Vitamin E and vitamin A contents, fatty acid profiles, and gross composition of harp and hooded seal milk through lactation

C. Debier, K.M. Kovacs, C. Lydersen, E. Mignolet, and Y. Larondelle

Abstract: This study reports fatty acid profiles and vitamin A and vitamin E contents of the milk of the harp seal (*Phoca groenlandica*) and hooded seal (*Cystophora cristata*) throughout the lactation period, as well as standard compositional analyses. The milk for this study was collected from harp and hooded seals breeding on the pack ice in the Gulf of St. Lawrence. Mother–pup pairs were serially captured, or point-sampled, in order to obtain milk samples during different lactation stages. Milk lipids showed the respective species' typical patterns during lactation, with a significant increase for harp seal milk and a relatively constant value for hooded seal milk. The profiles of most of the milk fatty acids remained relatively stable over the course of lactation in both species. Vitamin A content was also quite stable through lactation. By contrast, vitamin E content underwent a dramatic drop between the day of birth and early lactation for harp seals and from birth to midlactation for hooded seals. It then continued to decrease at a slower rate until the end of lactation in both species. The highest vitamin E content of colostrum reached 125 mg/kg of milk for harp seals and 200 mg/kg of milk for hooded seals. These concentrations subsequently dropped to 20–50 mg/kg of milk by early or mid lactation. These changes in this lipophilic, antioxidizing vitamin were not correlated with milk lipid content or its polyunsaturated fatty acid profiles.

Résumé : Cette étude traite de l'évolution du profil en acides gras ainsi que des teneurs en vitamine A et en vitamine E dans le lait de Phoque du Groenland (*Phoca groenlandica*) et de Phoque à capuchon (*Cystophora cristata*) au cours de la lactation. La composition grossière du lait est également présentée. Les échantillons de lait ont été recueillis sur des Phoques du Groenland et des Phoques à capuchon qui se reproduisent sur la banquise du golfe du Saint Laurent. Dans le but d'avoir des échantillons de lait à différents stades de lactation, les couples mères-petits ont fait l'objet de captures successives ou ponctuelles. Les concentrations en lipides du lait suivent l'évolution habituellement observée pour ces espèces, avec une augmentation considérable dans le lait de Phoque du Groenland et une teneur relativement constante dans le lait de Phoque à capuchon. Chez les deux espèces, le profil de la plupart des acides gras du lait reste assez stable au cours de la lactation. De même, le contenu en vitamine A est relativement stable pendant toute la période de la lactation. Par contre, la concentration en vitamine E du lait subit une chute spectaculaire entre le jour de la naissance et le premier tiers de la lactation chez les Phoques du Groenland et entre le jour de la naissance et le milieu de la lactation chez les Phoques à capuchon. Elle continue ensuite à diminuer à un taux plus lent jusqu'à la fin de la lactation. Les contenus en vitamine E du colostrum atteignent 125 mg/kg de lait chez le Phoque du Groenland et 200 mg/kg de lait chez le Phoque à capuchon. Les concentrations chutent ensuite jusqu'à 20–50 mg/kg de lait à partir du milieu de la lactation chez les deux espèces. Les variations des teneurs en vitamine E, molécule lipophile et antioxydante, au cours de la lactation, ne sont donc pas en corrélation avec le contenu lipidique du lait ni avec son profil en acides gras polyinsaturés.

Introduction

Lactation in phocid seals has received considerable research attention during the last two decades. This is largely due to the fact that phocids display several rather remarkable features concerning the transfer of energy to their young. One of the unusual features of the phocid seal lactation period is its duration. All phocid seals have very short lactation periods for their body mass compared with other mammals (e.g., Bowen et al. 1985; Kovacs and Lavigne 1986; Oftedal et al. 1987; Bowen 1991; Lydersen and Hammill 1993). Another odd trait of the phocid seal lactation period is that the females usually reduce their food intake, or fast entirely, while nursing their young. Thus, they support their own metabolic requirements and milk production largely or completely from their fat reserves (e.g., Riedman and Ortiz 1979; Bowen et al. 1987; Iverson et al. 1993; Lydersen and Kovacs 1996). The composition of seal milk is also unique. One of its outstanding features is its very high fat content (Oftedal et al. 1988; Costa 1991; Iverson et al. 1993; Lydersen et al. 1995, 1997).

