

Variation in Milk Production and Lactation Performance in Grey Seals and Consequences for Pup Growth and Weaning Characteristics

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ABSTRACT

Phocid seals are one of the few groups of mammals capable of sustaining the energetic demands of lactation entirely through body nutrient stores while fasting. Lactation performance of the female in turn influences the rate and pattern of pup growth. We examined variation in and patterns of milk composition and production, maternal energy output, and pup growth and energy deposition over the entire lactation period in 18 grey seal mother-pup pairs using hydrogen isotope (³H₂O and D₂O) dilution. Milk composition was independent of maternal mass and nutrient stores, indicating dependence on other physiological and genetic factors. Heavier females lactated longer ($r^2 = 0.653$, $P < 0.001$), had higher total milk outputs ($r^2 = 0.652$, $P < 0.001$), and produced larger pups at weaning ($r^2 = 0.417$, $P = 0.005$). While fatter females lactated for longer periods of time ($r^2 = 0.595$, $P < 0.001$), females with a larger lean body mass at parturition produced more milk ($r^2 = 0.579$, $P < 0.001$). Total milk energy output was the strongest predictor of pup weaning mass, which, along with the pup's efficiency of energy storage, accounted for 91% of the variation in weaning mass. Nevertheless, there was sufficient plasticity in milk composition and energy output that some smaller females produced relatively large pups. Few females appeared to deplete body nutrients to the point where it might limit the duration of lactation.

Introduction

Lactation is the most energetically demanding period faced by female mammals. Phocid seals (order Carnivora, family Phocidae) are one of the few groups of mammals capable of sustaining the energetic demands of lactation while fasting. Grey seal (*Halichoerus grypus*) females give birth to a single pup and lactate for only 16–18 d, during which time pups rapidly gain mass (2.8 kg d⁻¹; Bowen et al. 1992). Because females fast throughout lactation, body energy stores at parturition must support both maintenance metabolism and the total energetic and nutrient costs of milk production. Lactation performance of the female in turn influences the rate and pattern of pup growth (Iverson et al. 1993), which may affect the likelihood of pup survival during the extended postweaning fast (4 wk; Reilly 1991) and transition to nutritional independence.

Pup mass at weaning has often been used to measure lactation performance and to infer maternal reproductive success in phocid seals (e.g., Fedak et al. 1996; Arnbom et al. 1997). However, variations in and consequences of maternal performance of individual females in phocid species are poorly understood. There is considerable evidence that maternal mass at parturition accounts for much of the variability in pup weaning mass, both within and between populations (Anderson and Fedak 1987; Bowen 1991; Bowen et al. 1992; Kovacs and Lavigne 1992; Iverson et al. 1993; Reilly et al. 1996; Arnbom et al. 1997), yet few studies have determined which attributes associated with large maternal mass are most influential in producing a larger pup (Deutsch et al. 1994; Arnbom et al. 1997). It has been suggested that the major advantage of large maternal size is increased energy stores, particularly in the form of subcutaneous fat reserves. The potential role of protein reserves, however, has typically been overshadowed by the strong emphasis on the influence of fat stores. Fat may be the primary resource for energy transfer (via high milk fat content), but protein may also play a crucial role, for instance, in determining the limits of a female's fasting ability while under energetic stress (e.g., Cahill et al. 1979). Additionally, there is little information on the degree to which pup characteristics, such as body composition at birth and patterns of energy allocation during lactation, influence pup characteristics at weaning.

In this study we examined individual variation in patterns of milk composition and production and trends in pup growth, nutrient deposition, and energy allocation over the entire lactation period. We had three main goals: (1) to document the variability in lactation performance among females and to de-

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termine to what degree this variability could be understood through female characteristics; (2) to determine the consequences of variation in female lactation performance on pup growth; and (3) to investigate the effects of pup energy storage efficiency on weaning mass and body composition.

Material and Methods

Field Procedures

Eighteen grey seal mother-pup pairs were studied on Sable Island, Nova Scotia, Canada (43°55'N, 60°00'W), during January 1996 ($N=6$) and 1997 ($N=12$), as part of a larger study investigating the physiological regulation of milk lipid secretion and transfer. Females were chosen to represent the full range of initial body mass found in the population and, thus, to provide maximum contrast to examine the relative influence of maternal traits on milk production and lactation performance. Each pair was selected within the first few hours of birth as indicated by the presence of a freshly expelled placenta and the thin, wet appearance of the pup. Females were captured with a net and weighed (i.e., parturition mass) to the nearest 0.5 kg using a 300-kg Salter scale. Pups were weighed (i.e., birth mass) to the nearest 0.1 kg using a 50-kg Salter scale. Both mother and pup were given a uniquely numbered tag (Jumbo Rototag) in the hind flipper and marked with a matching number on the lower back with fluorescent paint.

Mother and pup were both given isotopically labeled water to determine total body water (TBW), water flux, milk intake, and energy output. Females were injected with a known quantity (5 g of 0.5 mCi mL⁻¹) of tritiated water (³H₂O). Blood samples (10 mL) were initially taken via the extradural vein at 1.0 and 1.5 h postinjection. In 1996, ³H₂O was injected intravenously (IV); however, there were several occasions in which the IV method proved difficult and the data suggested that several females had not equilibrated. A separate group of five grey seal females studied in October 1996 indicated that equilibration occurred within 30–90 min of ³H₂O administration using an intramuscular (IM) route (Mellish 1999). Therefore, the 12 females studied in January 1997 were injected IM and bled at 2.0 and 2.5 h postadministration. Females were then given an IV dose of the sedative diazepam (5 mL, 5 mg mL⁻¹) and milk samples were collected by suction using a 60-cm³ syringe with the tip removed, after the administration of 1.5 cm³ oxytocin to facilitate milk letdown. Two samples (~60 mL total) were stored in 30-mL Nalgene bottles.

Pups were intubated to remove and discard any milk in their stomachs (which delays equilibration) and then were given a weighed dose (at 3 g kg⁻¹) of 99.8% deuterium oxide (D₂O; Sigma Aldrich) by gastric intubation. Blood samples (10 mL) were taken at 2.0 and 2.5 h postadministration to determine D₂O concentration and to ensure that equilibration had occurred. Females and their pups were kept together, but pups were not allowed to suckle through a net for the entire 2.5 h

isotope equilibration period. This did not appear to be stressful since mothers and pups often slept much of this time.

After the initial sampling (day 0), mother-pup pairs were transported to a large, fenced enclosure (100 × 100 m) in the middle of the colony. The enclosure simulated a moderate- to low-density area typical of the main colony but ensured that pairs remained together and provided easy access to the animals throughout the course of study. Since the first sampling was so close to birth, a few pairs that appeared particularly flighty were placed in individual pens (5 × 3 m) to reduce the risk of abandonment by the female. The data obtained from these pairs were not in any way unusual. Each pair was resampled on days 5, 10, and 15 of lactation, with the exception of pair 3, which was sampled on day 6 instead of day 5 due to a severe winter storm. On days 5 and 10, each female and pup was weighed, a single blood sample (10 mL) was taken to determine isotope concentration, and 60 mL of milk was collected from the mother. Isotope was readministered to both mother and pup on day 15 (after a preadministration blood sample was taken) to determine final TBW. Females were allowed to leave the enclosure after the day 15 sampling, but there was ample room for the pair to separate if weaning occurred before this time. Several adult males were also resident in the enclosure throughout the study. Observed copulations between these males and the study females were recorded as evidence that lactation was about to end. Pups were considered weaned on the day the female departed or when the female was consistently separated from her pup by >30 m. Pups, but not mothers, were weighed at weaning, as maternal departure was unpredictable and usually had occurred during the night.

All samples (blood and milk) were stored in a cooler on ice during collection periods in the field (3–4 h). Blood samples were centrifuged and serum was collected into cryovials and frozen at -20°C. Milk samples were frozen at -20°C until analysis.

