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Prenatal and postnatal transfer of fatty acids from mother to pup in the hooded seal

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Abstract Unlike most mammals, hooded seal (*Cystophora cristata*) pups are born with a substantial layer of adipose tissue. Subsequently, during the brief lactation period of only 4 days, fasting mothers mobilize enormous amounts of lipid from blubber and secrete milk (60% fat) at rates of $10\,kg \cdot day^{-1}$. Pups gain $7\,kg \cdot day^{-1}$ due primarily to the deposition of fat in blubber. We measured blubber content and fatty acid composition of blubber and milk in hooded seal mother-pup pairs at birth and over the 4-day lactation period to examine the nature and source of fetal lipids, the incorporation of maternal blubber fatty acids into milk lipid, and patterns of fatty acid deposition in suckling young. The fatty acid composition of the blubber of the newborn was notably different from that of its mother. Fetal deposition was likely due to a combination of both fetal synthesis and direct placental transfer of maternal circulating fatty acids. The blubber of the newborn was characterized by high levels (>90% of total fatty acids) of saturated and monounsaturated fatty acids of primarily endogenous origin. In particular, the fetus appeared to have high Δ-9 desaturase activity as evidenced by the large amounts of 14:1n-5 (4.2%) and 16:1n-7 (37.0%) in newborn blubber compared to maternal blubber (0.2% and 14.1%, respectively). Nevertheless, essential and long-chain polyunsaturated fatty acids of the n-3 and n-6 families, which could only have originated by direct transfer from the mother, comprised >7% of pup blubber fatty acids and indicated greater rates of placental transfer than found in humans. In hooded seal mothers, rapid lipid transfer during the brief lactation period appeared to be facilitated by direct incorporation of mobilized fatty acids into milk. Although some differences in proportions of specific fatty acids were found between milk and maternal blubber, most of these differences declined over the course of lactation. However, selective mobilization of 20:5n-3 from maternal blubber into milk was apparent throughout lactation and resulted in elevated levels in pup blubber at weaning compared to maternal blubber. Ingested fatty acids were deposited directly and without modification into the blubber of pups, and by 4 days the fatty acid composition of pup blubber was virtually identical to that of the milk consumed.

Key words Placental transfer · Lactation · Fatty acids · Adipose tissue · Hooded seal Cystophora

Abbreviations *BHT* butylated hydroxytoluene · *BM* body mass · *EFA* essential fatty acid(s) · *FA* fatty acid(s) · *FFA* free fatty acid(s) · *LPL* lipoprotein lipase · *MUFA* monounsaturated fatty acid(s) · *PUFA* Polyunsaturated fatty acid(s) · *SFA* saturated fatty acid(s)

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Introduction

Mammalian reproduction relies heavily on the transfer of lipid from mother to young. Throughout pregnancy and lactation, large amounts of FA must be provided by the mother, either directly from dietary sources, from the mobilization of FA stored in adipose tissue, or

from biosynthesis. EFA are required by both the fetus and neonate for structural growth and brain development (Hahn 1972; Jensen 1988). Also, in many species non-essential FA serve as the primary source of energy in the first few hours after birth (Van Duyne 1966; Hahn 1978; Herrera et al. 1992) and FA continue to be the major source of energy during lactation (Oftedal 1984).

Prenatal transfer of FA from mother to young has been studied in only a few species and little is known about the EFA status and lipid metabolism of the fetus (Hornstra 1992). Although the majority of fetal lipids are thought to be derived via synthesis from glucose (Popjak 1954; Elphick et al. 1975; Jensen 1976), which is also apparently the primary energy substrate of the fetus (Van Duyne and Havel 1959; Harding 1970; Battaglia and Meschia 1978), some FA (e.g., EFA) must also cross the placental membrane. The term EFA is derived from the fact that certain FA cannot be biosynthesized by animals and must be provided from the diet for normal growth and metabolism. In this discussion, EFA refers to both the classical components [18:2n-6, 18:3n-3 and 20:4n-6; Burr and Burr (1929)] and their longer-chain derivatives, the n-3 and n-6 PUFA (Crawford et al. 1978; Karrick 1990; Nelson 1992).

