The Effect of Maternal Size and Milk Energy Output on Pup Growth in Grey Seals (*Halichoerus grypus*)

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Abstract

In phocid species in which females fast throughout lactation, maternal energy reserves at parturition must support lactation. Thus, differences in body size and energy stores may influence the magnitude of maternal energy transfer. We examined variation in milk energy transfer and its consequence for pup growth in grey seals over a wide range of maternal body sizes. Milk samples (n = 42) were collected from 28 females over the 16-d lactation period. Milk yields were measured in nine mother-pup pairs by deuterium oxide dilution. Milk fat and energy content increased dramatically over the first half of lactation. At mid- to late lactation milk composition averaged 71.1% dry matter, 59.8% fat, 9.2% protein, and 24.8 kJ/g (bomb calorimetry). Initial maternal mass (149–256 kg) and daily mass loss (3–9 kg/d) were correlated with daily milk output (1.3–4.1 kg/d). Milk energy output (29–92 MJ/d) in turn explained pup mass gain (0.8–2.8 kg/d, r^2 = 0.997). Pups with greater energy intakes had a greater ratio of fat to protein deposition and thus a greater efficiency of energy storage, increasing from 40% to 79%. Daily milk intake did not change over lactation, but growth rate was greater during the second half of lactation because milk energy intake was greater. Results of this study indicate that large females lose mass more rapidly, produce more milk, and wean larger, fatter pups than do small females. We conclude that differences in maternal mass and available energy stores may have substantial consequences for the pup and may account for much of the reported variation in pup growth both within and between populations of phocids such as the grey seal.

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Introduction

In phocid seals, rapid transfer of nutrients to young is achieved in a relatively brief and intense lactation period, during which many species fast (Bonner 1984; Bowen, Oftedal, and Boness 1985; Oftedal, Boness, and Tedman 1987a). In species that fast, the energy reserves that a female brings ashore prior to parturition must support the energetic costs of both maintenance and milk production. Hence, differences among phocid species in maternal body size and in energy stores at parturition are likely to influence measures of reproductive effort, such as pup birth mass and subsequent pup growth (Bowen 1991; Costa 1991). Variation in energy stores may also influence within-species relationships between maternal size and pup growth (see, e.g., Anderson and Fedak 1987; Le Boeuf, Condit, and Reiter 1989; McCann, Fedak, and Harwood 1989). Examination of the variation in milk output of mothers and growth and tissue deposition of their offspring may be valuable in interpreting life history patterns both within and between species.

Of the various methods used to study the energetics of lactation, the measurement of milk energy output over the course of lactation is the most direct assessment of total energy transfer from mother to pup. Estimates of milk yield derived from isotope dilution studies on pups have been reported in a number of phocid species, including harbor seals, *Phoca vitulina* (Oftedal, Bowen, and Boness 1991), hooded seals, *Cystophora cristata* (Oftedal, Bowen, and Boness 1993), northern elephant seals, *Mirounga angustirostris* (Ortiz, Le Boeuf, and Costa 1984; Costa et al. 1986), and Weddell seals, *Leptonychotes weddellii* (Tedman and Green 1987). However, these studies have generally used a single ratio of milk intake to water intake, an average metabolic rate, or an average tissue deposition rate, which precludes investigation of individual variation.

Milk energy output may be influenced by changes in milk energy content or milk yield. For instance, in some phocid species milk composition changes dramatically over lactation (e.g., southern elephant seals, *Mirounga leonina* [Bryden 1968; Peaker and Goode 1978], and northern elephant seals [Riedman and Ortiz 1979]). There is also evidence that pups grow at somewhat slower rates early in lactation (e.g., southern elephant seals [McCann et al. 1989], grey seals [Anderson and Fedak 1987; Bowen, Stobo, and Smith 1992b], and hooded seals [Bowen et al. 1985]). However, the interplay between these factors and the pattern of milk energy transfer in phocids has not been addressed.

Grey seals, *Halichoerus grypus,* provide an excellent opportunity to examine individual variation in energy transfer over the lactation period. Like many other phocids, grey seals do not feed during a short, intense lactation

period (16–18 d; Boness and James 1979), and weaning is abrupt. Further-more, grey seals are also found on both sides of the North Atlantic, where population differences are readily apparent (Mansfield 1977). In the United Kingdom, adult females average about 174 kg at parturition and typical growth rates of suckling pups are about 1.3–1.8 kg/d (Coulson 1960; Coulson and Hickling 1964; Boyd and Campbell 1971; Fedak and Anderson 1982; Kovacs and Lavigne 1986), whereas on Sable Island, Canada, adult females are 15%–30% larger and pups grow at about twice the rate, averaging 2.8 kg/d (Bowen 1991; Bowen et al. 1992b). Maternal size also varies substan-tially within populations (see, e.g., Mansfield 1977; Anderson and Fedak 1987). Pup growth rates may vary with maternal body size or age (Anderson and Fedak 1987; Bowen and Stobo 1991), with length of lactation period (Bowen and Stobo 1991), or with time during the breeding season (Boness, Bowen, and Iverson 1991).

We studied the energetics of lactation in grey seals on Sable Island, Nova Scotia, using isotope dilution methodology. Females representing a wide range of body sizes were used to examine the relationships among maternal size, milk energy output, and pup growth. One aim of our study was to determine the effects of changes in milk composition and individual vari-ability in pup tissue deposition on milk yield derived from isotope dilution. A second aim was to determine whether energy intake accounted for indi-vidual differences in pup growth.

Material and Methods

Field Procedures

Data were collected from January 15 to February 3, 1990, the latter portion of the 6-wk pupping season on Sable Island, Nova Scotia (43°55'N, 60°00'W). The rookeries were censused daily for new births. Thus, stage of lactation was known to within 1 d postpartum. Mother-pup pairs were individually marked with waterproof fluorescent paint (Lenmar Paint, Baltimore) and numbered hind-flipper tags (Rototag; Dalton, Heneley-on-Thames).

Milk samples ($n = 42$) were collected from 28 females at various times over the first 15 d of the approximately 16-d lactation period (Boness et al. 1991). Females were chemically immobilized with Telezol, a mixture of tiletamine hydrochloride and zolazepam hydrochloride, at a dose of ap-proximately 1.0 mg/kg estimated body weight. Telezol was injected into the lumbar muscles via an air-pressurized dart blown from a Teleinject blowpipe. Initial doses of Telezol were based on previous work (1.0–1.5 mg/kg; Boyd et al. 1990; Baker et al. 1990). We attempted to avoid deep

anesthesia (deeper than level 2; Boyd et al. 1990) by delivering a relatively low initial dose of Telezol followed by an intravenous booster as necessary at capture. The initial dose delivered by dart ranged between 0.66 and 1.45 mg/kg (average 0.91 ± 0.022 mg/kg, n = 62). The time between darting and capture varied between 5 and 20 min (average 12.2 ± 0.42 min). The total dose ranged between 0.74 and 1.46 mg/kg (average 0.95 ± 0.024 mg/kg). During 62 immobilizations, two females went to level 5 anesthesia after initial doses of 0.79 and 1.32 mg/kg. The first female recovered after artificial resuscitation, whereas the second died.

