase activity in lumbar fat pads and liver in pregnant rats and liver of their fetuses at day 21 of gestation.

Enzyme activity (relative to complete system)			
Conditions of assay:	Complete system	NaCl (2.44 M)	Protamine sulfate (4mg/ml)
<u>Pregnants</u>			
Lumbar fat pads	100	6.3±2.5	30.9±2.2
Liver	100	7.4±3.8	10.5±0.6
<u>Fetuses</u>			
Liver	100	3.2±1.4	4.7±1.8
<u>Virgin controls</u>			
Liver	100	25.6±4.1	31.0±0.5

The inhibitory characteristics of lipoprotein lipase were established in tissues from 21 day pregnant rats and their respective fetuses. Samples were assayed in the presence of either 4 mg/ml of protamine sulfate or 2.44 M NaCl. As shown in Table II, both protamine and NaCl produced an intense inhibition of lipase activity in the liver from fetuses, mothers, and adult virgin rats as well as in the lumbar fat pads from mothers. The fetus liver preparation shows the greatest inhibition (Table II) in spite of exhibiting higher activity with the complete system than that of the mother and control livers (Table I).

DISCUSSION

These findings indicate that, during late pregnancy and after parturition, there is a decline in lipoprotein lipase activity in the mother. This effect may be restricted to the adipose tissue since the enzyme activity in heart is not altered. These results are in agreement with those reported by other investigators (15,16) and may partially explain the well known hyper-

lipemia of pregnancy (17-21) in terms of a diminished uptake of triglyceride fatty acids by adipose tissue. Other factors also contribute to the augmented circulating lipids in the mother including the active, intrinsic metabolism of her adipose tissue (22,23).

In contrast with the low enzyme activity observed in livers of adult controls and mothers, lipoprotein lipase activity was present in the fetus liver with values becoming maximal just before birth, after which they disappeared. These findings should be interpreted with caution since the technique may also be measuring minor amounts of other lipases (9,10). The enzyme activity present in fetus liver showed all the inhibitory characteristics of the extrahepatic lipoprotein lipase in adults and not those of hepatic lipase (24,25). These findings could be explained in terms of the liver's role as a temporary lipid depository for the fetus and the newborn (5,6), substituting for the as yet unformed white adipose tissue (2,3). Although from present results it is not possible to determine whether the lipoprotein lipase of the fetus liver is the same as that found after birth in adipose tissue (3,4), its appearance in the later part of intrauterine life may be related to the presence of very low density lipoproteins in fetal circulation near term (26,27). Unlike extrahepatic tissues, the normal adult rat liver has traditionally been regarded as a VLDL exporter, not a utilizer, while these roles appear inversed in the fetal liver. This is not a unique situation since, for example, ketone bodies are utilized by fetal liver both as oxidative (28) and lipogenetic substrates (29), while they are not used by the liver in adults.

The need for adequate fuels in early extrauterine life and the absence of adipose tissue require the fetus to use its own liver as a storage tissue with the support of metabolites continuously supplied by the mother. This may account for the storage of triglycerides in the fetus as previously suggested for the storage of glycogen (30-33).

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