Placental transfer of FA is usually studied by examining temporary differences between maternal and fetal blood lipid concentrations, particularly since most mammalian young are born with little if any fat reserves that could be sampled (Widdowson 1950; McCance and Widdowson 1977; Pond 1977). However, results from such studies are often difficult to interpret due to the rapid clearance of FFA from both maternal and fetal circulations and transfer of circulating FFA back and forth between the mother and fetus (Hull 1975). Thus, whether all or only specific FA can cross the placenta is unclear, as is the extent to which this varies among species. In the non-ruminant species studied, the placenta is impermeable to the esterified FA of maternal plasma, but not to FFA; however, the net transfer of FFA to the fetus is much greater in the guinea pig and rabbit than in the rat and human (Hershfield and Nemeth 1968; Hummel et al. 1974; Elphick et al. 1975; Pavey et al. 1976; McCance and Widdowson 1977). In the human it is believed that long-chain PUFA do not readily cross the placenta even in unesterified form, resulting in a marginal or insufficient EFA status of the newborn (Hornstra et al. 1989; Hornstra 1992).

Although the fetus is apparently incapable of utilizing fat as a primary energy source, the newborn of most species are readily able to deposit or catabolize FA to obtain energy (Hull 1975; Hahn 1978). In most mammals, lactation is the period of maximum lipid transfer from mother to young, resulting in extensive lipid mobilization and depletion of maternal adipose tissue stores even when feeding (Young 1976; Bauman and Elliot 1983), as well as pronounced fat deposition in the young (Spray and Widdowson 1950; McCance and Widdowson 1977). However, mobilization of maternal adipose tissue FA into milk and subsequent postnatal deposition of milk FA in the neonate has rarely been studied.

Phocid seals (i.e., family Phocidae) serve as a good model in which to examine FA transfer during reproduction. Females of most species fatten intensively prior to parturition and fast throughout a lactation period which lasts less than 4 weeks; during this time the pup consumes only milk and lactation is terminated by abrupt weaning (Bonner 1984; Oftedal et al. 1987). The mobilization of maternal fat reserves and transfer of milk fat from mother to pup occurs at very high rates (Oritz et al. 1984; Costa et al. 1986; Tedman and Green 1987; Iverson et al. 1993b; Oftedal et al. 1993). Blubber deposition in the suckling pup is rapid and important for both thermoregulation and as a critical energy reserve during a postweaning fast of several weeks or months.

The ice-breeding hooded seal (*Cystophora cristata*) is a phocid species with the shortest lactation period of any mammal (Bowen et al. 1985). Females lactate for only 4 days, losing 8–10 kg per day and secreting large quantities of high-fat milk (60%) while fasting; during the suckling period pups gain 7 kg per day, approximately 70% of which is blubber (Oftedal et al. 1988; Bowen et al. 1987). Thus, the amount and rate of mobilization of maternal blubber FA into milk and of deposition of these milk FA into pup blubber during lactation must be phenomenal. Additionally, unlike most other phocids and mammals in general, hooded seal pups are born with a sizable layer of adipose tissue (as blubber), comprising about 17% of BM at birth (Bowen et al. 1987). Due to the complexity of marine FA, it is possible to distinguish between components of adipose tissue or milk which could have been readily biosynthesized by the mother or pup versus those that must or most likely come from the diet (Iverson 1993). Hence the characteristics of reproduction in the hooded seal, coupled with its marine diet, provide an excellent opportunity to examine important aspects of both prenatal and postnatal lipid transfer.

In this study, we measured the total blubber content and FA composition of blubber lipid and milk lipid from individual lactating hooded seal mothers and their pups at birth, mid, and late lactation (<1, 2, and 4 days postpartum, respectively). FA in hooded seal mothers and their pups were used to indicate (1) the nature and source of fetal lipids, (2) the degree of direct incorporation of maternal blubber FA into milk, and (3) the patterns of deposition of dietary FA in pups during intensive fattening.