Seal milk fat is very rich in long-chain polyunsaturated fatty acids (PUFAs) such as C20:5 n–3, C22:5 n–3, and C22:6

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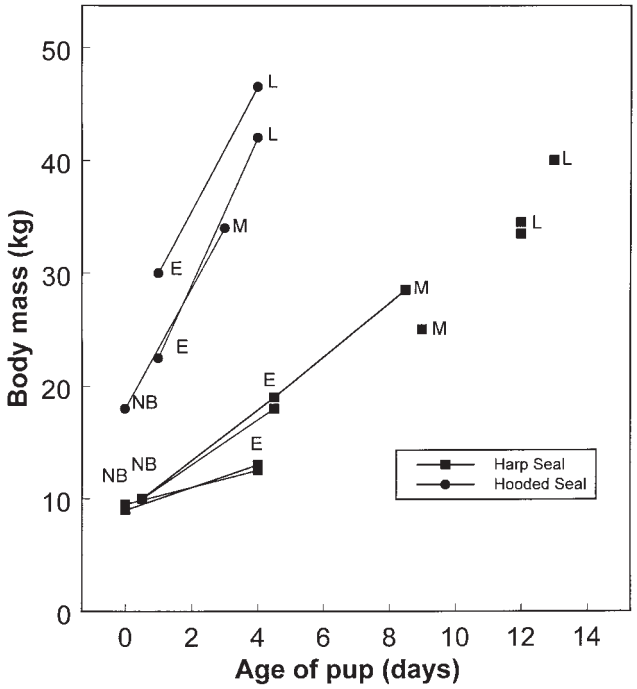
Fig. 1. Changes in pup body mass throughout lactation. Lactation stages for samples (newborn (NB), early (E), mid (M), and late (L)) are shown for each species.



n–3 (Ackman and Burgher 1963; Van Horn and Baker 1971; Riedman and Ortiz 1979; Iverson et al. 1992, 1995*a*, 1997; Iverson 1993). These PUFAs are derived directly from the seal's diet. Marine food chains are notable for their high levels of PUFAs, which originate from various forms of unicellular plankton and seaweed (Ackman 1980). These fatty acids remain essentially intact during digestion in monogastric animals such as seals (Iverson 1993). Fatty acids are released from adipose tissue into circulation in seal species that mobilize fat during lactation, owing to the very high activity of hormone-sensitive lipase and the low activity of blubber lipoprotein lipase (Allen 1976). Mammary lipoprotein lipase activity increases as lactation progresses, paralleling the increase in the milk fat content of seal milk (Iverson et al. 1995*b*).

Because seal milk, and thus the pup's diet, is very rich in PUFAs, the supply of antioxidizing molecules, such as vitamin E, through the milk is particularly important. By reacting with peroxyl radicals, vitamin E (α-tocopherol) acts as an antioxidizer, protecting unsaturated fatty acids in cellular membranes as well as in plasma lipoproteins. Vitamin E's antioxidizing function is effective at high oxygen concentrations and is of the utmost importance in lipid structures such as erythrocyte membranes. Only one study of the vitamin E content of seal milk has been published (Schweigert and Stobo 1994). This study reported high levels of vitamin E in grey seal milk compared with the milk of terrestrial mammals.

The present study investigates several aspects of the composition of harp and hooded seal milk through the lactation period. Because the duration and the primary aspects of the energetics of lactation in these two pack-ice-breeding phocids is well documented (e.g., Bowen et al. 1985; Kovacs and Lavigne 1985; Oftedal et al. 1988, 1993; Lydersen and Kovacs 1996), compositional analysis of the milk is dealt with only briefly here. Instead, this study focuses on fatty acid profiles and vitamin E content of the milk throughout the 12- and 4-day lactation period in harp and hooded seals, respectively. Levels of another fat-soluble vitamin, A, are also examined in comparison with vitamin E concentrations.

