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Lipoprotein lipase activity and its relationship to high milk fat transfer during lactation in grey seals

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Abstract Lipoprotein lipase regulates the hydrolysis of circulating triglyceride and the uptake of fatty acids by most tissues, including the mammary gland and adipose tissue. Thus, lipoprotein lipase is critical for the uptake and secretion of the long-chain fatty acids in milk and for the assimilation of a high-fat milk diet by suckling young. In the lactating female, lipoprotein lipase appears to be regulated such that levels in adipose tissue are almost completely depressed while those in the mammary gland are high. Thus, circulating fatty acids are directed to the mammary gland for milk fat production. Phocid seals serve as excellent models in the study of lipoprotein lipase and fat transfer during lactation because mothers may fast completely while secreting large quantities of high fat milks and pups deposit large amounts of fat as blubber. We measured pup body composition and milk fat intake by isotope (deuterium oxide) dilution and plasma post-heparin lipoprotein lipase activity in six grey seal (*Halichoerus grypus*) mother-pup pairs at birth and again late in the 16-day lactation period. Maternal post-heparin lipoprotein lipase activity increased by an average of four-fold by late lactation ($P = 0.027$), which paralleled an increase in milk fat concentration (from 38 to 56%; $P = 0.043$). Increasing lipoprotein lipase activity was correlated with increasing milk fat output (1.3–2.1 kg fat per day) over lactation ($P = 0.019$). Maternal plasma triglyceride (during fasting) was inversely correlated to lipoprotein lipase activity ($P = 0.027$) and may be associated with the direct incorporation of long-chain fatty acids from blubber into milk. In pups, post-heparin lipoprotein lipase activity was already high at birth and increased as total body fat content ($P = 0.028$) and the ratio of body fat: protein increased ($P = 0.036$) during lactation. Although pup plasma triglyceride increased with increasing daily milk fat intake ($P = 0.023$), pups effectively cleared lipid from the circulation and deposited 70% of milk fat consumed throughout lactation. Lipoprotein lipase may play an important role in the mechanisms involved with the extraordinary rates of fat transfer in phocid seals.

Key words Lactation · Milk fat transfer · Fat deposition · Lipoprotein lipase · Phocid seals

Abbreviations *FFA* free fatty acid · *HL* hepatic lipase · *LPL* lipoprotein lipase · *PH-HL* post-heparin hepatic lipase · *PH-LPL* post-heparin lipoprotein lipase · *VLDL* very low density lipoprotein

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Introduction

LPL is the key enzyme which regulates the uptake of circulating triglycerides by most tissues (Eckel 1989). LPL is a tissue-bound enzyme that acts at the luminal surface of the capillary endothelium where it hydrolyzes circulating triglyceride carried in chylomicrons (formed in the intestinal mucosa from dietary intake) and VLDL (formed primarily in the liver from endogenous sources of fatty acids). Although LPL is found in most extrahepatic tissues, the highest levels (and the most LPL-specific messenger RNA) have been

found in adipose tissue, lactating mammary gland, and cardiac and red muscle tissue (Robinson 1963; Hamosh and Hamosh 1985; Eckel 1989).

Under hormonal regulation, changing LPL activity among various tissues effectively serves to direct circulating triglyceride fatty acids according to the specific demands of each tissue (Scow et al. 1977; Hamosh and Hamosh 1985; Herzberg 1991). LPL activity in adipose tissue is highest during feeding, resulting in the uptake and storage of fatty acids in adipose tissue depots during periods of positive energy balance (Herzberg 1991). However, during fasting LPL activity in adipose tissue decreases sharply, halting the uptake of fatty acids, while hormone-sensitive lipase becomes active and mobilizes stored triglyceride fatty acids for transport in the plasma to other tissues (Allen 1976). The FFA released from adipose tissue may be either taken up by other tissues or, more likely, are incorporated first into VLDL in the liver and then transported to other tissues (Vance and Vance 1990). Because FFA may be taken up equally by all tissues, largely as a function of their concentration in blood (Scow and Chernick 1970), only those tissues which maintain or increase LPL activity can compete for triglyceride fatty acids (Robinson 1970). For instance, during fasting while adipose LPL is diminished, the maintenance of LPL activity in cardiac muscle allows it to utilize VLDL, thus providing a constant supply of triglyceride fatty acids to the heart (Hamosh and Hamosh 1985; Eckel 1989; Herzberg 1991).

Perhaps the most striking example of the regulation of triglyceride uptake by LPL is the role it plays in nutrient partitioning during lactation. The association of LPL activity and lipid uptake by the mammary gland during lactation has been studied primarily in guinea pigs, rats, and ruminants (Barry et al. 1963; Robinson 1963; McBride and Korn 1963, 1964; Annison et al. 1967; Hamosh et al. 1970; Scow et al. 1977; Moore and Christie 1979). In these species, LPL is active in adipose tissue throughout most of gestation. However, shortly before parturition, LPL activity in adipose tissue decreases sharply, while LPL in the mammary gland (previously non-existent) suddenly becomes active (McBride and Korn 1963; Hamosh et al. 1970). Under the influence of prolactin (Zinder et al. 1974), LPL activity in adipose tissue remains almost completely depressed in the lactating female, while LPL activity in the mammary gland increases after parturition and remains at high levels throughout lactation. Hence circulating triglyceride fatty acids carried in chylomicrons and VLDL are directed to the mammary gland during lactation. LPL activity appears to be correlated with degree of lipid uptake by tissues in general (Bezman et al. 1962; Scow et al. 1972; Eckel 1989), and in guinea pigs and rats, mammary LPL activity in vitro appears to be associated with the degree of uptake of lipid by the mammary gland (McBride and Korn 1963; Hamosh et al. 1970).

Thus, LPL plays a critical role during the lactation period for both mother and young. LPL is important to the lactating female for the uptake and secretion of long-chain fatty acids in milk. It is also essential to the suckling neonate in the assimilation of its high fat milk diet, where even in human milk (4% fat) lipid accounts for greater than 50% of total ingested calories. The shift in energy supply from mainly carbohydrates and FFA during fetal life to primarily milk triglyceride during early postnatal life, requires adequate mechanisms of triglyceride uptake by tissues (Hamosh and Hamosh 1985; Iverson et al. 1995). However, it is not known whether LPL directs the degree of milk lipid output by the lactating female or fattening in the suckling neonate. The relationship between LPL activity and fat transfer during lactation has not been thoroughly investigated and comparative information on LPL activity and lipid metabolism among mammals is limited.

