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Starvation enhances lipoprotein lipase activity in the liver of the newborn rat

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To determine to what extent lipoprotein lipase activity in the liver of the newborn rat depends on milk ingestion, its changes were studied during different nutritional conditions. Newborns were placed with nurse rats with or without ligated nipples and they were killed at 0, 8 or 24 h of life. Lipoprotein lipase in newborns liver was characterized by its inhibition in the presence of 1.0 M NaCl, its specific elution at 1.5 M NaCl on heparin-Sepharose 4B column and its requirement for serum in the assay mixture to manifest its activity. In fed animals lipoprotein lipase activity and triacylglycerol content in liver as well as circulating triacylglycerols and ketone bodies increased progressively after birth. When newborns were kept starved the change in enzyme activity was significantly enhanced, whereas the increase found after birth in the other parameters disappeared. Starvation produced reduction in circulating RIA-insulin levels in the newborn rats. Results show that liver lipoprotein lipase activity in the newborn rat is controlled by a mechanism which resembles that of the enzyme in the adult heart and indicate that its presence facilitates the uptake by the liver of fatty acids from circulating triacylglycerols for their oxydation rather than deposit.

Introduction

In the adult mammal, lipoprotein lipase (EC 3.1.1.34) activity is generally found in extrahepatic tissues but not in liver [1,2] and is responsible for the hydrolysis of triacylglycerol-rich lipoproteins, the products of which are taken up by the subjacent tissue [3–6]. In contrast, during the perinatal phase in the rat we detected the presence of liver lipoprotein lipase activity [7,8] which converts the newborn liver into a triacylglycerol utilizer instead of exporter, as occurs normally in adults. Coincident with that change, the liver content of triacylglycerol, which is low in the rat fetus, increases rapidly after birth [8,9] and we recently found that both liver lipoprotein lipase activity increase and triacylglycerol accumulation are parturition-dependent, since they do not occur in postmature fetuses [8]. As the mechanisms which controls these changes in the liver of the newborn rat are not yet established and it is well known that lipoprotein lipase activity in extrahepatic tissues is altered by nutritional conditions, in the present work we determined how starvation in the neonatal rat affects those parameters. Results indicate that newborn rat liver lipoprotein lipase increases with starvation, whereas liver triacylglycerols content decreases.

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Materials and Methods

Female Wistar rats from our own colony were mated and maintained in a controlled environment (23 ± 2°C, 12 h light-dark cycles). At day 22 of gestation (estimated by the appearance of spermatozoids in vaginal smears), rats were killed by cervical dislocation and fetuses rapidly excised and placed under a hot lamp. 10 min later fetuses were removed from their placentas and placed in litters of 8–10 pups per cage together with a nurse rat with or without ligated nipples [10] to obtain the fed or the starved condition without modifying maternal behavior. One group of newborn starved rats was fed from the 8th h of life, whereas another group of fed rats was starved from the same time by changing nurses with or without ligated nipples. At 0, 8 or 24 h of life the pups were beheaded and blood from the neck wound was collected in receptacles containing heparin. Livers were rapidly excised and placed in liquid N_2. Aliquots of whole blood were deproteinized [11] and supernatants were used for glucose [12] and ketone body [13] determinations. Other aliquots of blood were used for plasma separation where triacylglycerols [14] and RIA-insulin [15] were measured. For the latter, RIA-kits for rat insulin generously provided by Novo Industri A/S, Denmark, were used. Lipoprotein lipase activity was measured in acetone/diethyl ether extracts from aliquots of the liquid N_2 frozen livers as previously described [16,17], with minor modifications [7]. Protein concentration in aliquots of the enzyme preparations was measured [18] to make the proper enzyme activity calculations, which units were expressed as nmol of substrate transformed per unit of time. Partial purification of lipase activity was carried out by heparin-Sepharose affinity chromatography [19] in a pool of 5.7 g of livers from 24-h-old rats that were starved from birth. All purification steps were carried out at 4°C. The acetone/diethyl ether extract of the livers was homogenized in 25 ml 5 mM barbital buffer (pH 7.2) containing 0.5 M NaCl (buffer A) and centrifuged at 30000 × *g* for 30 min. The supernatant was immediately applied to a heparin-Sepharose 4B column (1 × 8 cm) equilibrated with buffer A. The column was then washed with 50 ml of the same buffer and elution of the bound liver lipases was carried out with 5 mM barbital buffer (pH 7.2) containing either 0.9 M NaCl during the first part and 1.5 M NaCl during the second. Fractions of 1.5 ml each were collected automatically and lipase activities measured in aliquots of the fractions after being properly diluted with 5 mM barbital buffer (pH 7.2) to obtain a final NaCl concentration of 0.16 M. Activity corresponding to hepatic triacylglycerol lipase was assayed as described by Ehnholm et al. [20], whereas that corresponding to lipoprotein lipase was measured following the same procedure as that used for the crude liver preparations [7]. Liver triacylglycerols were measured by extracting lipids with chloroform/methanol [21] and phospholipids were removed as described elsewhere [8]. Aliquots of the neutral lipid extracts were dried at 40°C under N_2 for alkali saponification and triacylglycerol determination [14]. Results are expressed as mean ± S.E. and statistical comparison of groups was done by Student's *t*-test.

