Individual variation in milk composition over lactation in harbour seals (*Phoca vitulina*) and the potential consequences of intermittent attendance

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Abstract: We studied milk composition over the course of lactation in 21 harbour seal (*Phoca vitulina* L., 1758) females on Sable Island, Nova Scotia. Milk fat content increased significantly from 40.8% ± 1.01% at parturition to 50.2% ± 1.39% at day 7 and then remained relatively constant throughout the remainder of lactation. Changes in dry matter mirrored changes in fat content. Protein content averaged about 9% over mid to late lactation. There was considerable between-individual variation in the composition of milk and how it changed over lactation, particularly in milk fat content (CV 9.1%–11.4%). In three females that were separated from their pups for 4–6 days, milk fat content declined by 20%–23% and milk protein content increased by 6%–11%. These changes in milk composition indicate that changes in mammary gland function occur rapidly following the onset of milk stasis in harbour seals. The rapid response of the mammary glands to separation suggests that, in direct contrast to the glands of otariids, the glands of harbour seals rely on regular evacuation to maintain normal function. These results suggest that there may be a significant physiological constraint on the duration that harbour seal females, and presumably other phocid seals, can forage without negatively affecting energy transfer to their pups.

Résumé : Nous avons étudié la composition du lait durant la période d'allaitement chez 21 phoques communs (*Phoca vitulina* L., 1758) femelles sur l'île de Sable, Nouvelle-Écosse. Le contenu lipidique du lait augmente significativement de 40,8 % ± 1,01 % à la mise bas à 50,2 % ± 1,39 % au jour 7; il reste ensuite relativement constant pendant le reste de la période d'allaitement. Les variations de la matière sèche reflètent celles du contenu lipidique. Du milieu à la fin de la période d'allaitement, le contenu protéinique est en moyenne d'environ 9 %. Il y a des variations individuelles importantes de la composition du lait et des changements de composition au cours de l'allaitement, particulièrement du contenu lipidique (C.V. 9,1 % – 11,4 %). Chez trois femelles séparées de leurs petits pendant 4–6 jours, le contenu lipidique du lait a diminué de 20 % – 23 % et le contenu protéinique a augmenté de 6 % – 11 %. Ces changements de composition du lait indiquent que les modifications de la fonction de la glande mammaire se produisent rapidement après la stase du lait chez les phoques communs. La réaction rapide des glandes mammaires à la séparation laisse croire que, à l'inverse de celles des otariidés, les glandes des phoques communs dépendent d'une évacuation régulière pour maintenir leur fonction normale. Ces résultats indiquent qu'il peut exister une contrainte physiologique significative sur la période de temps que les femelles du phoque commun, et probablement aussi d'autres phoques phocidés, peuvent partir à la recherche de nourriture sans affecter négativement le transfert d'énergie aux petits.

[Traduit par la Rédaction]

Introduction

In pinnipeds, lactation strategies are constrained by the spatial and temporal separation between giving birth on land or ice and acquiring nutrients for milk production at sea (Bonner 1984). Among phocid seals (family Phocidae), this constraint has led to the evolution of a brief (4–50 days, Bowen 1991), intense lactation period during which females secrete large volumes of energy-dense milk (40%–60% fat, Schulz and Bowen 2004) while supporting most or all of the energetic costs of lactation from body energy stores (Oftedal et al. 1987; Boness and Bowen 1996). Weaning is abrupt in phocids, and pups must rely on the blubber deposited during the suckling period to survive the post-weaning fast and transition to nutritional independence (Bowen 1991). Therefore, the ability of females to rapidly transfer sufficient energy to their offspring is a critical determinant of maternal reproductive success in phocid seals.

One of the factors essential to our understanding of variation in the pattern of energy transfer between phocid females and their offspring, both within and between species, is a thorough knowledge of milk composition and milk energy output over the course of lactation (Oftedal et al. 1987). To date, individual variation in aspects of lactation performance related to milk secretion and the consequences for offspring growth have been well characterized in only a few species of large-bodied (>150 kg) phocids that fast throughout the lacta-

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tion period (Mellish et al. 1999*a*, 1999*b*; Crocker et al. 2001). Small-bodied phocid seals (those weighing less than 100 kg, on average) remain poorly studied, and available data on milk composition and milk energy output are currently limited to estimates based on a small number of samples (Lydersen et al. 1992) and average values reported from an unpublished cross-sectional study (see Boness and Bowen 1996; Schulz and Bowen 2004). As a result, relatively little is known about variation in patterns of energy transfer and allocation over lactation in small-bodied phocids or the consequences for pup mass gain. Given that 6 of the 18 phocid species are small-bodied, this lack of data limits our ability to make inferences about the relative roles of factors such as body size and phylogeny in the evolution of pinniped lactation strategies (Schulz and Bowen 2004).

