The storage and synthetic pools of heparin-releasable lipoprotein lipase and hepatic triacylglycerol lipase in the growing puppy

John B. DAS, Indira D. JOSHI and Arvin I. PHILIPPART

Division of Paediatric General Surgery, Children's Hospital of Michigan, 3901 Beaubien Boulevard, Detroit, MI 48201, U.S.A.

(Received 24 May 1982/Accepted 4 June 1982)

Age-related changes in the activities of extrahepatic lipoprotein lipase and hepatic triacylglycerol lipase were determined during a primed/constant-rate infusion of heparin for 2 h in puppies between birth and 18 weeks of age. The early (storage) and late (synthetic) phases were measured. Both phases of hepatic triacylglycerol lipase activity were well developed in the first week, reflecting the metabolic maturity of the liver at birth. During the 18 weeks of study, the activity remained relatively unchanged except for a sharp peak at 12 weeks. Extrahepatic lipoprotein lipase activity was low in the first 4 weeks of suckling. Its storage pool increased 6-fold in the next 14 weeks, with a less marked rise in its late (synthetic) pool. Sustained increases in the activity of this enzyme were first noticed during weaning, when the insulin-secretory response matured. Endogenous insulin-secretory capacity rather than the fat content of the feed appeared significant in the postnatal development of lipoprotein lipase (clearing-factor lipase) activity.

Heparin, given as a primed/constant-rate infusion, elicits a two-phase response in the activities of the triacylglycerol hydrolases released from the luminal surface of capillary endothelium (Robinson, 1970; Brunzell *et al.*, 1972). An early phase with lipolytic activity peaking at 15 min is due to the release of enzymes from a storage site. The later phase exhibits a plateau of enzyme activity for the duration of the heparin infusion and represents ongoing synthesis of enzymes. Two major triacylglycerol hydrolases contribute to the post-heparin lipolytic activities. Lipoprotein lipase originates mainly in adipose tissue and muscle, and requires serum cofactors for its activation; it is inhibited by high concentrations of NaCl. The second enzyme is hepatic triacylglycerol lipase, which does not require serum cofactors; it is rapidly inactivated by sodium dodecyl sulphate, but is resistant to salt (Ehnholm *et al.*, 1975; Baginsky & Brown, 1979).

The age-related changes in the lipoprotein lipase activity of muscle, adipose tissue and lung in the rat pup have been described (Cryer & Jones, 1978). The measured activity was that of the extrahepatic lipoprotein lipase. Lipolytic activity measured in a tissue sample reflects only the static, stored pool of the enzyme. Little is learned about the dynamic, synthetic phase of enzyme activity, that will be brought to play in the clearance of circulating

triacylglycerols from plasma. In this study, we measured the storage and synthetic phases of the two major triacylglycerol hydrolases in canine pups during suckling and after weaning. We theorized that the emergence of the enzyme profiles would be in response to the high-fat content of mother's milk, and the endocrine events triggered by the metabolic fuel.

Materials and methods

Animals

Male and female beagle pups ($n = 17$) from four litters were studied. The pups were delivered in our animal facility and were nursed by their dams. They were weaned to a high-protein puppy diet in week 5 of life. Weaning was completed by the end of week 8, after which the pups were fed standard laboratory dog food.

The heparin-infusion study

A total of 34 infusion studies were successfully completed at six age levels from birth to 18 weeks of life, with two or more studies in puppies older than 2 weeks. The puppy was isolated in a servo-controlled radiant-heat warmer (core temperature, 37.5°C), and was starved for 2 h before the infusion study. Under intraperitoneal pentobarbital anaesthesia (25 mg/kg body wt.), an artery was cannulated for

blood sampling, and a major vein for the infusions. Adequate pulmonary ventilation was assured by a small animal respirator, breathing room air via an endotracheal tube.