Results

Newborn rats were studied at 0, 8 or 24 h after delivery by caesarean operation. Newborns were kept with nurse rats with or without ligated nipples to maintain them either fed or starved, respectively. Results of the conditions of the animals studied are summarized in Table I, where it is seen that starvation for either 8 or 24 h always produced a significant reduction in body weight values of starved vs. fed rats of the same age. Blood glucose concentrations decreased only slightly in fed neonates until 24 h after birth, whereas it was significantly reduced in rats that were kept starved during the first 24 h of life (Table I). A significant reduction in blood glucose concentration was also found in rats fed until the 8th h and then starved until the 24th h (post-starved) (Table I). Plasma RIA-insulin levels did not change during the first 24 h of life in fed animals, whereas starvation always produced a reduction that was significant and striking in those rats fed until the 8th h and then starved until the 24th h of life (Table I). Plasma triacylglycerol concentration was very low at birth and changed very slightly during the first 8 h of life in both fed and starved newborns (Fig. 1). This effect increased very sharply from the 8th to the 24th h of

TABLE I

BODY WEIGHT, BLOOD GLUCOSE AND PLASMA RIA-INSULIN LEVELS IN NEWBORNS KEPT UNDER DIFFERENT NUTRITIONAL SCHEDULES

Values are expressed as mean ± S.E. of seven animals per group. [a,b,c] Statistical comparisons versus neonates; [d,e,f] statistical comparisons versus the same group but fed; [a] or [d] $P < 0.05$; [b] or [e] $P < 0.01$; [c] or [f] $P < 0.001$.

	Time killed (h after birth)	Period of food intake (h)	Period of starvation (h)	Body weight (g)	Blood glucose (mg/dl)	Plasma RIA-insulin (μU/ml)
Neoantes	0	–	–	6.21 ± 0.04	125 ± 11	33.8 ± 2.1
Fed (8 h)	8	0–8	–	6.36 ± 0.09	113 ± 4	26.7 ± 4.6
Fed (24 h)	24	0–24	–	6.86 ± 0.22 [c]	103 ± 7	27.6 ± 3.5
Starved (8 h)	8	–	0–8	5.90 ± 0.07 [c,f]	120 ± 8	20.4 ± 3.1 [b]
Starved (24 h)	24	–	0–24	5.43 ± 0.16 [c,f]	26 ± 15 [c,f]	15.1 ± 6.9 [a]
Refed	24	8–24	0–8	5.86 ± 0.09 [b,e]	99 ± 7	24.1 ± 3.4 [a]
Post-starved	24	0–8	8–24	5.66 ± 0.15 [c,f]	76 ± 11 [c]	5.5 ± 1.3 [c,f]