Phocid seals provide an excellent opportunity to study the relationship between LPL activity and both milk fat output and neonatal fat deposition. Pinnipeds (seals and sea lions) must cope with the constraints imposed on the mother by the need to feed at sea but to give birth and suckle her pup on land. In many phocid species, females fast throughout a brief intense lactation period while secreting large quantities of high-fat milk derived primarily from the mobilization of adipose tissue (blubber) fatty acids (Iverson 1993; Iverson et al. 1995). Lactation is terminated by abrupt weaning as the mother departs to sea. During the lactation period, pups digest and deposit large amounts of milk lipid daily. Thus, growth is rapid and primarily due to the deposition of fat as blubber. Fattening is a critical factor in the life history of phocid neonates (Iverson et al. 1993), as weaned pups must rely on the blubber they have deposited during suckling to survive a post-weaning fast of several weeks or months before initiating foraging (Bowen 1991).

Typical of a large phocid species, female grey seals (*Halichoerus grypus*) build up large energy reserves as blubber prior to parturition (Fedak and Anderson 1982) and fast during an approximately 16-day lactation period (Boness et al. 1995). Grey seal pups consume about 4 $kg \cdot day^{-1}$ of milk that averages 60% fat. Both milk fat concentration and milk fat output change over lactation (Iverson et al. 1993). In the present study, we measured milk fat output using isotope dilution and patterns of heparin-releasable LPL and plasma lipids in grey seal mothers and their pups during early and late lactation. Since LPL is rapidly and quantitatively released into the circulation after heparin administration (Hamosh and Hamosh 1986; Eckel 1989), plasma PH-LPL is a sensitive indicator of endothelial lipase levels and triglyceride hydrolysis and clearing ability of tissues (Robinson 1970; Scow et al. 1973; Krauss et al. 1974; Nilsson-Ehle et al. 1980; Krauss et al. 1983; Hamosh and Hamosh 1985; Eckel 1989). Given the relative accessibility of serum or plasma post-heparin

lipolytic measurement, it is a useful tool in studies of functional lipase concentrations and lipid metabolism. We used PH-LPL activity as an index of total activity of tissues, and the nature of lipid metabolism during fasting and feeding in mothers and pups, respectively, to infer probable major sources of LPL activity.

Materials and methods

Field procedures

Data were collected in January and February of 1990 from grey seal mothers and their pups during the annual pupping season on Sable Island, Nova Scotia (43° 55′N, 60° 00′ W). All animal-handling procedures were approved by the animal care committees of Georgetown University, Washington, D.C., and Dalhousie University, Halifax. Newborn pups were identified by their thin wet appearance, the presence of fresh placentas, and fresh blood on both mother and pup. Newborn mother-pup pairs were individually marked with waterproof fluorescent paint (Lenmar Paint, Baltimore) and a numbered hind-flipper tag was applied to pups (Rototag; Dalton, Heneley-on-Thames). Plasma PH-LPL activity, PH-HL activity, and plasma neutral lipid composition were studied in mother-pup pairs near birth (0–1 days postpartum) and again late in the 16-day lactation period (14–15 days postpartum). Concurrently, body composition of the pups and milk lipid transfer from mother to pup over the course of the lactation period were studied in these same pairs, using isotope dilution (Iverson et al. 1993).

At 0–1 days postpartum mothers were chemically immobilized using Telezol (tiletamine hydrochloride and zolazepam hydrochloride) and weighed to the nearest kg on a 300-kg Salter scale; pups were weighted to the nearest 0.1 kg on a 50- or 100-kg Salter scale. An initial blood sample (10 ml) was taken from the extradural vein of both mother and pup and the pair was then placed in separate but contiguous pens. After evacuating stomach contents of pups, a preweighed amount of deuterium oxide (D_2O, 99.8% purity, Atomic Energy of Canada) was administered to the pup by gastric intubation and allowed to equilibrate for 3 h during which serial blood samples (5–10 ml) were taken, as described in Iverson et al. (1993). During the equilibration period, a bolus intravenous injection of heparin (100 IU per kg body mass) was administered to both mother and pup via the extradural vein. Post-heparin blood samples (5–10 ml) were subsequently taken from mother and pup at 10, 20, and 30 min following heparin injection to determine peak response and again from pups at about 1.75 h to examine half-life of circulating lipases. Difficulty in handling mothers precluded sampling them after the initial 30 min. In pups, blood sampling was coordinated such that the same sample could be used for both D_2O analysis and post-heparin lipase activity. Blood samples for D_2O analysis were collected into plain vacutainers (serum), whereas samples for lipase and lipid analyses were collected into heparinized vacutainers (plasma). Blood samples were kept cool until return to the field laboratory (4–8 h) and then centrifuged at room temperature. Serum and plasma were collected into airtight vials and stored frozen at − 5 °C initially, and at − 70 °C (plasma samples) thereafter.

Following the initial equilibration period, pairs were released and observed to make sure the mother-pup bond was intact. We recaptured the pairs at approximately 5-day intervals to weigh both mother and pup and to obtain a blood sample from the pup for D_2O analysis. At the final recapture (14–15 days postpartum) we repeated all procedures conducted at the initial capture: mother and pup were weighed and blood sampled, D_2O was administered to the pup, and heparin (100 IU per kg body mass) was given to both mother and pup. Blood sampling procedures and processing followed that as described above. When possible a milk sample was collected from the mother of the pair during captures; these samples were part of a larger set of milk samples collected over the entire lactation period and analyzed for proximate composition (Iverson et al. 1993).

Analysis of post-heparin lipase activity and plasma neutral lipid composition

Both endothelial lipases, LPL and HL, are rapidly released into the circulation after the administration of heparin, but each can be measured with great specificity. LPL is characterized by an alkaline pH optimum of about 8.0, activation requires apolipoprotein C-II as co-factor, and LPL is completely inhibited by high ionic strength (e.g., 1 $mol \cdot l^{-1}$ NaCl) (Hamosh and Hamosh 1985). In contrast, HL does not require serum co-factors and is unaffected by NaCl (Krauss et al. 1973; Eckel et al. 1988). These characteristics, and confirmation that LPL and HL are immunologically distinct, has come from the purification of these enzymes using the production of specific antibodies (Eckel et al. 1988; Ikeda et al. 1989).

LPL and HL activities in post-heparin plasma samples were quantitated approximately 6 months after collection by measuring the hydrolysis of tri-^{3}H-olein, essentially as described by Nilsson-Ehle and Schotz (1976), and expressed as micromoles FFA released per ml plasma per hour. A stable substrate emulsion was prepared containing approximately 200 µmol triolein (99%, Sigma), 30 µCi tri-^{3}H (9,10)-oleylglycerol (Amersham, England), and 12 mg lecithin (L-α-phosphatidylcholine, Sigma), emulsified in 3.3 ml glycerol. The mixture was placed in an ice bath and sonicated for two periods of 1 min each using a Polytron PT10-35 (Brinkmann Instruments, Westbury, NY) and allowed to stand for 3 days prior to use. The triglyceride concentration of the final emulsion was determined using the hydroxamic acid ester assay of Rapport and Alonzo (1955).