The harbour seal (*Phoca vitulina* L., 1758) is a small-bodied phocid with females weighing an average of 85 kg at parturition (Ellis et al. 2000). As a result, most females do not carry sufficient body energy stores to fast throughout the 24 day lactation period (Bowen et al. 1992, 2001). Consequently, females exhibit a foraging-cycle lactation strategy somewhat analogous to that observed in otariid seals (fur seals and sea lions), with females beginning short foraging trips, lasting an average of 6.5 h, at around 8 days postpartum (Boness et al. 1994; Thompson et al. 1994; Bowen at al. 2001). Pups generally suckle every 3–4 h and the duration of suckling bouts increases over the course of lactation (D. Boness, personal communication). As part of a larger study examining the relationships between maternal foraging effort, food intake, and lactation performance in harbour seals (Bowen et al. 2001), we obtained milk samples from individual females over the course of lactation with two objectives: (1) to compare the overall patterns of change in milk composition to those previously observed in large-bodied phocid species and (2) to examine individual variation in the composition of milk over lactation.

Methods

Field sampling

We obtained three or four milk samples from each of 21 harbour seal females over the course of the lactation period during May and June of 1990 (n = 13) and 1991 (n = 8) on Sable Island, Nova Scotia, Canada (43°90′N, 60°00′W). On the day of parturition, each mother–pup pair was captured on the beach as previously described (Bowen et al. 1992) and weighed to the nearest 0.1 kg. A milk sample (approximately 60 mL) was collected by suction using a 60 cm^3 syringe with the tip removed, following a 1.5 cm^3 intramuscular injection of oxytocin (Vétoquinol N.-A. Inc., Quebec; 20 IU·mL^{-1}) to facilitate milk letdown. Milk samples were stored in 30 mL Nalgene bottles at –20 °C until analysis. Prior to release, pups were given an individually numbered hind-flipper tag and the pair was marked with a fast-drying fluorescent paint (V-285, Lenmar Inc., Baltimore, Maryland) to permit identification from a distance. Pairs were observed the day following initial capture to verify that mothers and pups were still together, and daily thereafter. Observations of separation and fostering were recorded.

At each recapture, females and pups were weighed and a milk sample was collected as described above. Due to some constraints on field captures, the timing of recaptures differed slightly between the two years. In 1990 we planned to sample females four times over the course of lactation: on the day of birth (day 0) and during early (day 4 or day 7 postpartum (pp)), mid (day 14 pp), and late lactation (day 19–21 pp). All 13 females were sampled at parturition, during early lactation, and, with the exception of one female that could not be relocated (D290), again at mid lactation. Four females were not sampled during late lactation: two had lost their pups in mid lactation (D1190, D1490), one weaned at day 17 (D1590), and one could not be recaptured (D590). In 1991, all eight females were sampled three times: on the day of birth, during mid lactation (at day 10 or day 14 pp), and during late lactation (day 19–21 pp).

All sampling protocols were conducted in accordance with the requirements of the Canadian Council of Animal Care and were approved by the Dalhousie University Committee on Laboratory Animals, protocol No. 90-028.

Sample analyses

All milk samples (n = 71) were analysed for proximate composition. Total milk fat and dry matter were analysed in duplicate. Dry matter was determined following forced convection drying 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 International 1980). Single replicates were analysed for protein by macro-Kjeldahl. Samples were not analysed for carbohydrate content, as it has been previously shown that this is a very minor component of phocid milks (Iverson 1993; Iverson and Oftedal 1995). The energy content of the milk was calculated using values of 39.3 MJ·kg^{-1} and 23.6 MJ·kg^{-1} for fat and protein, respectively.

