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Fat transfer and energetics during lactation in the hooded seal: the roles of tissue lipoprotein lipase in milk fat secretion and pup blubber deposition

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Abstract Hooded seals (*Cystophora cristata*) lactate for 3.6 days during which females simultaneously fast and transfer large amounts of energy to their pups through fat-rich milk. Pups grow rapidly, principally due to blubber deposition. Lipoprotein lipase (LPL), the primary enzyme responsible for tissue uptake of triglyceride fatty acids, may strongly influence both maternal milk fat secretion and pup blubber deposition. We measured the energetic costs of lactation (using hydrogen isotope dilution, $^3H_20$), milk composition, prolactin, and LPL activity (post-heparin plasma LPL [PH LPL], blubber, mammary gland and milk; U) in six females. PH LPL and blubber LPL were measured in their pups. Females depleted 216.3 MJ · day^{-1} of body energy and fat accounted for 59% of maternal mass loss and 90% of postpartum body energy loss, but maternal body composition changed little. Maternal blubber LPL was negligible (0.0–0.2 U), while mammary LPL was elevated (1.8–2.5 U) and was paralleled by changes in prolactin. Estimated total mammary LPL activity was high (up to 20,000 U · animal^{-1}) effectively favoring the mammary gland for lipid uptake. Levels of total blubber LPL in pups increased seven-fold over lactation. Pups with higher PH LPL at birth had greater relative growth rates ($P = 0.025$). Pups with greater blubber stores and total blubber LPL activity had elevated rates of fat deposition ($P = 0.035$).

Key words Lactation · Lipoprotein lipase (LPL) · Prolactin · Milk composition · Hooded seal *Cystophora cristata*

Abbreviations *DEE* daily energy expenditure · *FFA* free fatty acid · *HL* hepatic lipase · *LPL* lipoprotein lipase · *PH* post-heparin · *PH LPL* post-heparin plasma lipoprotein lipase · *TG* triglyceride · *VLDL* very low density lipoprotein

Introduction

Phocid seals spend the majority of their life at sea, but are tied to either land or ice for the birth and suckling of their young. During this time, females fast and lactation periods are typically reduced, usually lasting less than 50 days (Bonner 1984; Oftedal et al. 1987). The hooded seal (*Cystophora cristata*) represents the most extreme example of this, with a lactation period of less than 4 days (Bowen et al. 1985) which is characterized by very rapid energy transfer in milk and equally rapid blubber deposition in the pup. Hooded seal females produce up to 10 kg · day^{-1} of milk that is comprised of 61% fat (Oftedal et al. 1988, 1993). Pups gain up to 7 kg · day^{-1}, the majority of which is fat deposited directly from milk into a layer of blubber (Iverson et al. 1995a). This blubber fat is critical to the pup's ability to survive the subsequent month-long postweaning fast (Bowen et al. 1987). Giving birth on unstable and unpredictable pack ice is thought to have strongly influenced the evolution of an abbreviated suckling period, but the physiological capacity and regulation of these phenomenal rates of lipid metabolism in both mothers and pups is not understood. Additionally, while it is proposed that hooded seal females achieve a significant reduction in the total

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overhead costs of lactation by reducing its length, direct energetic costs to the mother have not yet been studied.

Lipoprotein lipase (LPL) was identified over three decades ago as a major enzyme involved in the regulation of lipid metabolism and has since been studied extensively in humans and small laboratory mammals (Bezman et al. 1962; Högstedt and Lindquist 1963; Wing et al. 1966a; Rath et al. 1974; Taskinen and Nikkilä 1977; Planche et al. 1980; Chajek-Shaul et al. 1985; Jackson and McLean 1991). Tissue LPL activity is rapidly modified in response to the immediate energetic need of the tissue (Wing et al. 1966b; Planche et al. 1980; Chajek-Shaul et al. 1985) by hormones such as insulin, cortisol, and prolactin (Borensztajn et al. 1972; Zinder et al. 1974; Spooner et al. 1977; Ostrom 1990; McNamara 1994; Ottosson et al. 1994). During periods of feeding, adipose tissue LPL increases resulting in tissue storage of triglyceride (TG) fatty acids; during fasting, adipose tissue LPL declines sharply, halting the uptake and further storage of fatty acids.

During lactation, regulation of tissue LPL may play a particularly important role. In non-lactating guinea pigs and rats, the mammary gland is dormant and accordingly LPL levels are negligible (McBride and Korn 1963; Robinson 1963; Ottway and Robinson 1968; Hamosh et al. 1970). However, just prior to parturition, mammary LPL activity increases dramatically with a reciprocal decrease in adipose tissue LPL activity, even when feeding (McBride and Korn 1963; Robinson 1963; Hamosh et al. 1970; Ramírez et al. 1983). The increase in mammary gland activity, which is thought to be influenced by prolactin (Ramírez et al. 1983; Hang and Rillema 1997), diverts circulating TG carried in lipoproteins (both chylomicrons from diet and very low density lipoproteins, VLDL, produced by the liver) to the mammary gland for incorporation into milk. While free fatty acids (FFA) are available to all tissues, only those organs with active LPL can compete for TG fatty acids. This process has been demonstrated in rats, mice, rabbits and guinea pigs, typically by sacrificing the animal and excising the entire mammary gland and/or fat pad for analysis (McBride and Korn 1963; Robinson 1963; Zinder et al. 1974; Scow et al. 1977; Spooner et al. 1977; Ramírez et al. 1983). These previous studies suggested that mammary LPL activity may be correlated with the degree of lipid uptake by the mammary gland (McBride and Korn 1964; Scow et al. 1977). However, this relationship and the metabolic transition in adipose and mammary tissue has not been examined in other species.

Hooded seals are an excellent model for studying the role of LPL in facilitating milk fat transfer. Given that LPL is thought to regulate both milk fat output and fat storage in adipose tissue, it was hypothesized (Iverson et al. 1995a) that characteristics of tissue LPL activity may be related to patterns of milk fat secretion by hooded seal mothers and blubber deposition by their pups. While Iverson et al. (1995b) proposed that LPL may play an important role in determining rapid transfer of milk fat in phocid seals, they were only able to indirectly infer characteristics of tissue LPL in grey seals (*Halichoerus grypus*), which are similar to hooded seals in both maternal parturition mass and pup mass. In the present study, we measured LPL activities in individual tissues of mothers and pups over lactation in relation to estimates of nutrient transfer. The objectives of this study were threefold: (1) to investigate how maternal nutrient and energy stores are depleted during lactation, (2) to measure circulating lipid and prolactin levels, and whole-body, mammary, and adipose tissue LPL activities in mothers in relation to milk fat content and output, and (3) to measure whole-body and blubber LPL activities in pups to examine how these may influence rates of growth and blubber deposition.

Materials and methods

Field procedures

Six mother-pup pairs were studied during the March 1997 breeding season on the pack ice in the southern Gulf of St. Lawrence, Canada (46°34′–46°42′N, 61°51–63°09′W). Females with newborns were identified initially from a helicopter and, subsequently upon close examination, by the presence of a fresh placenta and blood on the surrounding ice. While it was clear when pups were newborn, it was usually not possible to discern their age within 0.5 days of their 3.6-day lactation period. Females were captured on the day of birth (day 0) in a net and a blood sample (10 ml) was immediately taken for plasma lipid and hormone analysis. Females were then weighed to the nearest 0.5 kg using a 300-kg Salter scale suspended from an aluminum tripod. A blood sample (10 ml) for plasma lipid analysis was also taken immediately from pups, after which they were weighed in a canvas bag to the nearest 0.1 kg with a 100-kg Salter scale. Mothers were injected intramuscularly (i.m.) with a precisely weighed dose (averaging 5 g of 0.5 $mCi \cdot m1^{-1}$) of tritiated water (^3H_2O) to determine total body water. Blood samples (10 ml) were taken via the extradural vein after equilibration (2.0 h and 2.5 h post-injection) as determined previously for grey seal females (Mellish 1999). It was originally intended that body composition and water turnover in their pups would be measured (using deuterium oxide dilution) by other investigators in a concurrent study. However, the isotope quality and quantity used resulted in unmeasurable levels in pup sera, and thus body composition, water turnover, and milk intake data were not available. Therefore, we used relationships derived previously for hooded seal pups (Oftedal et al. 1993) to estimate these data.