The assay system was carried out in a total volume of 200 µl and incubated in a Dubnoff shaking water bath at 37 °C. Pooled volumes of plasma from several seals were used to evaluate the optimal assay system for total lipolytic activity. These were also compared with samples of post-heparin plasma obtained from adult rats, which had been analyzed previously (Zaidan et al. 1984). Since LPL requires apoC-II, which is found in chylomicrons, VLDL, and high-density lipoproteins (Hamosh and Hamosh 1985; Eckel 1989), heat-inactivated serum is usually added to LPL assay systems as a source. Although this was necessary for optimal activity in rat plasma, it did not produce higher rates of hydrolysis with seal plasma. Apparently, sufficient apoprotein was already present in seal plasma, and hence additional inactivated serum was not added to the assay system. The pH optimum for the assay was tested from pH 5.0 to 9.5 using both phosphate and TRIS-HCl buffers (0.2 $mol \cdot l^{-1}$). The reaction in both seal and rat plasma followed a similar pH curve with the optimum at pH 8.0 as found in other studies (Nilsson-Ehle and Schotz 1984; Zaidan et al. 1984); however, the phosphate rather than the TRIS-HCl buffer produced optimal activity. Enzyme activity in seal plasma increased in a linear fashion with increasing amounts of enzyme preparation (10–100 µl plasma). Total FFA released was linear with incubation time (5–60 min). When expressed as activity ($\mu mol \cdot ml^{-1} \cdot h^{-1}$) FFA peaked at about 8% hydrolysis (15 min), after which the rate declined due to end-product inhibition as has been reported previously (Nilsson-Ehle and Schotz 1984).

Based on the above observations, the final substrate solution for assay was prepared daily from 1 part concentrated emulsion: 4 parts 0.2 $mol \cdot l^{-1}$ phosphate buffer, pH 8.0:1 part water. The reaction tube for total lipolytic activity contained 100 µl assay substrate, 60 µl 3.5% (w/v) bovine serum albumin and 40 µl post-heparin plasma. The assay for HL activity followed the same procedures as that for total activity, except that 1 $mol \cdot l^{-1}$ NaCl was contained in the final assay system. Incubations were carried out in duplicate or triplicate for 15 min and stopped by the addition of 3.25 ml methanol/chloroform/heptane [1.41:1.25:1, v/v/v; Belfrage and Vaughan (1969)] and 1.05 ml 0.1 $mol \cdot l^{-1}$ potassium carbonate buffer, pH 10.5. The mixture was vortexed and centrifuged. A 0.5-ml aliquot of the methanol-water upper phase was assayed with 5 ml Scinti Verse

II (Fisher Scientific) for radioactivity in a scintillation counter (Beckman LS 7500, Irvine CA) after correcting for quenching. The theoretical upper phase contained 2.45 ml and about 76% of the ^{3}H-oleic acid released (Nilsson-Ehle and Schotz 1976). The total counts of the assay system, blank runs, and the exact percent recovery of ^{3}H-oleic acid in the upper phase was tested in five replicates each on each day of analysis. Additionally, a pooled sample of seal plasma, frozen in daily aliquots, was run each day as a standard and used as a correction factor between experiments. LPL activity in each sample was calculated by subtracting the HL activity from total lipolytic activity.

Plasma neutral lipid composition was determined only in the initial plasma samples taken from mothers and pups prior to administration of heparin, since heparin affects circulating blood neutral lipid composition (Nordoy et al. 1977; Dhanireddy et al. 1981). Measured aliquots of plasma were extracted according to the method of Folch et al. (1957) as modified by Iverson (1988). The total lipid content of plasma was determined gravimetrically and measurement of neutral lipid classes was performed using thin-layer chromatography and quantitative densitometry on a Shimadzu CS-910 Dual Wavelength TLC scanner according to Iverson et al. (1992). Concentrations of lipid classes were expressed as $mg \cdot dl^{-1}$ plasma or converted to $\mu mol \cdot ml^{-1}$ using preliminary data on the fatty acid composition of grey seal plasma and milk (S.J. Iverson, unpublished data).

Determination of body composition of pups and milk lipid transfer

Total body fat content of pups and milk intake (i.e., maternal milk output) over the course of lactation were determined as a subset of a larger study described in Iverson et al. (1993). Briefly, free water was collected from blood sera by heat distillation and D_2O concentration was determined by quantitative infrared spectrophotometry (Oftedal and Iverson 1987). Equilibration concentration of D_2O was used to calculate initial (0–1 days postpartum) and final (14–15 days postpartum) body water pool size of pups. Body composition of pups was determined from the relationships of percent body water to percent fat-free or lean body mass, and protein to lean body mass, as derived from carcass analyses (Iverson et al. 1993). Fractional water turnover rate was determined for each pup from the linear regression of the decline in ln(serum D_2O concentration) against time elapsed since isotope administration. Total water intake of each pup during early and late lactation was calculated using water turnover rates during these periods and changes in body water pool size. Water intakes were converted to milk intakes from the free water content of milk consumed and estimated metabolic water production. We used the milk composition data from the large number of milk samples analyzed in Iverson et al. (1993), which included the samples collected from the females in this study. Since our aim was to directly compare LPL data with milk fat output, we used the milk composition of each mother on the day of the PH-LPL study (i.e., days 0–1 and 14–15) for milk intake calculations. In the few cases where this was not possible, we used the population average on the day of study.

Data comparisons and statistical analyses

Five mother-pup pairs were initially included in this study. However, several days after the initial sampling period one mother deserted her pup, precluding evaluation of milk intake or late post-heparin sampling in this pair. Therefore, an additional pair for which we already had birth data and milk intake information was included in the post-heparin sampling at 14–15 days. In another pair, a mother weaned her pup and departed from the rookery 1 day prior to our sampling at day 14. Hence, we were unable to sample the mother, but normal milk intake and post-heparin data were obtained from the pup. The plasma lipid from this pup, however, represented a fasting state ($\geq$ 24 h) and thus could not be compared with the other pups that had milk in their stomachs. Finally, late plasma lipid data was unavailable for one pup due to breakage and loss of the pre-heparin sample. As a result of these occurrences, sample sizes for analyses were not always paired or equal. Thus, data were analyzed using non-parametric tests in order to account for small sample sizes and uneven repeated sampling of animals. The Mean-Whitney *U*-test was used for unpaired comparisons and the Spearman's Rank Correlation was used to test the correlation of paired variables. Data were tested using StatView for the Macintosh. Mean values are presented as mean $\pm$ SEM throughout.