Methods

Milk samples were collected from harp and hooded seals on the drifting pack ice in the Gulf of St. Lawrence, Canada, in March 1996. Initially, the harp and hooded seal breeding areas were located by fixed-wing aircraft. Thereafter, a helicopter was used to get to the study sites daily, weather permitting. The study areas were marked with fluorescent dye powder to increase their visibility from the helicopter, and VHF transmitters were placed on the ice among the study pairs so that these areas could be found again in the drifting pack. Harp and hooded seal mothers were captured using suitably sized A-frame nets, while pups of both species were hand-captured. Harp seal mothers and pups were weighed using appropriate 200 ± 0.5 and 50 ± 0.5 kg Salter spring scales, respectively. Hooded seal mothers and pups were weighed using a 500 ± 0.5 kg Dilon dynamometer and a 100 ± 0.5 kg Salter spring scale, respectively. The age of the pups was determined on the basis of body mass, color of the pelt, appearance, and relative fatness (Kovacs and Lavigne 1985, 1992). Milk samples (~50 mL) were taken from each female about 10 min after an intramuscular injection of 20 IU of oxytocin. Milk was collected from the teats using an adapted syringe and was subsequently transferred to 30- or 60-mL plastic cryo-jars. These were filled completely to minimize air contamination and wrapped in aluminum foil to prevent exposure to light. They were frozen at –20°C within a few hours of collection. All animal-handling procedures were carried out in accordance with the principles and guidelines of the Canadian Council for Animal Care.

Eight harp seal mother–pup pairs were used in this study. One pair was captured on the day of birth as well as at early and mid stages of lactation. Three pairs were captured at both the newborn and early stages. Four pairs were handled at only one (mid or late) stage of lactation. Three hooded seal mother–pup pairs were used in this study. One pair was captured on the day of birth and at midstage and two pairs were captured at the early and late stages. The distributions of pup ages in the samples are shown in Fig. 1.

Chemicals used during the analyses of the milk were of analytical grade. Before analysis, milk samples were homogenized with a Potter–Thomas mixer. Dry matter and ash content were determined by means of standard procedures (Association of Official Analytical Chemists (AOAC) 1995) except that the milk samples were placed on circular AP40 047 05 glassfiber filters (Millipore, Bedford, Mass.) on glass plates to facilitate the release of water during dry-matter determination. Crude protein content was determined by the Kjeldahl method (AOAC 1995), using 6.38 as a conversion factor. Lipids were extracted from milk by adapting the methods of Radin (1981) and Schweigert and Stobo (1994). A 3-g sample was extracted 3 times with 10 mL of an hexane–isopropanol mixture (3:2 v/v). The extraction products were then filtered, combined, and mixed with 10 mL of an NaCl solution (0.1 M) to remove nonlipid substances that were only partially soluble in the organic solvent. Phase separation was obtained within a few minutes. Lipid content was determined by drying the organic extract for 10 min in a rotavapor and then drying it for a further 2 h in an air oven.

Fatty acid profiles were obtained by gas–liquid chromatography of the fatty acid methyl ester derivatives. The lipid extract was

obtained via the method mentioned above, except that the final drying step (2 h in an air oven) was avoided to prevent oxidation of the PUFAs. Derivatives were isolated via saponification and esterification with KOH in methanol (0.1 M) and HCl in methanol (1.2 M). The fatty acid derivatives were then extracted with hexane and put through a GC 6000 Vega gas–liquid chromatograph (Carlo Erba, Milan, Italy). The chromatograph was equipped with an Omegawax 320 (30 m × 0.32 mm) capillary column (Supelco, Bellefonte, Pa.), a flame ionization detector, and a DP 800 recorder (Carlo Erba). Helium was used as the carrier gas and the temperature program was as follows: 3 min at 130°C; gradual heating to 220°C for 9 min; 35 min at 220°C; gradual cooling to 130°C for 4–5 min. Individual peaks were identified by referring to a fatty acids methyl esters standard solution (189.19, Sigma, St. Louis, Mo.), analyzed under the same operating conditions.