Sample Analysis

Female serum samples ($N=126$) were distilled in triplicate using the method of Ortiz et al. (1978). Distilled serum was counted for ³H activity using a Beckman LS 5000CE scintillation counter. Pup serum samples ($N=126$) were distilled using the method of Oftedal and Iverson (1987). Distillates from pup serum were analyzed in triplicate for D₂O concentration on a single-beam, Fourier transform, infrared spectrophotometer (Perkin-Elmer FT-IR Paragon 1000), using gravimetrically prepared standards and distilled water as reference.

Milk samples ($N=68$) were analyzed for proximate composition (dry matter, protein, and fat content). Dry matter and protein were analyzed in duplicate using forced convection drying and macro-Kjeldahl methods, respectively. Total fat content was determined gravimetrically following duplicate extractions using a modified version of Folch et al. (1957), containing an

increased ratio (54 : 1 rather than 18 : 1) of solvent to milk (but maintaining the appropriate aqueous phase volume). This method was used to ensure complete lipid extraction. Samples were not analyzed for carbohydrate content as it has been shown previously that this is a minor component in grey seal milk (Iverson et al. 1993; Oftedal and Iverson 1995).

Data Analysis and Calculations

Isotope dilution space (D) of both females and pups was calculated on day 0 and again after isotope readministration on day 15 as given in Iverson et al. (1993). Dilution space was then corrected for overestimation due to isotope loss to non-exchangeable body compartments using the specific relationship derived for pinnipeds by Bowen and Iverson (1998):

$$\text{TBW (kg)} = 0.003 + (0.968 \times D).$$

Total body fat (%TBF) and body protein (%TBP) were calculated for both mother and pup on day 0 and day 15 using the equations derived for grey seals by Reilly and Fedak (1990):

$$\%TBF = 105.1 - (1.47 \times \%TBW),$$

$$\%TBP = (0.42 \times \%TBW) - 4.75.$$

Intermediate TBW at day 5 and day 10 were estimated by interpolation, assuming that the decrease in pool size in each female was linearly proportional to her mass loss and that the increase in pool size in each pup was linearly proportional to its mass gain. These TBW estimates were used to calculate intermediate body composition values and to correct intermediate isotope concentrations for changes not attributable to water turnover as described by Iverson et al. (1993).

Total daily water intake (TWI, kg) and daily milk intake (MI, kg) were calculated as described in Oftedal and Iverson (1987). The MI was estimated from the following equation:

$$MI = 100 \times \frac{TWI + (1.07 \times F_d) + (0.42 \times P_d)}{\%W_m + (1.07 \times \%F_m) + (0.42 \times \%P_m)},$$

where F_d is daily fat deposition, P_d is daily protein deposition, W_m is water in milk (%), F_m is fat in milk (%), and P_m is protein in milk (%). Values F_d and P_d were calculated from differences between initial and final body composition.

Milk and energy intakes of each pup were calculated using its mother's milk composition and data on each pup's tissue and energy deposition over the period of study. The energy content of tissues and milk was calculated using the values of

39.3 MJ kg⁻¹ (9.39 Mcal kg⁻¹) and 23.6 MJ kg⁻¹ (5.65 Mcal kg⁻¹) for fat and protein, respectively (Blaxter 1989), and were used to calculate daily rates of energy loss in females and gain in pups.

Data for all pairs and sampling times were used in analyses, with the exception of pairs 11 and 16. Female 11 was our smallest mother and terminated lactation on day 12; however, the pair was not resampled until day 15 since we were initially unsure that the pup had been weaned. This pair was therefore excluded from day 15 or late lactation (days 10–15) observations. However, data from 0–10 days (83% of their lactation period) were used to represent overall lactation characteristics for this pair and to estimate pup weaning mass at day 12. Pair 16 ceased suckling after day 5 and was excluded from any analysis past that point. Pup 16 was not reequilibrated at day 5; therefore, body composition was predicted from the relationship between body mass and TBW derived from the other pups at day 5. An average milk composition value from the study females at day 15 was used for pair 8, as the pair had just weaned and insufficient milk for analysis was obtained.

Changes in milk composition and body mass over the course of lactation were analyzed using mixed-effects repeated-measures regressions. Comparisons of day 0 and day 15 body compositions were performed using repeated-measures ANOVA (rmANOVA). Early, mid, and late lactation refer to days 0–5, 5–10, and 10–15, respectively. Rate variables such as mass change, daily water flux, and milk transfer are reported for these three lactation periods; however, only differences between the early and late periods were tested statistically, given the shared reliance of these measures on an estimated body water pool value for each individual at days 5 and 10. Given the number of seals in our study, the statistical power to correctly reject the null hypothesis at the 5% level for a medium-effect size (Cohen 1977) varied from about 0.25 to 0.4. Therefore, failure to reject the null hypothesis in our study should not be taken as strong evidence for no effect. Models to predict aspects of maternal milk output, lactation length, and pup weaning mass were developed using stepwise regression. Statistical analyses were performed using StatView 4.1 for the Macintosh, SPSS V 7.0, and Splus 4.5. Values are presented as mean \pm standard error (SE) unless otherwise stated.

Results

Milk Composition

Milk composition changed significantly over the course of lactation (Table 1). Water content varied inversely with fat content and dry matter. Average milk protein content was significantly higher at parturition than at other times during lactation (Table 1), but there was little change thereafter. The energy content

Table 1: Proximate composition of milk over the course of lactation in 18 grey seal females

Component	Day 0 (N = 18)	Day 5 ^a (N = 18)	Day 10 (N = 17)	Day 15 (N = 15)	<i>P</i> ^b
Water (%)	50.0 ± 1.12	36.4 ± 1.23	30.7 ± .61	31.0 ± .96	<.001
Dry matter (%)	50.0 ± 1.12	63.6 ± 1.23	69.3 ± .61	69.0 ± .96	<.001
Protein (%)	10.1 ± .39	9.0 ± .21	9.1 ± .17	9.3 ± .20	.047
Fat (%)	34.5 ± 1.33	48.7 ± 1.48	53.7 ± 1.22	54.0 ± 1.22	<.001
Energy (MJ kg ⁻¹)	15.9 ± .47	21.3 ± .58	23.3 ± .48	23.4 ± .47	<.001

Note. Milk samples were collected four times during the lactation period from 15 of the 18 females studied; two females were sampled three times (days 0, 5, and 10) and a single female was sampled twice (days 0 and 5).

^a All samples were taken on day 5 with the exception of one that was taken on day 6 due to extreme weather conditions.

^b Differences tested by mixed-effects, repeated-measures regression using the 15 animals with four sampling points each. Changes in all constituents were best fit by quadratic equations (mixed-effects, repeated-measures regressions, $P < 0.05$): water (%) $y = 50.27 - 3.30x + 0.14x^2$; dry matter (%) $y = 49.73 + 3.30x - 0.14x^2$; fat (%) $y = 34.10 + 3.31x - 0.13x^2$; protein (%) $y = 10.03 - 0.25x + 0.01x^2$; energy (MJ kg⁻¹) $y = 15.77 + 1.24x - 0.05x^2$.

of milk increased by 47% over the course of lactation, primarily reflecting the increase in milk fat and decrease in water content.

Despite the overall changes in milk composition during lactation, there was considerable variation in the composition of milk and how it changed among individual females (Fig. 1). On day 0, there was a twofold range in milk protein (7%–13%; Fig. 1). In some females (e.g., females 4 and 5), milk protein decreased substantially at day 5, followed by a slight increase, while in others (e.g., females 12 and 18) almost the reverse pattern was observed. Milk fat content increased in all individuals but decreased in seven females during late lactation (Fig. 1). Variability in milk composition was not related to initial body size or body composition of females. Average fat content of milk was independent of maternal parturition mass ($P > 0.5$, $N = 17$) and both relative and absolute body fat content ($P > 0.5$). Average milk protein content also was not related to either parturition mass ($P > 0.5$) or body protein content (relative, $P = 0.246$; absolute, $P = 0.394$).