On loss of mobility and coordination, females were captured with a net that was fastened between two aluminum poles hinged at the head and open at the rear. Each female was administered an intramuscular injection of oxytocin (15–30 IU). Prior to sampling, the teats were rinsed with fresh water and wiped dry. Milk was evacuated by suction with a 60-cm^3 syringe with the end cut off, collected into 30-mL Nalgene bottles, and stored frozen at −20°C until analysis. In three females, one mammary gland was completely evacuated in sequential 30-mL aliquots (180–450 mL per collection). Comparison of the first 30-mL to the sixth 30-mL aliquot of these females did not reveal any significant difference in dry matter, fat, or protein content ($P > 0.3$; paired t-tests). Thus, approximately 120 mL milk was collected from most females and considered to be representative.

Mass transfer and milk intake were studied over the course of lactation in nine mother-pup pairs starting on the day of birth (day 0) or 1 d postpartum. Immobilized females were weighed to the nearest 1 kg on a 300-kg Salter scale that was suspended from the apex of an aluminum tripod. Stomach contents of pups were evacuated by gastric intubation using a 3/8-inch (9.5-mm) veterinary stomach tube. The pup was then weighed ("empty" body mass) to the nearest 0.1 kg on 50- and 100-kg Salter scales during early and late lactation, respectively. A preweighed amount (3.7 ± 0.18 g/kg body mass; error is standard error of the mean [SEM]) of deuterium oxide (D_2O, 99.8% purity, Atomic Energy of Canada) was delivered from a 60-cm^3 syringe by gastric intubation using a 12 French stomach tube. Syringe and stomach tube were rinsed with two 5-cm^3 quantities of water, and air was blown through the tube as it was withdrawn to ensure complete isotope delivery. Mother and pup were then held in separate but contiguous pens to permit isotope equilibration prior to suckling. Sequential blood samples (10 cm^3) were taken, with 18-gauge needles (38–89 mm), from the extradural vein of pups at approximately 2, 2.5, and 3 h following isotope administration. Pairs were subsequently released and observed until we were sure the mother-pup bond was intact as evidenced by suckling or other attentive maternal behavior.

We recaptured the nine mother-pup pairs at about 6–7 d, 11 d, and 14–15 d following isotope administration. However, two females departed early from the rookery and were thus studied only until days 10 and 12 postpartum, respectively; female 8 died during anesthesia on day 7. At each recapture, both mother and pup were weighed and a blood sample was taken from the pup. At the final recapture, the pup was bled and given a preweighed amount (1.5 ± 0.16 g/kg [± SEM]) of D_2O to measure final body water pool. Blood sampling procedures were the same as during the initial equilibrations. Pup 8 was not re-equilibrated at day 7.

All blood samples were centrifuged in the field laboratory, and serum was transferred to airtight cryovials and stored frozen at −20°C.

Laboratory Analyses and Calculations

Milk samples were analyzed in duplicate. Dry matter was measured by drying in a forced convection oven for 5 h at 100°C. Total milk fat was determined gravimetrically following sequential petroleum ether and diethyl ether extractions using the standard Roese-Gottlieb procedure for milks (AOAC 1975). Total nitrogen was determined by a macro-Kjeldahl method and converted to crude protein by using the conversion factor for milk protein of 6.38 (AOAC 1975). Ash content was measured by incineration of dried samples in a muffle furnace. Gross energy (kJ/g, where kJ = kcal X 4.184) was determined by adiabatic bomb calorimetry using cotton of known energy content as a carrier for the dried milk (Parr Adiabatic Calorimeter, 1241 oxygen bomb).

Total free water was collected from blood sera by heat distillation, and D_2O concentration was determined by quantitative infrared spectrophotometry on a Perkin-Elmer ratio-recording infrared spectrophotometer (Perkin-Elmer, Norwalk, Conn.) as described previously (Oftedal and Iverson 1987; Oftedal, Iverson, and Boness 1987b).

Equilibration was considered to have occurred when the isotope level in two sequential blood samples during the equilibration period had reached a plateau. Initial equilibrated isotope concentration (C_i, %) was then used to calculate initial body water pool size (P_i, kg),

$$P_i = \frac{gD_2O \text{ administered}}{10 \times C_i},$$ (1)

and final pool size (P_f, kg) at the last recapture was calculated as

$$P_f = \frac{gD_2O \text{ readministered}}{10 \times (C_r - C_f)},$$ (2)

where C_r is the re-equilibration concentration and C_f is the isotope concentration immediately prior to readministration. Intermediate pool sizes (P_t) were calculated at time t for each pup, with the assumption that its change in pool size was linear over lactation. Given the variability among individuals, calculations for each pup were based on its initial and final data to minimize error in estimates.

After equilibration, isotope concentrations measured in subsequent blood samples (C_t) were corrected (C_t^*) for changes in pool size according to the formula

$$C_t^* = C_t \times \frac{P_t}{P_i}, \tag{3}$$

since increases in body water pool cause a decline in isotope that is not due to turnover (Dove and Freer 1979; Nagy and Costa 1980; Oftedal and Iverson 1987). Fractional water turnover (k) of each pup was determined as the slope of the linear regression of ln (C_t^*) against time elapsed since isotope administration. Total daily water intakes (TWI, kg/d) were assumed to be constant over time and were calculated as described previously (Oftedal and Iverson 1987):

$$\text{TWI} = (k \times P_{avg}) + \Delta P, \tag{4}$$

where P_{avg} is the average pool size for each pup and ΔP is the daily gain in pool size.

Conversion of TWI to milk intake (MI) for each pup required estimation of both free water content of milk consumed and metabolic water production from catabolism of milk fat and protein. This was calculated according to a modification of the equation developed by Oftedal et al. (1987b):

$$MI = 100 \times \frac{\text{TWI} + 1.07F_D + 0.42P_D}{\%W_M + 1.07\%F_M + 0.42\%P_M}, \tag{5}$$

where F_D and P_D are daily deposition (kg) of fat and protein, respectively, and $\%W_M$, $\%F_M$, and $\%P_M$ are the average water, fat, and protein content of milk, respectively. Milk sugar was assumed to be negligible. Equation (5) is derived from a series of equations in which the catabolism of 1 kg each of fat and protein is assumed to yield 1.07 kg and 0.42 kg water, respectively (Van Es 1969), and the amounts (kg) of fat and protein catabolized are estimated as the difference between intake and deposition of each component.

