0031-3998/85/1901-0023$02.00/0
PEDIATRIC RESEARCH

Vol. 19, No. 1, 1985
Printed in U.S.A.

Lipid Clearing in Premature Infants during Continuous Heparin Infusion: Role of Circulating Lipases

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ABSTRACT. The nature of the lipases released into the circulation during low level continuous infusion of heparin (1 unit/ml total parenteral nutrition) and after bolus heparin injection (10 units/kg) was investigated in a group of 11 low birth weight infants (gestational age 27–34 wk, and postnatal age of 7–26 days) receiving total parenteral nutrition with Intralipid (0.5 g/kg). Hepatic lipase and extrahepatic lipoprotein lipase were differentiated with the aid of an antibody specific for human hepatic lipase. The data show that continuous low level heparin infusion leads to a constant baseline postheparin lipolytic activity of 0.77 ± 0.18 μmol free fatty acids released per milliliter serum per hour. Bolus heparin injection leads to peak lipolytic activity levels of 3.77 ± 0.46 μmol free fatty acids per milliliter serum per hour, 10 min after injection. About two-thirds of the total postheparin lipolytic activity was of the hepatic type during low level continuous infusion or after bolus injection of heparin. (*Pediatr Res* 19: 23–25, 1985)

Abbreviations

PHLA, postheparin lipolytic activity
TPN, total parenteral nutrition
LPL, lipoprotein lipase
HL, hepatic lipase
FFA, free fatty acids

Low birth weight infants are often unable to feed orally and are maintained on TPN for extended periods of time. A major source of energy during TPN is Intralipid, a 10% soybean oil emulsion containing high levels of unsaturated fatty acids (18). Intralipid is cleared from the circulation in a similar way to that of naturally occurring chylomicrons (8, 19), *i.e.* the lipid is hydrolyzed at the capillary endothelial surface prior to uptake of triglyceride-fatty acids by the tissues. The lipases active at the capillary endothelium are readily released into the circulation by heparin administration, where they are called PHLA. PHLA contains two distinct lipases of different origin (16): lipoprotein lipase (9, 14), released from the endothelium of adipose tissue, heart, lung, and skeletal muscle, and HL (14, 15), released mainly from liver capillaries. These two lipases differ in that LPL requires apoprotein C-II for optimal activity, and is inhibited by high salt concentrations and by protamine sulfate, while HL does not require the protein cofactor and is not inhibited by high salt concentration. The main substrates for LPL are chylomicrons and very low density lipoproteins (9, 14). It appears that the HL acts on other types of lipoproteins, high-density lipoproteins (15, 17) and perhaps small very low-density lipoproteins (5). The purpose of this study was to measure the contribution of LPL and HL to the PHLA of premature infants.

Received November 16, 1983; accepted July 11, 1984.
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This study was presented at the Annual Meeting of the Society for Pediatric Research, Washington, DC, May 1983 and published in abstract form (Pediatr Res 17:297A, 1983).

METHODS

Subjects. A group of 11 nonicteric premature infants receiving TPN were studied at Louisiana State University Hospital in Shreveport, LA. The standard medical care in the intensive care nursery of this hospital includes the continuous administration of low levels of heparin, 1 unit/ml iv fluids. The amount of heparin given was directly related to the amount of parenteral fluids administered. TPN with Intralipid (Cutter, Berkeley, CA) was not started until 5–10 days after birth, or until phototherapy was discontinued. Intralipid administration was started at a rate of 0.25 g/kg infused during 8–12 h for the first 2 days. The amount of lipid infused was increased by increments of 0.25 g/kg every other day to a maximum of 2–2.5 g/kg given over a 16-h period daily. The infants had a gestational age of 27–34 wk and a postnatal age of 7–26 days at the time of study (Table 1); birth weights ranged between 720–1740 g, only one infant was small for gestational age. None of the infants was heparinized, and all were stable at the time of study. On the day of study, the infants were given a bolus heparin injection (10 units/kg) for maximal release of endothelial lipases, followed immediately by infusion of Intralipid 0.5 g/kg, for 4 h. No other changes in iv fluids were made, including infusion of the small standard amounts of heparin. Blood specimens were obtained at 0, 10, 20, 30, 120, and 240 min of infusion. The 0 time samples were obtained prior to the bolus heparin injection. The blood specimens were placed on ice, and the serum was immediately separated by centrifugation in a refrigerated centrifuge in order to avoid *in vitro* lipolysis. All serum specimens were stored at −70° C until analysis. These studies were approved by the Committee on Human Research of the Medical Center. Informed consent was obtained from the parents before enrollment of the infants in the study.