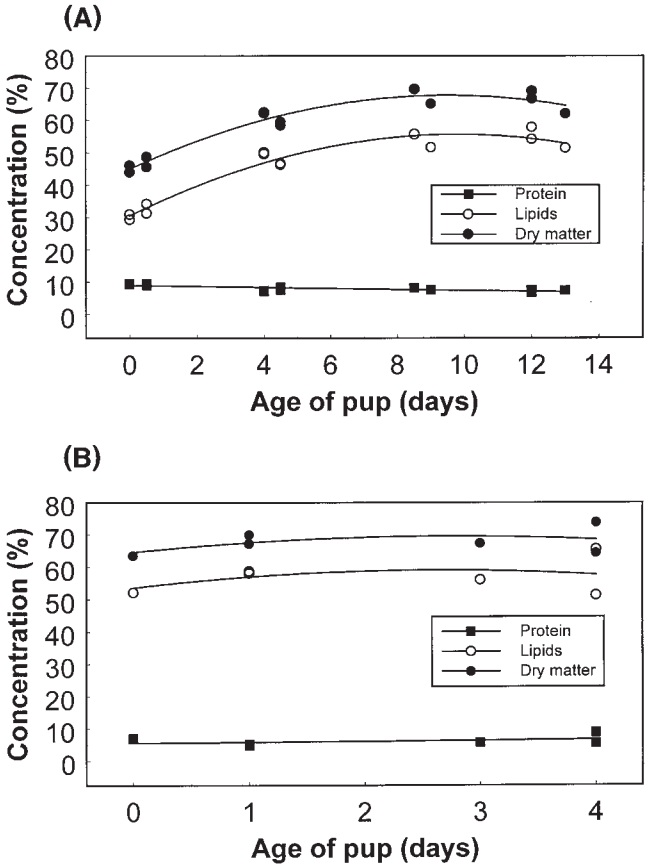
Vitamin A and E contents were quantified according to COST (1991), with slight modifications for seal milk. A 4-g milk sample was saponified under nitrogen with ethanolic KOH (40 mL KOH, 50% w/v; 120 mL ethanol, 96%; 2 mL Na_2S, 12 g/100 mL; 100 mg hydroquinone) at 80°C for 30 min. Fat-soluble vitamins were then extracted from the saponified sample twice with 150 mL of stabilized diethyl ether (7 mg butylated hydroxytoluene/L). The organic phases were pooled and washed with NaCl solution (50 g/L, 100 mL) and MilliQ water (100 mL several times) until a neutral pH was obtained. This material was then filtered before being dried with a rotavapor at 35°C. The residue was reconstituted in 10 mL of methanol. Vitamin A and E analyses were performed by high-performance liquid chromatography (HPLC) at 40°C. The chomatograph was composed of a pump (Perkin–Elmer, Norwalk, Conn.), an oven, a SP 8450 UV/visible detector, and a SP 4290 recorder (Spectra-Physics, San Jose, Calif.) and was equipped with a Supelcosil LC 18 inverse phase column (Supelco). The mobile phase was a mixture of methanol and water (95:5 v/v). Individual peaks were identified by referring to a standard solution of retinyl acetate (1.08520, Merck, Darmstadt, Germany) and tocopheryl acetate (95250, Fluka, Buchs, Switzerland). This solution was subjected to extraction and HPLC under the same operating conditions as the unknown samples except for the quantities of chemicals used for saponification (10 mL KOH, 100 mg hydroquinone, 25 mL ethanol 96%) and extraction (2 × 100 mL of diethyl ether). Recovery values for vitamins A and E were 100.6 and 99.6%, respectively.