Data analyses

To account for the serial correlation in the data collected within females, changes in the proximate composition of milk over the course of lactation were analysed using linear mixed-effects models (SPSS 11.5, SPSS Inc., Chicago). We fitted a model with time period as the fixed effect and female as the random effect using the AR1 covariance structure. Differences among time periods were tested using pairwise, Bonferroni-adjusted comparisons. Data from all females were used in the analyses with the exception of the final sampling points for D1190 (day 12) and D1490 (day 14) and the last two sampling points for D890 and D1290. Both D1190 and D1490 were sampled following the loss of their pups at day 8 and day 11, respectively. Since the samples obtained following the loss of the pups would not be representative of continuous lactation, their proximate values were not used. Similarly, D890 and D1290 were separated from their pups for 2 (day 14–16) and 6 (day 8–14) days, respectively, and, therefore, samples obtained following separation were not used.

Results

At parturition, females averaged 85.6 ± 2.35 kg (n = 21) and represented the full range of initial body masses found in the population (65.5–105.3 kg). Pups averaged 10.9 ±

Table 1. Proximate composition of milk over the course of lactation in 21 harbour seal (*Phoca vitulina*) females.

Component	Day 0 (n = 21)	Day 4 (n = 2)	Day 7 (n = 11)*	Day 10 (n = 5)	Day 14 (n = 11)†	Day 19–21 (n = 15)	P
Water (%)	46.2±0.95a	40.8±3.02ab	37.4±1.30b	39.0±1.92b	37.1±1.30b	38.3±1.12b	<0.000
Dry matter (%)	53.8±0.95a	59.2±3.02ab	62.6±1.30b	61.0±1.92b	62.9±1.30b	61.7±1.12b	<0.000
Fat (%)	40.8±1.01a	46.7±3.22ab	50.2±1.39b	49.3±2.05b	50.9±1.39b	49.0±1.19b	<0.000
Protein (%)	9.9±0.22a	8.8±0.69ab	9.2±0.30ab	8.9±0.44ab	8.3±0.30b	9.6±0.26a	0.001
Energy (MJ/kg)	18.4±0.39a	20.4±1.24ab	21.9±0.54b	21.5±0.79b	22.0±0.54b	21.5±0.46b	<0.000

Note: Values are the estimated marginal means ± SE calculated using linear mixed-effects modelling. Values followed by different letters are significantly different at the 0.05 level.

*Includes Day 6 values for D590.

†Includes Day 13 values for D1590.

0.20 kg (n = 21) at birth and pup mass gain averaged 0.61 ± 0.034 kg·day^{-1} (n = 17) between 0 and 19–21 days pp, with pup mass averaging 23.2 ± 0.77 kg at 19–21 days pp. Lactation length averaged 23.5 ± 0.74 days in females that did not lose their pup prematurely (n = 17). We were able to obtain weaning mass for 9 pups, which averaged 23.8 ± 1.34 kg.

Milk composition changed significantly over the course of lactation (Table 1). Fat content increased significantly from day 0 to day 7 and then remained relatively constant throughout the remainder of lactation. Changes in dry matter mirrored the changes in fat, and water content changed inversely. Changes in protein content were relatively minor over lactation. Protein appeared slightly elevated at day 0 relative to the remainder of lactation, but only the estimated mean for day 14 was significantly lower. Although protein appeared to be elevated at day 19–21, only 4 of the 21 females had elevated protein content at this time. Therefore, we believe this result represents sampling error rather than biological significance. Changes in energy content primarily reflected the changes in milk fat, with a significant increase from day 0 to a plateau by day 7.

Among the 17 females with no known separations during lactation, there was considerable between-individual variation in milk composition and how it changed over the course of lactation (Fig. 1). Milk fat content among females at day 0 ranged from 30.6% to 47.2% (CV 11.4%). Milk fat content increased following parturition in all but one female (D1590), although the magnitude of change was highly variable among females. For example, D1390's milk fat increased by 18% between day 0 and day 7, an increase threefold greater than that observed in the milk of D990 over the same period (Fig. 1). The level of individual variation in milk fat content remained high over mid lactation (day 10–14), ranging from 43.2% to 60.4% (CV 9.1%, Fig. 1). In general, milk protein content declined following parturition, with increases observed in only 2 of the 17 females (D490, D590).