Preparation of purified human HL and of the rabbit antibody against this enzyme. HL was prepared from freshly drawn human postheparin plasma by affinity chromatography on heparin-

Table 1. *Clinical data on infants*

Infant	Sex	Gestational age/wk	Birth wt (g)	Intralipid received prior to study (g)	At study: Wt (g)	At study: Age (days)	At study: Intralipid infused (g)
BO	F	27	720	15.6	780	26	0.39
TA	F	28	1420	1.2	1280	7	0.64
BR	F	29	1020	4.2	900	11	0.45
SN	F	30	1410	19.5	1260	19	0.63
SH	F	30	1320	11.0	1020	19	0.51
WI	F	30	1220	21.5	1160	17	0.58
SM	M	27	780	5.2	660	11	0.33
JA*	M	29	720	5.6	680	17	0.33
McG	M	32	1740	3.8	1480	7	0.74
BA	M	32	1260	0	1680	26	0.84
GA	M	34	1700	0	1620	9	0.81

* Small for gestational age.

Sepharose. The enzyme was eluted by a gradient of NaCl in 20 mM Tris-Cl pH 8.5 containing 20% (w/v) glycerol. The peak of HL activity eluted at about 0.7 M NaCl. To remove traces of LPL the material was then mixed with anti-LPL immunoglobulins prepared by adsorption on protein A-Sepharose of a rabbit antiserum raised against bovine milk LPL. This antiserum cross-reacted with human LPL (10). After 4 h at 4° C the mixture was diluted with 3 volumes of the buffer with glycerol and then applied to a column of N-desulfated, acetylated heparin-Sepharose (4). The enzyme was eluted as described above for the first column. The final preparation of HL had a specific activity of 150 μmol fatty acids released/min/mg protein and showed two bands on polyacrylamide gel electrophoresis in sodium dodecyl sulfate. This material was used for immunization of a rabbit; about 50 μg was used for each injection. Serum from several bleedings were combined and used as anti-HL antiserum in this study. This antiserum could completely inhibit HL activity in human postheparin plasma but did not affect the activity of LPL purified from human postheparin plasma, bovine milk, or rat postheparin plasma. On double diffusion in agarose plates against human preheparin plasma no precipitates were seen, demonstrating that the antiserum did not react with any major plasma protein.

Specimen analysis. All the sera were analyzed for lipolytic activities, triglyceride, and free fatty acid (FFA) levels according to methods previously described (10, 20). PHLA, LPL, and HL activities, were measured by hydrolysis of tri-9, 10(n) [^{3}-H] oleylglycerol (Amersham, England) in a gum arabic stabilized emulsion (10). Lipolytic activity was expressed as μmol FFA produced per milliliter serum per hour. LPL activity was measured after inactivation of HL by rabbit antibody against purified human HL. The reaction was stopped and the FFA produced were extracted and quantitated as described by Belfrage and Vaughan (2).

Serum FFA were measured by the [^{63}]$NiCl_2$ micromethod (11) on 10 μl of serum, and triglycerides were quantitated enzymatically using 3 μl serum in a Micro Centrifugal Analyzer (13) (Instrumentation Laboratories, Lexington, MA).

RESULTS

Data on the infants studied (six girls and five boys), gestational age, birth weight, age at study, and amount of Intralipid received prior to the study are given in Table 1.