In the absence of direct data on fat and protein deposition in grey seal pups, we used relationships of body water to lean body or fat-free mass (LBM), and protein to LBM derived from carcass analysis of harp seal, *Phoca groenlandica*, pups (data provided by G. A. J. Worthy [personal communication] and from Worthy and Lavigne [1983]). We calculated that at birth harp seals contain 71.1% water, 18.9% protein, 3.7% fat, and 96.3% LBM (n = 3), and at weaning 37.6% water, 11.5% protein, 47.2% fat, and 52.8% LBM (n = 2). Thus, ratios of BW:LBM and protein:LBM at birth were 0.738 and 0.196, respectively, and near weaning were 0.712 and 0.218, respectively. These values were used to calculate percentage of fat (%F) and protein (%P) at initial (indicated by subscript i) and final (indicated by subscript f) equilibrations, and F_D and P_D (kg/d) for each pup over the days of study (d), where M represents pup body mass (kg):

$$\%LBM_i = \frac{\%BW_i}{0.738}$$

and

$$\%LBM_f = \frac{\%BW_f}{0.712};$$

(6)

$$\%F_i = 100 - \%LBM_i$$

and

$$\%F_f = 100 - \%LBM_f;$$

(7)

$$F_D = \frac{\left(\dfrac{\%F_f \times M_f}{100}\right) - \left(\dfrac{\%F_i \times M_i}{100}\right)}{d};$$

(8)

$$\%P_i = \%LBM_i \times 0.196$$

and

$$\%P_f = \%LBM_f \times 0.218;$$

(9)

$$P_D = \frac{\left(\dfrac{\%P_f \times M_f}{100}\right) - \left(\dfrac{\%P_i \times M_i}{100}\right)}{d}.$$

(10)

Since milk composition changed substantially over lactation (see Results, fig. 1), the composition of milk consumed by each pup was estimated by integrating under the curve for each component ($\%W_M$, $\%F_M$, $\%P_M$) and dividing this by the days between the measurement period (e.g., 0–15 or 1–7 d postpartum).

Calculated milk intakes were converted to energy intakes by using the average caloric content (kJ) of milk as determined by bomb calorimetry. Rates of mass gain by pups and mass loss by females over lactation were determined by regression. Energy content of pup mass gain was calculated from deposition rates by using the factors of 39.3 MJ/kg (9.39 Mcal/kg) fat and 23.6 MJ/kg (5.65 Mcal/kg) protein (Blaxter 1989).

Although the periods of measurement differed somewhat among several pairs in the milk intake study (table 1), only pair 8 was considered to be abnormal: This pair was studied for less than half the lactation period because the female died during anesthesia; pup birth mass and growth rate were also low. Thus, we excluded this pair in calculating mean values for normal pairs in tables 1–4. However, we used the data from this pair in examining the relationships between milk and energy output and pup growth (figs. 2–6). All other pairs were used to assess characteristics of milk energy transfer and rates of change over the lactation period. Only the subsample of pairs studied up to 14–15 d ($n = 6$) were used to assess characteristics (e.g., mass or pool size) near weaning. Of these six pairs, female 3 was not weighed with her pup at the final capture (14 d), as she had departed the rookery the night before the planned recapture. Although the entire 14 d of study were used in comparing this pup to other pups (growth rate and milk intake), in all comparisons involving both females and pups (e.g., rates of maternal mass loss to pup mass gain to milk intake), only the 0–7-d data points for this mother and pup were used. Throughout, summary values are presented as mean ± SEM.

Results

Milk Composition

Milk composition changed substantially over lactation. Data for all components other than ash were fit by second-order polynomial regressions (fig. 1). The most dramatic changes occurred over the first half of lactation (0–7 d postpartum), after which levels changed more gradually. Water content averaged 45.0% ± 2.09% at parturition and decreased to 33.0% ± 1.44% at 7 d and 28.6% ± 1.30% at 15 d postpartum (fig. 1a). Dry matter content increased from 55.0% ± 2.09% at birth to 71.4% ± 1.30% at 15 d (fig. 1b).
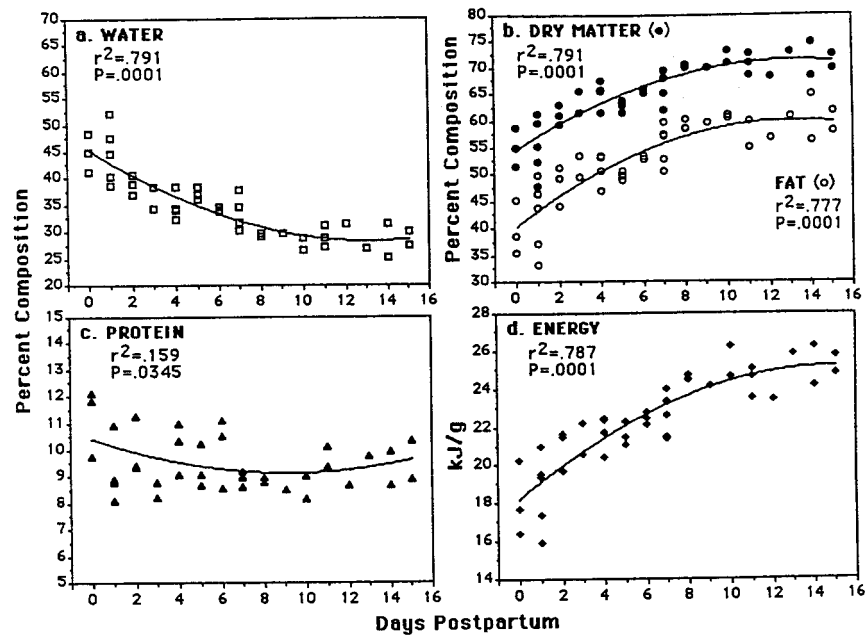
Fig. 1. Changes in milk composition of grey seals over lactation (n = 42). Second-order polynomial regression equations for changes of components over days postpartum were (a) water content (calculated as 100 − dry matter %): y = 45.44 − 2.60x + 0.10x²; (b) dry matter: y = 54.56 + 2.60x − 0.10x²; fat: y = 40.19 + 3.08x − 0.12x²; (c) protein: y = 10.42 − 0.28x + 0.02x²; (d) energy: y = 18.25 + 0.92x − 0.03x².

Fat content mirrored and accounted for most of the changes in dry matter, increasing from 39.8% ± 2.85% (72.2% of dry matter) at parturition to 55.6% ± 1.66% (83.0% of dry matter) at 7 d and 60.0% ± 1.86% (84.1% of dry matter) at 15 d (fig. 1b). Changes in protein were relatively minor (fig. 1c), although protein was slightly elevated at birth (11.2% ± 0.75%) compared with the rest of lactation (9.4% ± 0.14%). Gross energy content of milk increased from 18.1 ± 1.12 kJ/g at day 0 to 25.3 ± 0.50 kJ/g at 15 d (fig. 1d). Ash content accounted for 0.69% ± 0.015% of milk throughout lactation. Thus, on average milk contained 61.1% dry matter, 48.0% fat, 9.8% protein, and 20.6 kJ/g during the first week postpartum and 71.1% dry matter, 59.8% fat, 9.2% protein, and 24.8 kJ/g in the second week.