In preparation for tissue biopsy, females were administered diazepam (5 ml of 5 $mg \cdot ml^{-1}$) intravenously (i.v.) to permit safer handling. A 4-cm × 4-cm area on the posterior flank was then shaved, cleaned with alcohol and prepodyne, and locally anesthetized with 2 cc of lidocaine. Subsequently, a small incision (approx. 1 cm) was made using a sterile no. 11 scalpel blade. A biopsy (approx. 300–500 mg) was taken using a modified (lengthened and with the blade crimped on either side) 6-mm core tool (Acupunch, CDMV) through the full depth of the blubber layer and into the mammary gland (approx. 8–9 cm). The biopsy was immediately divided with a scalpel blade into blubber and mammary tissue, and each tissue was separately wrapped in aluminum foil and placed in a cooler. The incision was closed with three interrupted sutures using an absorbable suture and sprayed with topical antibiotic (Topazone). Oxytocin (1.5 ml of 20 $IU \cdot ml^{-1}$, Austin) was then given i.m. to facilitate milk let-down, after which milk samples (2 × 30 ml) were collected by suction using a 60-cc syringe with the end removed. Heparin (0.01 $ml \cdot kg^{-1}$ body mass of 10,000 $U \cdot ml^{-1}$ Hepalean, Organon Teknika) was then administered i.v. to the female to promote lipase release from the capillary endothelium

(Jackson and McLean 1991). Blood samples (5 ml, 15% EDTA vacutainers, Becton Dickson) were taken via the extradural vein at 20 min and 30 min post-heparin administration (optimal lipase release time as determined for grey seals; Iverson et al. 1995b).

A similar site (3 cm × 3 cm) was prepared on the pup, as described above. A biopsy was taken through the full depth of the blubber layer (approx. 100–200 mg) using a 6-mm core tool and stored in aluminum foil in a cooler. The biopsy site was closed with two interrupted sutures and sprayed with topical antibiotic. Heparin (0.1 ml·kg^{-1} body mass of 1000 U·ml^{-1} Hepalean, Organon Teknika) was then administered i.v., with blood samples (5 ml) taken via the extradural vein 20 min and 30 min after heparin administration.

Females were kept in their capture net adjacent to their pups for the entire 2.5 h isotope-equilibration period to prevent suckling. Pairs were released after the equilibration period and observed to ensure that the mother-pup bond was intact. A VHF radio transmitter and green fluorescent dye were placed on the ice pan used by each pair to facilitate relocation.

On day 3 of the 3.6-day lactation period, pairs were relocated from a helicopter and subsequently recaptured and sampled again as described above (the other side of the body was biopsied). An incisor was extracted from mothers to determine maternal age as described in Bernt et al. (1996). All samples (blood, milk, and other tissues) were stored on ice in a cooler while in the field (< 4 h). Upon return to the field laboratory, plasma was collected from blood samples after centrifugation at 2000 rpm for 20 min. Aliquots of plasma, milk, and tissues collected for LPL analysis were stored in 2-ml cryovials submerged in liquid nitrogen for approx. 2 weeks prior to analysis. All other samples were frozen (−20 °C) until analysis.

Sample analysis

Milk samples were analyzed in duplicate for dry matter and protein using forced convection drying, and macro-Kjeldahl methods, respectively. Milk fat content was determined in duplicate using a modified version of Folch et al. (1957), with an increased ratio of solvent to milk to ensure complete lipid extraction. Female serum samples for determination of total body water were distilled in triplicate using the method of Ortiz et al. (1978) and counted for ^{3}H activity with a Beckman LS 5000CE scintillation counter. Maternal serum samples were analyzed in duplicate for prolactin concentration by I^{125} radioimmunoassay with human prolactin as reference. FFA were analyzed in duplicate for females and singly for pups using a WAKO NEFA-C enzymatic kit (WAKO Chemicals USA, Baltimore, Md.). Circulating TG were determined in duplicate for females and singly for pups using a Sigma Triglyceride GPO-Trinder enzymatic kit (Procedure no. 337, Sigma Diagnostics, Oakville, Ont.).

Tissue LPL and post-heparin plasma LPL (PH LPL) activities were assayed by measuring the rate of hydrolysis (rate of FFA release) of ^{3}H-labeled triolein contained in an emulsion (Hernell et al. 1975). A number of modifications were made to optimize this assay. An appropriate volume of the substrate emulsion was prepared immediately prior to each assay, consisting of 15.6 µl ^{3}H-labeled triolein ([9,10-^{3}H(N)], 0.5 mCi·ml^{-1}, NEN Research Products) and 2.6 ml unlabeled triolein (C18:1, [cis]–9; Sigma) in chloroform (0.027 g triolein per ml chloroform), and evaporated to dryness. Gum arabic (10%, 2.6 ml), Tris buffer (pH 8.0, 1.2 ml) and distilled water (1.3 ml) were added. The mixture was sonicated on ice with a Cole Parmer 4710 series ultrasonic cell disrupter for two 3-min periods separated by a 1-min rest. The emulsion was kept on ice and used within 2 h of preparation.

Blubber and mammary tissues were prepared prior to analysis generally according to Hamosh et al. (1970). Blubber biopsies were rinsed with saline and weighed directly in a 5 ml glass tissue homogenizer. Samples were thoroughly homogenized with a Teflon pestle in approx. 4 ml cold acetone, after which 50 µl 20% fatty acid free bovine serum albumin (BSA, Sigma) was added. Mammary samples were quickly weighed in 2-ml Nalgene cryovials and placed back into liquid nitrogen. After a minimum of 30 min, the mammary sample was homogenized in an ice-cold 1-oz ceramic mortar. After complete homogenization, the mammary tissue was re-suspended in 8–10 drops of cold saline followed by the addition of approx. 4 ml cold acetone. A 250-ml side arm flask was rinsed with acetone and attached to a buchner funnel equipped with Whatman no. 5 (4.25 cm, Fisher Scientific) filter paper. The homogenates (both blubber and mammary) were then each filtered using vacuum aspiration at maximum strength and rinsed three times with 2 ml cold 1:1 acetone: ether, and four times with 2 ml cold ether. Dry precipitate was scraped off the filter paper with a sterile no. 22 scalpel blade and weighed in a disposable borosilicate culture tube (16 mm × 100 mm). The powder was placed in a cold desiccator and stored in a freezer (−20 °C) until 1 h prior to assay, at which time the sample was re-suspended in a 0.025 M NH_4OH buffer, pH 8.1 (10 µl per mg precipitate) containing 1.0 U·ml^{-1} heparin which stabilizes the enzyme.

Reactions were carried out in 16 mm × 100 mm disposable borosilicate glass culture tubes (Fisher Scientific). For plasma and milk samples, each assay tube contained 32 µl 20% BSA, 50 µl substrate, 30 µl saline, 48 µl distilled water, 20 µl heat-inactivated (20 min at 62.5 °C) seal plasma (as a source of apo CII activator), and 20 µl test plasma. Since plasma post-heparin (PH) lipolytic activity represents activity of both LPL and hepatic lipase (HL), PH HL was also assayed in plasma samples with 32 µl 20% BSA, 50 µl substrate, 30 µl saline, 8 µl distilled water, 20 µl heat-inactivated seal plasma, 40 µl 5 M NaCl (to inhibit LPL activity) and 20 µl test plasma. For tissue samples, each assay tube contained 32 µl 20% BSA, 50 µl substrate, 48 µl distilled water, 20 µl heat-inactivated seal plasma, and 50 µl re-suspended tissue powder. Total reaction volume for all samples was 200 µl. Each sample was assayed in triplicate and added to the reaction tube immediately prior to incubation. Plasma and milk samples were incubated for 15 min at 37 °C in a shaking water bath, whereas tissue samples were incubated for 60 min. These incubation times were determined by previous optimization trials with grey seal plasma, milk, and tissue using 10, 15, 20, 30 and 60 min incubations. The reaction was stopped with 3.25 ml methanol:chloroform:heptane (1.4:1.25:1) and 1.25 ml 140 mM K_2CO_3·boric acid (pH 10.6). The mixture was centrifuged for 20 min at 2000 rpm, after which 1.0 ml of the upper phase (FFA in chloroform) was removed using a pipette, added to 10 ml Scintiverse II (Fisher Scientific) and counted for 10 min in a Beckman LS 5000CE scintillation counter. The total upper phase volume (from which the 1.0 ml was taken) and the percent recovery in the upper phase of all FFA released were determined for calculation of total FFA released. Blank counts (assay tubes containing no sample) were measured in triplicate for each assay and subtracted from each sample's total FFA count. Total counts originally present in each assay were also measured in triplicate to relate assay counts to µmol FFA released.