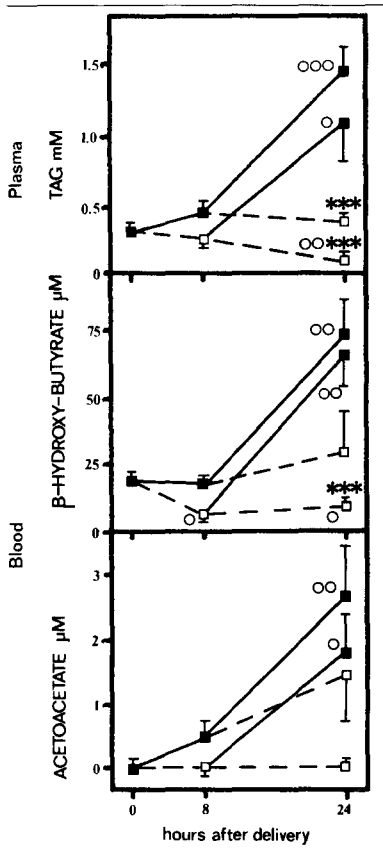

Fig. 1. Plasma triacylglycerols (TAG) and blood β-hydroxybutyrate and acetoacetate concentrations in newborn rats. Animals were studied either fed (■) or starved (□) for different periods after birth. Each point represents the mean ± S.E. of seven rats per group. Statistical comparisons versus animals killed at birth (0 time) are shown by ○ and these comparing starved versus fed rats of the same age, by ★: ○ or ★, $p < 0.05$; ○○ or ★★, $p < 0.01$; ○○○ or ★★★, $p < 0.001$.

life but was completely abolished when the animals were starved (Fig. 1). Parallel changes to those of plasma triacylglycerols occurred in blood ketone body concentrations (β-hydroxybutyrate and acetoacetate), with minor changes during the first 8 h of life and a sharp rise from the 8th until the 24th h in fed but not in starved animals (Fig. 1).

Lipoprotein lipase activity was assayed in the liver of the newborn rats. Inhibitory characteristics of the enzyme measured have been previously assayed [8] and shown to contain lipoprotein lipase characteristics similar to those in the adult extrahepatic enzyme. For further verification, two other experiments were carried out. In the first one, acetone/diethyl ether liver extracts taken from newborns at birth (0 h) and from 24-h-old newborns starved from birth were assayed for lipoprotein lipase activity in the presence or absence of 1.0 M NaCl. The degree of inhibition of the assayed activity was 79.6 ± 3.9% ($n = 6$) for preparations from newborns of 0 h and 84.6 ± 3.6% ($n = 5$) for 24-h-old starved rats. In the second experiment acetone/diethyl ether liver extract from 24-h-old newborns starved from birth was passed through a heparin-Sepharose 4B column. As shown in Fig. 2, the lipase activity was resolved into two clearly separated peaks. The first one eluted at 0.9 M NaCl and contained a lipase activity that corresponded to the typical hepatic triacylglycerol lipase found in adult liver according to its reactivity with the proper assay method [20]. In this peak a high amount of protein was also

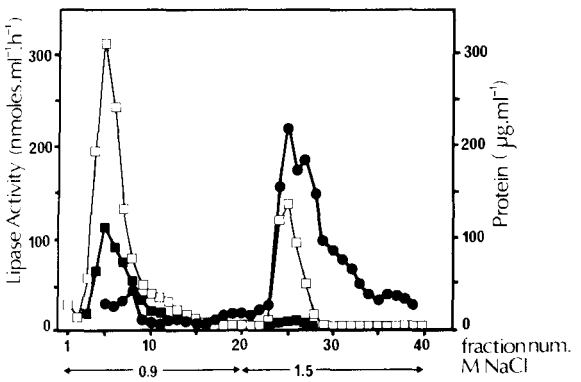

Fig. 2. Heparin-Sepharose chromatography of liver acetone/diethyl ether extract from 24-h-old rats starved from birth. The column (1 × 8 cm) was eluted with barbital buffer (5 mM, pH 7.2) containing either 0.9 or 1.5 M NaCl as indicated in the fig. The fractions (1.5 ml) were assayed for hepatic triacylglycerol lipase (■) and lipoprotein lipase (●) activities and protein concentration (□).

found but negligible lipase activity was found when assayed for lipoprotein lipase (Fig. 2). The second peak eluted at 1.5 M NaCl and showed no lipase activity when assayed for hepatic triacylglycerol lipase, an intense activity when assayed for lipoprotein lipase and a smaller amount of protein than the first peak. When the enzyme assay was carried out using a reaction mixture without serum, aliquots of this second peak showed a lipoprotein lipase activity that was always 31.5% below the value found in the same samples assayed with the complete medium. Lipoprotein lipase activity measured in crude extracts from liver of newborn rats progressively increased after birth and the effect was much greater in animals starved from birth or from the 8th h of life than in age-matched fed rats (Fig. 3). The concentration of triacylglycerols in liver increased progressively after birth in fed newborns but starvation completely abolished this increase in animals starved from birth or from the 8th h of life (Fig. 3). The direct dependence of this parameter on food intake is clearly seen in rats starved until the 8th h and then fed until the 24th h, at which time liver triacylglycerols concentration was significantly greater than that of rats of the same age starved for the entire 24 h period (Fig. 3).