Maternal Size, Mass Loss, and Pup Mass Gain

Initial maternal mass ranged from 149 to 256 kg and rates of mass loss varied from 3.1 to 9.3 kg/d (table 1). Maternal mass loss (n = 7) was generally

TABLE 1

Grey seal mother-pup pairs and mass changes over lactation

Pup ID-Sex	Duration of Study (Days Post-partum)[a]	No. of Cap-tures	Maternal Initial Mass (kg)	Maternal Mass Loss (kg/d)	Pup Initial Mass (kg)	Pup Mass Gain (kg/d)	Index of Mass Transfer[b] (%)
2-F	0–14	4	256	9.3	18.5	2.78	29.9
3-F	0–14	3	214	3.6	15.5	1.46[c]	40.4
5-F	0–15	4	240	4.0	18.5	1.58	39.4
6-F	0–14	4	221	4.4	17.7	1.77	40.2
7-F	0–14	3	215	5.2	17.0	2.56	49.4
11-M	1–14	3	208	5.7	21.0	2.63	46.0
12-M	1–12	3	155	3.5	18.0	1.49	42.6
13-M	0–10	3	149	3.1	14.5	1.06	33.8
8-M[d]	1–7	2	155	4.3	13.3	.78	18.1
Mean[d]			207	4.9	17.6	1.92	40.2
SEM			13.3	.70	.70	.229	2.20

[a] Only pups captured on the day of birth (day 0) were considered newborn ($n = 6$).
[b] 100 × pup mass gain/maternal mass loss.
[c] Only data from 0–7 d were used in this comparison for pair 3, since the mother was not weighed subsequently. See tables 2–4 for complete pup data.
[d] Pair 8 was excluded from general means and SEM in tables 1–4 but included in examinations of relationships between mass changes, milk energy output, and pup growth in figs. 2–6 (see text).

linear over lactation. The range of final mass of females was equally large, with females ($n = 5$) losing between 25% and 50% of their initial body mass over the first 14–15 d of lactation. Mass gain of pups varied threefold (table 1). Thus, there was a large range (41.5–56.5 kg) in mass of pups at 14–15 d postpartum, with an average mass of 48.7 ± 2.77 kg ($n = 6$). In those weighed more than twice ($n = 8$), pup mass gain was also described by a linear regression over lactation.

The differences in initial maternal mass explained much of the variability in pup mass gain over lactation ($r^2 = 0.488$, $P = 0.0364$; fig. 2c). In the mother-pup pairs studied from day 0 ($n = 6$; table 1), pup birth mass was also correlated with initial maternal mass ($r^2 = 0.808$, $P = 0.0149$). Although the variation in maternal mass loss was partly explained by her initial mass, this was not significant at the 0.05 level ($r^2 = 0.404$, $P = 0.0658$; fig. 2a). However, maternal mass loss during lac-
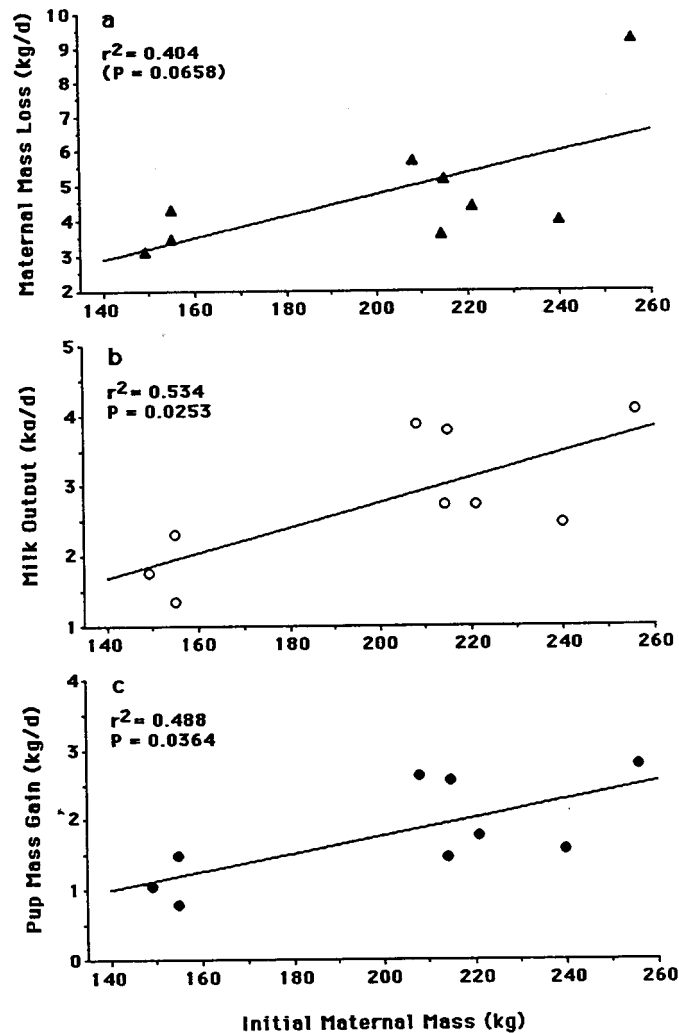
Fig. 2. The relationship of (a) maternal mass loss, (b) milk output, and
(c) pup mass gain over lactation to initial maternal mass in grey seals
(n = 9). For maternal mass loss, y = 0.030x − 1.320; for milk output,
y = 0.018x − 0.781; for pup mass gain, y = 0.013x − 0.771.

tation was significantly correlated with mass gain of her pup (r^2 = 0.583,
P = 0.0167; fig. 3). The mass transfer index (100 × pup mass gain/
maternal mass loss) was variable but averaged 40.2% among normal
pairs (n = 8). Although mass loss by female 8 was average, her mass
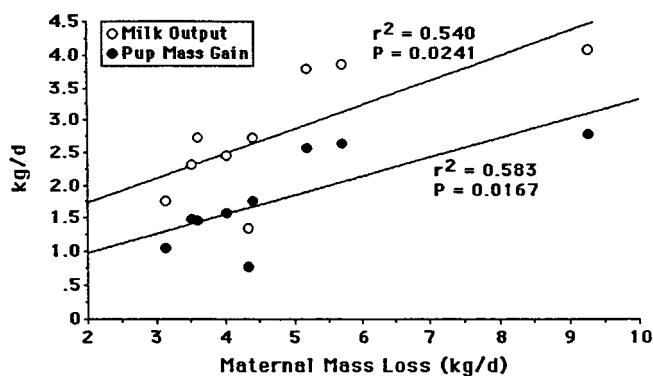transfer index was extremely low at 18.1% because her pup's mass gain
was low (table 1).

Fig. 3. The relationship of milk output and pup mass gain to maternal mass loss over lactation in grey seals (n = 9). For milk output, y = 0.372x + 0.996; for pup mass gain, y = 0.291x + 0.395.

Isotope Dilution and Body Composition

Isotope concentrations in the final two blood samples taken at 2.5 and 3 h following isotope administration were within 1% of one another, indicating that equilibration had occurred in all pups administered D_2O at the beginning (n = 9) and seven of eight pups at the end of the intake study. Deuterium oxide concentrations had not equilibrated (10% apart) within 3 h for one pup (pup 11) at the final capture. For equilibrated pups, initial and final body water pools averaged 11.9 ± 0.49 kg at 0-1 d (n = 9) and 20.9 ± 0.89 kg near weaning at 14-15 d (n = 5). The individual variation in pool size of pups over the course of lactation was largely explained by a linear relationship between body water pool and body mass (r^2 = 0.945, P = 0.0001; fig. 4). Thus, we used this general equation (fig. 4) to estimate final pool size for the two pups (pups 8 and 11) that did not have final equilibration values.