PH LPL activity was calculated as total plasma lipase activity (higher of the two samples taken, i.e., 20 min or 30 min PH) minus PH HL activity. All units are expressed as µmol FFA released·h^{-1}·ml plasma^{-1} or·g tissue^{-1} (U).

Data analysis and calculations

All pairs were sampled on the day of parturition and again on day 3 of lactation, with the exception of Pair 1. This pair was resampled on day 2 of lactation due to impending weather conditions which would not have allowed sampling of this pair on day 3. Although all rates of mass and tissue loss were calculated based on the interval between sampling for each pair in hours, day 2 data for Pair 1 are included in day 3 averages. In addition, we were not able to obtain sufficient milk from Female 1 on the day of parturition for proximate composition analysis.

Isotope dilution space, total body water and body composition were calculated for females on the day of parturition and again on day 3 of lactation (as described in Iverson et al. 1993; Mellish 1999) and using the conversion of dilution space to total body water as derived in Bowen and Iverson (1998). Total body fat and protein were converted to energetic equivalences using the values of 39.3 MJ·kg^{-1} and 23.6 MJ·kg^{-1}, respectively (Blaxter 1989). Since

pup isotope data were not available, estimates of blubber content and rates of fat deposition were made using predictive relationships between body mass and body water content obtained from both newborn hooded seal pups ($r^2 = 0.85$) and 3-day-old hooded seal pups ($r^2 = 0.68$) in Oftedal et al. (1993). We also inferred milk intake rates for these pups based upon the relationship between mass gain and energy intake ($r^2 = 0.82$) measured in Oftedal et al. (1993). The terms initial and final refer to day 0 and day 3, respectively.

Data were generally examined using non-parametric tests to account for departure from normality given our small sample sizes. Changes within individuals over time were measured using a Wilcoxon signed rank test and relationships between variables were tested using Spearman rank correlation (rho). However, predictive relationships were estimated using simple, least squares regression. Values are expressed as mean ± SE unless otherwise stated. Relative variation is given by the coefficient of variation (cv). All statistical analyses were performed using StatView 4.1 for the Macintosh.

Results

Maternal and neonatal body mass and composition

Female body mass on the day of parturition averaged 235.3 kg, ranging from 203.0 kg to 270.0 kg (Table 1). Daily mass loss ranged from 5.1 kg·day^{-1} to 12.3 kg·day^{-1} (mean 8.7 ± 1.02 kg·day^{-1}), with an average mass on day 3 of 211.2 kg. Although larger females tended to have both greater absolute (rho = 0.771, $P = 0.085$) and relative mass losses (rho = 0.543, $P = 0.225$), neither relationship was significant. In total, females lost 7.2–13.3% of their initial body mass (mean 10.1 ± 1.04%) during the 3-day study period.

Table 1 Individual mass changes in six hooded seal mother-pup pairs. Pair 1 was resampled on day 2 due to an impending severe winter storm. Ages were determined using incisors. (*F* female, *M* male)

Animal	Mothers			Pups		
	Day 0	Day 3	Age (years)	Day 0	Day 3	Sex
1	236.0	219.0	22	24.8	35.7	M
3	203.0	188.0	19	22.0	30.0	F
4	213.5	188.0	10	22.7	35.5	M
5	230.0	208.5	27	10.7	19.0	F
6	259.5	225.0	15	28.2	44.5	F
7	270.0	238.5	22	22.8	41.0	F
Mean	235.3	211.2	19.2	21.9	34.3	
SE	10.55	8.33	2.44	2.42	3.67	

The range of pup masses on day 0 was fairly narrow among five pups, averaging 24.1 ± 1.13 kg. However, a sixth pup was exceedingly small at only 10.7 kg, but born to a medium-sized female (Table 1). Therefore, the correlation between pup birth mass and maternal parturition mass was not statistically significant (rho = 0.657, $P = 0.142$). Daily mass gain ranged from 2.7 kg·day^{-1} to 6.1 kg·day^{-1} (mean 4.5 ± 0.59 kg·day^{-1}). Larger pups at birth tended to have greater rates of absolute mass change (rho = 0.771, $P = 0.085$), however this was not significant. Relative mass gain was also independent of initial pup mass (rho = −0.086, $P > 0.5$). In fact, the very small pup at day 0 (Pup 5) had the highest relative daily mass gain (27.8%·day^{-1}). Pup mass at day 3 ranged from 19.0 kg to 44.5 kg, representing an increase of 58.7 ± 7.13% of their initial body mass (range 36.4–79.8%). Pup mass at day 3 was weakly correlated with initial maternal mass (rho = 0.771, $P = 0.084$), but removal of Pup 5 increased the strength of the correlation (rho = 0.900, $P = 0.072$).

In females, total body water calculated from isotope dilution was strongly predicted by body mass, both on day 0 ($r^2 = 0.954$, $P < 0.001$, $y = 13.752 + 0.465x$) and on day 3 ($r^2 = 0.816$, $P = 0.013$, $y = -6.338 + 0.593x$). Females averaged 52.4% water, 28.1% fat and 17.3% protein at parturition (Fig. 1). Relative body composition changed only slightly but significantly ($P = 0.046$) over the study period, with females composed of 54.7% water, 24.7% fat and 18.2% protein on day 3 (Fig. 1). Female 5 actually maintained her body composition during the study period (water: 53.1% vs 53.0%, fat: 27.0% vs 27.1%, and protein: 17.6% vs 17.5%). From day 0 to day 3, absolute maternal fat stores changed significantly (from 66.3 ± 4.19 kg to 52.3 ± 3.16 kg, $P = 0.028$), as did total body water (from 123.1 ± 5.02 kg to 115.4 ± 4.02 kg, $P = 0.028$) and protein stores (from 40.5 ± 1.62 kg to 38.4 ±

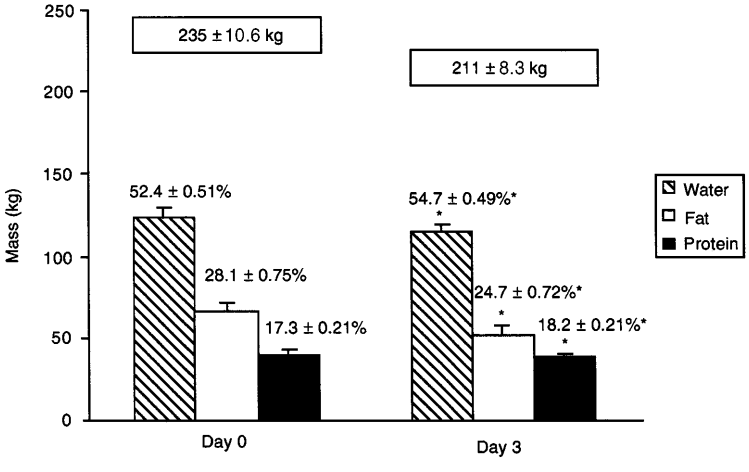

Fig. 1 Body mass and composition at parturition and 3 days postpartum in six hooded seal females. *Bars* represent absolute values (kg), with the percentage of each component indicated above. Relative water, fat and protein content all differed significantly between day 0 and day 3 ($P = 0.046$). Mass, absolute fat content and absolute protein content also changed significantly ($P = 0.028$)

1.30 kg, $P = 0.028$; Fig. 1). As expected, larger females had significantly larger fat stores and protein reserves at parturition than smaller females (rho = 0.943, $P = 0.035$ and rho = 0.886, $P = 0.048$, respectively), but relative body composition was independent of body mass (rho = 0.468, $P = 0.277$). Larger females at parturition maintained both larger protein stores and larger fat reserves (rho = 0.943, $P = 0.035$ for both) at day 3 than did smaller females. Fat accounted for 59.2 ± 9.11% of daily maternal mass loss, but loss rate was highly variable (range 2.0–8.0 $kg \cdot day^{-1}$). Daily protein loss was also variable (range 0.3–1.8 $kg \cdot day^{-1}$) and accounted for only 8.4 ± 2.60% of total daily mass loss. Fat and protein accounted for 89.5 ± 4.69% and 10.5 ± 4.69% of total body energy loss, respectively. Females depleted 306–963 MJ of body energy reserves by day 2 ($n = 1$) or day 3 ($n = 5$). These losses corresponded to total daily energy expenditures (DEE) of 216.3 ± 31.89 $MJ \cdot day^{-1}$ ($n = 6$).