Body Mass Changes

Maternal mass ranged from 138.0 to 234.0 kg at parturition (mean 196.6 ± 6.02 kg, $N = 18$; Fig. 2). Females lost mass at an average of 4.1 kg d⁻¹ over the course of lactation (Table 2). Overall, heavier females at parturition lost mass at a faster rate (maximum 5.0 kg d⁻¹) than did smaller females (minimum 3.3 kg d⁻¹, $r^2 = 0.427$, $P = 0.005$, $N = 17$). Maternal mass at day 15 averaged 137.6 ± 4.91 kg (range 92.0–162.5 kg, $N = 16$), representing a loss of 50–75 kg or $31.4\% \pm 0.85\%$ (range 25.8%–38.1%) of maternal parturition mass.

Pup birth mass ranged from 12.5 to 19.5 kg (mean 16.6 ± 0.50 kg, $N = 18$; Fig. 2) and was independent of maternal parturition mass ($P = 0.282$) or pup sex (Mann-Whitney *U*-test, $P = 0.190$). Pup growth rates increased significantly over the course of lactation (mixed-effects, repeated-measures quadratic

regression, $P < 0.001$, $N = 16$; Table 3). Overall pup growth rates were not significantly related to either maternal mass at parturition ($P = 0.173$) or to pup sex ($P = 0.626$). However, approximately half of the variation in overall pup growth rates (range 0.8 – 2.2 kg d⁻¹) was explained by variation in daily maternal mass loss ($r^2 = 0.536$, $P < 0.001$). More of the variation in pup mass gain was explained by maternal mass loss during midlactation ($r^2 = 0.386$, $P = 0.008$) than during either early ($r^2 = 0.246$, $P = 0.036$) or late lactation ($r^2 = 0.197$, $P = 0.085$). Pair 5 behaved abnormally throughout the study, in that both mother and pup appeared lethargic and the pup appeared to suckle only from a single teat. This pup had the lowest growth rate at all time periods (0.3 , 0.8 , and 1.2 kg d⁻¹) despite typical mass loss (2.0 , 3.8 , and 4.1 kg d⁻¹) by the female. Therefore, the relationships between pup mass gain and maternal mass loss in mid and late periods of lactation were strengthened with the removal of this pair (mid $r^2 = 0.428$, $P = 0.006$; late $r^2 = 0.220$, $P = 0.078$).

Pup mass at day 15 (41.5 ± 1.19 kg, $N = 16$) was positively related to initial maternal mass ($r^2 = 0.279$, $P = 0.036$). Again, the strength of this relationship was increased with the removal of pair 5 ($r^2 = 0.375$, $P = 0.015$). There was no significant difference in the day 15 mass of male and female pups when initial maternal mass was used as a covariate (one-way ANCOVA, $P > 0.5$).

The overall mass transfer efficiency ($100 \times$ pup mass gain/maternal mass loss) between mothers and pups averaged $40.1\% \pm 1.62\%$, but ranged more than twofold among pairs (23.3% – 54.2%) and was significantly lower during early lactation ($27.4\% \pm 2.32\%$, rmANOVA, $P < 0.001$) than in either mid ($44.8\% \pm 2.54\%$, $N = 17$) or late ($48.9\% \pm 3.06\%$) lactation. However, several pairs differed from this general pattern. During early lactation, pair 13 had a mass transfer efficiency of only 8%. Pair 5 had a low mass transfer efficiency throughout lactation (e.g., 15.0% early, 29.6% late), which was associated

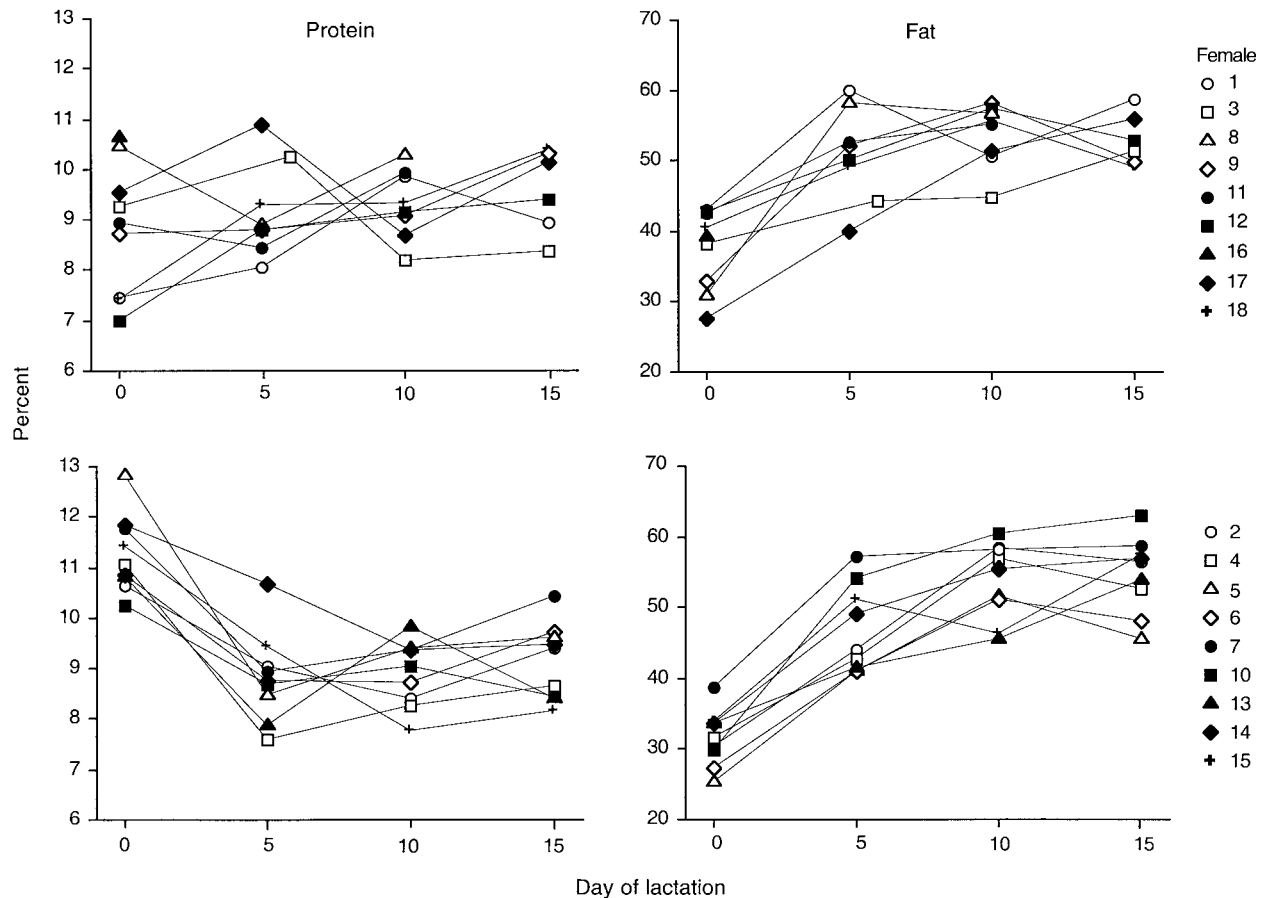


Figure 1. Individual changes in milk protein content (%) and milk fat content (%) during lactation in 18 grey seal females. Females are plotted in two panels to enable viewing of all data points. Females 8 and 11 were only sampled three times, and female 16 was sampled twice.

with both low maternal daily mass loss (3.3 kg d^{-1}) and the lowest pup growth rate (0.8 kg d^{-1}).

Maternal and Neonatal Body Composition

Total body water was strongly related to body mass in both mothers and pups at birth and late lactation (Fig. 3). In mothers, the relationship was equally strong at both 0 and 15 d postpartum, whereas the relationship was stronger and less variable in newborn pups than in 15-d-old pups (Fig. 3).

Maternal body composition changed significantly over the course of lactation (Fig. 2). Heavier females had larger absolute fat and protein stores at parturition than lighter females ($r^2 = 0.533$ and 0.649 , respectively, $P < 0.001$ for both), but relative composition (% fat and % protein) was independent of initial body mass ($P = 0.418$ for both). Heavier females at parturition also maintained greater absolute protein stores ($r^2 = 0.607$, $P = 0.001$) and absolute fat stores ($r^2 = 0.304$, $P = 0.022$) at day 15 than did smaller females.