In the females that lost their pups (D1190, D1490) or became separated from their pups for 6 days (D1290), milk composition changed substantially, with a 20%–23% decline in milk fat and a 6%–11% increase in protein following 4–6 days of separation (Fig. 2). After the successful reunion with her pup, D1290's milk fat content increased to a level at day 21 that was more than 7% higher than the level prior to separation, with a corresponding 12% decrease in the level of milk protein (Fig. 2). Consistent with reduced milk intake over this period, the 6 day separation of D1290 from her pup was associated with a decrease in the rate of pup mass gain from 0.40 kg·day^{-1} (day 0–7) to 0.21 kg·day^{-1} (day 7–14). Similar changes in milk composition were observed for D890 (Fig. 2), which had a known period of separation of 2 days between day 14 and day 16 of lactation. As with the milk composition of the females separated from their pups for longer periods, this brief period of separation was associated with decreased milk fat content, increased protein content (Fig. 2), and a decrease in pup mass gain from 0.73 kg·day^{-1} (day 0–7) to 0.57 kg·day^{-1} (day 7–16). Similar to the changes observed in the milk composition of D1290, D890's milk fat content increased rapidly following the reunion with her pup on day 16, and this increase was accompanied by a rapid decline in protein content.

Discussion

The overall changes in the milk composition of harbour seals during lactation (Table 1) are similar to the patterns previously reported for other phocid seals. The rapid increase in milk fat content during early lactation was similar to that observed over a comparable period in both grey seals (*Halichoerus grypus* (Fabr., 1791), Iverson et al. 1993) and harp seals (*Phoca groenlandica* Erxleben, 1777, Oftedal et al. 1996); however, the magnitude of the increase was less dramatic in harbour seals, with only a 9% increase in milk fat content compared with the 20% increase from similar levels at parturition observed in both the grey seal and the harp seal. Although the milk fat content at mid lactation in harbour seals (50%) was lower than the values reported for grey (60%, Iverson et al. 1993), harp (57%, Oftedal et al. 1996), and hooded seals (*Cystophora cristata* (Erxleben, 1777); 61%, Oftedal et al. 1988), it was comparable to the peak milk fat content estimated for the southern elephant seal (*Mirounga leonina* (L., 1758); 51%, Peaker and Goode 1978; 52%, Carlini et al. 1994), the northern elephant seal (*Mirounga angustirostris* (Gill, 1866); 50%, Riedman and Ortiz 1979), and the Weddell seal (*Leptonychotes weddellii* (Lesson, 1826); 51%, Tedman 1985) and was consistent with the average value reported for harbour seals from an unpublished cross-sectional study (50%, see Boness and Bowen 1996; Schulz and Bowen 2004). The slightly elevated level of protein at parturition also corresponded to the patterns previously reported for phocid seals (Peaker and Goode 1978; Iverson et al. 1993; Oftedal et al. 1988, 1996; Mellish et al. 1999*a*) and was likely a result of the higher protein content typical of colostrum. Protein content remained relatively low throughout lactation, averaging 9% over mid to

Fig. 1. Changes in milk fat and milk protein content during lactation in 17 harbour seal (*Phoca vitulina*) females.