As expected, although changes in body mass of pups predicted changes in pool size, body water did not constitute a constant proportion of body mass between early and late lactation. Body water content averaged 70.2% ± 0.74% of body mass (coefficient of variation [CV] = 2.6%) in the six newborn pups (table 1) and constituted between 39.3% and 51.2% of mass at 14-15 d (44.5% ± 2.04%, n = 5, CV = 10.3%). Using equations (6)-(10), we estimated that pups contained 4.9% ± 1.01% fat and 18.6% ± 0.19% protein at birth (n = 6) and 28.1%-44.9% fat and 12.0%-15.7% protein at 14-15 d. The estimated composition of gain differed greatly among pups. Both daily gain in pool size and fat deposition in normal pups varied threefold, and protein deposition varied twofold (table 2). Fat constituted 44%–
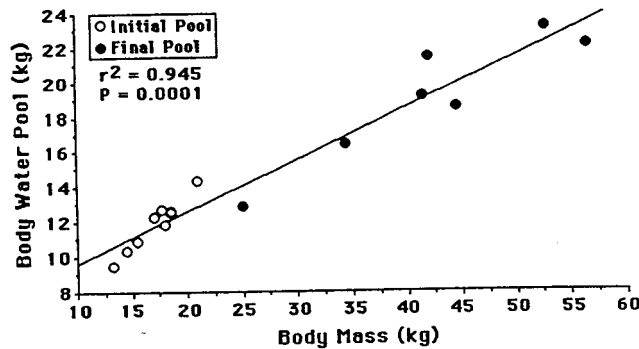
Fig. 4. Isotope equilibration measurements of initial (n = 9) and final body water pool size (n = 7) as a function of body mass in suckling grey seal pups (y = 0.30x + 6.71).

63% of gain and protein constituted 9%–14% of gain. Greater rates (kg/d) of mass gain (x) were accompanied primarily by greater rates of fat deposition (F_D = 0.70x − 0.26, r^2 = 0.958, P = 0.0001, n = 9), although protein

TABLE 2

Components of mass change over lactation in grey seal pups

	Mean ± SEM	Range
Daily mass gain (kg/d)	2.00 ± .220	1.06–2.78
Initial body composition:		
Body water (%)	69.4 ± .78	65.8–72.2
Body fat (%)	5.9 ± 1.06	2.2–10.9
Body protein (%)	18.4 ± .21	17.4–19.1
Gain per day:		
Gain in water (kg/d)	.55 ± .059	.26–.78
Fat deposited (kg/d)	1.15 ± .147	.64–1.71
Protein deposited (kg/d) . .	.21 ± .018	.12–.27
Percent of mass gain:		
Water % of gain	28.2 ± 1.52	24.8–37.8
Fat % of gain	57.8 ± 2.12	44.3–62.8
Protein % of gain	10.8 ± .53	9.1–13.7
Energy stored (MJ/d)	50.1 ± 6.11	28.0–73.0

Note. N = 8.

deposition also increased (P_D = 0.06x + 0.10, r^2 = 0.703, P = 0.0047, n = 9). As x increased, the estimated ratio of fat to protein in gain also increased (F_D/P_D = 1.96x + 1.35, r^2 = 0.563, P = 0.0199, n = 9). Although pup 8 was quite different in its pattern of gain (24% fat and 23% protein), its ratio of fat to protein in gain was explained by its very low growth rate. In normal pups, the amount of energy stored daily varied widely (28–73 MJ; table 2) and fat accounted for 89.6% ± 0.82% of energy stored (range 84.6%–92.0%, n = 8). Pup 8 stored only 11 MJ daily, and fat accounted for only 61.9% of energy storage.

In pups sampled more than twice (n = 8) the semilogarithmic decline in (C_t^*) with time was linear. Fractional water turnover rate averaged 0.064 in normal pups (n = 8; table 3) and was 0.060 in pup 8. Average daily water loss (0.64–1.25 kg/d) and average daily water gain (ΔP, 0.26–0.78 kg/d; table 2) were used to calculate the pup's average daily water intake (eq. [4]). Water intake varied twofold among all pups (table 3).

Milk and Energy Intakes

The ratio of milk intake to water intake averaged 1.91 ± 0.052 in normal pups but ranged from 1.62 to 2.09 (n = 8); the ratio for pup 8 (study period 1–7 d) was 1.34. Daily milk intakes varied from 1.77 to 4.06 kg/d among normal pups (n = 8; table 3; CV = 27.6%); milk intake of pup 8 was low at 1.35 kg daily. However, the ratio of milk intake to mass gain was relatively constant (CV = 4.5%), averaging 1.54 (n = 8; table 3), with the exception

TABLE 3

Water, milk, and energy intake of suckling grey seal pups

	Mean ± SEM	Range
Fractional water turnover (k)	.064 ± .0026	.054–.076
Water intake (kg/d)	1.57 ± .128	.95–1.99
Milk intake (kg/d)	3.03 ± .295	1.77–4.06
Milk intake/kg gain	1.54 ± .025	1.46–1.67
Energy intake (MJ/d)	68.7 ± 6.87	38.6–92.0
Energy intake/kg gain	34.7 ± .41	33.1–36.5
Efficiency of energy storage (%)[a]	72.1 ± 2.35	57.8–79.4

Note. N = 8.
[a] 100 × energy stored/energy consumed.

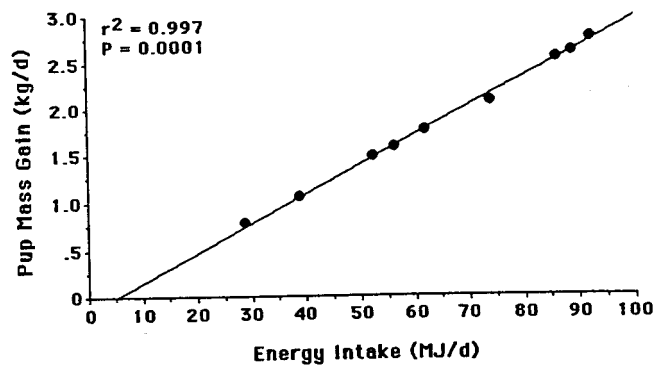Fig. 5. The relationship between daily mass gain and daily energy intake in suckling grey seal pups over lactation (n = 9; y = 0.031x − 0.156).

of pup 8, in which milk intake per unit of mass gain (1.73) was high. Milk intake (x) was highly correlated with mass gain (y) in all pups (y = 0.735x − 0.229, r^2 = 0.998, P = 0.0001, SE_b = 0.013, n = 9). Daily energy intakes varied from 39 to 92 MJ/d, but energy intake per gain was relatively constant at 33.1–36.5 MJ/kg (table 3). Although pup 8 had a very low daily energy intake of 29 MJ/d, its energy intake per unit of mass gain (36.9 MJ/kg) was similar to that of other pups. Energy intake and pup mass gain were also highly correlated (fig. 5; r^2 = 0.997, SE_b = 0.003). Although energy intake was correlated with energy stored (r^2 = 0.972, P = 0.0001), there was large variation in the efficiency of energy storage (energy stored/energy consumed; table 3) because pups with greater absolute rates of energy intake gained proportionately more fat than protein (fig. 6) and thus deposited
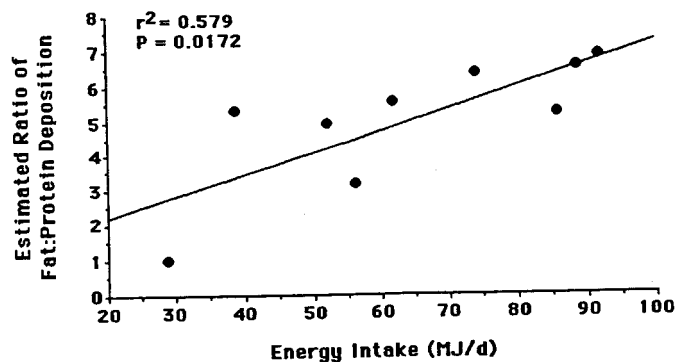