Using the relationship between pup body mass and total body water from Oftedal et al. (1993), we estimated that the pups in this study were 15.9 ± 3.10% fat (3.8 ± 1.00 kg) and 20.7 ± 0.88% protein (4.4 ± 0.38 kg) on day 0. By day 3, they were approximately 35.2 ± 5.10% fat (13.0 ± 2.68 kg) and 15.2 ± 1.46% protein (5.0 ± 0.17 kg). Therefore, estimated fat deposition (up to 5.0 $kg \cdot day^{-1}$) accounted for the majority of pup mass gain (70.6 ± 10.00%). There was a tendency for pups who had larger estimated fat stores at birth to have faster growth rates, although this relationship was not significant at the 5% level (rho = 0.771, $P = 0.085$). Estimated protein deposition was minimal (0.2 ± 0.08 $kg \cdot day^{-1}$) and accounted for only 5.1 ± 2.86% of daily growth. Pup 5 was almost half the mass at birth than any hooded seal previously studied and therefore it was difficult to predict body composition, especially at birth. With the exception of Pup 5, estimated fat deposition averaged 37.3 ± 4.02 times the estimated rate of protein deposition.

Milk composition

Proximate milk composition did not change significantly over the course of lactation (Table 2). Average milk fat content and therefore average dry matter and energy content were slightly higher on day 3 than on day 0, but this was not significant. There was a significant decrease in milk protein content between days 0 and 3 (6.7% to 5.4%, $P = 0.043$).

While levels of most milk components did not change significantly during lactation, there was considerable variation in milk composition both among and within individual females (Fig. 2). For example, the milk fat content of two females (Females 4 and 7) was considerably lower than that of the other females, especially on day 0. As a result, milk fat content was much more variable on day 0 (cv 17.5%) than at day 3 (cv 5.3%). In contrast, the milk protein content of two females (Females 5 and 7) was noticeably higher than the other females' milk on day 0. Therefore, milk protein content was also more variable at the beginning of lactation (cv 17.0%) than at the end of lactation (cv 8.2%). Female 7 was clearly sampled very close to parturition as indicated by a particularly fresh placenta, which would be consistent with the high protein, low fat content of her milk (i.e., colostrum). Female 5 had the unusually small pup that may have been a premature birth, which might also correspond to high initial milk protein values. Finally, the variation in milk energy content during

Table 2 Proximate composition of milk over lactation in six hooded seal females

Component	Day 0 ($n = 5$)	Day 3[1] ($n = 6$)	P[2]
Water (%)	33.8 ± 1.86	31.3 ± 0.76	0.225
Dry matter (%)	66.2 ± 1.86	68.7 ± 0.76	0.225
Protein (%)	6.7 ± 0.51	5.4 ± 0.18	0.043
Fat (%)	51.7 ± 4.04	56.7 ± 1.24	0.225
Energy ($MJ \cdot kg^{-1}$)	21.9 ± 1.55	23.5 ± 0.50	0.225

[1] Milk for proximate composition analysis from Female 1 was only available on day 2 and was included in day 3 averages
[2] Wilcoxon signed rank test

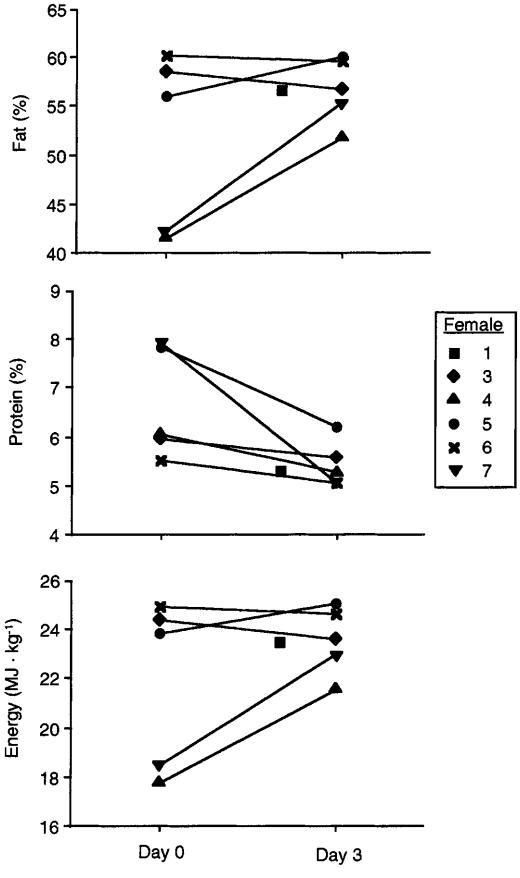

Fig. 2 Individual changes in proximate milk composition in six hooded seal females during lactation. Only milk protein changed significantly ($P = 0.043$). Milk for proximate composition analysis for Female 1 was only available on day 2

lactation (cv 15.8% to 5.3%) generally reflected the variation observed in milk fat content.

As expected, milk composition was independent of maternal characteristics such as body mass or composition. Average milk fat content was unrelated to average maternal mass (rho = 0.086, $P > 0.5$) or fat stores (absolute rho = 0.086, $P > 0.5$; relative rho = −0.029, $P > 0.5$). Average milk protein content was similarly unrelated to maternal mass (rho = −0.086, $P > 0.5$) or protein stores (absolute rho = −0.029, $P > 0.5$; relative rho = −0.371, $P = 0.406$).

Maternal LPL activity, prolactin and milk fat content

Maternal PH LPL activity averaged 10.1 ± 4.33 U at parturition and 4.3 ± 2.53 U on day 3, however this apparent decrease was not significant ($P = 0.345$; Fig. 3) as patterns were variable among females. PH LPL remained constant in Female 1 and Female 3, and doubled in Female 5. In contrast to PH LPL, average mammary LPL activity tended to increase between day 0 (1.8 ± 0.63 U) and day 3 (2.5 ± 0.72 U), but again this was not significant ($P > 0.5$). The pattern was variable among females, as mammary LPL was relatively constant in two females and decreased substantially in Female 3. Blubber LPL activity was negligible in all females at the beginning of the study (0.17 ± 0.119 U) and unmeasurable in four of the six females by the end of the study (0.02 ± 0.015 U; $P = 0.345$). Milk LPL activity was also negligible on day 0 (0.06 ± 0.060 U), but increased almost 100-fold by day 3 (5.8 ± 2.00 U; $P = 0.043$).

Maternal plasma prolactin concentrations were low near parturition (1.1 ± 0.08 $ng \cdot ml^{-1}$), and increased slightly but not significantly by day 3 (1.6 ± 0.23 $ng \cdot ml^{-1}$; $P = 0.104$). Among females, average prolactin levels over lactation were positively correlated with average mammary LPL activity (Fig. 4), although data from one female (Female 1) were anomalous. This female was resampled out of sequence (1 day earlier than the other females). The removal of this female increased the significance level from $P = 0.064$ to $P = 0.046$ (rho = 1.000). Although initial milk fat content tended to be greater in females with higher initial mammary LPL activity ($n = 5$, rho = 0.700; Fig. 5), this again was not statistically significant ($P = 0.162$). The relationship between mammary LPL activity and milk fat content on day 3 was stronger but also not significant ($P = 0.085$).

Given the amount of individual variation among females, it is useful to compare changes in levels of mammary LPL with those of circulating prolactin and milk fat content. Despite the small sample size, there were similarities in the changes of mammary LPL, prolactin and milk fat content during lactation (Fig. 5).