Results

Characteristics of lipolytic activity

Virtually no lipolytic activity was detected in pre-heparin plasma samples from grey seal mothers and pups, but substantial activity was present after heparin administration. HL activity accounted for only a negligible portion of total peak post-heparin lipolytic activity in both mothers and pups at both sampling times. In the samples taken between 10 and 30 min after heparin administration, HL activity remained constant and averaged 2.5% (0.1 ± 0.02 $\mu mol \cdot ml^{-1} \cdot h^{-1}$) and 1.7% ($0.4 \pm 0.11$ $\mu mol \cdot ml^{-1} \cdot h^{-1}$) of total lipolytic activity in mothers at early ($n = 5$) and late ($n = 4$) lactation, respectively, and 2.4% (0.8 ± 0.17 $\mu mol \cdot ml^{-1} \cdot h^{-1}$) and 1.5% ($0.8 \pm 0.08$ $\mu mol \cdot ml^{-1} \cdot h^{-1}$) of total lipolytic activity in pups at birth ($n = 5$) and late lactation ($n = 5$), respectively. Henceforth, only LPL activity is reported, after subtracting HL activities from total activities.

The time-course of peak PH-LPL activity was studied in all animals (Fig. 1). In both mothers and pups, plasma LPL activity rapidly increased after heparin administration and generally peaked at 20 or 30 min. In pups, PH-LPL activity had almost returned to preheparin levels by 105 min. Peak activity levels in both mothers and pups were higher at 14–15 days postpartum than near parturition and levels were higher in pups than in mothers at both time periods (Fig. 1). Because average peak LPL activity in mothers or pups did not significantly differ between 20 and 30 min post-heparin, we used the higher of the two values to represent the PH-LPL activity of individuals.

Post-heparin LPL activity and milk fat output in mothers

Summary data for mothers and pups are presented in Tables 1 and 2, respectively. Mothers averaged 218 kg at parturition (0 day) or 1 day postpartum and lost an average of 5.6 ± 1.01 kg per day during the lactation period (Table 1). Near parturition, PH-LPL activity was relatively low at 6.1 $\mu mol \cdot ml^{-1} \cdot h^{-1}$; however, levels had increased approximately fourfold by late lactation (Table 1). Among individual females sampled

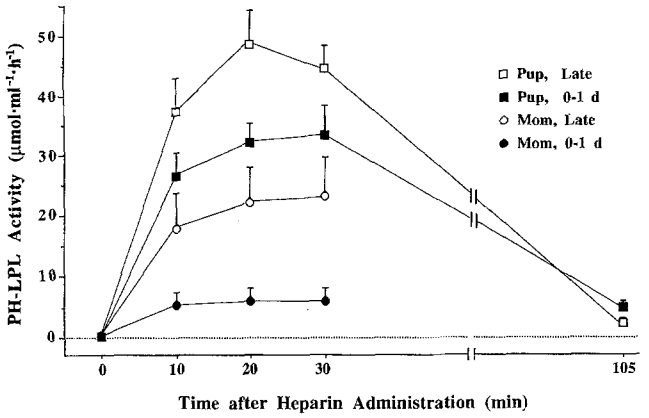

Fig. 1 Time-course of plasma post-heparin LPL activity measured in grey seal mothers near parturition ($n = 5$) and late lactation ($n = 4$) and in their pups near birth ($n = 5$) and late lactation ($n = 5$)

early and late, PH-LPL levels increased between two- and tenfold. In these same mothers, milk fat content averaged 38% near parturition and 56% at late lactation. Average daily milk output did not differ between periods; however, average daily milk fat output increased by about 1.7-fold between early and late periods (Table 1).

Maternal PH-LPL activity was directly associated with several aspects of milk output. As described in Iverson et al. (1993), milk composition in grey seals (including the females in this study) changed dramatically over lactation, primarily due to a large increase in the concentration of fat from early to late lactation. PH-LPL activity measured in mothers during days 0–1 and days 14–15 postpartum were proportional to these changes in milk fat concentration (Fig. 2). Additionally, maternal PH-LPL activity was directly correlated with an increase in total daily milk fat output ($P = 0.019$; Fig. 3).

Mothers fasted several days before and throughout lactation and no significant differences were found in the composition of maternal plasma neutral lipids between early and late periods (Table 3). Although maternal plasma triglyceride tended to decrease and FFA tended to increase between early and late periods, these differences were not significant. However, a decrease in plasma triglyceride concentration was significantly correlated with increased PH-LPL activity ($P = 0.027$; Fig. 3) and differences in PH-LPL activity among individuals explained the large variation in late maternal plasma triglyceride. During early and late lactation, respectively, triglyceride fatty acids comprised about 66 and 39% of the circulating fatty acids most available to tissues (triglyceride plus FFA). The concentration of cholesterol (54–63 $mg \cdot dl^{-1}$) and cholesterol ester (204 $mg \cdot dl^{-1}$) in maternal plasma remained relatively constant between periods (Table 3).

Post-heparin LPL activity, body composition, and milk fat intake in pups

Near birth pups averaged 18 kg and contained about 1 kg (5.5%) of body fat (Table 2). By 14–15 days postpartum (i.e., near weaning), pups averaged 50 kg and

Table 1 Body mass, LPL activity, and milk fat output of grey seal mothers in this study

Lactation stage (days postpartum)	Body mass kg	PH-LPL $\mu mol \cdot ml^{-1} \cdot h^{-1}$	Milk fat %	Milk output[a] $kg \cdot day^{-1}$	Milk fat output[a] $kg \cdot day^{-1}$
Early (0–1 d)	218 ± 15.1	6.1 ± 2.21	37.7 ± 2.29	3.2 ± 0.37	1.3 ± 0.16
n	5	5	4	5	5
Late (14–15)	146 ± 11.4	23.6 ± 6.51	55.8 ± 4.50	3.7 ± 0.28	2.1 ± 0.21
n	4	4	4	5	5
P	0.019	0.027	0.043	ns	0.016

[a] Milk and milk fat output calculated as $kg \cdot d^{-1}$ on days 0–1 versus $kg \cdot d^{-1}$ on days 14–15 using the isotope turnover data from the pup and the actual milk composition of the mother on these days when available, otherwise the population average for that day was used

Table 2 Body mass, LPL activity, and milk fat intake of grey seal pups in this study

Lactation stage (days postpartum)	Body mass kg	Body fat content kg	PH-LPL $\mu mol \cdot ml^{-1} \cdot h^{-1}$	Milk fat intake[a] $kg \cdot day^{-1}$	Milk gain[a] $kg \cdot day^{-1}$
Early (0–1 days) $n = 5$	18.1 ± 0.91	1.0 ± 0.21	32.6 ± 2.64	1.3 ± 0.16	1.9 ± 0.31
Late (14–15 days) $n = 5$	50.1 ± 2.90	19.7 ± 2.29	50.8 ± 3.78	2.1 ± 0.21	2.7 ± 0.17
P	0.009	0.009	0.009	0.016	0.017