This study was designed as a longitudinal analysis of a series of milk samples taken from individual mothers during early, mid, and late lactation. Unfortunately, inclement weather caused significant break-up of the ice pack and some mother–pup pairs were lost or were found on ice pieces that were so small that capture of the mothers was not possible when resampling was attempted. Thus, the milk samples analyzed in this study are a mixture of longitudinal samples supplemented by cross-sectional, point samples for stages where the sample size would have been too small. This mixed sampling regime caused some limitations in the analysis of this material. For milk constituents that changed dramatically according to lactation stage, and where inter-individual variability was small, curvilinear best-fit regression techniques were employed despite the lack of complete independence or balance among the samples from the different lactation stages. Because the data for fatty acids were more variable among individuals and less predictable through time, the results are simply graphically presented and described.

Results

The gross composition of harp and hooded seal milk through the lactation period is shown in Fig. 2. Total dry matter in harp seal milk increased substantially during early lactation and then stabilized. Protein content decreased only slightly from colostrum to mature milk. Lipids followed the same basic pattern as dry matter, the most significant increase occurring during the first 4 days of lactation. Ash content (not shown) varied only slightly, decreasing from 0.79% at the newborn stage to 0.62% by late lactation. Lactation stage had little influence on the gross composition of hooded seal milk. Ash content (not shown) stayed relatively constant: 0.54% at the newborn stage and 0.46% by late lactation.

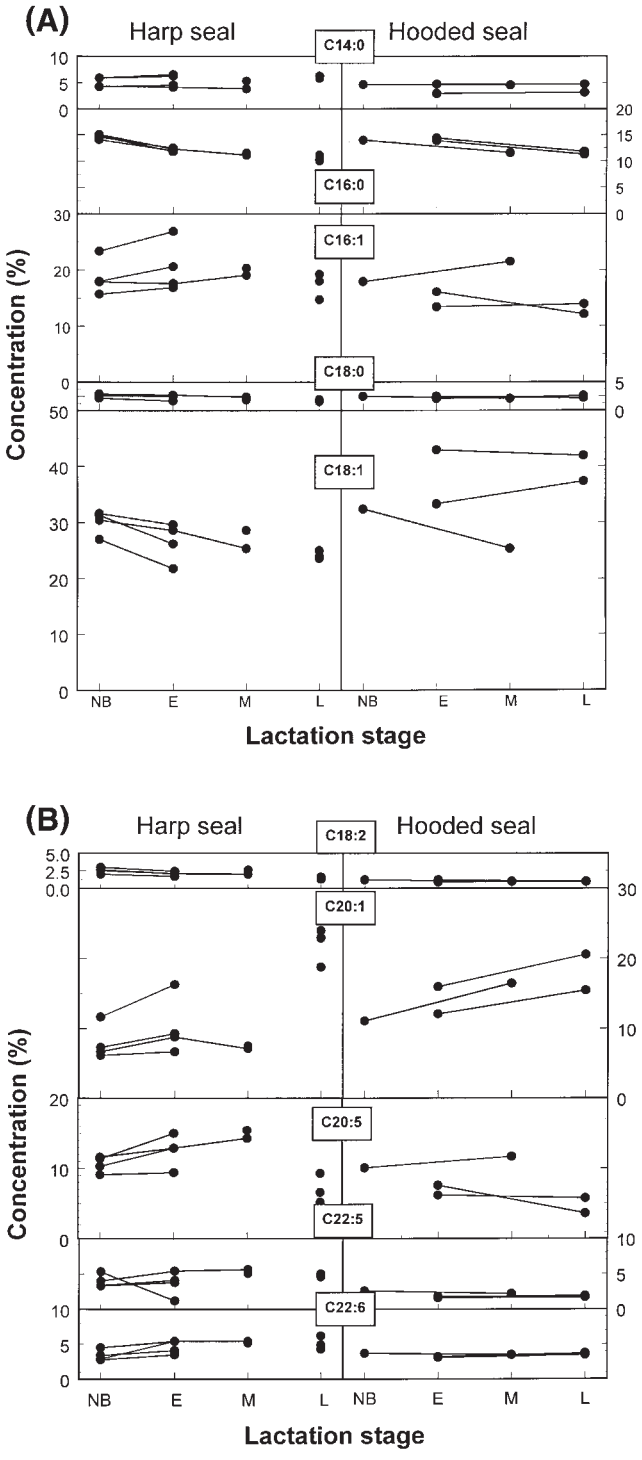
Fig. 2. Changes in the gross composition of milk throughout the lactation period. (A) Harp seals: dry matter, $y = 45 + 4.8x - 0.25x^2$; lipids, $y = 30.5 + 5.2x - 0.26x^2$; protein, $y = 9 - 0.16x$. (B) Hooded seals: dry matter, $y = 64.6 + 3.6x - 0.66x^2$; lipids, $y = 53.6 + 4.2x - 0.79x^2$; protein, $y = 5.6 + 0.26x$.