We have previously shown that in infants who receive TPN without "on line heparin," lipolytic activity is not detectable in the circulation prior to heparin administration and disappears completely 120 min after bolus heparin injection (7). The continuous infusion of small amounts of heparin (1 unit/ml iv fluid) produced a detectable baseline PHLA (Fig. 1). Administration of a bolus of 10 units/kg of heparin, led to the previously observed (7, 20) maximal release of lipolytic enzymes within 10 min after heparin injection (Fig. 1). Under our assay conditions LPL constitued about one-third of the total PHLA, while HL was the major component, two-thirds of the circulating PHLA (Table 2). Lipase activity levels (PHLA and LPL) were higher with Intralipid as substrate in the assay system (7, 20) than with the triolein-gum-arabic emulsions. The proportional contribution of LPL and HL to total PHLA in the infants' serum was, however, not changed by the type of lipid emulsion used.

The triglyceride concentration rose gradually during Intralipid infusion from the baseline value of 56 mg/dl to stabilize at about 110 mg/dl at 2 h of infusion. The FFA values reached a 1 μmol/

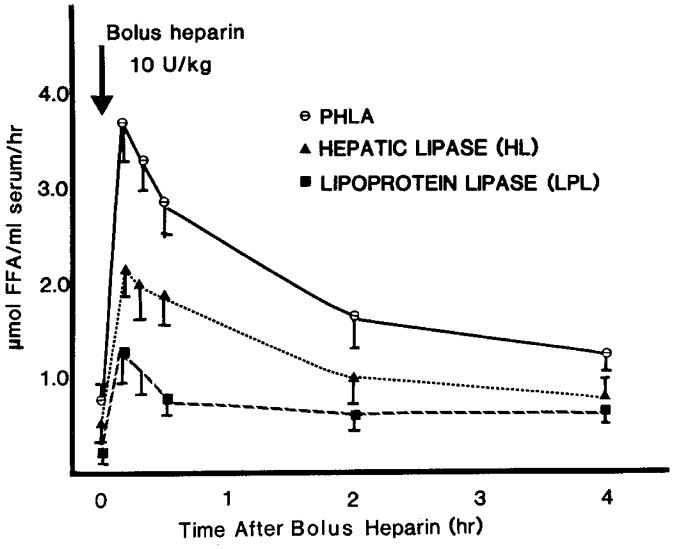

Fig. 1. Total PHLA, LPL, and HL activities in serum of very low birth weight infants maintained on TPN with Intralipid. All infants received continuous on line heparin (1 unit/ml iv fluids). At the beginning of the 4-h study period each infant received a bolus injection of 10 units/kg heparin, for maximal release of endothelial LPL and HL. Intralipid was infused at a rate of 0.5 g/kg/4 h, starting at 0 time. Lipase activity was measured before and after inhibition of HL by specific antibody.

Table 2. *HL, triglyceride, and FFA levels during Intralipid infusion**

Time after bolus heparin (10 units/kg) injection (min)	HL (% of PHLA)	Triglyceride (mg/dl)	FFA (μmol/ml)
0	61 ± 13.3 (29–94)	56.4 ± 9.4 (37.4–138.8)	0.69 ± 0.09 (0.34–1.32)
10	65 ± 8.7 (33–89)	84.3 ± 11.4 (53.0–166.5)	1.03 ± 0.14 (0.48–1.87)
20	66 ± 9.2 (38–93)	82.8 ± 13.4 (49.7–181.8)	0.92 ± 0.13 (0.54–1.86)
30	66 ± 6.7 (34–85)	89.8 ± 11.9 (51.7–178.3)	1.01 ± 0.10 (0.41–1.75)
120	65 ± 8.5 (35–90)	118.6 ± 13.0 (57.4–201.9)	1.04 ± 0.14 (0.54–1.85)
240	55 ± 8.3 (18–71)	110.4 ± 14.4 (58.6–207.1)	1.10 ± 0.21 (0.63–2.46)

* Data are mean ± SEM and ranges (in parentheses).