[a] Milk fat intake on days 0–1 versus days 14–15 calculated as described in Table 1 and methods
[b] Mass gain measured over the first-half versus the second-half of lactation

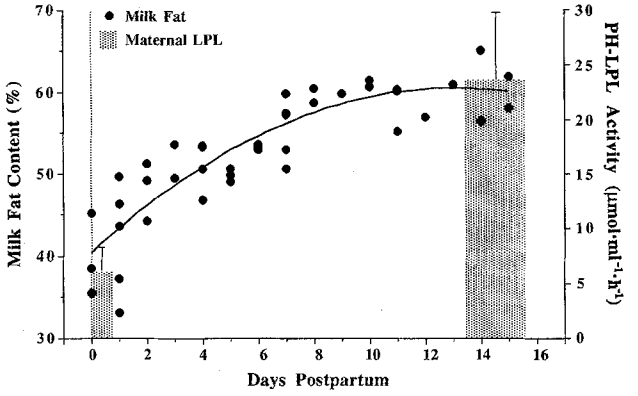

Fig. 2 Changes in maternal post-heparin LPL activity in relation to changes in milk fat content over lactation in grey seals. LPL data from Table 1; milk composition data from Iverson et al. (1993), including the females in the present study

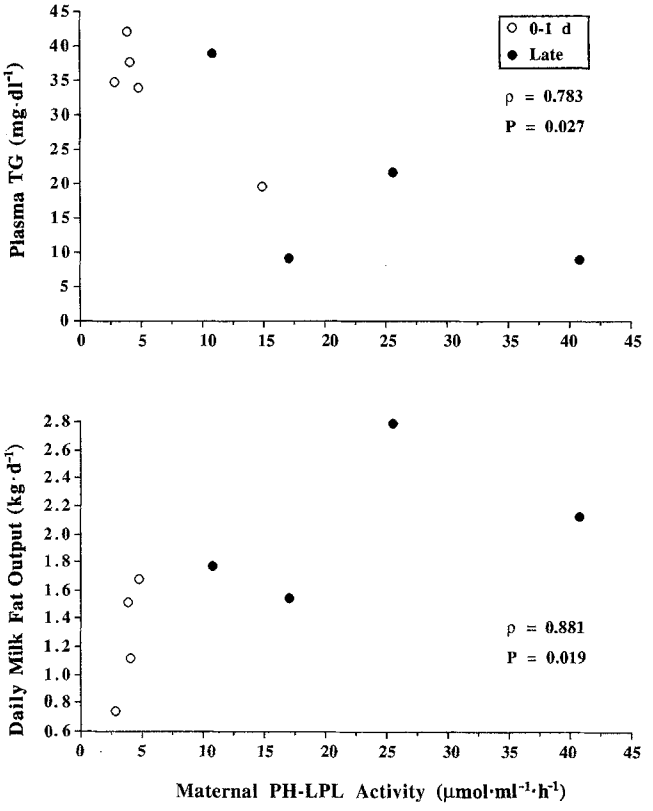

Fig. 3 Maternal plasma triglyceride concentration and daily milk fat output in relation to maternal plasma post-heparin LPL activity over lactation in grey seals. Data tested by Spearman's Rank Correlation (ρ = rho)

contained 20 kg (39%) fat. Mass gain from birth to weaning was comprised of 57 ± 3.5% fat, 29 ± 2.4% water, and 10 ± 0.8% protein ($n = 5$). PH-LPL activity in pups was high near birth at 33 $\mu mol \cdot ml^{-1} \cdot h^{-1}$ and increased significantly between early and late lactation ($P = 0.009$; Table 2). The increases in PH-LPL activity were directly correlated with the increases in total body fat content of pups during suckling ($P = 0.028$, Fig. 4). Pup PH-LPL activity also increased with an increasing body fat : protein ratio ($P = 0.0365$).

Average daily milk fat intake in pups increased by about 1.6-fold between early and late lactation and was associated with an increase in daily mass gain (Table 2). Increased milk fat intake was highly correlated with increased mass gain ($P = 0.007$) but was only weakly correlated with the increased PH-LPL ($P = 0.053$). Changes in plasma neutral lipids of suckling pups primarily reflected the increase in milk fat intake between early and late lactation, but triglyceride fatty acids comprised 90–94% of circulating fatty acids throughout (Table 3). Triglyceride concentration in pups increased from 172 to 263 $mg \cdot dl^{-1}$ between

Table 3 Plasma neutral lipids of grey seal mothers and suckling pups

Lactation stage (days postpartum)	TG $mg \cdot dl^{-1}$	FFA $mg \cdot dl^{-1}$ ($\mu mol \cdot ml$)	% of plasma Fatty acids in TG[a] mol %	Cholesterol $mg \cdot dl^{-1}$	Cholesterol ester $mg \cdot dl^{-1}$
Mothers:[b]					
Early (0–1 days) $n = 5$	33.7 ± 3.77	15.8 ± 0.85 (0.56 ± 0.030)	66.4 ± 3.11	62.8 ± 6.08	204.5 ± 13.42
Late (14–15 days) $n = 4$	19.7 ± 7.09	32.8 ± 10.43 (1.16 ± 0.368)	39.1 ± 16.68	53.9 ± 6.87	204.3 ± 12.24
P	ns	ns	ns	ns	ns
Pups:[c]					
Early (0–1 days) $n = 5$	171.6 ± 26.21	19.9 ± 7.75 (0.70 ± 0.274)	90.1 ± 2.99	50.5 ± 4.67	168.8 ± 4.46
Late (14–15 days) $n = 3$	263.0 ± 31.99	14.7 ± 4.45 (0.41 ± 0.157)	94.3 ± 1.94	96.6 ± 3.97	238.3 ± 0.78
P	0.025	ns	ns	0.025	0.025

[a] 100 × moles of fatty acids in plasma TG/total moles of plasma fatty acids in TG plus FFA
[b] All fasting
[c] All within several hours of suckling (see methods)

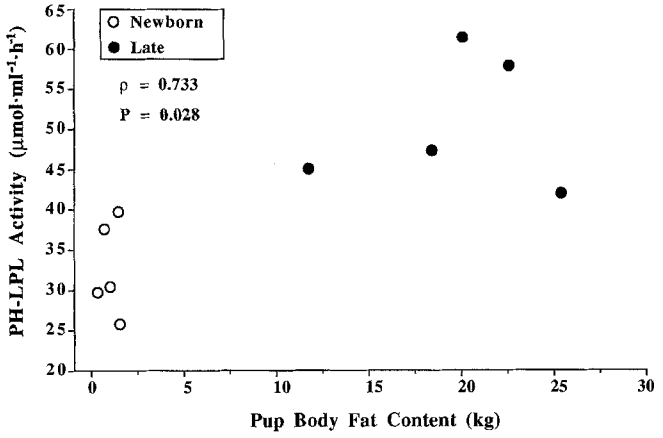

Fig. 4 Plasma post-heparin LPL activity in relation to increasing total body fat content over lactation in suckling grey seal pups. Data tested by Spearman's Rank Correlation (ρ = rho)