Fat accounted for $62.5\% \pm 3.37\%$ of overall daily maternal mass loss, while protein accounted for only $7.7\% \pm 1.04\%$ of mass loss ($N = 17$). Rates of maternal fat loss ($2.6 \pm 0.15 \text{ kg d}^{-1}$) and protein loss ($0.3 \pm 0.05 \text{ kg d}^{-1}$) did not change over the course of lactation (rmANOVA, $P > 0.5$) and were unrelated to initial maternal mass ($P > 0.1$ for both), absolute fat and protein stores ($P > 0.05$ for both), or relative amounts of these tissues ($P > 0.05$ for both). Females lost an average of $18.1 \pm 1.75 \text{ kg}$ water, $39.1 \pm 2.34 \text{ kg}$ fat, and $4.6 \pm 0.71 \text{ kg}$ protein by day 15 ($N = 16$). Females depleted $49.9\% \pm 2.23\%$ of their initial body energy stores by day 15 ($N = 16$) and an estimated $56.7\% \pm 2.54\%$ (range 44%–85%) of energy stores by the end of lactation ($N = 17$). The relative amount of body energy used by females was independent of maternal mass at parturition ($r^2 = 0.055$, $P = 0.365$). Over the course of lactation, fat and protein accounted for $92.3\% \pm 1.25\%$ and $7.7\% \pm 1.25\%$, respectively, of total body energy loss.

Pup body composition also changed significantly over the

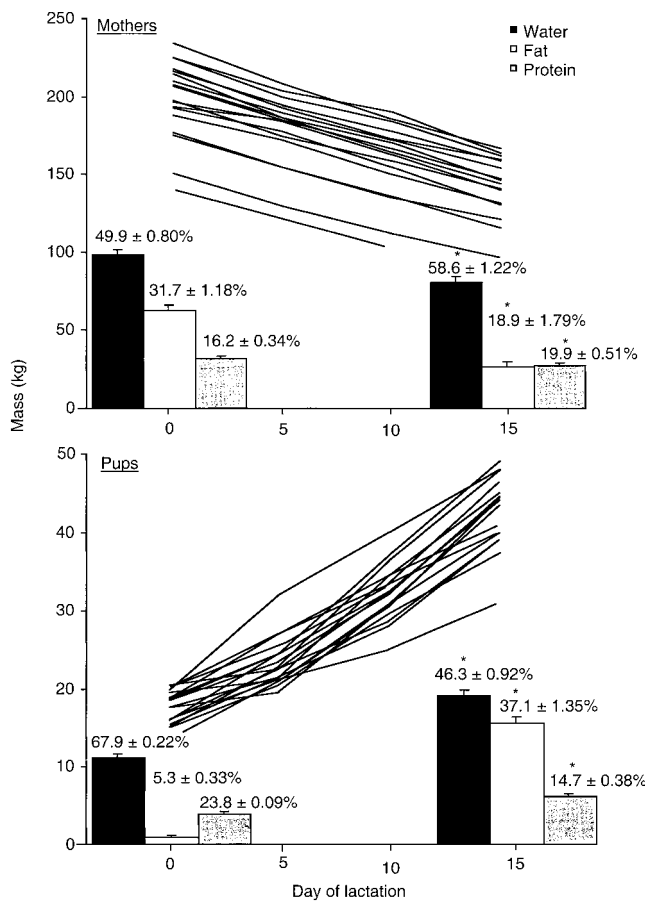


Figure 2. Mass and body composition changes of 18 grey seal mother-pup pairs during lactation. Daily mass loss in females was linear with time ($N = 16$), while daily pup mass gain differed significantly during lactation and was best fit by quadratic equations (mixed-effects, repeated-measures regression; see text and Tables 2 and 3). Bars represent absolute values (kg), with the percentage of each component indicated above. Both absolute and relative fat, water, and protein content for both mother and pup differed significantly between day 0 and day 15 (paired t -tests $P < 0.001$, $N = 16$).

course of lactation (Fig. 2). Relative fat (1.9%–8.2%) and protein content (23.0%–24.8%) at birth were unrelated to pup body mass ($P = 0.144$) or pup sex (Mann-Whitney U -test, $P = 0.236$). However, relative protein content was much less variable than fat content ($CV = 2\%$ vs. 26% , respectively), and therefore there was a stronger relationship between birth mass and absolute protein stores (3.0–4.6 kg, $r^2 = 0.984$, $P < 0.001$) than with absolute fat stores (0.3–1.5 kg, $r^2 = 0.464$, $P = 0.001$).

At day 15, heavier pups contained absolutely more fat ($r^2 = 0.672$, $P < 0.001$) and protein ($r^2 = 0.488$, $P = 0.002$) than lighter pups. Heavier pups also tended to be relatively fatter

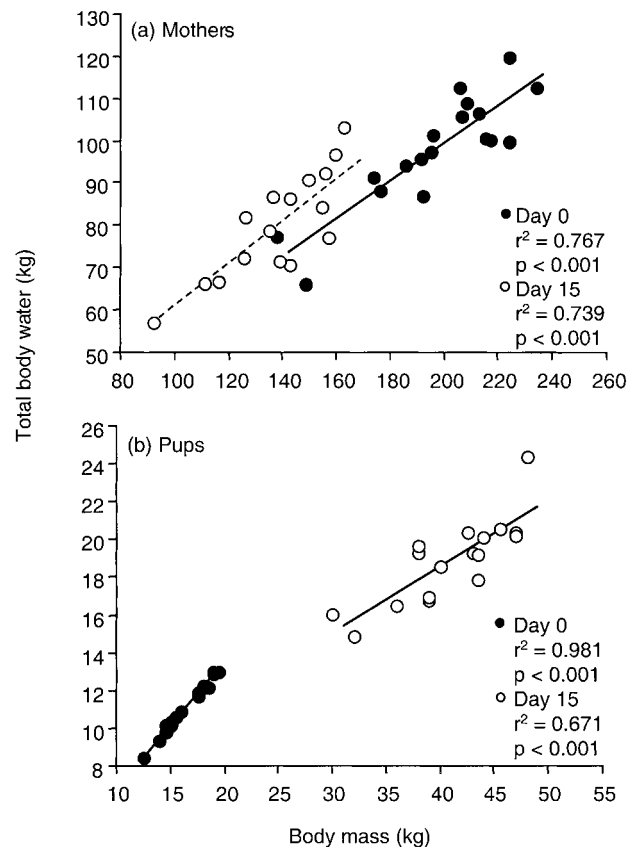


Figure 3. Relationship between total body water (kg) and body mass in 18 grey seal mother-pup pairs during lactation (day 15, $N = 17$; pair 11 was included at 15 d although they were 3 d postlactating). For mothers (a), day 0: $y = 9.485 + 0.45x$, day 15: $y = 11.073 + 0.506x$; for pups (b), day 0: $y = 0.877 + 0.626x$, day 15: $y = 4.269 + 0.357x$.

than lighter pups, although this was not significant ($r^2 = 0.199$, $P = 0.072$). Absolute fat and protein stores varied by factors of 2.5 (7.9–19.6 kg) and 1.7 (4.7–8.0 kg), respectively, among 15-d-old pups. Fat and protein accounted for $59.4\% \pm 2.12\%$ and $8.3\% \pm 0.60\%$ of mass gain and $91.9\% \pm 0.81\%$ and $8.1\% \pm 0.81\%$ of energy stored, respectively. Pup mass at day 15 was positively related to both initial maternal mass (see above) and initial maternal protein stores ($r^2 = 0.341$, $P = 0.014$) but not to initial maternal fat stores ($r^2 = 0.188$, $P = 0.082$).