The major fatty acids in both harp and hooded seals milk are C16:0, C16:1, C18:1, C20:1, and C20:5 (Fig. 3). The percentage of PUFAs (C18:2, C20:5, C22:5, and C22:6) was 22% (range 18–28%) in harp seal milk and 15% (range 11–18%) in hooded seal milk. The tendency for hooded seal milk to have a lower percentage of PUFAs can also be seen when each PUFA is considered separately. Fatty acid profiles in harp and hooded seals milk did not appear to undergo dramatic changes during the course of lactation (Fig. 3). Only C20:1 showed a clear tendency to increase as lactation proceeded.

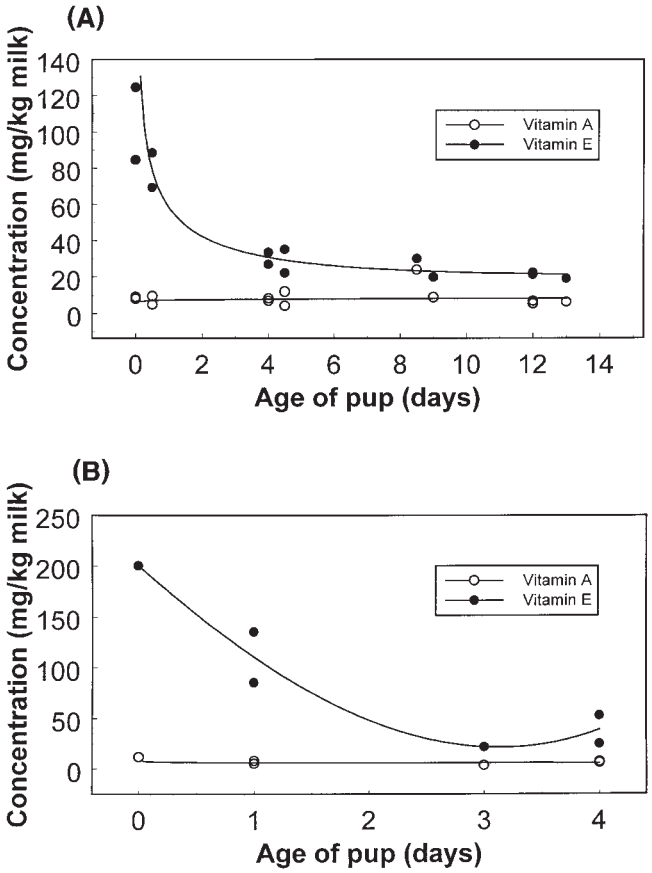
The vitamin E and A contents of the milk of harp and hooded seals are shown in Fig. 4. Vitamin E content underwent a considerable drop between the newborn and early stage in harp seal milk and between the newborn and mid

Fig. 3. Fatty acid profiles, from C14:0 to C18:1 (A) and from C18:2 to C22:6 (B), in harp and hooded seal milk throughout lactation. Points referring to recaptured females are joined. C22:1 was not quantified.



stage in hooded seal milk and then continued to decline more slowly throughout lactation in both species. When vitamin E content is expressed per kilogram of lipids, it appeared to decrease more sharply in harp seal milk than in hooded seal milk, since the lipid content of the milk of the former species increased as lactation proceeded. In contrast

Fig. 4. Changes in vitamin A and E contents of milk of harp seals (A) and hooded seals (B) during lactation.



to the changes observed in vitamin E content, the vitamin A content of the milk of both harp and hooded seals remained stable throughout lactation.