All infants received continuous infusion of heparin (1 unit/ml infusion fluids). At the beginning of the 4-h study period each infant received a bolus injection of 10 units/kg heparin. Intralipid 0.5 g/kg was infused during the 4-hr study period starting at 0 time.

ml level at 10 min post Intralipid infusion and remained stable throughout the 4 h of the study (Table 2).

DISCUSSION

This study shows that an average of 63% of total PHLA released into the circulation of premature infants is of hepatic origin.

We have shown previously that a continuous infusion of very small levels of heparin (1 unit/ml iv fluid) maintains an appreciable and constant level of PHLA in the circulation (20). This in turn stabilizes the triglyceride level at about 100 mg/dl (20) as compared to 180 mg/dl in infants who receive only a single bolus heparin injection without continuous heparin infusion (7). In the present study, in which all infants also received continuous heparin infusion (1 unit/ml iv fluid), the serum triglyceride concentration at the end of 4 h of Intralipid infusion was 110 mg/dl, which is one-third lower than the triglyceride levels of infants who received Intralipid without continuous on line heparin infusion (7). The improvement in triglyceride clearing is associated with an increase in circulating FFA. The molar ratio of FFA to albumin at peak PHLA levels was 3.28 ± 0.32, lower that 4, the level at which albumin-bound bilirubin is displaced and could cause encephalopathy (6).

The peak level of lipolytic activity released 10 min after bolus heparin injection (Fig. 1) is similar in infants who receive continuous low level heparin infusion (1 unit/ml iv fluid) and infants who receive TPN without continuous heparin administration (20) indicating that lipase reserves are not depleted by continuous low level heparin administration.

The ratio of HL to LPL in serum PHLA is similar in our group of very low birth weight infants (Table 2) to that recently reported in older children (1). Using similar techniques to differentiate between the two lipases, Asayama *et al.* (1) report that hepatic lipase amounts to 62% of serum PHLA in 8 to 9-yr-old children. The ratio between the lipase activities is also similar to that reported for adults (14, 16), but both activities seem to be lower in infants.

We believe that the circulating, heparin-released lipases are responsible for the enhanced rate of triglyceride clearing in premature infants who receive TPN with Intralipid and heparin. Normally, LPL hydrolyses triglyceride-rich lipoproteins, and presumably also Intralipid, at the capillary endothelium (9, 14). The activity of LPL varies widely between tissues and this determines where the lipid is taken up. After its release to the blood by heparin, LPL catalyzes the same hydrolysis but it can no longer direct the uptake of the fatty acids to the appropriate tissues. Thus, the increased rate of triglyceride clearing is achieved at the expense of a loss of control over where the fatty acids are taken up.

There was a higher activity of HL than of LPL in the blood after heparin administration. What contribution this HL makes to hydrolysis of the Intralipid triglycerides in the circulating blood is not clear. In an *in vitro* system the HL is fully capable of attacking Intralipid, but in blood this activity is subject to strong inhibition by high-density lipoproteins (3), which are a preferred substrate for HL. An interesting, but unresolved, question is whether the HL may attack the Intralipid phospholipids, some of which are present as liposome-like structures (4).

More studies are required on the effect of long-term heparin and Intralipid administration to premature infants before any recommendations for best clinical management can be made. We may assume that efficient utilization of Intralipid in very low birth weight infants could be achieved by administration of Intralipid together with low levels of heparin and carnitine, the former to improve lipid clearing and the latter to facilitate the oxidation of the FFA released. The possible risk of hemorrhagic diathesis in premature infants given continuous heparin infusion has also to be considered, although we did not encounter any hemorrhagic complications with this small amount of heparin.

Acknowledgement. This study was supported by NIH Grant HD-15631, and by Grant 13X-727 from the Swedish Medical Research Council. We thank Ms. Marguerite Starry for secretarial help.

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