Milk and Energy Transfer

Overall daily milk intakes of pups varied from 1.9 to 3.6 kg d^{-1} (Table 3), and these intakes largely predicted pup growth rates ($r^2 = 0.695$, $P < 0.001$, $N = 17$). The significant increase in rates of milk intake between early and late lactation (Table 3)

Table 2: Water flux and energy output of 18 lactating grey seal females

	Early (N = 18)	Mid (N = 17)	Late (N = 16)	Overall (N = 17)	P ^a
Mass loss (kg d ⁻¹)	4.2 ± .23	4.1 ± .14	4.3 ± .19	4.1 ± .11	NS ^b
Water efflux (kg d ⁻¹)	2.95 ± .278	3.87 ± .292	4.53 ± .305	4.13 ± .283	.002
Milk water output (kg d ⁻¹)	1.00 ± .052	.97 ± .063	.98 ± .048	1.06 ± .043	NS
Milk energy output (MJ d ⁻¹)	43.9 ± 3.46	63.7 ± 3.14	74.4 ± 3.66	59.2 ± 2.40	<.001
Energy expenditure (MJ d ⁻¹)	107.7 ± 8.55	106.0 ± 6.45	111.0 ± 4.54	108.4 ± 5.08	NS

Note. Values are means ± SE. Daily energy expenditure was calculated from tissue loss (see “Material and Methods”).

^a Differences between early and late periods tested by repeated-measures ANOVA for all components except mass loss (see “Material and Methods”).

^b Rate of mass loss estimated using mixed-effects, repeated-measures regression. Best fit was the linear equation: $y = 203.7 - 4.7x$.

also explained the differences in pup mass gain between periods (Fig. 4a). Daily milk fat intake by pups increased by 74% over the course of lactation, which corresponded to a 76% increase in both fat and energy deposition, respectively (Table 3). Hence, fat intake was a significant factor in predicting daily fat deposition, both overall ($r^2 = 0.769$, $P < 0.001$) and during early and late periods (Fig. 4b). Although milk protein intake increased by 32% (Table 3), it was correlated with protein deposition only in early lactation (Fig. 4c).

Overall daily milk energy intake ranged from 35.3 to 73.2 MJ d⁻¹ (Table 3) and was the most significant factor in determining overall rates of daily pup mass gain ($r^2 = 0.854$, $P < 0.001$). Increased daily milk energy intakes (Table 3) explained most of the variation in pup mass gain during all periods of lactation (91% early, 79% mid, 80% late; $P < 0.001$). Pups had a higher mass storage efficiency ($100 \times$ mass gain/milk intake) during mid ($62.8\% \pm 2.62\%$) and late lactation ($64.4\% \pm 2.27\%$) than during early lactation ($47.7\% \pm 2.72\%$, rm-ANOVA, $P < 0.001$). In contrast, energy storage efficiency

($100 \times$ energy stored/energy intake) was similar during early and late periods and appeared to be highest during the mid period (Table 3). Overall, the proportion of energy intake by pups allocated to energy storage (52.3%–77.9%) was unrelated to birth mass, initial protein content, or initial fat content ($P > 0.5$ for all).

Overall daily maternal milk output was not significantly related to either maternal parturition mass ($r^2 = 0.146$, $P = 0.130$) or average body mass ($r^2 = 0.117$, $P = 0.180$). However, with the removal of two previously mentioned outliers (pairs 5 and 13), the strength of these relationships increased ($r^2 = 0.316$, $P = 0.029$ and $r^2 = 0.316$, $P = 0.029$, respectively). While daily milk output was not predicted by initial maternal fat stores ($r^2 = 0.012$, $P > 0.5$), it was significantly related to initial protein stores ($r^2 = 0.228$, $P = 0.052$). By day 15, females ($N = 16$) had produced a total of 42.8 ± 1.78 kg milk, representing 15.7 ± 0.72 kg water, 20.0 ± 0.89 kg fat, and 3.9 ± 0.18 kg protein. Subtracting these values from measured total body losses, overall females used 18.8 kg fat but only 2.1 kg water and 0.6 kg

Table 3: Water turnover and milk intake in 18 grey seal pups during lactation

	Early (N = 18)	Mid (N = 17)	Late (N = 16)	Overall (N = 17)	P ^a
Mass gain (kg d ⁻¹)	1.2 ± .12	1.8 ± .13	2.1 ± .12	1.7 ± .09	<.001 ^b
k^c08 ± .003	.06 ± .005	.05 ± .004	.07 ± .003	<.001
Milk intake (kg d ⁻¹)	2.34 ± .143	2.87 ± .155	3.20 ± .168	2.83 ± .114	.003
Milk fat intake (kg d ⁻¹)99 ± .082	1.46 ± .073	1.72 ± .086	1.35 ± .054	<.001
Milk protein intake (kg d ⁻¹)22 ± .011	.26 ± .014	.29 ± .012	.27 ± .011	<.001
Milk energy intake (MJ d ⁻¹)	43.9 ± 3.46	63.7 ± 3.14	74.4 ± 3.66	59.2 ± 3.46	<.001
Energy stored (MJ d ⁻¹)	29.2 ± 3.28	45.7 ± 2.81	51.4 ± 3.28	41.8 ± 2.16	<.001
Storage efficiency (%)	63.2 ± 3.81	71.1 ± 1.71	68.9 ± 2.59	70.2 ± 1.68	NS

Note. Values are means ± SE. Energy stored was calculated as (kg fat deposited \times 39.3) + (protein deposited \times 23.6). Storage efficiency was calculated as $100 \times$ (energy stored/energy intake).

^a Differences between early and late periods tested by repeated-measures ANOVA for all components except mass gain (see “Material and Methods”).

^b Rate of mass gain estimated using mixed-effects, repeated-measures regression. Best fit was the quadratic equation $y = 16.4 + 1.05x + 0.04x^2$.

^c Fractional water turnover.

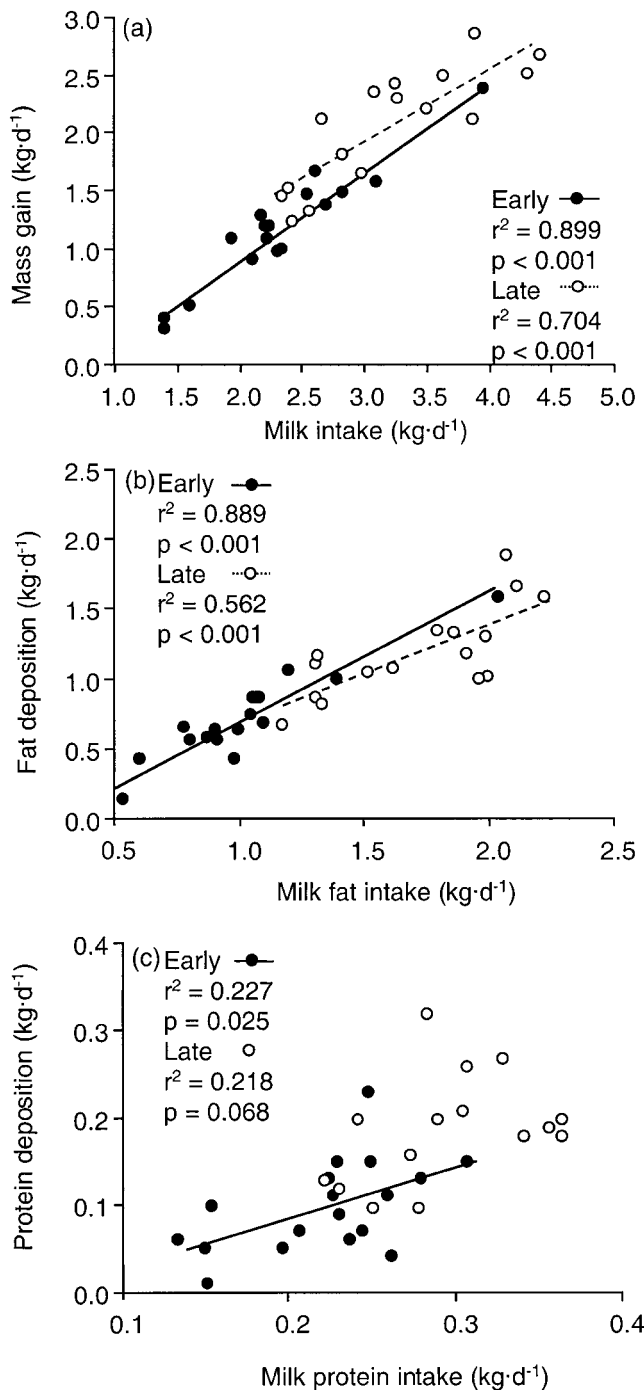


Figure 4. Relationships of (a) daily mass gain to daily milk intake, (b) daily fat deposition to daily milk fat intake, and (c) daily protein deposition as a function of milk protein intake in grey seal pups during early ($N=18$) and late ($N=16$) periods. (a) early $y = -0.632 + 0.766x$; late $y = 0.040 + 0.632x$; (b) early $y = -0.234 + 0.926x$; late $y = 0.003 + 0.697x$; (c) early $y = -0.030 + 0.579x$.

protein above that directly exported in milk (i.e., for maintenance expenditures).