Fig. 6. The estimated ratio of fat to protein deposition in mass gain as a function of daily energy intake in suckling grey seal pups (n = 9; y = 0.062x + 0.983).

more energy. As a consequence, the efficiency of energy storage was significantly greater with greater rates of both mass gain ($r^2 = 0.533$, $P = 0.0256$) and milk intake ($r^2 = 0.545$, $P = 0.0232$). Hence, the efficiency of energy storage was lowest (39.7%) in pup 8 (mass gain 0.78 kg/d, energy intake 29 MJ/d) and highest (79.4%) in pup 2 (mass gain 2.78 kg/d, energy intake 92 MJ/d).

Given that pup mass gain was highly correlated with milk and energy intakes (table 3; fig. 5) and that the fat and energy content of milk increased dramatically between the first and second halves of lactation (fig. 1), we had reason to suspect that the daily intake of milk or energy might differ over the course of lactation. We therefore separately analyzed components of mass gain, water turnover, milk intake, and energy intake for the early (averaging 0–7 d postpartum) and late (averaging 7–13 d postpartum) periods of lactation in those pups that were recaptured at 6–7 d and subsequently ($n = 8$). For consistency, in each pup we used a two-point model for both early (0 d to first recapture) and late (first recapture to final recapture) periods, ignoring any intermediate recaptures. Calculations of body water pool, water turnover, and average composition of milk consumed by each pup were performed as described previously.

Many characteristics of growth and intake differed between early and late lactation (table 4). Pup mass gain was significantly greater (2.23 kg/d) during the second half of lactation than during the first half (1.68 kg/d, $P < 0.05$). Water turnover rate was lower in late lactation ($P < 0.01$), but, because average pool size was greater during this period, daily water intake did not differ between periods (table 4). Despite differences in average milk composition between early and late periods, the milk-intake-to-water-intake ratio did not differ significantly (1.84 ± 0.088 and 2.00 ± 0.082 early and late, respectively, $P = 0.065$) and thus daily milk intake also did not differ (table 4). However, milk intake per kilogram of mass gain was significantly lower in the later period ($P < 0.05$; table 4). The higher energy content of milk in the later period (fig. 1) resulted in a higher daily energy intake during the second half of lactation (76.3 MJ/d) than during the first (58.3 MJ/d, $P < 0.01$), but energy intakes per mass gain were similar for both periods (36.1 MJ/kg early and 35.0 MJ/kg late; table 4).

In summary, most of the variability in pup growth, both among individuals and between lactation stages, could be explained by milk and energy intakes. Differences in maternal mass (table 1) in turn explained much of the variability in the rate of milk output over lactation (i.e., milk intake by pups). Initial female body size in our study ranged from 149 to 256 kg, which is similar to the range in the population on Sable Island. Initial maternal mass accounted for much of the variability in milk output ($r^2 = 0.534$, $P = 0.0253$;

TABLE 4

Comparison of mass gain, milk intake, and energy intake as a function of lactation stage in grey seal pups

Lactation Stage (Days Postpartum)	Mass Gain (kg/d)	Water Turnover	Water Intake (kg/d)	Milk Intake (kg/d)	Milk Intake/kg Gain	Energy Intake (MJ/d)	Energy Intake/kg Gain
Early (0–7 d)	1.68 ± .246	.076 ± .0049	1.51 ± .136	2.78 ± .320	1.73 ± .074	58.3 ± 6.77	36.1 ± 1.46
Late (7–13 d)	2.23 ± .260	.054 ± .0029	1.55 ± .124	3.15 ± .321	1.44 ± .053	76.3 ± 7.95	35.0 ± 1.21
P Value (paired t)	.0328	.0039	NS	NS	.0192	.0094	NS

Note. $N = 8$. Values are means ± SEM.

fig. 2b) and thus in pup mass gain (fig. 2c). Similarly, maternal mass loss during lactation was significantly correlated with milk output ($r^2 = 0.540$, $P = 0.0241$) and thus with pup mass gain (fig. 3).

Discussion

Milk Composition

Changes in the milk composition of grey seals over lactation (fig. 1) were similar to patterns observed in the northern elephant seal (Riedman and Ortiz 1979), with the most rapid changes occurring prior to midlactation. Changes in milk fat content are more extreme in the elephant seal (12% at birth to about 50% in late lactation) than in the grey seal (40% at birth to 60% in late lactation), however. In both species milk protein levels remained at about 9% for most of lactation. Although milks of other phocid species have been shown to increase somewhat in fat content over lactation (Oftedal et al. 1987a), only milk of the southern elephant seal appears to exhibit similar patterns and extremes in change (e.g., 9%–51% fat; Peaker and Goode 1978). At an average of 59.8% fat during mid- to late lactation (8–15 d), milk fat content of the grey seal is comparable to that of the hooded seal (61.0% fat at mid- to late lactation; Oftedal, Boness, and Bowen 1988) and higher than that reported for other phocids (47%–54%; Oftedal et al. 1987a).

In a study of milk composition of grey seals from North Rona, Scotland, Baker (1990) obtained mean values that were generally similar to ours, except that protein content averaged 12% (9%–17%), which is substantially higher than that of grey seal milk in this study or in milks reported for other phocids (5%–9%; Oftedal et al. 1987a). However, Baker's protein values may be biased, given that the colorimetric method used (the biuret method) is influenced by the types of protein present (Gornall, Bardawill, and David 1949) and hence is only accurate if calibrated to the specific proteins in the samples analyzed. It seems unlikely that large population differences would occur in the proximate milk composition of a species (Iverson 1993).

There was good agreement of our bomb calorimetry data with that using calculations of energy content from energetic equivalents of either 9.39 (Bernard and Oftedal 1989) and 5.65 or earlier values of 9.11 and 5.86 (Oftedal and Iverson 1987) kcal/g fat and protein, respectively ($r^2 = 0.970$, $P = 0.0001$ for both), confirming that carbohydrate was a minor component. During mid- to late lactation, the energy content of grey seal milk averaged 24.8 ± 0.24 kJ/g (5.93 kcal/g), which again is comparable to that of the hooded seal at about 25.3 kJ/g (6.05 kcal/g; data from Oftedal et al. [1988], recalculated with 4.184 × 9.39 kcal/g fat).