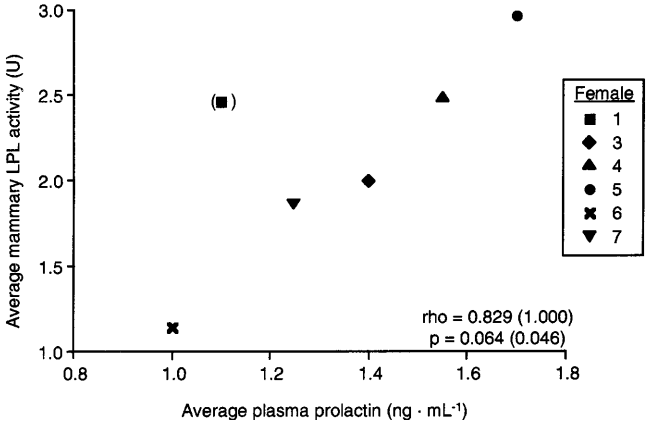

Fig. 4 Relationship between average maternal plasma prolactin concentration and average mammary LPL activity during lactation in six hooded seal females. Values for rho and P in parentheses represent test with removal of Female 1

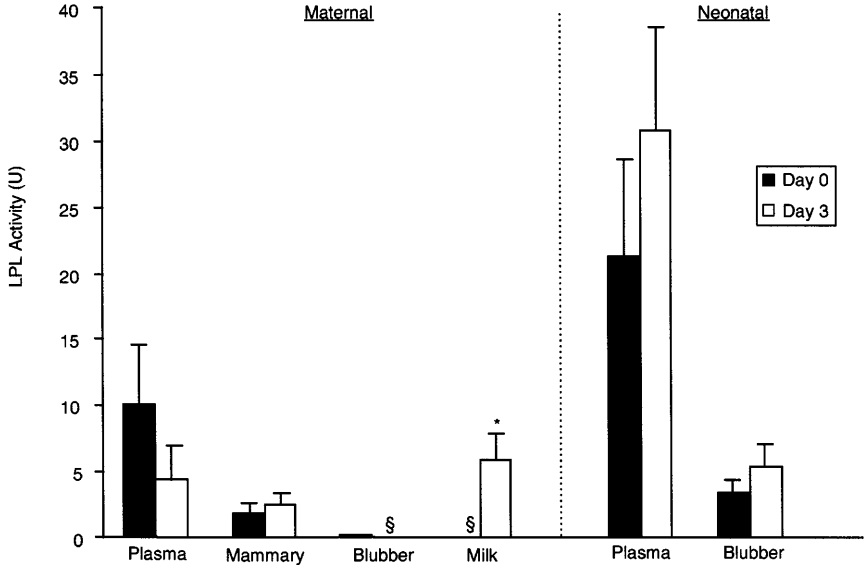

Fig. 3 Changes in post-heparin plasma lipoprotein lipase (PH LPL) and tissue lipoprotein lipase (LPL) activity during lactation in six hooded seal mother-pup pairs. *Only milk LPL activity changed significantly between day 0 and day 3 ($P = 0.043$). §Levels of maternal blubber LPL activity (day 3) and milk LPL activity (day 0) were unmeasurable

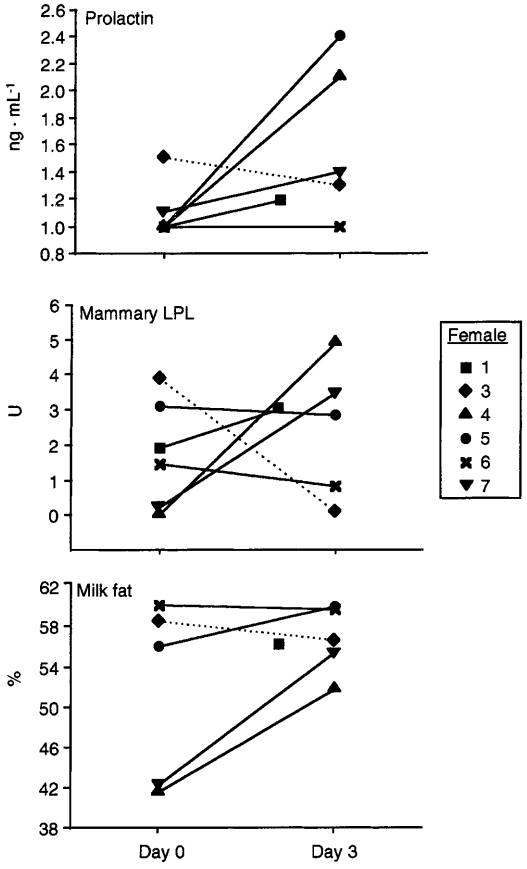

Fig. 5 Maternal prolactin, mammary LPL activity and milk fat content during lactation in six hooded seal females. None of the observed changes were significant

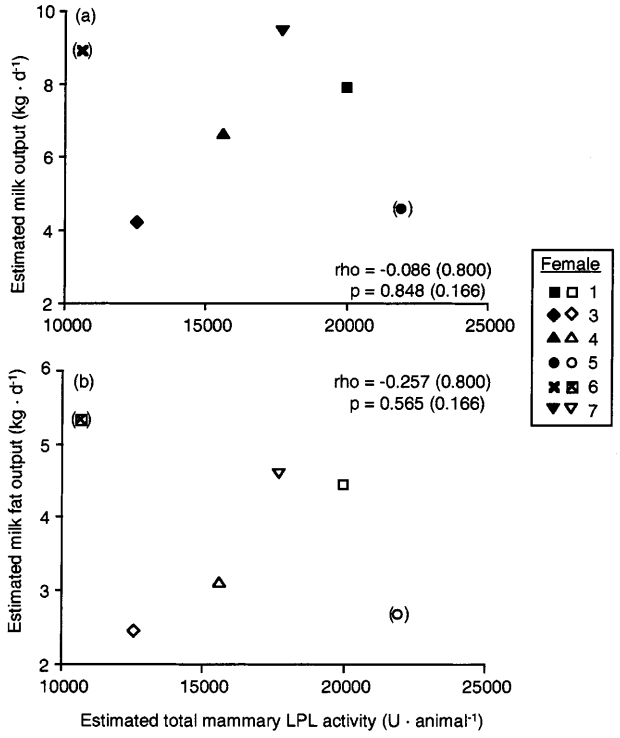

Fig. 6 Relationships between **a** daily milk volume and **b** milk fat output with total mammary LPL activity in six hooded seal females. Values for rho and *P* in parentheses represent test with removal of points in parentheses

Two individuals (Females 4 and 7) exhibited large increases in mammary LPL from day 0 to day 3. These changes were accompanied by simultaneous increases in both milk fat content and circulating prolactin. In contrast, relatively little change occurred in mammary LPL in two other females (Females 5 and 6) and correspondingly, milk fat content and prolactin also exhibited little change. While we did not have day 0 milk composition data for Female 1, both prolactin and mammary LPL increased in parallel. Female 3 was distinct in that her mammary LPL, prolactin, and milk fat content all decreased (Fig. 5).

Using the relationship between pup growth and milk intake (r^2 = 0.822, Oftedal et al. 1993) and each female's individual milk composition, we estimated daily milk output (6.9 ± 0.89 kg·day^{-1}) and milk fat (3.8 ± 0.48 kg·day^{-1}) and energy (157.5 ± 0.19.9 MJ·day^{-1}) output and compared them to levels of mammary LPL. To include the effect of body mass, we estimated total mammary LPL levels based on lean body mass using data for mammary masses across three species of phocid seals, including hooded seals (S.J. Iverson and W.D. Bowen, personal observation). Based on all six females, there was no evidence of a positive trend between either milk output or milk fat output and total mammary LPL activity among females (Fig. 6). However, Female 5 had the unusually small pup which was likely unable to consume milk at a rate comparable to the milk production capacity of its mother. In contrast, Female 6 had consistently low mammary LPL activity (<1.5 U) and may have been sampled both close to parturition and at weaning when activity is likely to be lowest. This is supported by the fact that her pup had greatly decreased PH LPL and blubber LPL on day 3, suggesting that it had stopped suckling.

Neonatal LPL activity and growth

Pup PH LPL activity was high near birth at 21.2 ± 7.29 U and tended to increase at day 3 (30.8 ± 7.58 U, P = 0.345; Fig. 3). Average initial pup PH LPL was double that of maternal PH LPL on day 0, and seven times the maternal PH LPL on day 3. Pup blubber LPL activity was elevated on day 0 (3.4 ± 0.79 U), but did not increase significantly at day 3 (5.4 ± 1.54 U; P = 0.345). Pup blubber LPL averaged twice that of maternal mammary activity throughout the suckling period. Although average blubber LPL activity in pups appeared to increase only 1.6-fold (but not significantly), this was activity measured on a per gram tissue basis. However, the size of blubber tissue in pups increases greatly over lactation

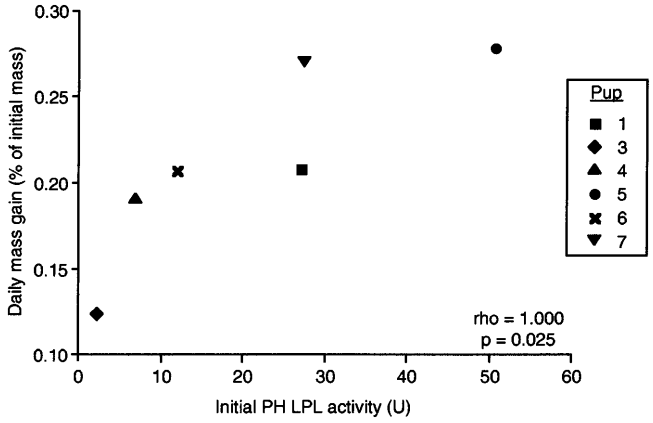

Fig. 7 Relationship between initial PH LPL and daily mass gain (as % of initial mass) during lactation in hooded seal pups