Females depleted 1,171–2,430 MJ of body energy reserves by day 15 ($N=16$) and an estimated 1,049 to 3,407 MJ by the end of their respective lactation periods ($N=17$). These losses corresponded to total daily energy expenditures (DEE) of 78.2–162.2 MJ d⁻¹ (Table 2). Rates of total DEE were unrelated to average maternal body mass ($P=0.146$) or average lean body mass (LBM; $r^2=0.172$, $P=0.098$). Overall, 55.9% \pm 2.71% of DEE was due to the energy secreted in milk. Although rates of total DEE did not change with lactation stage, milk energy output of females nearly doubled between early and late lactation (Table 2). Thus, females were significantly more efficient at producing milk in late lactation than during early lactation using both a mass index ($100 \times$ milk output/mass loss: early 57.4% \pm 3.64%, late 75.4% \pm 3.08%, $P=0.011$) and an energy index ($100 \times$ milk energy/body energy loss: early 43.6% \pm 3.64%, late 67.5 \pm 2.79%, $P<0.001$). Overall daily rates of milk energy output were not significantly related to either average mass ($P=0.197$) or LBM ($P=0.097$). However, excluding females 5 and 13 as outliers, these relationships were both significant ($r^2=0.502$, $P=0.003$ and $r^2=0.339$, $P=0.023$, respectively).

Lactation Length and Total Milk Transfer

Lactation length varied almost twofold among females, with the smallest female lactating for the shortest length of time and the largest female lactating for the longest length of time (see Appendix). A stepwise regression model with maternal parturition mass, daily mass loss, and daily milk energy output as independent variables indicated that maternal parturition mass alone explained the largest proportion of the variation in lactation length ($y = 3.255 + 0.069x$, $r^2 = 0.653$, $P < 0.001$; Table 4). In a separate model, with initial maternal mass partitioned into initial fat or protein content, fat content explained a larger amount of the variation in lactation length ($r^2 = 0.595$, $P < 0.001$) than did protein content ($r^2 = 0.281$, $P = 0.029$; Table 4). Rates of daily fat or protein loss had no significant effect on lactation length ($P > 0.1$ for both).

Total milk production and milk energy output of each female was estimated from observed lactation lengths. Total milk production and milk fat production varied twofold (see Appendix). Maternal mass at parturition explained 65.2% and 62.0% of the variation in total milk and milk fat production, respectively ($P < 0.001$ for both). However, despite the predominance of fat in milk, initial maternal protein content and LBM explained more of the variation in total milk output ($r^2 = 0.532$ and 0.579, respectively, $P < 0.001$) than did initial fat stores ($r^2 = 0.300$, $P = 0.023$). Total milk protein output was more strongly related to protein content at parturition ($r^2 = 0.594$, $P < 0.001$), while total milk fat production was only weakly related to initial maternal fat stores ($r^2 = 0.287$, $P = 0.027$). The twofold varia-

Table 4: Selected forward stepwise regression models for predicting lactation duration in grey seal females and weaning mass in their pups

Model	Significant Terms	r^2	F	df	P	SEE
Lactation length: ^a						
1	Parturition mass	.653	28.242	1, 15	<.001	1.364
2	Protein stores	.281	5.873	1, 15	.029	1.963
3	Fat stores	.595	22.059	1, 15	<.001	1.474
Pup weaning mass: ^b						
4	Total milk energy intake	.884	114.139	1, 15	<.001	2.097
5	Total milk energy intake and energy storage efficiency	.905	77.046	2, 14	<.001	1.838

^a Model 1 variables: maternal parturition mass (kg), daily mass loss (kg), and daily milk energy output (MJ); model 2 and 3 variables: initial maternal protein or fat stores in place of parturition mass.

^b Model 4 and 5 variables (maternal parturition mass removed; see text): pup birth mass (kg), total milk energy intake (MJ), maternal energy allocation to milk energy output (% efficiency), and pup energy allocation to energy storage (% efficiency).

tion in total milk energy output (647–1,431 MJ) was also significantly related to initial maternal mass ($r^2 = 0.634$, $P < 0.001$) and was more strongly related to initial maternal protein content or LBM ($r^2 = 0.522$ and 0.568 , respectively, $P < 0.001$) than to fat content ($r^2 = 0.288$, $P = 0.027$).

Pup Weaning Mass

Pup weaning mass (41.8 ± 1.45 kg) was positively related to maternal parturition mass and initial maternal protein content but not to maternal fat stores (Fig. 5). However, maternal parturition mass and protein content explained only 41%–42% of the variability in pup weaning mass. Hence, a stepwise regression was used to construct a model to predict weaning mass (Table 4). Variables initially used in the analysis were maternal parturition mass, pup birth mass, total milk energy intake (MJ), maternal energy allocation to milk energy output (% efficiency), and pup energy allocation to energy storage (% efficiency). Since the primary effect of maternal parturition mass was on daily milk output and lactation length, maternal mass was removed from the final model. Pup birth mass had no effect on the final model. Weaning mass was best predicted by total milk energy intake (88.4%, $y = 15.641 + 0.026x$; $N = 17$; Table 4). The addition of pup energy storage efficiency increased the strength of the model to explain 90.5% of the variation in pup weaning mass ($y = 4.964 + 15.568 \times$ energy allocation to storage $+ 0.026 \times$ total energy intake, $N = 17$; Table 4). Heavier pups at weaning were produced by females with greater total energy outputs, and these energy outputs were partially predicted by maternal mass and protein stores (Fig. 6). In addition, pups that allocated a larger fraction of energy to growth tended to be heavier at weaning.

Discussion

Milk Composition

Cross-sectional studies have shown that milk fat content increases dramatically over the course of lactation in some phocid seal species, such as the southern elephant seal (*Mirounga angustirostris*, 9%–51%; Peaker and Goode 1978), northern elephant seal (*Mirounga leonina*, 12%–50%; Riedman and Ortiz 1979), and grey seal (40%–60%; Iverson et al. 1993). Our longitudinal study confirms this pattern of change within individuals but also shows that there is considerable variability in the magnitude and pattern of change among females (Fig. 1). For instance, milk fat content differed by almost a factor of 2 on the day of parturition (range 25%–43%), and values were lower than previously reported (40%; Iverson et al. 1993). This difference may have resulted from sampling females closer to parturition, as several samples had the thin consistency, yellowish color, and elevated protein levels of colostrum. Milk fat content actually doubled during lactation in some females (e.g., females 10 and 17; Fig. 1), while these changes were much less pronounced in other females (e.g., female 18). As expected, the sources of variation in milk composition among females could not be explained by obvious maternal characteristics such as maternal mass, body composition, or body nutrient stores. Small females were capable of producing milk of similar energy content as much larger females (see Appendix). Data for other species have also shown that milk volume, rather than composition, is influenced by such factors as nutritional condition or chronic malnourishment (White and Luick 1984; Oftedal 1985; Iverson 1993). Milk fat content, in particular, is thought to be under the influence of such physiological mediators as prolactin (DaCosta et al. 1995) and lipoprotein lipase (Iverson et al. 1995; Mellish et al. 1999; J. E. Mellish and S. J. Iverson, unpublished data). Additionally, age, parity, and genotype of