Isotope Dilution Methodology

Initial and final equilibration data in this study indicated that individual changes in pool size of pups could be predicted from the relationship between body mass and pool size (fig. 4). Dilution of D_2O may overestimate body water content compared with carcass analysis, but recent studies have shown this overestimate to average only 2.8% in grey seals (Reilly and Fedak 1990), less than 2% in ringed seal, *Phoca hispida,* pups (Lydersen, Hammill, and Ryg, in press), and 1% in hooded seal pups (Oftedal et al. 1993). Thus, we believe our estimates of pool size are reliable.

Body water content can be used to estimate components of body composition (eqq. [6]–[10]) on the basis of evidence that the water and protein contents of LBM are approximately constant, particularly among individuals of a given species and age (Widdowson 1950; Reid, Wellington, and Dunn 1955; Reid et al. 1963; Roubicek 1969; Blaxter 1989). Despite differences in rates and patterns of growth, ratios of water to LBM (0.709–0.740) and protein to LBM (0.200–0.232) obtained from carcass analysis of pups of both phocid and otariid species appear to be similar (Oftedal et al. 1987b; Reilly and Fedak 1990; Oftedal et al. 1993). Although ratios of water to LBM and protein to LBM have been reported for grey seal pups (0.739 and 0.232, respectively, $n = 2$; Reilly and Fedak 1990), ratios used in the present study were based on a larger data set from the harp seal, which included both newborns and older pups (Worthy and Lavigne 1983; G. A. J. Worthy, personal communication). Using the values of Reilly and Fedak would not have resulted in a significant difference in our average milk intakes (3.08 kg/d vs. 3.03 kg/d; table 3).

Using individual, rather than average, rates of fat and protein deposition to calculate milk intake for each pup (eq. [5]) reduced the variability (r^2 = 0.997 vs. 0.938) in the relationship between milk energy intake and growth (fig. 5), thus increasing the precision of predictions. This finding may be characteristic of species, such as most phocids, in which the rate of fat deposition is both high and variable. Although the ratio of milk to water intake is expected to vary with differences in milk composition, our results show that this ratio may also differ among individuals in relation to growth rate, composition of gain, and intake rates. The relationship of milk to water intake is not simple, however, as demonstrated by the finding that this ratio did not differ significantly between early and late lactation (1.8 vs. 2.0) even though milk composition changed substantially (table 4). Although milk was lower in water later in lactation, this was counteracted by the greater contribution of metabolic water from milk fat catabolism.

Pup Growth and Milk Energy Intake

On the day of birth pups averaged 17.0 kg (table 1), which is about 16% larger than that reported for British populations (Coulson 1960; Coulson and Hickling 1964). Average maternal mass at parturition (216 kg) was also about 24% larger than reported for British populations (174 kg; Fedak and Anderson 1982). Pup birth mass in this study averaged 8.0% of initial maternal mass, which is similar to values reported for several populations of grey seals (8.4%–8.6%) and within the range of many other phocids (Bowen 1991).

Although the sample in the present study was too small to address possible differences between male and female pups, in a more comprehensive study of pup growth on Sable Island, Bowen et al. (1992*b*) found that male pups do not grow more rapidly than female pups. Mass gain of pups we studied was highly variable (table 1) and averaged 32% lower (1.9 kg/d) than the population mean for Sable Island (2.8 kg/d, Bowen et al. 1992*b*). Our study was conducted late in the breeding season after the peak period (1.5 wk) in which 75% or more of births occur. Pup growth on Sable Island after the peak of pupping has been found to be about 30% lower than growth of pups born during the peak, possibly owing to adult male harassment of females and interruptions of suckling (Boness et al. 1991). Nevertheless, the variability in pup growth rates in our study was highly correlated with milk and energy intakes, which in turn were largely accounted for by maternal size and mass loss. Thus, we believe our results can be used to predict values for the grey seal population generally. The average Sable Island grey seal pup gaining 2.8 kg/d probably consumes about 4.1 kg milk daily or 95 MJ/d.

Energy intake also explained the lower growth rates observed in pups during early lactation (table 4). Although similar amounts of milk were consumed over lactation, it required less milk for a pup to gain 1 kg mass during the second half of lactation than during the first half (table 4), because milk became more energy dense (fig. 1). Reduced growth rates early in lactation have previously been reported in grey seals from North Rona (Anderson and Fedak 1987) and Sable Island (Bowen et al. 1992*b*) and in southern elephant seals (McCann et al. 1989), which may be in part due to the lower energy content of milk near parturition. Thus, in species where energy density of the milk increases significantly over lactation, the latter half of lactation may be more critical in attaining normal weaning mass. It is not known whether the rate of milk or energy intake is linear over lactation in the northern elephant seal (see, e.g., Ortiz et al. 1984; Costa et al. 1986), but, since milk composition changes (Riedman and Ortiz 1979) in a pattern like that of the grey seal, similar relationships among pup growth, energy intake, and energy deposition may exist. Our results suggest that extrapo-

lations from one portion of lactation to another may be inaccurate. Thus, comparisons among species or populations should be made for similar portions of lactation.

Grey seals are born with a body fat content (5%) similar to that of the harp seal (6%–7%; Worthy and Lavigne 1983) and lower than that of the Weddell seal, harbor seal, and hooded seal (8.5%, 11%, and 14%, respectively; Tedman and Green 1987; Bowen, Oftedal, and Boness 1992a; Oftedal et al. 1993). Although early postnatal growth is primarily LBM in most mammals (Blaxter 1989), phocid pups are exceptional in that their suckling period is brief, during which growth is primarily fattening (Worthy and Lavigne 1983; Oftedal et al. 1989). In our study, mass gain, the ratio of fat to protein in gain, and the efficiency of energy storage increased as energy intake increased, demonstrating the predominance of fattening at high fat and energy intakes. Although there may be a limit to skeletal and muscle tissue growth, it is not known whether there is a limit to rates of fat deposition by pups (Iverson, Sampugna, and Oftedal 1992). The high efficiency of energy retention in phocid pups is presumably facilitated by the efficient digestion of milk triglycerides (Iverson et al. 1992) and the direct incorporation of milk fatty acids into lipid stores (Iverson 1988; Iverson 1993). Our results suggest that pups with greater energy intakes are likely to be fatter at weaning and thus may be better prepared for the postweaning fast.

Although variable (40%–79%), the average efficiency of energy storage in grey seal pups in the present study (72%) was somewhat lower than that reported in the northern elephant seal (82%; Costa et al. 1986), another species with high energy intake rates and relatively low metabolic costs of pups during the suckling period. However, the method of estimating body composition in elephant seal pups may have overestimated fat and energy deposition (Oftedal et al. 1993). We may have underestimated the average efficiency of grey seal pups, because we measured pups late in the pupping period when growth rates (and presumably intakes) are reduced (see, e.g., Boness et al. 1991). Fedak and Anderson (1982) estimated an efficiency of energy storage for grey seal pups on North Rona that was lower (62%), but this value was based on indirect estimates of energy intake and is not directly comparable to our estimate for Sable Island. However, a lower efficiency of energy storage might be expected in the British population given the lower overall growth rates; in our study, lower growth rates were associated with lower efficiencies of storage (e.g., fig. 6).