The values of circulating levels of RIA-insulin,

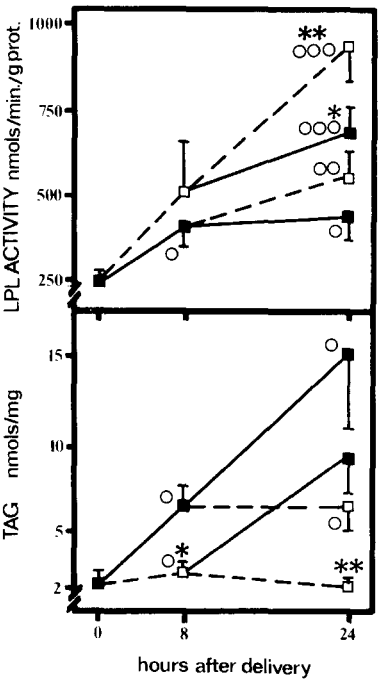

Fig. 3. Lipoprotein lipase activity and triacylglycerols (TAG) concentration in liver of newborn rats. Animals were studied either fed (■) or starved (□) for different periods after birth. Each point represents the mean ± S.E. of seven rats per group. Statistical comparisons versus animals killed at birth (0 time) are shown by ○ and those comparing starved versus fed rats of the same age, by ★: ○ or ★, $p < 0.05$; ○○ or ★★, $P < 0.01$; ○○○ or ★★★, $P < 0.001$.

glucose and triacylglycerols were plotted against liver lipoprotein lipase activity of individual animals from all the groups; the compiled values showed that there was always a negative and significant ($P < 0.01$) linear correlation (data not shown).

Discussion

This study documents the presence in the newborn rat liver of two distinct lipolytic enzymes. One of these resembles the hepatic triacylglycerol hydrolase, according to its elution on heparin-Sepharose 4B column [22,23]. The other corresponds to that previously described by us [7,8] and exhibits properties similar to those of the extrahepatic lipoprotein lipase of the adult [1,24,25].

Its characterization as lipoprotein lipase was established by its inhibition by 1 M NaCl and protamine, its elution at 1.5 M NaCl on heparin-Sepharose 4B column and its requirement for serum in the assay mixture as source of apolipoprotein cofactors (presumably, apolipoprotein C-II). Present findings indicate that the lipoprotein lipase activity which appears in the liver around birth in the rat is not induced by milk ingestion, as previously suggested by us [8], as it is present in animals starved from birth. This activity is, however, altered by nutritional factors and the increase found in the starved versus fed newborn rats is similar to that found for the heart enzyme in adults [26–28] and opposite to that in adipose tissue [3,6,28,29]. This similar response to food deprivation in the newborn liver lipoprotein lipase and the adult heart enzyme indicates that their physiological role may be also similar, facilitating the uptake of fatty acids derived from plasma triacylglycerols contained in circulating triacylglycerol-rich lipoproteins for their oxydation rather than for deposit. Inverse changes of circulating triacylglycerols and ketone body concentrations and liver lipoprotein lipase activity are consistent with this hypothesis, although it requires greater experimental support.

Recent studies in neonatal mice [30] suggest that the presence of liver lipoprotein lipase around birth is genetically controlled but its increase with starvation indicates that its activity is also modulated by hormonal factors and/or substrate availability. Observed inverse correlations between neonatal liver lipoprotein lipase activity and circulating glucose, RIA-insulin and triacylglycerols do not demonstrate a cause/effect relationship between these factors but do show that they are interrelated in the neonatal liver in a similar manner as for the adult heart enzyme. Rat heart lipoprotein lipase is known to be reduced by glucose administration [26] and enhanced by both counter-insulin factors such as corticosteroids [31,32] and reductions of plasma triacylglycerols [33]. Thus, it seems that the increase in liver lipoprotein lipase activity in the starved newborn rat represents a compensatory response to the decrease in circulating triacylglycerols and the actual mechanism of its induction remains to be established.

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