Heparin-releasable lipolytic activities were measured during a 2-h infusion of heparin. After a priming dose of 100 units/kg body wt. given as a pulse, heparin diluted in 0.15 M-NaCl was infused at 50 units/kg body wt. per h. A concurrent infusion of dextrose (5–8 mg/kg body wt. per min) was given to ensure a background of sugar and insulin optimal for the continued synthesis of the lipolytic enzymes (Parkin *et al.*, 1980). Arterial blood samples (0.7 ml) were taken before, and at 15, 60 and 120 min during the heparin infusion, for the estimation of lipolytic activities (Baginsky & Brown, 1979), blood sugar (Peterson & Young, 1968) and plasma immuno-reactive insulin with porcine insulin as standard (Hales & Randle, 1963). The samples were collected in chilled polypropylene tubes. The plasma was separated immediately and stored at -20°C for up to 1 month before analyses in triplicate.

The methodology and the reagents were essentially those described by Baginsky & Brown (1979), with a few minor modifications. The substrate was prepared fresh before each set of assays. Omission of the gum arabic enabled us to reduce the trioleoylglycerol content of the emulsion without impairing the sensitivity of the assay. Phosphatidylcholine was added to the assay mixture for maximal activation of the lipoprotein lipase in the presence of serum cofactors (Chung *et al.*, 1973). After release of the labelled fatty acids, the incubation was terminated by the addition of a benzene/chloroform/methanol (10:5:12, by vol.) mixture (Pittman *et al.*, 1975). This final modification assured efficient extraction of the non-esterified fatty acids into the aqueous phase, unaffected by the serum albumin and trioleoylglycerol in the medium.

The substrate was unlabelled trioleoylglycerol (Applied Science, State College, PA, U.S.A.) mixed with tri[1- ^{14}C]oleoylglycerol (New England Nuclear, Boston, MA, U.S.A.) to yield a specific radioactivity of 0.123 Ci/mol. Amounts of substrate sufficient for 45 assays were freshly prepared by adding 4.5 ml of fatty acid-free albumin (1.25 g/5 ml) and 4.5 ml of 1 M-Tris/HCl (pH 8.2 for lipoprotein lipase or pH 8.8 for hepatic triacylglycerol lipase) to the mixture of trioleoylglycerol (4 μCi of label in 28.68 mg of unlabelled trioleate) and 2.9 mg of phosphatidylcholine, previously dried under N_2 . The mixture was emulsified with a sonicator (Branson Instruments Inc., Danbury, CT, U.S.A.) for 2 min at setting no. 7.

The assay mixture (0.45 ml/assay) contained 0.72 μmol of trioleoylglycerol, 0.072 μmol of phosphatidylcholine, 100 μmol of Tris/HCl and 25 mg of albumin. In the assay for lipoprotein lipase the pH was 8.2; 0.05 ml of pooled serum from starved dogs

and 50 μmol of NaCl were also added. In the assay for hepatic triacylglycerol lipase, the pH was 8.8; 375 μmol of NaCl was added to the mixture, and the serum cofactors were omitted.

Assay for lipoprotein lipase

Post-heparin plasma (0.1 ml) was added to 0.1 ml of 50 mM-sodium dodecyl sulphate (Eastman Kodak, Rochester, NY, U.S.A.) in 0.2 M-Tris (pH 8.2), incubated for 30 min at 37°C and then diluted further with 0.8 ml of 0.2 M-Tris. Hepatic triglyceride lipase-inactivated plasma (0.05 ml) was added to 0.45 ml of assay medium (which had been pre-incubated for 30 min at 37°C). This mixture was incubated for 60 min at 37°C . The assay medium contained 0.05 ml of starved serum and 50 μmol of NaCl. The incubation was terminated with the addition of 7.5 ml of benzene/chloroform/methanol and 1.75 ml of 0.2 M-NaOH. After thorough mixing and subsequent centrifugation, 1.0 ml of the upper phase was transferred to 10 ml of scintillation fluid (Pittman *et al.*, 1975). Radioactivity was measured in a Packard TriCarb scintillation spectrometer (Packard Instruments, Downer's Grove, IL, U.S.A.). Enzyme activity was expressed as μmol of non-esterified fatty acids released per 1 ml of plasma in 60 min at 37°C .