Maternal Size, Mass Loss, and Milk Energy Transfer

The relationship between maternal size and milk energy output explained much of the variability in growth and efficiency of energy storage in grey

seal pups. In a phocid such as the grey seal, the energy that a female brings ashore prior to parturition must be sufficient to support lactation. At a rate of mass loss of up to 9 kg/d and a milk output of up to 92 MJ/d (table 1), lactation is expensive and blubber lipid will become depleted (see, e.g., Bowen et al. 1992a). Thus, if a female has low energy reserves, it seems likely that lactation will be difficult to sustain. Conversely, the greater her absolute reserves, the greater contribution a female may be able to make to her pup over the lactation period. The large range of initial maternal body size in our study allowed us to examine the influence of initial mass and thus presumably extent of energy stores on milk output and pup growth. Our results show that larger females had greater milk output and produced faster-growing pups (fig. 2b and c). Faster-growing pups were also fatter and thus likely better prepared for the postweaning fast that is characteristic of this and other phocid species. This suggests that larger mothers may produce not only larger but better-quality offspring.

Maternal mass loss tended to increase with increasing initial mass (fig. 2a), but the large variation in this relationship suggested that other costs associated with lactation and maintenance may vary among females. For instance, late in the breeding season, encounters with males may be more prevalent (Boness et al. 1991), and in more densely populated rookeries energetic expenditure associated with defense of pups during agonistic encounters with other females may be higher. ⹋

Data from the present study on maternal size, energy transfer, and pup growth in grey seals can be compared with data from other phocid species in which lactation is essentially a "closed system," that is, where females primarily fast throughout lactation and where pups are relatively inactive. A positive relationship between maternal size and pup growth rate has been reported in several such phocid species. In southern elephant seals, maternal body mass at parturition is a strong indicator of both subsequent maternal mass loss and pup mass gain (McCann et al. 1989). In grey seals on North Rona, Anderson and Fedak (1987) found a positive correlation between maternal size and mass gain, but only for female pups. Kovacs, Lavigne, and Innes (1991) also reported that growth rate of harp seal pups was correlated with maternal mass loss but not with initial maternal size. However, in the latter two studies, maternal masses at parturition were not measured, but extrapolated from rates of maternal mass loss using descriptive stages to estimate pup age and hence days postpartum. Although it appears that maternal mass loss may explain pup mass gain in phocids that fast during lactation (see, e.g., fig. 3), the extent to which maternal size at parturition may influence these characteristics across species is not clear from existing data. Data from southern elephant seals and harbor seals indicate that larger

females do have greater absolute fat and energy reserves (Fedak et al. 1989; Bowen et al. 1992*a*).

Among phocids that fast during lactation, it appears that females lose a similar proportion of initial body mass over the lactation period. To compare our data with those of other populations or species, we use only those four mother-pup pairs (pairs 2, 5, 6, and 7) that were studied for most of lactation (i.e., 0–14 or 15 d, table 1). If we assume that lactation is approximately 16 d on Sable Island (see, e.g., Boness et al. 1991), the average 233-kg female of these four pairs would lose about 39% (92 kg) of her initial body mass over lactation. This is comparable with that estimated for grey seals on North Rona (38%–39%; Fedak and Anderson 1982; Anderson and Fedak 1987) and similar to the percentage total mass loss in northern and southern elephant seals at 42% and 37%, respectively (Costa et al. 1986; McCann et al. 1989).

This average grey seal female in our study would expend 1,183 MJ in milk output or about 6.3 MJ/kg average body mass over lactation. However as stated previously, our study was conducted late in the breeding season, when pup growth rates were somewhat reduced. Thus, the average pup of these four pairs gained only 2.2 kg daily. Although we have evidence that the average body mass of breeding females on Sable Island is near 233 kg at the beginning of lactation, late in the breeding season harassment by male grey seals may prevent females from efficiently delivering milk energy at their full potential (see, e.g., Boness et al. 1991). Assuming a pup growth rate of 2.8 kg/d (Bowen et al. 1992*b*) and an energy intake of 95 MJ/d (fig. 5), a female grey seal on Sable Island may actually expend about 1,526 MJ over lactation, or closer to 8.0 MJ/kg average body mass. In comparison, on the basis of isotope studies, the much larger (429 kg average body mass) northern elephant seal expends about 2,580 MJ in milk energy output over a longer lactation period of 26.5 d (Costa et al. 1986). On a body mass basis, this is lower (6.0 MJ/kg) than in the grey seal. Estimates of milk energy output for a British population (North Rona) of grey seals were based on mass changes, with the assumption that there were constant energy values for pup mass gain and rates of energy conversion to storage (Fedak and Anderson 1982; Anderson and Fedak 1987). These studies report that pups growing at a rate of 1.6–2.0 kg/d would consume 69–79 MJ/d, which is equivalent to 40–43 MJ/kg pup mass gain. On the basis of our results, this appears to overestimate milk energy output by 16%–23%. If grey seal pups on North Rona do not face significantly higher metabolic costs, they may actually consume closer to 58–68 MJ/d (see, e.g., fig. 5), which is equivalent to 35 MJ/kg mass gain (see, e.g., table 3). Thus, over the 18-d lactation period (used by Anderson and Fedak [1987]), we calculate that grey seal

females on North Rona may expend an average of about 1,133 MJ in milk output over lactation, or about 8.1 MJ/kg average mass, which on a body size basis is the same as our average estimate for the Sable Island population.

Although the gross composition of milk in mammals is species specific (Jenness 1974; Oftedal 1984), the rate of milk synthesis and secretion depends on, among other things, the availability of metabolic substrates to the mammary gland (Mepham 1983). Phocids secrete extremely concentrated milks at high rates. Hence, maternal body size (i.e., absolute fat and energy stores) may have a profound impact on the ability to sustain milk output in species that fast during lactation. Although all phocids deplete fat stores during lactation, the influences of body size may be less pronounced within species that supplement the use of these stores with feeding (e.g., the harbor seal; Bowen et al. 1992*a*). Despite high rates of milk energy output in all phocids, species differ in the extent to which pups fatten. For instance, in species in which pups are relatively inactive during brief lactation periods (e.g., the grey seal, hooded seal, harp seal, and the elephant seals), energy intake may translate directly into growth rate, whereas in species where pups begin swimming during longer lactation periods (e.g., the harbor seal, Weddell seal, and monk seal), the relationship between energy intake and growth may be reduced and relatively variable, as has been demonstrated in otariids (Costa and Gentry 1986; Oftedal et al. 1987*b*).

Data from the present study clearly demonstrate that differences in energy transfer can be used to predict differences in pup growth as well as explain differences observed in maternal mass loss in a species where mothers fast during a brief lactation period. Differences in maternal mass and available energy stores may account for much of the reported variation in pup growth, both within and between populations, in phocids such as the grey seal and southern elephant seal (reviewed in Bowen 1991). Thus, these differences in maternal energy stores may in turn effect pup survival (Coulson and Hickling 1964). Unfortunately, for most species, data on maternal mass and energy stores at parturition are unknown. Previous studies have also suggested that other factors, such as maternal age and experience, rookery density, and timing of pup births, can also influence pup growth. Further study is needed to assess the relative importance of maternal size and these other factors on energy transfer and pup growth during lactation.

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