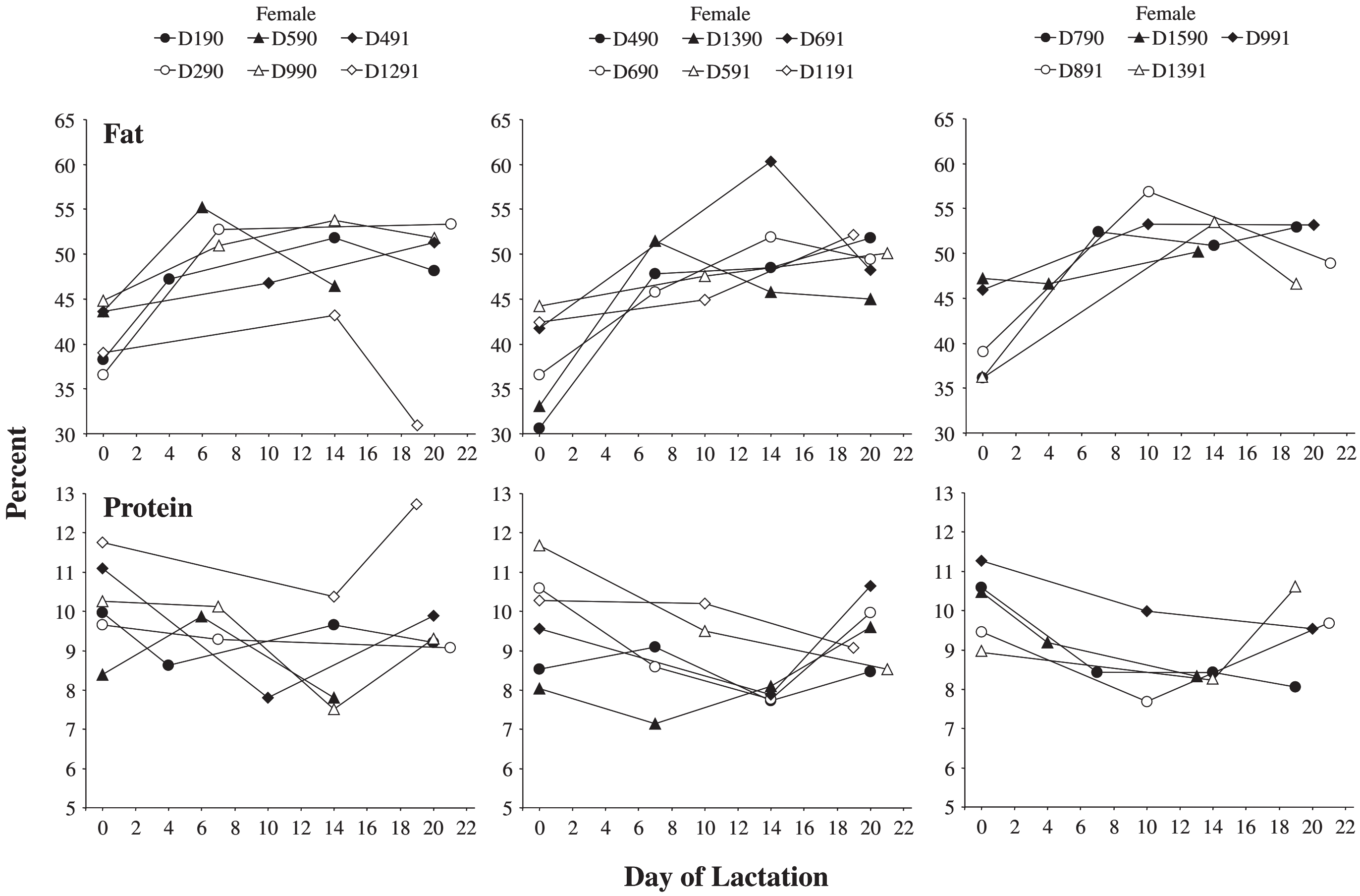
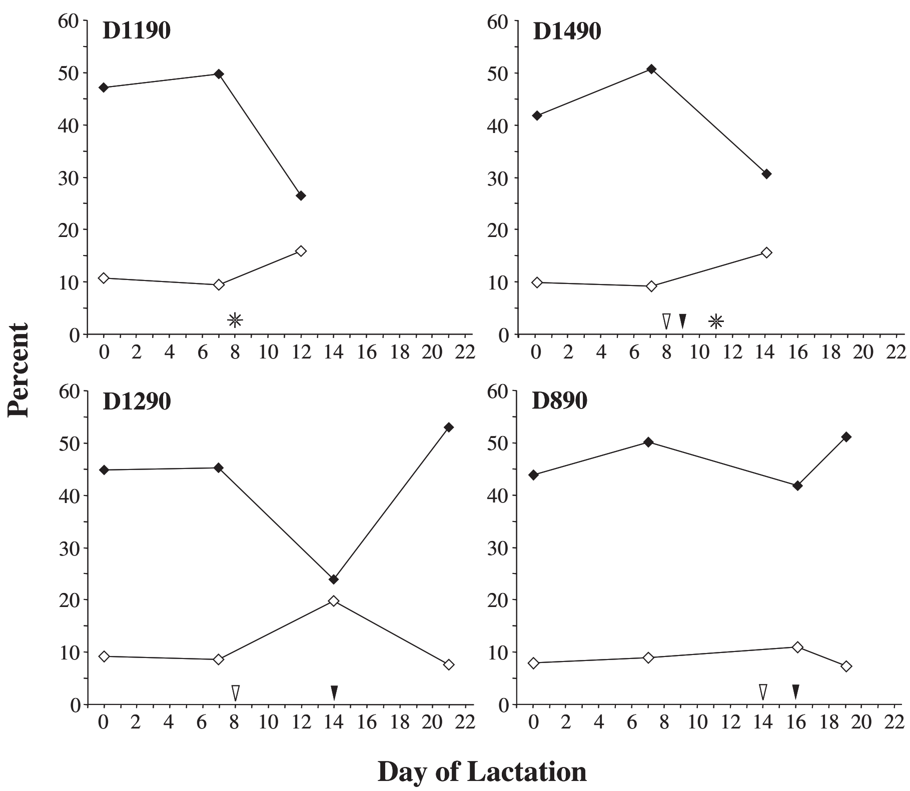