Assay for hepatic triacylglycerol lipase

Post-heparin plasma (0.1 ml) was diluted with 0.9 ml of 0.2 M-Tris (pH 8.8). Dilute plasma (0.05 ml) was immediately incubated with a 0.45 ml assay mixture containing 375 μmol of NaCl (with serum cofactors omitted) for 60 min at 37°C . Termination of incubation, separation of the aqueous phase and scintillation spectrometry of the labelled fatty acids released were similar to those in the assay for lipoprotein lipase.

Specificity of the assays

Lipoprotein lipase is characterized by its specific needs for serum cofactors and an assay medium of low ionic strength with a pH of 8.2. In our assays for lipoprotein lipase, replicate samples of all preparations were routinely run in which the cofactors were omitted from the assay mixture. Residual activities measured in these samples were allowed for in the calculation of the 'true' activity of lipoprotein lipase in the samples incubated with cofactors. In the assay for hepatic triacylglycerol lipase, addition of sodium dodecyl sulphate during incubation quenched all enzyme activity in the assay medium (pH 8.8). Thus each enzyme activity was measured efficiently at its specific pH, without any contribution from the other.

Statistical analysis

The results are expressed as means $\pm$ S.D. The statistical significance of the difference between pairs

of age groups was computed by the *t*-test for unpaired samples with significance set at *P* < 0.025.

Results and discussion

Table 1 shows the age-related changes in lipoprotein lipase and hepatic triacylglycerol lipase during the heparin infusion. Enzyme activity from the storage pool (early phase) was measured in the plasma sample 15 min after the priming dose, whereas the activities in the 60 and 120 min samples represented the continued release of enzymes from sites of synthesis (late phase).

Lipoprotein lipase activity was lowest during the first week of life. The storage pool increased 7-fold over the 18 weeks of study. The increase in synthetic capacity was less marked. In the second week of life, there was a small peak in enzyme activity, but the number of observations at this age was too small (*n* = 3) to warrant a statistical inference. The lipolytic activity in the synthetic pool was low until weaning, when there was a sharp rise in enzyme activity after 60 and 120 min of infusion. The activities in the storage and synthetic pools continued to increase over the 10 weeks after weaning.

Hepatic triglyceride lipase activity was well developed at birth. The activity in the storage and the synthetic pool remained essentially unchanged during the period of suckling and weaning. There was a transient sharp peak in the activities of both pools in week 12 of life (*P* < 0.001).

Table 2 shows blood sugar and immunoreactive insulin values. During the heparin infusion, dextrose was infused at a rate equalling that of endogenous glucose production in the liver of the newborn pup (Kornhauser *et al.*, 1970). In the newly born, blood sugar continued to rise during the dextrose infusion. However, by the time of weaning, blood sugar homeostasis was well established, indicating that endogenous glucose production could be turned off, whereas the rate of peripheral glucose utilization equalled the infusion rate. These responses are dependent on the animal's ability to release and respond to insulin. The age-related decrease in blood sugar levels of starved animals and increase in the insulinogenic index [change in immunoreactive insulin (μ -units/ml)/change in blood sugar (mg/dl)] reflect the functional maturation of the insulin-secretory system.

In the present study, the postnatal age-related changes in the storage and synthetic pools of the two heparin-releasable triacylglycerol hydrolases were studied. It was anticipated that the development of enzyme activities would be in response to the high-fat content of the milk ingested. But we found a low lipoprotein lipase activity in the first 4 weeks of life, when the suckling pup was totally dependent on mother's milk. A rapid increase in lipolytic activity

Table 1. Activities of extrahepatic lipoprotein lipase and hepatic triacylglycerol lipase during primed/constant-rate infusion of heparin in canine pups. Lipolytic activities were measured in plasma at 15 min (storage pool), and at 60 and 120 min (synthetic pool) during the heparin infusion. Enzyme activity is expressed as μ mol of non-esterified fatty acid/h per ml of plasma at 37°C (means $\pm$ s.d.; no s.d. given if *n* < 4). The numbers of independent studies at each age group are given in parentheses.