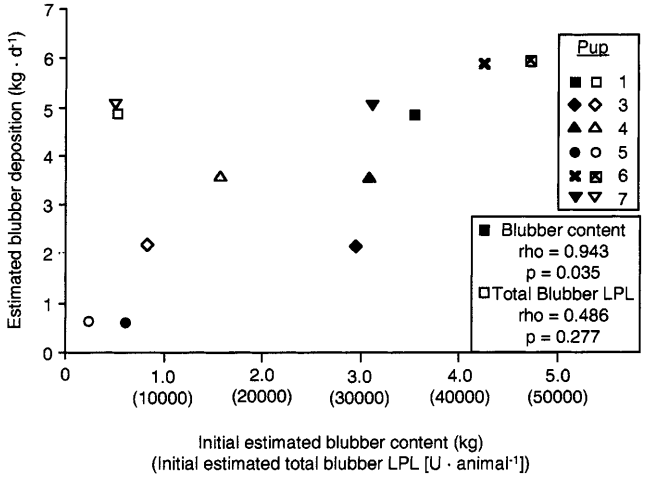

Fig. 8 Estimated daily fat deposition as a function of fat content and total blubber LPL activity near birth in hooded seal pups. See Results for estimations of body fat content and total blubber LPL activity

and should be more reflective of the total ability of a pup to take up and store TG fatty acids. Using estimates of total blubber tissue (see above), we estimated the total blubber LPL in pups. These estimates suggested that total blubber LPL activity ($U \cdot animal^{-1}$; see Materials and methods) increased up to 16-fold from day 0 to day 3 (mean 13988 ± 6864.9 $U \cdot animal^{-1}$ to 61828 ± 21802.5 $U \cdot animal^{-1}$, respectively; $P = 0.028$).

Pups with greater PH LPL activities at birth had significantly higher relative growth rates during the 3-day study period ($P = 0.025$, Fig. 7). The smallest pup (Pup 5) not only had the highest relative growth rate but had twice the initial PH LPL activity of any other pup. The wide range in estimated blubber content at birth (0.6–7.8 kg) explained a considerable amount of the variation in estimated daily fat deposition (Fig. 8). When translated into total blubber LPL activity (2404–46997 $U \cdot animal^{-1}$), the same was generally true. However, two animals which appeared to have particularly low total LPL levels at birth were exceptions (Fig. 8).

Table 3 Plasma neutral lipids during lactation in hooded seal mother-pup pairs. (*FFA* free fatty acid, *TG* triglyceride)

	Day 0 ($n = 6$)	Day 3[1] ($n = 6$)	P[2]
Mothers			
TG ($mg \cdot dl^{-1}$)	28.5 ± 2.90	21.1 ± 2.18	0.075
FFA ($mg \cdot dl^{-1}$)	55.5 ± 6.96	78.6 ± 10.7	0.046
%FA as TG[3] (mol %)	34.0 ± 4.20	22.0 ± 3.14	0.028
Pups			
TG ($mg \cdot dl^{-1}$)	144.7 ± 21.06	207.8 ± 71.19	0.600
FFA ($mg \cdot dl^{-1}$)	107.9 ± 19.47	96.9 ± 29.72	0.600
%FA as TG (mol %)	56.3 ± 3.44	66.2 ± 5.49	0.173

[1] Pair 1 was resampled on day 2 but data were included in day 3 averages
[2] Wilcoxon signed rank test
[3] 100 mol fatty acids in plasma TG/total moles of plasma fatty acids in TG plus FFA

Maternal and neonatal plasma neutral lipids

Maternal plasma TG ranged from 20.4 $mg \cdot dl^{-1}$ to 39.2 $mg \cdot dl^{-1}$ near parturition and tended to decrease by day 3, ranging from 13.2 $mg \cdot dl^{-1}$ to 26.6 $mg \cdot dl^{-1}$ ($P = 0.075$; Table 3). Conversely, maternal plasma FFA increased significantly between day 0 (range 31.5–75.8 $mg \cdot dl^{-1}$) and day 3 (range 28.1–100.1 $mg \cdot dl^{-1}$; $P = 0.046$). As a result, the relative amount of FA carried as TG (100 × moles of fatty acids in plasma TG/total moles of plasma fatty acids in TG plus FFA) decreased significantly ($P = 0.028$; Table 2). An average of 28% of circulating fatty acids in females were carried in TG.

In contrast to females, pup plasma neutral lipids did not change significantly over the study period ($P > 0.5$; Table 3). Intake of large volumes of high fat milk likely resulted in elevated plasma TG (range 68.8–557.8 $mg \cdot dl^{-1}$) and FFA (range 23.7–198.2 $mg \cdot dl^{-1}$). Approximately 61% of circulating fatty acids in pups were carried in TG. The proportion of pup plasma fatty acids carried as TG varied two-fold among pups (range 42.7–84.9%), but did not differ between days ($P = 0.173$).

Discussion

Although hooded seals are remarkable in their extreme lactation characteristics and represent an excellent model for studying energetics and the role of LPL in facilitating milk fat secretion and neonatal fat deposition, research conducted in their natural environment is difficult for several reasons. The breeding grounds are situated on remote and unstable pack ice that make recapture of individual mother-pup pairs extremely difficult and assessment of exact birth and weaning times nearly impossible. We were fortunate to recapture all pairs, but inclement weather forced the resampling of

one pair (Pair 1) a day earlier than the other pairs. Given that the lactation period lasts only 3.6 days, a difference of only a few hours can represent quite different stages of lactation. Particularly at either end of lactation, physiological changes (e.g., prolactin, LPL activity) are likely to occur rapidly due to initial onset or to mammary involution. Because of this, we may have sampled some pairs closer to parturition than others, and similarly may have sampled some pairs closer to weaning than others. We have attempted to point out those cases where enzyme or hormone levels suggest that a pair may have been closer to a particular stage than others.

Maternal body composition and energetic investment

Body energy stores of female hooded seals at parturition (28% fat, 17% protein) were comparable to those of other large phocid species (grey seals, 32% fat, 16% protein, J.E. Mellish, S.J. Iverson and W.D. Bowen personal observation; southern elephant seals *Mirounga leonina*, 31% fat and 15% protein, Fedak et al. 1996; northern elephant seals *Mirounga angustirostris*, 34% fat and 16% protein, Costa et al. 1986). However, both the daily and the total energetic cost incurred by hooded seal females during lactation differs dramatically from these other species. Hooded seal females rapidly depleted body energy reserves with an average DEE of 216.3 $MJ \cdot day^{-1}$. This is twice the DEE measured in similarly-sized lactating grey seals (Mellish 1999). Assuming that hooded seals have a mass-specific resting or standard metabolic rate (SMR) similar to that measured in grey seals (averaged from $n = 6$, Lavigne et al. 1986; Boily and Lavigne 1997), this represents a sustained DEE of $11.8 \times SMR$, or higher than ever reported for any vertebrate (Hammond and Diamond 1992). However, subtracting estimates of milk energy exported in these females (157.5 $MJ \cdot day^{-1}$), energy costs associated with fuel metabolism (58.8 $MJ \cdot day^{-1}$) were only $3.2 \times SMR$, which is similar to that estimated for lactating grey seals (Mellish 1999).

Although hooded seal females have extraordinarily high daily energetic costs, their total lactation costs are remarkably low. Northern elephant seals (up to 600 kg) lactate for 26 days, losing 42% of their initial mass and 58% of their initial fat stores (Costa et al. 1986). During the 16–18-day lactation period, grey seal females (200 kg) lose 35% of their initial mass and 68% of their initial fat reserves (J.E. Mellish, S.J. Iverson and W.D. Bowen personal observation), and are often visibly emaciated by the end of lactation. In contrast, the total estimated postpartum energetic contribution of females in this study constituted only 9–17% of initial maternal mass and 11–38% of initial fat reserves. Although some costs of pup development are higher in hooded seals than in other phocids, such as prepartum energetic investment (12% of maternal mass compared to $<9\%$ in other phocids, Bowen 1991; Costa 1991; Oftedal et al. 1993) and daily milk energy output (157–187 MJ vs 59–69 MJ and 96 MJ in grey seals and northern elephant seals, respectively, Costa et al. 1986; Iverson et al. 1993; Oftedal et al. 1993; J.E. Mellish, S.J. Iverson and W.D. Bowen personal observation), hooded seals lactate for only 3.6 days (Bowen et al. 1985). The components of mass loss in hooded seals are similar to other fasting species, where 59% of the mass loss and 90% of energy loss is derived from fat stores (grey seals, 63% and 92%, respectively; J.E. Mellish, S.J. Iverson and W.D. Bowen personal observation). However, relative maternal body composition changed little over the course of lactation (Fig. 2). Protein losses in hooded seals (0.8 $kg \cdot day^{-1}$), although higher on an absolute daily basis than in other species (e.g., grey seals, 0.3 $kg \cdot day^{-1}$; J.E. Mellish, S.J. Iverson and W.D. Bowen personal observation), were minimal on a relative basis and therefore little total protein was used. Perhaps most importantly, hooded seal females used only 22% of total body energy during lactation whereas grey seal females expend 57% or more of total body energy to produce a pup of similar size (J.E. Mellish, S.J. Iverson and W.D. Bowen personal observation).