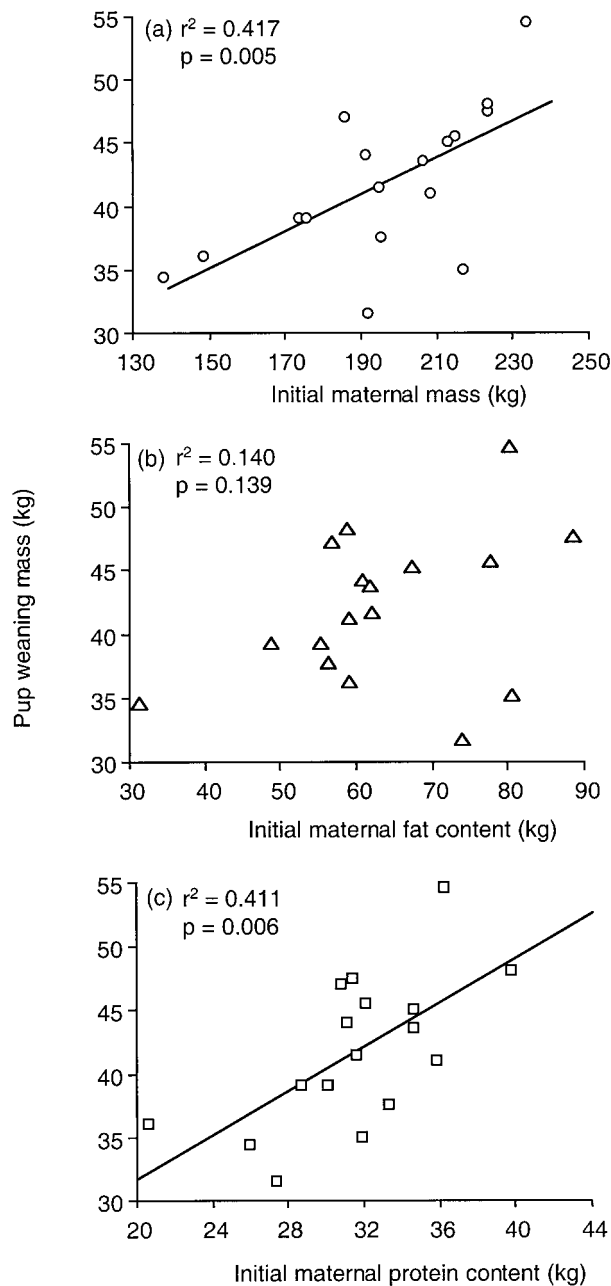


Figure 5. Pup weaning mass as a function of (a) initial maternal mass, (b) initial maternal fat content, and (c) initial maternal protein content in 16 grey seal mother-pup pairs. Pair 16 was excluded due to early termination of lactation. Initial maternal mass $y = 12.931 + 0.147x$; initial maternal protein content $y = 14.401 + 0.867x$.

females may play a role in determining mammary gland performance (Künkele and Kenagy 1997), but this information was not known for the females in our study.

The consequences of this individual variation in milk composition (especially fat and energy) for pup growth and weaning

mass are not necessarily straightforward, because daily milk output and lactation length can vary in magnitude but be independent of milk fat content (see below, Appendix).

Maternal Mass, Nutrient Stores, and Lactation Length

The process of weaning in most phocids is abrupt, with the female leaving the breeding colony to forage at sea (Bowen 1991). However, the factors controlling the length of lactation of individual females and possible cues for weaning are still unclear. Behavioural data indicate that in some phocid species, it is the female that increasingly terminates suckling in late lactation (e.g., harbour seal; Renouf and Diemand 1984). As grey seal females on Sable Island fast completely throughout lactation, critical depletion of body nutrient reserves could be responsible for this change in maternal behaviour and the termination of lactation. Lactation length varied almost twofold in this study (see Appendix), and this variation was largely explained (65%) by differences in maternal mass at parturition (Table 4). Despite a wide range of initial body masses, relative fat stores were not correlated with initial body mass in grey seals. Thus, on a relative basis, females appear similarly prepared to support the costs of lactation. However, on an absolute basis, larger females have more protein and fat stores and thus can support higher milk protein and fat outputs during a longer period of fasting (i.e., lactation length).

Females lost 35% of their initial body mass over the course of lactation, which is similar to that found previously for grey seals (38%–39%; Fedak and Anderson 1982; Iverson et al. 1993) and southern and northern elephant seals (37% and 42%, respectively; Costa et al. 1986; McCann et al. 1989). Fat was rapidly mobilized at rates of up to 4.7 kg d^{-1} and accounted for 92% of maternal energy loss, which is characteristic of fasting-adapted species (e.g., petrels 83%–90%, Groscolas et al. 1991; penguin species 85%–94%, Chappell et al. 1993, Cherel et al. 1993; lactating polar bears 93%, Atkinson and Ramsay 1995; lactating hooded seals 70%–98%, Mellish et al. 1999). In contrast, protein losses never exceeded 0.8 kg d^{-1} and comprised only 8% of energy loss. Although females lost 39.1 kg fat and 4.6 kg protein by 15 d, much of this loss was due to direct export of these nutrients in milk. Subtracting that exported in milk, females lost a total of 18.8 kg fat and only 0.6 kg protein by 15 d, presumably due to oxidation. Therefore, fat provided 98% of the fuel for maternal energy metabolism, whereas protein provided only 2%. Therefore, grey seal females show remarkable protein conservation despite the energetic demands of lactation. Additionally, the oxidation of 18.8 kg fat would have yielded approximately 20.1 kg water, contributing substantially to both maternal water balance and milk water output during fasting.

On average, grey seal females lost approximately 68% of their initial fat stores and 16% of their initial protein stores by the end of lactation. In most animals, a loss of 90% of initial body

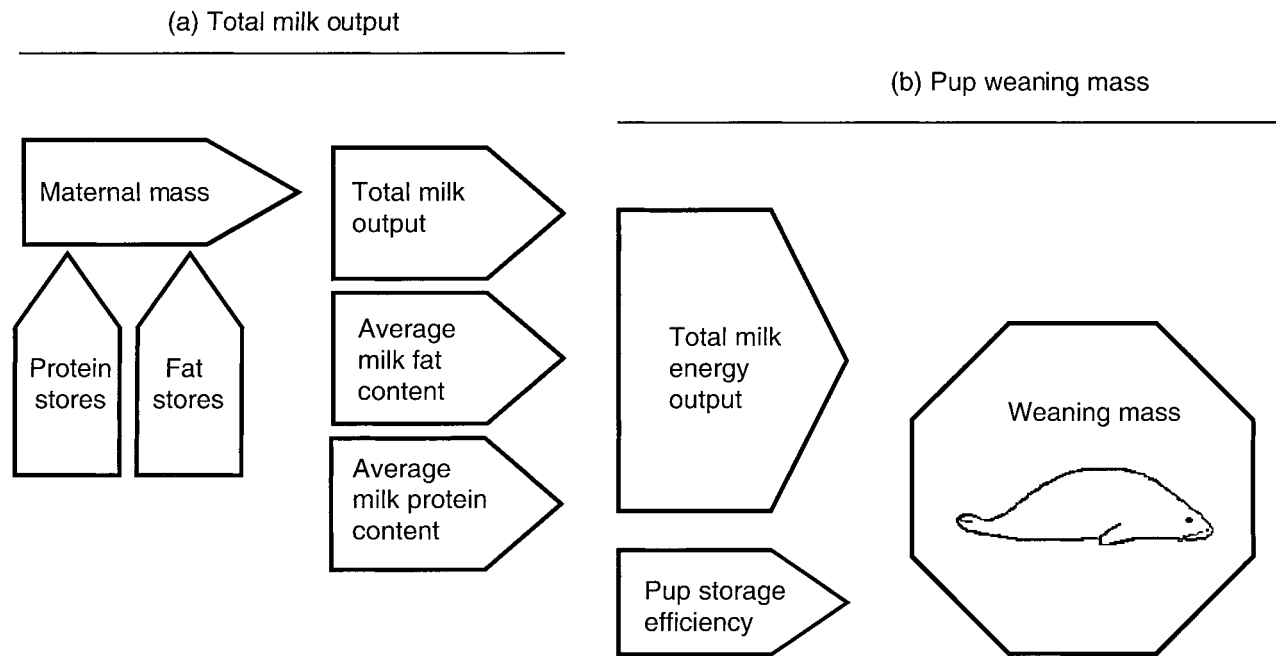


Figure 6. Representation of relationships involved in predicting weaning mass in neonatal grey seals. (a) Total milk output was best described overall by $y = -11.415 + 0.301 \times \text{initial mass}$ ($r^2 = 0.652$, $P < 0.001$); initial maternal protein content alone explained 53% of total milk output while fat stores alone explained 30%. Average milk fat content and milk protein content were unrelated to maternal mass or nutrient stores (see "Results"). Total milk energy output is a function of the average milk fat and protein content multiplied by total milk output. (b) Pup weaning mass was best predicted by $y = 4.964 + 15.568 \times \text{pup energy allocation to storage} + 0.026 \times \text{total milk energy output}$ ($r^2 = 0.905$, $P < 0.001$).