Age	Wt. (kg)	Heparin infusion (min)	Lipoprotein lipase activity			Hepatic triacylglycerol lipase activity		
			15	60	120	15	60	120
6 days (7)	0.41 $\pm$ 0.08	...	2.18 $\pm$ 0.66	0.96 $\pm$ 0.61	1.09 $\pm$ 0.68	4.30 $\pm$ 0.17	2.34 $\pm$ 0.16	1.62 $\pm$ 0.53
2 weeks (3)	0.93		3.41	1.94	0.83	4.57	3.23	2.12
4 weeks (4)	1.50 $\pm$ 0.16		2.76 $\pm$ 0.57	0.56 $\pm$ 0.23	0.38 $\pm$ 0.21	3.24 $\pm$ 0.87	2.02 $\pm$ 0.60	1.22 $\pm$ 0.44
8 weeks (7)	2.67 $\pm$ 0.42		7.19 $\pm$ 1.29	3.97 $\pm$ 1.26	2.57 $\pm$ 0.81	4.48 $\pm$ 0.33	3.02 $\pm$ 0.82	1.99 $\pm$ 0.93
12 weeks (7)	4.03 $\pm$ 0.73		12.07 $\pm$ 3.25	5.71 $\pm$ 1.48	4.00 $\pm$ 0.66	8.29 $\pm$ 0.59	4.69 $\pm$ 0.94	3.87 $\pm$ 0.99
18 weeks (6)	6.04 $\pm$ 0.86		16.31 $\pm$ 6.20	9.10 $\pm$ 6.40	4.56 $\pm$ 1.67	5.73 $\pm$ 1.33	3.68 $\pm$ 2.80	2.81 $\pm$ 2.33

Table 2. *Changes in blood sugar and immunoreactive insulin during concurrent infusions of heparin and dextrose in canine pups*

During the heparin infusion, dextrose was infused concurrently at a rate of 5 mg/kg body wt. per min up to the age of 8 weeks, and at 8 mg/kg body wt. per min thereafter. Abbreviations: B.S., blood sugar (mg/dl); Δ B.S., blood sugar peak above starvation level during dextrose infusion; IR-Ins, immunoreactive insulin (μ -units/ml of plasma); Δ IR-Ins, insulin peak above starvation level during dextrose infusion. Results are means $\pm$ S.D. for the numbers of independent observations given in parentheses (no S.D. is given if $n < 4$).

Age		Dextrose and heparin infusions:				Insulinogenic index (Δ IR-Ins/ Δ B.S.)
		0min	15min	60min	120min	
6 days (7)	B.S.	114 $\pm$ 30	136	204	193 $\pm$ 40	0.042
	IR-Ins	7.2 $\pm$ 1.9			10.5 $\pm$ 0.7	
2 weeks (3)	B.S.	123		141	185	0.043
	IR-Ins	5.3			8.0	
4 weeks (4)	B.S.	124 $\pm$ 31	110	149	143 $\pm$ 10	0.158
	IR-Ins	6.3 $\pm$ 0.9			9.3 $\pm$ 2.2	
8 weeks (7)	B.S.	86 $\pm$ 17	90	113 $\pm$ 15	128 $\pm$ 13	0.289
	IR-Ins	8.1 $\pm$ 1.7		18.1 $\pm$ 5.8	20.1 $\pm$ 5.2	
12 weeks (7)	B.S.	85 $\pm$ 8	106 $\pm$ 21	118 $\pm$ 22	117 $\pm$ 24	0.328
	IR-Ins	11.0 $\pm$ 4.0		22.0	21.5 $\pm$ 6.1	
18 weeks	B.S.	91 $\pm$ 6	102 $\pm$ 10	128 $\pm$ 28	134 $\pm$ 33	0.314
	IR-Ins	10.5 $\pm$ 1.7		20.0 $\pm$ 7.0	24.0 $\pm$ 10.0	