From our data, it is clear that the direct energy cost of lactation, relative to body reserves, in hooded seal females is substantially less than in other phocids. Why should this be the case? Reduction in total overhead costs by rapid and brief nutrient transfer to offspring is clearly energetically advantageous. But it could also be that hooded seal females face a trade-off between the energy expenditure for reproduction and other aspects of their annual energy budget. For example, unlike other phocids, immediately after lactation hooded seal females migrate to northern ice floes where they then moult (Sergeant 1976). Thus, energy costs associated with immediate migration followed by reduced food intake during the moult must be met from energy stored prior to parturition or from that acquired during the relatively brief migration. Perhaps uncertainty associated with food resources during migration has also selected for females that retain sufficient reserves to minimize the risk of starvation.

Maternal LPL activity, prolactin and milk fat secretion

Lactation is an energetically demanding process that requires many metabolic adjustments. In particular, substantial amounts of energy must be directed to the mammary gland for milk synthesis. In the larger phocid species, lactation is also energetically intense, as females support milk production and metabolic requirements solely from stored energy. In the hooded seal, the mammary gland must shift from a dormant state to a tissue capable of producing more than 10 $kg \cdot day^{-1}$ of 60% fat milk (Oftedal et al. 1993). As the primary enzyme responsible for lipid uptake (Hamosh and Hamosh 1985), mammary LPL has been shown to be essential for milk production in rats (Hamosh et al. 1970; Scow et al.

1977; Ramírez et al. 1983), mice (Zinder et al. 1974; Jensen et al. 1994), rabbits (Falconer and Fiddler 1970) and guinea pigs (McBride and Korn 1963; Robinson 1963). Iverson et al. (1995b) proposed that mammary LPL and the concurrent changes in prolactin and other regulatory hormones should be equally if not more important for successful milk production in the larger phocid species, and that phocid females may have a particularly efficient system of lipid transfer.

Unfortunately, data on either PH LPL (as an indicator of whole-body LPL activity) and/or LPL activity in specific tissues are for the most part limited to small laboratory species, humans, and a single study on the grey seal. In the present study, hooded seal maternal PH LPL in early lactation was similar to that found for grey seals at 0–1 days postpartum (6.1 U, Iverson et al. 1995b). PH LPL in lactating hooded seal females did not change significantly as lactation progressed. Iverson et al. (1995b) showed substantial increases in grey seal PH LPL and suggested that these may reflect mammary LPL increases given the likelihood of reduced blubber LPL. Despite the apparent differences between species, these differences can be understood in terms of what is known of their respective lactation characteristics. Milk fat content and milk output appear to remain constant throughout the brief hooded seal lactation period (Oftedal et al. 1993), while both have been shown to increase significantly between the early and later stages of lactation in grey seals (Iverson et al. 1993; J.E. Mellish 1999).

Theoretically, a fundamental metabolic shift from high LPL activity in adipose tissue (i.e., energy storage state) and low mammary LPL, to low adipose LPL and high mammary LPL activity must occur to facilitate milk fat output. This pattern has been shown for rats (Hamosh et al. 1970; Scow et al. 1977; Ramírez et al. 1983) and guinea pigs (McBride and Korn 1963; Robinson 1963). In the lactating hooded seal, this metabolic shift appears to be pronounced as evidenced by almost immeasurable levels of adipose LPL and high levels of mammary LPL throughout lactation (Fig. 3). Direct comparisons of tissue (and PH LPL) activity between species are difficult given the different assay methods used among studies, such that levels of U are not equivalent. Nevertheless, some relative comparisons can be made. For instance, the mammary LPL activity we measured in the hooded seal (1.8–2.5 U, Fig. 3) was much lower than reported in rats (50–120 U, Hamosh et al. 1970; Zinder et al. 1974), however, concurrent blubber LPL activity in the rat was much higher (2–16 U, Hamosh et al. 1970; Zinder et al. 1974). Lactating rats not only feed during lactation, but often increase food intake to offset the high energetic demands of milk production. Consequently, one might expect some blubber LPL activity in lactating rats as any excess energy from food intake will need to be stored for later use. Therefore it may be necessary for mammary LPL to be much higher in lactating rats to facilitate preferential lipid uptake by the mammary gland. In the fasting hooded seal, blubber LPL activity would compete with the mammary gland for TG fatty acid uptake. As a result, the relative difference in tissue activity (i.e., mammary vs adipose) may be a determining factor in the fasting hooded seal for energy allocation among tissues and their ability to produce such large volumes of high-fat milk. Perhaps more importantly, activity per gram tissue may not be as meaningful as total mammary LPL in understanding total fat uptake by the mammary gland. Since mammary gland size in phocid seals is much larger on a body mass basis than in other mammals (up to 2.2-fold, S.J. Iverson and W.D. Bowen personal observation; Oftedal et al. 1987), the efficiency of mammary lipid uptake by LPL may be greater in phocid seals than in other species.

Unlike most other mammals, milk LPL was unmeasurable in five of six females on the day of parturition. However, it increased dramatically to 2–14 U in five of six females on day 3. Milk LPL has no known function in the neonate and may simply be a convenient disposal route for excess enzyme (Jensen et al. 1994). While milk LPL tends to be high throughout lactation in most other species (Jensen et al. 1994), mammary LPL may be more highly conserved in the fasting hooded seal females, as protein synthesis in general declines with food deprivation. As a result, milk LPL may be negligible during the majority of lactation and mammary LPL is only released into milk as hooded seals approach the end of milk production.

For LPL activity to facilitate preferential lipid uptake, a significant proportion of plasma fatty acids must be transported as TG in lipoproteins (i.e., VLDL). FFA can cross the capillary endothelium unaided and therefore are equally available to all tissues. In hooded seal females, up to 49% of total circulating plasma fatty acids were carried as TG. Although re-esterification of mobilized fatty acids into TG may seem an unnecessary step especially in hooded seals which must transfer lipid so rapidly, the prevalence of TG may allow mammary LPL to compete for TG fatty acids over other tissues. Lactating grey seal females also show a similar pattern, but with generally higher levels (up to 66%) of plasma fatty acids carried in the bloodstream as TG (Iverson et al. 1995b). However, the differences between the two species in circulating TG levels could in part be due to more rapid mammary uptake of TG in hooded seals. This may be supported by the finding of a decline in TG over lactation (Table 3) coupled with a possible increase in mammary LPL (Fig. 3). Increased mammary LPL activity has also been shown to be coupled with decreased TG over lactation in other species (Hamosh et al. 1970; Watson et al. 1993). Hooded seal females tended to have higher FFA concentrations (56–79 $mg \cdot dl^{-1}$) during lactation than grey seal females (16–32 $mg \cdot dl^{-1}$; Iverson et al. 1995b), which corresponds to the greater rates of blubber mobilization in hooded seal females compared to grey seal females (5.1 $kg \cdot day^{-1}$ vs 2.6 $kg \cdot day^{-1}$, J.E. Mellish, S.J. Iverson and W.D. Bowen personal observation).