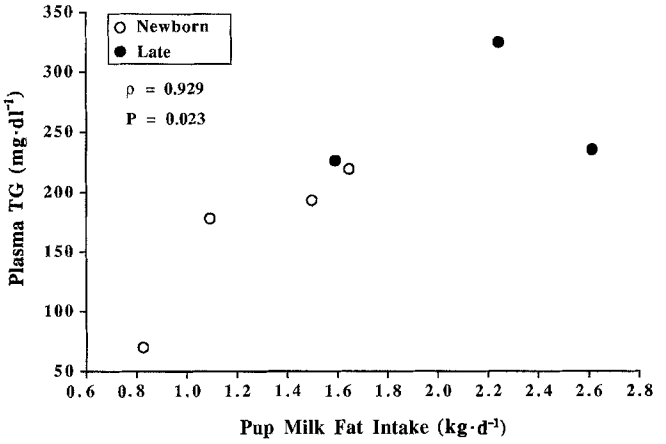

Fig. 5 Plasma triglyceride concentration in relation to milk fat intake over lactation in suckling grey seal pups. Data tested by Spearman's Rank Correlation (ρ = rho)

periods and was directly correlated with increased total daily milk fat intake (Fig. 5). The average concentration of cholesterol and cholesterol ester in pups increased about 1.9-fold and 1.4-fold, respectively, between early and late periods (Table 3). Although plasma triglyceride increased with increasing milk lipid intake in pups, pups deposited $70 \pm 4.7\%$ of the milk fat consumed ($100 \times$ daily fat deposited per daily fat consumed) throughout lactation.

Discussion

Post-heparin lipolytic activity in grey seals

In the present study we administered heparin intravenously at 100 $U \cdot kg^{-1}$ body weight. This dosage has been used in previous studies with humans and rats and results from dose-response studies have suggested 100 $U \cdot kg^{-1}$ to be optimal for release of both LPL and HL (Krauss et al. 1974; Kuusi, Ehnholm and Nikkila 1980), although in humans there was no significant difference between dosages of 50 and 100 $U \cdot kg^{-1}$ (Thompson et al. 1986). Some studies suggest that rapidly repeated doses of heparin (within 24 h) may begin to perturb circulating lipase and lipid levels (Nordoy et al. 1977; Bengtsson and Olivecrona 1980; Das et al. 1982); however, the evidence for this is contradictory (Zaidan et al. 1984; Thompson et al. 1986). In any case, our administrations of heparin in the same individuals were 13–15 days apart and thus unlikely to be affected by repetition. Additionally, we measured blood lipid composition only in pre-heparin plasma.

The assay conditions for PH-LPL and PH-HL used in the present study were similar to those of previous studies (see methods), with the primary exception that an additional source of apoC-II was not required for optimal lipolytic measurement in seal plasma. Our assay system (with the addition of an apoC-II source) measured a similar total peak post-heparin activity (36–42 $\mu mol \cdot ml^{-1} \cdot h^{-1}$) in adult rat plasma as that found previously [40–58 $\mu mol \cdot ml^{-1} \cdot h^{-1}$; Zaidan et al. (1984)]. Unfortunately, field conditions precluded the processing and freezing of seal blood samples immediately after collection which may have caused some loss of activity. However, samples for lipase measurement were collected into heparinized tubes, which helps to stabilize LPL and HL activity even at increased temperatures (Eckel et al. 1988; Eckel 1989; Ikeda et al. 1989), and were stored frozen after initial centrifugation. Although some loss of activity may have occurred during storage, all samples were treated under equal conditions such that relative activities and changes were directly comparable within our study. Since LPL assays among investigators are variable and since our absolute activity values may have been underestimated, comparisons with values from other studies are made with some caution.

Peak post-heparin activity generally occurred at 20 or 30 min in mothers and pups (Fig. 1), similar to the peak found in some primates (Rudel and Star 1990). In contrast, in studies of rats and humans, peak post-heparin activity occurs or is assumed to occur at 10 min following intravenous injection (e.g., Krauss et al. 1974; Dhanireddy et al. 1981; Das et al. 1982; Zaidan et al. 1984; Thompson et al. 1986). The nature of post-heparin lipolytic activity in grey seals also differed from previous studies in that PH-HL comprised only a negligible proportion (1–2%) of total lipolytic activity in both mothers and pups. In contrast, HL activity represents 60–70% of total post-heparin activity in infant and adult humans (Hamosh and Hamosh 1986; Thompson et al. 1986; Eckel et al. 1988), 11–34% in several non-human primates (Rudel and Star 1990), 54% in suckling dogs (Das et al. 1982), and about 20% in neonatal and adult rats (Zaidan et al. 1984). PH-HL

activity averaged 0.1–0.4 $\mu mol \cdot ml^{-1} \cdot h^{-1}$ in grey seal mothers in comparison to between 2 and 16 $\mu mol \cdot ml^{-1} \cdot h^{-1}$in adult humans and non-human primates (Thompson et al. 1986; Eckel et al. 1988) and about 10 $\mu mol \cdot ml^{-1} \cdot h^{-1}$ in adult rats (Zaidan et al. 1984), after similar doses of heparin. Although the PH-HL values from grey seal mothers in the present study are extremely low compared to other studies, post-heparin lipolytic activity has not been studied in lactating or fasting females of other species. It is possible that during fasting maternal liver requires little HL [or LPL, e.g. Ramirez et al. 1993) since it can readily utilize FFA mobilized from adipose tissue. PH-HL activity was also somewhat low in suckling grey seal pups at 0.8 $\mu mol \cdot ml^{-1} \cdot h^{-1}$, in comparison to about 2 $\mu mol \cdot ml^{-1} \cdot h^{-1}$ in human low birth weight infants (Hamosh and Hamosh 1986), and about 4 $\mu mol \cdot ml^{-1} \cdot h^{-1}$ in suckling dogs and rats (Das et al. 1982; Zaidan et al. 1984). Although HL may function in the liver in an analogous role to LPL, it appears to make a very low contribution to total heparin releasable lipase activity in lactating grey seal mothers and suckling pups.