Discussion

Phocid seals are notable among mammals for their rapid neonatal growth rates and short periods of maternal care (Kovacs and Lavigne 1986). This fast early growth and precocial independence is facilitated, in part, by the fat-rich nature of the milk. The extreme values for fat content in the milk of some phocid species have stimulated considerable research in the last two decades. Gross composition through lactation has been measured for a considerable number of pinniped species (e.g., Riedman and Ortiz 1979; Lavigne et al. 1982; Oftedal et al. 1988; Lydersen and Hammill 1993; Carlini et al. 1994; Lydersen et al. 1995; Lydersen and Kovacs 1996; Lydersen et al. 1996, 1997), including both harp and hooded seals. Most of the measures of gross composition of harp and hooded seal milk in this study are similar to previously published values for these species.

The fatty acid profiles of pinniped milk include chains of 14–22 carbon units and are characterized by a high proportion of long-chain PUFAs (Ackman and Burgher 1963; Jangaard and Ke 1968; Van Horn and Baker 1971; Riedman and Ortiz 1979; Iverson et al. 1992; Puppione et al. 1992; Iverson 1993; Iverson et al. 1995*a*, 1997). In this study, the fatty acid profiles of harp and hooded seal milk correspond

to this general pattern, with high concentrations of C16:0, C16:1, C18:1, C20:1, and C20:5 (Fig. 3). Percentages of milk PUFAs, calculated at each stage of lactation, were higher in harp seals than in hooded seals. Jangaard and Ke (1968) observed similar differences in C22:5 and C22:6 concentrations when they examined harp and hooded seal depot fat and milk at a single stage in lactation. The differences in C22:5 and C22:6 concentrations between harp and hooded seal blubber and milk may be partly due to dietary differences. Another explanation may be higher activity of the C20:5 elongation enzyme in harp seals. The present study shows that the fatty acid profile fluctuates only slightly through lactation in both species. These findings are in general agreement with the analyses of harp, northern elephant, and hooded seals by Van Horn and Baker (1971), Riedman and Ortiz (1979), and Iverson et al. (1995*a*), respectively. Generally speaking, long-chain PUFAs do not appear to be more concentrated in harp and hooded seal colostrum than in the milk, in contrast to the milk of terrestrial mammals such as humans (Boersma et al. 1991; Goedhart and Bindels 1994). A high concentration of C22:6 in the colostrum has been described as important in the development of mammalian central nervous systems (Giovannini et al. 1991, 1992; Goedhart and Bindels 1994; Zuppa et al. 1997). C20:5, C22:5, and C22:6 concentrations in harp and hooded seals are much higher generally throughout lactation than in humans, and thus may meet the needs of the neonates without initial inflation.

Despite the considerable amount of research attention that phocid seal milk has received, very little work has been done on its nutrient content in terms of vitamin composition. This study reports vitamin E and A contents of harp and hooded seal milk that are 3–15 times higher than in human milk, depending on the stage of lactation (Ostrea et al. 1986; Boersma et al. 1991; Holland et al. 1991). Nevertheless, when expressed per kilogram of lipids, the vitamin E content of harp and hooded seal milk was up to 2 times lower than in human colostrum and mature milk. Schweigert et al. (1990) found that vitamin E concentrations in grey seal blubber were only 1/10 of that found in human adipose tissue and suggested that this low level may be explained by the large amount of unsaturated fatty acids in seal blubber. Vitamin E is degraded during the chain-breaking reaction. This hypothesis may also explain the relatively low fat-specific values for vitamin E in seal milk.