Fig. 2. Changes in milk fat (◆) and milk protein (◇) content in four harbour seal females following periods of prolonged separation from their pups. ▽, pair noted as separated; ▼, pair reunited; *, pup noted as missing.



late lactation, which is within the range typical of phocid seals (5%–12%) and pinnipeds in general (5%–14%; Schulz and Bowen 2004).

Although the changes observed in milk fat and protein content over lactation within individual harbour seal females were generally consistent with the overall patterns, there was considerable variation among females in the composition of their milk at parturition and in the magnitude of the change in components following parturition (Fig. 1). For example, milk fat content varied by as much as 17% among females on day 0 and the rate of increase varied up to threefold among individuals following parturition. Substantial individual variation in milk composition has also been previously documented in grey seals (Mellish et al. 1999*a*). How milk composition is regulated remains poorly understood, and the factors that may be responsible for the level of individual variation observed among both harbour seals and grey seals are unclear. Although factors such as the level of an individual's body nutrient stores have been shown to influence milk yield in phocid seals (Mellish et al. 1999*a*; Crocker et al. 2001) and in mammals in general (see Iverson 1993), there is no evidence to suggest that they affect the proximate composition of milk in phocids (Iverson 1993; Mellish et al. 1999*a*) or any other mammal (Jensen 1995). Among domestic species, factors such as genotype and parity are known to have a significant influence on individual milk composition (Maijala and Hanna 1974; Peris et al. 1997; Sevi et al. 2000); however, these factors were not known for the females in our study and their potential contribution to the variation observed in wild populations remains to be determined.

In most mammals, prolonged separation from suckling initiates mammary gland involution, resulting in changes in mammary epithelial cell activity, gene expression, and gland morphology, the timing and extent of which vary by species (Lascelles and Lee 1978; Capuco and Akers 1999; Wilde et al. 1999). Among otariid seals, females alternate periods of attendance, during which they suckle pups every few hours (Oftedal et al. 1987; Bowen 1991), with foraging bouts, which can last from days to weeks depending on the species and stage of lactation (Gentry and Kooyman 1986; Campagna and Le Boeuf 1988; Higgins et al. 1988; Higgins and Gass 1993; Gales and Mattlin 1997; Francis et al. 1998; Mattlin et al. 1998; Georges and Guinet 2000; Melin et al. 2000; Arnould and Hindell 2001). Although the mechanism is not understood, otariid seals show no loss of mammary gland function during these periods of separation, returning with full mammae and, in some species, higher milk fat content than at departure (Costa and Gentry 1986; Arnould and Boyd 1995; Goldsworthy and Crowley 1999; Ochoa-Acuna et al. 1999; Georges et al. 2001).