Lipoprotein lipase activity and milk fat secretion in lactating grey seals

Lactation represents the greatest energetic cost of reproduction in female mammals (Blaxter 1962; Millar 1977; Oftedal 1985), far exceeding that of gestation and maintenance requirements. Large amounts of nutrients are required for the synthesis and secretion of milk, such that maternal metabolism is altered to efficiently partition nutrients to the mammary gland during lactation (Patton and Jensen 1976; Bauman and Currie 1980; Williamson 1980; Bauman and Elliot 1983). Of the major changes in tissue metabolism that occur, those involving adipose tissue and lipid metabolism may be the greatest in magnitude (Bauman and Elliot 1983). In particular, the changes in tissue LPL during lactation effectively direct circulating triglyceride, carried in chylomicrons and VLDL, from storage in adipose tissue to milk secretion by the mammary gland. The onset of mammary LPL activity, and the inverse correlation of LPL activities in mammary gland and adipose tissue, is believed to be regulated by prolactin (Scow et al. 1973; Zinder et al. 1974; Spooner et al. 1977). Since prolactin continues to be secreted in response to suckling, mammary LPL levels remain high throughout lactation, while adipose tissue LPL remains almost completely depressed (Hamosh and Hamosh 1985). The mechanisms of milk secretion in pinnipeds are similar to those of other mammals (Tedman 1983) and prolactin also appears to be essential for the maintenance of lactation (Boyd 1991); hence, tissue LPL activities in seals may be similarly regulated. In phocid species in which females fast throughout lactation, mediation of lipid metabolism among tissues is undoubtedly important, since a major component of their reproductive strategy relies on extensive deposition and subsequent mobilization of fat reserves for milk synthesis. On Sable Island, Nova Scotia, grey seal mothers lose about 92 kg, or 39%, of their initial body mass during the 16-day lactation period (Iverson et al. 1993) due primarily to the depletion of blubber lipid (e.g., Fedak et al. 1989; Bowen et al. 1992). During this time lactating females secrete a total of about 35 kg of fat in milk (Iverson et al. 1993) and it appears that milk fatty acids are derived almost entirely from direct uptake of fatty acids mobilized from blubber (e.g., Iverson 1993; Iverson et al. 1995).

Evidence from the present study supports the idea that LPL may play a significant role in the regulation of milk fat secretion by lactating phocids. Increases in total heparin releasable LPL in lactating females were directly associated with increased milk fat concentration and increased milk fat output (Figs. 2, 3) as well as with decreased circulating triglyceride (Fig. 3). Previous studies in guinea pigs and rats have shown that mammary LPL activity is correlated with uptake of chylomicron triglyceride (Robinson 1963; McBride and Korn 1964; Scow et al. 1977) and with decreased circulating triglyceride throughout lactation (Hamosh et al. 1970; Ramirez et al. 1983). Although we were not able to measure individual tissue activities, we can make some inferences about the nature of total PH-LPL in grey seal mothers based on their physiological status. In a fasting animal, energy metabolism depends almost entirely on lipid catabolism (Allen 1976), such that LPL in adipose tissue of lactating grey seal females should be virtually absent, regardless of the effects of prolactin, while hormone sensitive lipase actively mobilizes lipid from stores. Thus, the maternal PH-LPL we measured would have originated from sources other than adipose tissue. Of the tissues which may have contributed to PH-LPL, it is likely that mammary LPL represented a major portion of total PH-LPL, since regulation by prolactin would likely increase mammary LPL over all other tissues (Ramirez et al. 1983; Hamosh and Hamosh 1985). Also, grey seal mothers must have rapid uptake of circulating fatty acids in order to produce up to 2 kg of milk fat daily (e.g., Table 1, Fig. 3). We cannot compare our values of maternal PH-LPL with previous studies since PH-LPL activities have not been measured in other species that fast during lactation.

It might be argued that during fasting the FFA mobilized from maternal adipose tissue could be taken up directly by the mammary gland rather than being reesterified and incorporated into VLDL, thus lessening the need for mammary LPL. However, in ruminants even when circulating FFA are present, it appears that the triglyceride fatty acids of chylomicrons and VLDL are preferentially taken up and secreted by the mammary gland over FFA (Annison et al. 1967; Moore and Christie 1979; Gooden and Lascelles 1973).

Indeed FFA appear to be largely incorporated into VLDL in the liver prior to uptake by the mammary gland (McBride and Korn 1964; Annison et al. 1967; Vance and Vance 1990). Our finding that triglyceride fatty acids accounted for 39–66% of circulating fatty acids in a fasting animal (Table 3) suggests synthesis of VLDL. Only when FFA are present in large concentrations (>0.3 μmol · ml^{-1}) during fasting, do they appear to be taken up significantly by the ruminant mammary gland (Moore and Christie 1979). In the present study, levels of maternal circulating FFA were variable and relatively high (0.6–1.2 μmol · ml^{-1}) throughout lactation, although this may have been influenced in part by chemical immobilization at capture, since increases in plasma FFA concentration have been found in response to anesthesia or even mild excitement (Annison et al. 1967). If the FFA levels we measured were typical of lactating grey seals, this suggests that a significant fraction of FFA may be taken up by mammary tissue in fasting phocids. Even so, since all tissues compete equally for FFA released from adipose tissue, only tissues which have LPL can utilize triglyceride fatty acids. During lactation, the mammary gland is likely to be the primary tissue containing LPL due to the secretion of prolactin. Hence, the mammary gland can maximize use of VLDL synthesized in the liver, thus further enabling this tissue to take up the massive amounts of fatty acids necessary for milk fat secretion in phocid seals. The correlation of PH-LPL with all aspects of milk fat output in this study supports this hypothesis.

Maternal PH-LPL in grey seals increased significantly from early to late lactation (Table 1, Fig. 2). This could reflect an absence of adipose tissue activity and a delay in the rise of mammary gland activity near parturition, as has been found previously (Hamosh et al. 1970), and may explain the slightly elevated levels of maternal circulating triglyceride at this time (Table 3). Mammary LPL in rats increases during the first few days of lactation and remains at high levels thereafter (Hamosh et al. 1970), while in guinea pigs it reaches a maximum at 2 h postpartum (McBride and Korn 1963). In humans, LPL levels in the mammary gland (as indicated by LPL measured in milk) may increase over lactation and this is accompanied by increases in milk fat concentration from colostrum to mature milk (Mehta et al. 1982). We do not know whether PH-LPL activity levels in grey seal mothers were intermediate during mid-lactation, in parallel with milk fat concentration (Fig. 2), since interim PH-LPL levels were not measured.