The vitamin E and A patterns found in harp and hooded seals in this study were quite similar. The colostrum of both harp and hooded seals contained large amounts of vitamin E compared with milk produced late in lactation, while the vitamin A content stayed relatively constant (Fig. 4). Thus, these two fat-soluble vitamins followed quite different patterns. The only other study on vitamins E and A in seal milk was performed on grey seals (Schweigert and Stobo 1994). The results of this work were similar to those of this study: the vitamin E concentration dropped significantly, while the vitamin A content remained relatively stable at about 3.0 mg/kg of milk throughout lactation. However, the actual vitamin E and A concentrations documented for the grey seals were 2.5–4 times lower than those obtained for harp and hooded seals in the present study. These differences may be real interspecific differences, but variation in the collection protocols and analytical techniques may also have played a role. Schweigert and Stobo (1994) collected grey seal milk via dissection of the mammary glands of dead animals shot 10 min after an oxytocin injection, while in this study milk was extracted from living animals.

The consistent vitamin A concentrations through lactation in seal milk are somewhat unexpected. The vitamin A content drops by a factor of 2.5 between colostrum and mature milk in humans (Holland et al. 1991). The reason for the difference in the pattern of vitamin A concentrations between human milk and seal milk is unclear. The pattern of vitamin E concentrations observed through lactation in this study appears to be very similar to that observed in a variety of other mammal species (Kobayashi et al. 1975; Jansson et al. 1981; Chappell et al. 1985; Syvajoya et al. 1985; Ostrea et al. 1986; Hidiroglou 1989; Pehrson et al. 1990; Boersma et al. 1991). In humans, for example, the vitamin E concentration is 3–4 times higher in colostrum than in mature milk (Syvaoja et al. 1985; Boersma et al. 1991; Holland et al. 1991). Boersma et al. (1991) suggested that a progressive increase in the size of the milk fat globule in humans would have an unfavorable effect on the membrane constituents of the milk fat globule, such as vitamin E. This hypothesis may be relevant to the vitamin E patterns observed in the milk of harp and hooded seals. Several studies in newborn calves, sheep, rats, and humans have indicated inefficient placental transfer of vitamin E (Martin and Hurley 1977; Hidiroglou 1989; Jain 1989; Mino 1992; Njeru et al. 1994); the situation may be the same for seals. The high vitamin E concentration in colostrum represents an important supply of this vitamin to newborns (Kobayashi et al. 1975; Ostrea et al. 1986; Rajaraman et al. 1997). Several roles have been suggested for vitamin E in newborn mammals. First, vitamin E is beneficial to the development of the immature immune system of the newborn (Peplowski et al. 1981; Hayek et al. 1989; Eicher et al. 1994; Nemec et al. 1994; Rajaraman et al. 1997). Secondly, newborns can be regarded as very sensitive to oxidative stress. Studies of humans showed greater lipid peroxydation, as well as greater susceptibility of erythrocyte membranes, to oxidative damage in newborn infants than in adults (Wispe et al. 1985; Jain 1989). This higher oxidative stress is due, in part, to low vitamin E concentrations, as well as to the reduced activity of antioxidizing enzymes, at birth (Jain 1989). The vitamin E furnished by the colostrum is therefore important to combat this stress. Lydersen and Kovacs (1996) showed that in harp seals, pups put on almost exclusively blubber during the first days of life, but put on more muscle tissue (protein) during the second half of lactation. The high vitamin E ingestion during early lactation may thus be important to protect the lipid deposits against oxidation.

The differences observed between the patterns of concentration of the two fat-soluble vitamins, A and E, in seal milk in relation to each other and to the lipid content of the milk are currently not understood. Further studies are needed on the vitamin contents of seal milk to determine whether the traits observed here are common to a broader array of seal species. Additionally, studies of the changes in plasma lipoproteins and retinol-binding proteins throughout lactation would enhance our understanding of the mechanisms of trans-

fer of lipids and fat-soluble vitamins from seal mothers to their pups during lactation.

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