Among phocid seals, the females of at least five species are known to spend some portion of lactation at sea (Tedman and Bryden 1979; Boness et al. 1994; Lydersen 1995; Lydersen and Kovacs 1996; Bowen et al. 1999; Gjertz et al. 2000); however, how periods of separation may affect mammary gland function in these species has never been investigated. Our results demonstrate that, in direct contrast to otariids, periods of prolonged separation have a significant effect on mammary gland function in harbour seals. In the females that lost or became separated from their pups for more than 2 days, milk composition changed dramatically, with a 20%–23% decline in milk fat and a 6%–11% increase in protein after 4–6 days of separation (Fig. 2). Because females were not sampled until several days after separation, how quickly the changes in milk composition occurred is unknown; however, the reduced milk fat and increased protein content observed for D890 following a known separation of 2 days (Fig. 2) indicate that changes in mammary gland function occur rapidly following the onset of milk stasis in harbour seals. Such a rapid response to separation is consistent with data from studies of initiated involution in other species (Calvert et al. 1985; Grigor et al. 1986) and may explain some of the individual variation observed in the mid-lactation milk composition of the harbour seal females that sustained lactation (Fig. 1). Although our observation records were not detailed enough to establish attendance patterns, the reduced milk fat content observed in D590, D790, and D1390 during mid lactation (Fig. 1) may be indicative of periods of separation that were of sufficient duration to initiate changes in mammary gland function. The rapid response of the mammary glands to separation suggests that while the lactation strategy of harbour seals bears some similarity to that exhibited by otariids, their mammary gland physiology does not, and that the glands of harbour seals, and potentially all phocids, rely on regular evacuation to maintain normal function.

The rapid recovery of milk composition following the reunion with pups in both D890 and D1290 (Fig. 2) suggests that the changes in milk composition following separation were a result of changes in epithelial cell activity and not a consequence of the degeneration of mammary alveolar structure. In rodents, clear indications of mammary gland involution begin to appear within 24 h of pup removal (Quarrie et al. 1995), and widespread apoptosis is evident within 4 days (Quarrie et al. 1996). As a result, involution of the mammary glands is only partially reversible after 48 h of separation (Wilde et al. 1999). In ruminants, apoptosis is initiated within a time frame similar to that observed in rodents (Tatarczuch et al. 1997); however, the degeneration of alveolar structure occurs at a much slower rate, with recovery from separation still possible following several weeks of milk stasis (Wilde et al. 1999). The ability of D1290 to fully recover after 6 days of separation suggests that harbour seals are similar to ruminants in that degeneration of alveolar structure occurs slowly following the onset of milk stasis and, as a result, the mammary gland is capable of tolerating periods of prolonged separation without sustaining irreversible loss of function. Further study will be needed to determine whether this slow degeneration of gland structure in response to milk stasis is consistent across phocid species or whether it represents an intermediate character that has evolved in those species that sustain periods of separation as part of their lactation strategy.

The rapid changes observed in the mammary gland function of harbour seals following separation suggest that there is a physiological constraint on the duration that females can forage without negatively affecting the transfer of energy to their pups. Although we were unable to measure milk output in the present study, data from studies of initiated involution in other species suggest that periods of prolonged separation should affect not only milk composition but also subsequent milk output (Calvert et al. 1985; Wilde et al. 1999). In phocids, milk energy output, which is a function of both the composition of the milk and the amount of milk produced, is a significant determinant of pup mass gain (Mellish et al. 1999*a*; Crocker et al. 2001). If the effect of separation on milk composition in harbour seals is as rapid as the changes in D890 suggest, and milk output is affected by milk stasis in the same way as it is in other species, extended foraging bouts may result in the loss of energy transfer to the pup not only as a direct result of reduced suckling frequency (as observed in both D890 and D1290) but also as a result of the subsequent decreases in milk energy content and milk output. This constraint may be particularly important for the reproductive success of younger, lighter females that rely more heavily on foraging to meet the energetic requirements of lactation (Bowen et al. 2001) and that are, therefore, more likely to experience a greater frequency of prolonged separations from suckling.

In summary, our results demonstrate that both the pattern of change in overall milk composition and the level of individual variation observed among harbour seal females are consistent with results from other phocid species. While the sources of individual variation in milk composition remain poorly understood in phocid seals, the substantial changes observed in milk composition following the separation of mother–pup pairs suggest that at least some of the variation observed among harbour seal females over mid and late lactation may relate to maternal attendance patterns. Further study is needed to determine how quickly the changes in mammary gland function and structure occur following separation in phocid seals and whether or not the timing and extent of changes are consistent among species. Our results also highlight the need for caution when using cross-sectional sampling to study aspects of milk secretion and the consequences for offspring growth in phocid species in which females may separate from pups for extended periods during lactation. Depending on normal suckling frequency, separations of mother–pup pairs for extended periods may result in high levels of variation in both milk composition and milk output.

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