Lipoprotein lipase activity and fat deposition in suckling grey seal pups

Like most mammalian neonates (Widdowson 1950; McCance and Widdowson 1977), grey seal pups are born with relatively little fat [5%, Table 2; Iverson et al. (1993)]. However, unlike most other mammals where neonatal growth consists primarily of increases in lean body tissue, growth in phocid pups is exceptionally rapid and due primarily to deposition of fat in blubber (Worthy and Lavigne 1983; Oftedal et al. 1989). Grey seal pups on Sable Island consume approximately 22 Mcal · day^{-1} of milk and 95% of this daily energy intake is fat (Iverson et al. 1993). After the brief 16-day lactation period, pups remain onshore during a prolonged fast of up to 1 month, during which they rely primarily on stored fat in blubber for both energy needs and water balance (Ortiz et al. 1978; Nordoy and Blix 1985; Worthy and Lavigne 1987; Reilly 1991). Hence, fattening during the suckling period is crucial to survival of offspring. The fastest growing pups (i.e., those with the greatest milk consumption rates) are weaned with a proportionately greater fat content and thus may be better prepared for the postweaning fast (Iverson et al. 1993). Clearly, pups must divert a large portion of ingested milk triglyceride towards storage in adipose tissue.

In the present study, pups deposited up to six times as much fat as protein in mass gain. The ability of the pup to rapidly digest and deposit such large quantities of dietary lipid (up to 2 kg · day^{-1}) is remarkable and requires well-developed mechanisms of lipid metabolism from birth. On a body mass basis, the lipid intakes of grey seal pups are up to tenfold higher than in human infants and three- to fivefold higher than in suckling rats or dogs. Previous studies have shown that lipid digestion in phocid pups is rapid, at least in part due to extensive gastric hydrolysis by gastric lipase (Iverson et al. 1992) and to the presence of milk lipase, which probably aids intestinal lipolysis (Iverson 1988; Iverson et al. 1991). It is also clear that in these rapidly fattening animals, milk fatty acids are deposited directly and without modification into blubber stores (Iverson et al. 1995).

From the present study, it appears that the normal process of reesterification of fatty acids from the intestine into chylomicron triglyceride (Nelson 1992) occurs in phocid pups. In recently suckled grey seal pups, circulating fatty acids were carried primarily as triglyceride (90–94%, Table 3) and chylomicrons were readily apparent as evidenced by milky white serum (S.J. Iverson, pers. obs.). Hence, subsequent uptake of circulating fatty acids by tissues in the pup must depend largely on LPL. Unfortunately, we cannot directly compare PH-LPL activity with pup triglyceride uptake because triglyceride levels were quite variable and most likely a function of how much and when the pup had last suckled (e.g., Fig. 5). However, it appears that pups do have well-developed mechanisms of triglyceride clearing from birth. Even after recent suckling of high fat milk, pups did not appear to be hyperlipemic at an average plasma triglyceride concentration of 172–263 mg · dl^{-1} (Table 3, Fig. 5), possibly due to high levels of LPL. Our PH-LPL values for grey seal pups

were 20 times higher than in suckling dogs, despite the fact that our values may be somewhat underestimated as discussed previously. PH-LPL levels in grey seal pups were found as high as 40 and 62 $\mu mol \cdot ml^{-1} \cdot h^{-1}$ near birth and late lactation, respectively. In comparison, plasma PH-LPL activity in suckling dogs, after the same dose of heparin ($100\ U \cdot kg^{-1}$), averaged 2 $\mu mol \cdot ml^{-1} \cdot h^{-1}$) in the first week and 3 $\mu mol \cdot ml^{-1} \cdot h^{-1}$ during peak lactation (Das et al. 1982). PH-LPL values available for the suckling rat show an increase from about 8 to 35 $\mu mol \cdot ml^{-1} \cdot h^{-1}$ over lactation, but these values may not be directly comparable to ours since heparin was administered at a much higher dose of $500\ U \cdot kg^{-1}$ (IP route). In adult animals, which usually exhibit higher PH-LPL levels than neonates, PH-LPL activity has been measured at 14–18 $\mu mol \cdot ml^{-1} \cdot h^{-1}$ in non-human primates and 1–10 $\mu mol \cdot ml^{-1} \cdot h^{-1}$ in humans after 100 U heparin $\cdot kg^{-1}$ (Rudel and Star 1990; Thompson et al. 1986; Eckel et al. 1988).

Pup adipose tissue might be expected to have relatively high LPL activity compared to other tissues, given the deposition of up to 1.7 kg of fat daily (Iverson et al. 1993). Although we were not able to evaluate the relative importance of the various tissues which likely contain LPL, it was apparent that total PH-LPL activity increased directly with increasing fat content of pups (Fig. 4) and with an increasing body fat:protein ratio. Clearly, other tissues must have contributed to PH-LPL activity. However, given that pups gained almost six times as much fat as protein, that 70% of the fat consumed was deposited in blubber (see results), and that fat comprises up to 45% of body mass near weaning (Iverson et al. 1993), it is likely that LPL released from adipose tissue represented the major portion of the PH-LPL we measured in pups. If this is the case, this would be similar to adult rats, where the main contributor to total LPL activity is white adipose tissue (Planche et al. 1980). In suckling rats, muscle LPL is believed to be the primary source of total LPL throughout lactation (Planche et al. 1980). This might be expected since postnatal growth in suckling rat pups, were body fat content declines and protein content increases (Spray and Widdowson 1950), is quite different from that observed in phocid pups.

Clearly, grey seal pups are quite able to deposit fat from birth. In the rat, LPL is active in pre-adipocytes from birth and may play a regulatory role in lipid filling during the nursing period (Hamosh and Hamosh 1985). At 5% body fat, grey seal pups are born with about five times more body fat than rat pups (McCance and Widdowson 1977). This difference may in part explain the high PH-LPL activities in grey seal pups near birth. If adipose tissue LPL in pups is relatively high at birth this may facilitate rapid fattening. Since LPL activity in pups also appears to increase with increasing body fat content, a fat pup may be able to fatten more readily. If so, this might be particularly important in another phocid, the hooded seal (*Cystophora cristata*), where fattening must occur in only 4 days of lactation (Bowen et al. 1985). Hooded seal pups are born with a substantial blubber layer (17% of body mass), perhaps enabling these pups to begin depositing a phenomenal 4–5 $kg \cdot day^{-1}$ of milk triglyceride fatty acids directly into blubber (Iverson et al. 1995). In adult humans and rats, increases in body fat correlate with increased adipose tissue LPL; however, it is not known whether an increase in tissue LPL precedes or simply follows fattening (Eckel 1989).

In conclusion, in grey seals mammary LPL may be extremely important to the mother for uptake of circulating triglyceride fatty acids, even when fasting. LPL may be critical in the process of fat deposition in blubber by the neonate. To further understand the role of LPL in lipid metabolism in phocid seals, it will be essential to establish the relationship between PH-LPL levels and individual tissue activities in mothers and pups, and to determine the relative activity of different tissues with different stages of lactation.

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