Prolactin is thought to trigger the decrease in adipose LPL activity and reciprocal increase in mammary LPL activity at parturition (Falconer and Fiddler 1970; Zinder et al. 1974; Ramírez et al. 1983). Many species have elevated prolactin levels during lactation, such as rabbits (203 $ng \cdot ml^{-1}$, Kermabom et al. 1994), horses (25 $ng \cdot ml^{-1}$, Neuschaefer et al. 1991), and sows (43 $ng \cdot ml^{-1}$, Dusza and Krzymowska 1981). Published values for pinnipeds during lactation tend to be much lower (southern elephant seal, 6 $ng \cdot ml^{-1}$, I.L. Boyd personal observation; Antarctic fur seal *Arctocephalus gazella*, 5 $ng \cdot ml^{-1}$, Boyd 1991). However, all pinniped values have been measured against human standards and therefore may not reflect true values, but may instead only represent relative patterns of change. It has been shown that prolactin is essential for milk production in both these species (Boyd 1990, 1991). Circulating prolactin levels in hooded seals tended to parallel changes in mammary LPL (Fig. 5), supporting the notion that prolactin may also play an important role in this species. However, levels were even lower (1.3 $ng \cdot ml^{-1}$) than measured in other pinnipeds. If prolactin levels are truly low in the hooded seal, the frequent suckling stimulus of the pup (every 25 min, Perry and Stenson 1992) and subsequent release of prolactin from the pituitary may reduce the need for the sustained hormone secretion found in other mammals (Terkel et al. 1972; Yamamuro and Sensui 1994; Shanti et al. 1995). Frequent milk removal can increase prolactin sensitivity in the mammary gland (i.e., number of receptors) and milk yield, particularly when prolactin concentrations are low (<2 $ng \cdot ml^{-1}$, Knight et al. 1990).

Milk fat content is particularly high in hooded seals, and remains relatively constant over lactation as compared to other phocid species (e.g., grey seal, 35–60%; Iverson et al. 1993; J.E. Mellish 1999; northern elephant seal, 15–55%, Riedman and Ortiz 1979). As expected, milk composition and energy density were independent of maternal characteristics such as relative body composition or absolute energy stores both near parturition and at the end of lactation. Instead, milk fat content is likely to be regulated by species-specific physiological mediators, and may indeed be a function of mammary LPL activity, as some females with higher mammary LPL activities (per gram) on the day of parturition also tended to produce milk with a higher fat content. The lack of correlation between milk fat content and mammary LPL levels at day 3 may be due to several factors: (a) small sample size, (b) the potential for some females to be closer to weaning than others with reduced mammary LPL levels but not necessarily emptied mammary glands, and (c) once lactation is established, milk composition and milk output in some species is independent of the hormonal triggers and enzyme levels required for its initiation (Ostrom 1990).

As this study was longitudinal in design, we were unable to directly measure mammary mass and therefore relied on previous morphometric analyses (see Results) to estimate total mammary LPL in individual females. Oftedal et al. (1993) showed that the relationship between pup growth and milk intake is very strong in hooded seals (82%), which allowed the estimation of milk output and milk fat production. Given these indirect estimates of both mammary mass and milk output, as well as the variability associated with our small sample size, we were not able to show a direct relationship between total estimated mammary LPL activity and milk output or milk fat output in all females. However, in four of the six females, the relationship appeared to be strong (Fig. 6). Thus, given that total estimated mammary LPL was independent of maternal mass, this suggests that mammary LPL activity may indeed be a primary factor in the facilitation of rapid milk fat output in hooded seals.

Neonatal LPL activity and rapid growth

Unlike most other mammals, hooded seal pups are born with a substantial blubber layer (14% vs <5%, Widdowson 1950; Oftedal et al. 1993). Also unlike other mammalian neonates, growth consists largely of fat deposition (82%, Oftedal et al. 1993). After less than 4 days of nursing, pups are weaned and undergo an extended fast of 4–6 weeks prior to learning how to forage. Given the brief suckling period, harsh climate and extended nature of the post-weaning fast, a thick subcutaneous blubber layer is critical for pup survival (Bonner 1984; Bowen et al. 1987; Oftedal et al. 1989).

To maximize fattening during the limited suckling period, hooded seal pups must be physiologically equipped at birth to rapidly and efficiently digest and assimilate large volumes of milk lipid. Neonatal rats develop hyperlipemia in the first few days after birth, which has been attributed to a high fat milk (10–12%) and delayed development of tissue lipolytic activity (Hamosh and Hamosh 1986). Hooded seal pups, however, are capable of processing approximately 6 $kg \cdot day^{-1}$ of milk lipid quickly and efficiently (Oftedal et al. 1993). Despite the high rate of lipid intake, lipid removal is sufficiently efficient to prevent hyperlipemia (Table 3). Approximately 88% of ingested lipid is deposited directly in blubber (Oftedal et al. 1993; Iverson et al. 1995a). In the present study, the potential for rapid plasma lipid clearance was evident in newborns, with PH LPL activities that were comparable to or exceeded that of their mothers (Fig. 3). PH LPL is similarly elevated in newborn grey seals (32.3 U, Iverson et al. 1995b), which consume up to 2.5 $kg \cdot day^{-1}$ milk fat. Absolute growth may be partially limited by pup intake capacity (i.e., smaller gut size in smaller pups, such as Pup 5), and therefore absolute values of PH LPL may not be directly associated with growth patterns. However, on a relative basis, pups with higher PH LPL activity at birth exhibited greater relative growth rates (Fig. 6).

In neonatal mammals LPL is most active at the site of maximum growth. In the rat, growth is largely due to increased lean body mass and hence skeletal tissue is the principal site of lipid uptake (Planche et al. 1980). Growth in phocid pups is almost exclusively due to blubber deposition (hooded seals 82%, Oftedal et al. 1993; grey seals 58%, Iverson et al. 1993) which led Iverson et al. (1995b) to hypothesize that adipose tissue should be a site of elevated enzyme activity in newborns of these species. Again, although results from different labs are not directly comparable, our data indicate that this assumption is correct, in that pup blubber LPL activity on a per gram basis near birth was double that of maternal mammary LPL activity and 4.3 times adipose activity in obese humans (Taskinen and Nikkilä 1977). Research has suggested that obesity in humans is a self-perpetuating condition, as LPL activity is positively correlated with fat cell size (Taskinen and Nikkilä 1977). In hooded seals, relative blubber LPL (U) increased up to 2.8 times in less than 3 days. PH LPL increased in a parallel fashion to relative blubber LPL activity, suggesting that blubber was a primary source of heparin-releasable LPL. Using relationships between total body water and total body mass (Oftedal et al. 1993), we were able to estimate pup body fat content, and therefore an indice of total blubber LPL activity ($U \cdot animal^{-1}$). Indeed, total blubber LPL increased even more dramatically during the suckling period, probably as a function of both adipocyte filling and cell proliferation. While blubber LPL activity per gram increased only 1.6 times during the study period, estimates of total blubber LPL activity increased an average of 6.1 times, and up to 16 times. In fact, total estimated blubber LPL activity near birth was comparable to our estimate of total maternal mammary LPL activity at parturition, and three times higher than mammary LPL activity at the end of the suckling period. Suckling rats have much higher relative inguinal white adipose LPL activities (approx. 25 U), but adipose does not exceed 1.0% of total body mass (Planche et al. 1980). Therefore, on a mass-specific basis, hooded seal pups have a 300% greater fattening potential than that of rats at a comparable stage of development.

Up to 85% of hooded seal plasma lipids were carried in TG form, similar to that found for suckling grey seal pups (90–94%, Iverson et al. 1995b). Therefore, despite the need for the most rapid transfer of digested lipids possible, it appears that phocid pups do directly re-esterify digested milk lipids into chylomicrons as do other species. This conclusion was reinforced by the consistently cloudy appearance of pup sera. As a result, LPL must presumably be responsible for the vast majority of lipid uptake, as opposed to unfacilitated uptake of plasma FFA. Given the high estimated total blubber LPL activity, likely adipocyte proliferation, and rapid accumulation of blubber, it is probable that the primary destination for plasma lipids is the subcutaneous blubber layer. This indicates that hooded seal pups have a high potential for rapid fattening which increases dramatically concurrent with increases in body fat.

In conclusion, our data suggest that tissue LPL may be of considerable importance in both mother and pup, as evidenced by its elevation near parturition in maternal mammary and pup blubber tissue. Given the relatively large size of the mammary glands in hooded seals, total mammary activity appears to be particularly high. Concurrent maternal blubber LPL activity is negligible, effectively directing circulating lipids to the mammary gland and perhaps in part explaining the capacity for extremely high milk fat output. Hooded seal pups are born with a metabolically active blubber layer, enabling high lipid uptake from birth (i.e., relative and total blubber LPL), which further increases with continued fattening and adipose cell proliferation. It appears that relative tissue (mammary vs blubber) LPL activity is more predominant in lipid redirection and mammary lipid uptake in the lactating female, while high absolute tissue LPL activity in the pup facilitates the rapid blubber deposition required for the postweaning fast.

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