was seen subsequent to the maturation of the glucoregulatory system. At this time, blood sugar homoeostasis could be achieved with a brisk insulin response in the face of a dextrose infusion. The progressive increase in lipoprotein lipase activity could also be related to the known changes in body composition occurring during postnatal growth. In the newborn dog, adipose tissue accounts for 6% and muscle for 20% of body weight (Widdowson, 1968). The growth of muscle mass continues past weaning into the phase of sexual maturity when muscle equals 40% of body weight (Gunn, 1978). Body fat, on the other hand, reaches the adult proportion of 15% of body weight by week 4 (Sheng *et al.*, 1977). As the capillary bed in muscle increases with growth, the potential sites for storage of lipoprotein lipase on endothelium will also increase. It has been shown in the suckling rat that the clearance of plasma triacylglycerols is largely dependent on muscle lipoprotein lipase (Planche *et al.*, 1980), and that pancreatectomy and the consequent loss of endogenous insulin depresses the activity of this enzyme in skeletal muscle (Linder *et al.*, 1976).

The existence of a two-pool system for triacylglycerol hydrolases releasable by heparin has been described for the isolated rat heart (Aktin & Meng, 1972) and in the post-heparin plasma of man (Brunzell *et al.*, 1972). The short half-life of 20–60 min for lipoprotein lipase in most tissues (Chajek *et al.*, 1978) lends credence to the view that the two pools we measured are separate, and that the activities seen at 60 and 120 min of heparin infusion are due to the slow release of enzymes from sites of synthesis.

References

- Aktin, E. & Meng, H. C. (1972) *Diabetes* **21**, 149–156
- Baginsky, M. L. & Brown, W. V. (1979) *J. Lipid Res.* **20**, 548–556
- Brunzell, J. D., Smith, N. D., Porte, D., Jr. & Bierman, E. J. (1972) *J. Clin. Invest.* **51**, 16a–17a
- Chajek, T., Stein, O. & Stein, Y. (1978) *Biochim. Biophys. Acta* **528**, 456–465
- Chung, J., Scanu, A. M. & Reman, F. (1973) *Biochim. Biophys. Acta* **296**, 116–123
- Cryer, A. & Jones, H. M. (1978) *Biochem. J.* **174**, 447–451
- Ehnholm, C., Shaw, W., Greten, H., Lengfelder, W. & Brown, W. V. (1975) in *Atherosclerosis III: Proc. Int. Symp. 3rd*, 557–560
- Gunn, H. M. (1978) *Res. Vet. Sci.* **24**, 277–282
- Hales, C. N. & Randle, P. J. (1963) *Biochem. J.* **88**, 137–146
- Kornhauser, D., Adam, P. A. J. & Schwartz, R. (1970) *Pediatr. Res.* **4**, 120–128
- Linder, C., Chernick, S. S., Fleck, T. R. & Scow, R. O. (1976) *Am. J. Physiol.* **231**, 860–864
- Parkin, S. M., Walker, K., Ashby, P. & Robinson, D. S. (1980) *Biochem. J.* **188**, 193–199
- Peterson, J. I. & Young, D. S. (1968) *Anal. Biochem.* **23**, 301–316
- Pittman, R. C., Khoo, J. C. & Steinberg, D. (1975) *J. Biol. Chem.* **250**, 4505–4511
- Planche, E., Boulange, A., DeGasquet, P. & Tonnu, N. T. (1980) *Am. J. Physiol.* **238**, E511–E517
- Robinson, D. S. (1970) *Compr. Biochem.* **18**, 51–116
- Sheng, H. P., Kamonsakpithak, S., Naiborhu, A., Chuntanaukoon, S. & Huggins, R. A. (1977) *Growth* **41**, 139–146
- Widdowson, E. M. (1968) in *Growth and Development of Mammals* (Lodge, G. A. & Lamming, G. M., eds.), pp. 224–233, Plenum Press, New York