fat stores and 30% of initial body protein stores is considered critical to continued survival (Cahill et al. 1979; Cherel et al. 1987; Castellini and Rea 1992). In fasting rats and penguins, protein degradation increases at this point and is thought to stimulate the onset of foraging behaviour (Koubi et al. 1991; Cherel et al. 1994). In only two grey seal females (females 7 and 10) were these boundaries exceeded by the end of lactation. An additional two females (females 13 and 14) approached, but did not exceed, these limits. Thus, some grey seal females appear to sustain lactation up to and potentially beyond the point where maternal welfare could be compromised. Two of these females (females 4 and 13) had lower than average milk fat contents (Fig. 1; Table 1) and may have required extra time to produce even a medium-sized pup, thus incurring higher overhead costs. However, as most of the females terminated lactation before reaching these critical levels, it is unlikely that the duration of lactation is typically limited by maternal fat or protein content.

Characteristics of Pup Mass Gain and Milk Intake

Pup growth rates increased significantly over lactation (Fig. 3). Fat deposition (up to 1.9 kg d^{-1}) accounted for the majority of pup mass gain, and this deposition was primarily determined

by milk intake, coupled with the increasing energy content of milk ($r^2 = 0.890$). In an earlier study on Sable Island, significant differences in milk intake over lactation were not observed, and therefore increased growth rates were attributed to changes in milk energy density alone (Iverson et al. 1993). However, in the previous study, milk composition of individual females was unknown, the sample size was smaller ($N = 8$), and sampling intervals were broader (early 0–7 d, late 11–14 d) than those used here.

Grey seal pups doubled and even tripled their birth mass by weaning (range 1.7–3.6 times). Overall, fat deposition accounted for 66% of mass gain and 92% of energy stored, while protein deposition accounted for only 8% of mass and energy gain to weaning. Therefore, whereas variation in pup birth mass was primarily due to differences in protein stores, variation in weaning mass was primarily due to differences in fat content.

Energy Expenditure and Export

Grey seal females in our study had similar daily energy losses throughout lactation (Table 2). These losses (108 MJ d^{-1}) were similar to those measured in grey seal females on North Rona ($100\text{--}126 \text{ MJ d}^{-1}$; Fedak and Anderson 1982; Reilly et al. 1996) but were less than those measured in northern elephant seals

(163 MJ d⁻¹; Costa et al. 1986) or southern elephant seals (184 MJ d⁻¹; Fedak et al. 1996). However, relative to initial maternal body mass, the daily energy loss of grey seals (0.55 MJ d⁻¹ kg⁻¹) exceeded that reported for elephant seal females (0.32–0.35 MJ d⁻¹ kg⁻¹). Grey seal females allocated an average of 56% of their daily energy expenditure to milk production, similar to other estimates for grey seals (57%; Fedak and Anderson 1982) and northern elephant seals (59%; Costa et al. 1986).

Heavier grey seal females did not have greater daily energy expenditures; however, excluding two anomalous females (females 5 and 13), heavier females exported greater amounts of energy in milk daily than did smaller females. Heavier females had larger LBM and, therefore, a greater mass-specific ability to store energy. However, mass and LBM only accounted for approximately 50% and 34%, respectively, of the variation in daily milk energy output, and therefore some small females were able to produce greater amounts of milk energy than would be predicted by mass or LBM alone. As indicated earlier, other factors such as age, parity, mammary gland size and capacity, hormones, and enzymes responsible for lipid mobilization (e.g., Iverson et al. 1995; Künkele and Kenagy 1997; Mellish et al. 1999) appear to be involved in the regulation of energy expenditure and export.

Predicting Pup Weaning Mass

Total milk energy intake, which was generally associated with larger mothers at parturition, accounted for much of the variation (88.4%) in pup weaning mass. Nevertheless, it is evident even in our small group of animals that there is substantial variation in the ability of females to produce a large, fat pup at weaning. Although large maternal size (and LBM) appears to be advantageous by increasing energy storage capacity, milk output, and the duration of lactation, over one-third of the variation in total milk energy output and one-half of the variation in pup weaning mass are unexplained by maternal mass or its gross constituents of fat and protein. The best single predictor of pup weaning mass (88%) remains total milk energy output, which is primarily a function of milk fat output ($r^2 = 0.997$). However, while total milk output is partially predicted by maternal mass, milk fat content (which also dictates

milk fat output) is a wild card that does not relate to maternal mass or energy stores (e.g., see Appendix) but is instead likely linked to maternal genetic or phenotypic traits. It appears that females can achieve a fat pup at weaning through several different paths (i.e., longer lactation duration or higher milk fat content and/or greater daily milk energy output; see Appendix).

In summary, larger grey seal females tended to lactate longer, have higher total milk outputs, and produce larger pups at weaning. While fatter females lactated for longer periods of time, females with larger protein stores (i.e., greater LBM) at parturition produced more milk. Total milk energy output, which was a function of primarily maternal protein but also fat stores, was the strongest single predictor of pup weaning mass. However, there appears to be sufficient plasticity in milk and energy output and lactation performance among individual females that, nevertheless, allows some smaller females to produce relatively large pups. Furthermore, pup weaning mass may also be influenced by characteristics of pup performance, such as energy storage efficiency (Fig. 6). Underlying physiological characteristics such as mammary capacity and neonatal fattening ability (Mellish et al. 1999; J. E. Mellish and S. J. Iverson, unpublished data) likely account for some of the unexplained variability in lactation performance.

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Appendix

Table A1: Maternal attributes contributing to total milk fat output in 17 grey seal females

Female	Parturition Mass (kg)	Lactation Length (d)	Average Milk Fat (%)	Total Milk Output (kg)	Total Milk Fat Output (kg)	Total Milk Protein Output (kg)
1	224.0	17	53.0	54.4	28.9	4.7
2	186.0	15	47.3	50.6	23.9	4.7
3	215.0	18	44.6	58.9	26.3	5.3
4	195.0	17	46.0	50.3	23.1	4.5
5	192.0	20	40.8	38.4	15.7	3.9
6	191.5	15	41.8	52.4	21.9	5.0
7	213.0	18	53.2	48.2	25.6	4.9
8	174.0	15	48.5	38.3	18.6	3.8
9	176.0	15	48.2	41.0	19.8	3.8
10	234.0	21	51.9	63.4	33.0	5.8
11	138.0	12	51.7	29.6	14.9	2.7
12	148.5	15	50.8	34.2	17.4	2.9
13	217.0	19	43.6	42.6	18.6	3.9
14	195.5	17	48.7	44.4	21.6	4.6
15	224.0	19	47.2	58.5	27.6	5.4
17	206.5	17	43.7	60.9	26.6	6.0
18	208.5	16	48.6	43.8	21.3	4.0
Mean	196.4	17	47.6	47.6	22.6	4.5
SE	6.36	.5	.92	2.37	1.20	.22
CV	13.4	13.3	8.0	20.5	19.5	20.5

Note. Female 16 was not included because she ceased lactation after day 5.

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