Materials and methods

Field procedures

Tissue samples were collected from hooded seals on the pack ice approximately 180 km off the southeast coast of Labrador in March

1984 during the course of morphometric studies [Bowen et al. (1987); experimental research permit issued by the Canadian Department of Fisheries and Oceans, St. John's, Newfoundland]. In addition to total BM, standard morphometric data were taken from all animals, including sculp mass, defined as the mass of skin and attached blubber minus the anterior and posterior flippers (Bowen et al. 1987). To examine the transfer of FA from mother to pup during gestation and lactation, we examined the FA composition of animals that had already been taken for the cross-sectional study by Bowen et al. (1987). Blubber samples were available from three mother-pup pairs at each of three stages: newborn, 2 and 4 days postpartum. When logistically possible, milk had also been collected from the mother of each pair; however, in cases where this had not been possible, we used milk and blubber samples from additional mothers such that three maternal blubber/milk pairs and three mother/pup blubber pairs were available for each lactation stage (Table 1).

Newborn pups were identified by the presence of fresh placentas, blood on the ice, and their thin wet appearance; a subset of these pups were individually tagged and dye marked (Bowen et al. 1985). Pups believed to be within several hours of birth were intubated at capture to determine whether suckling had taken place. Stage of lactation was subsequently determined by relocation of marked pups of known age. Milk was obtained from chemically immobilized females or from females immediately postmortem (Oftedal et al. 1988). Blubber samples, excised from the sternal region, were obtained postmortem. Blubber FA composition in seals has previously been found to be homogeneous among different locations on the body and across different cross-sectional areas of blubber depth (Winter and Nunn 1950; Jangaard and Ke 1968). Hence, we assumed our blubber sample was representative of the entire blubber layer. At the time of collection, 1-ml aliquots of milk and approximately 1-g samples of blubber were placed in 5 ml 2:1 chloroform/methanol containing 0.01% BHT as an antioxidant. All samples were stored frozen until analysis.

Lipid extraction and analysis.

Samples were extracted in 18 ml 2:1 chloroform/methanol with 0.01% BHT by the Folch method (Folch et al. 1957) as modified by Iverson (1988). During the process of extraction, blubber samples were homogenized repeatedly in a glass tissue homogenizer (Potter Elvehjem type) equipped with a Teflon pestle, until reduced to a thin transparent film. FA methyl esters were prepared directly from aliquots of the chloroform extract (corresponding to 40 mg lipid) by the addition of 3.0 ml Hilditch reagent (0.5N H_2SO_4 in methanol) and then storing tightly sealed under N_2 in the dark at room temperature for 72 h. This method produced similar rates of transesterification as other methylating reagents (e.g., methanolic HCl at high temperatures) but was preferred as it eliminated the risk of an effect of oven heating on PUFA as well as artifact production and alkali isomerization reported with the use of basic reagents. Following transesterification, methyl esters were extracted into hexane and purified by preparative thin layer chromatography (Iverson et al. 1992), concentrated under N_2, and brought up to volume (25 mg · ml^{-1}) with high purity isooctane.

Analyses of FA methyl esters were performed according to Iverson et al. (1992) using temperature-programmed gas liquid chromatography on a Hewlett-Packard (Avondale, Pa., USA) model 5880 gas chromatograph fitted with a Monarch series flexible glass capillary column (25 m × 0.25 mm i.d.) coated with SP-2340 (Quadrex Corp., New Haven, Conn., USA). Identifications of FA and isomers were determined from known standard mixtures (Nu Check Prep., Elysian, Minn., USA) and silver-nitrate chromatography (Iverson 1988; Iverson et al. 1992). FA are designated by shorthand IUPAC nomenclature of carbon chain length:number of double bonds and location (n-x) of the double bond nearest to the terminal methyl group.

Statistical analyses.

FA concentrations are reported herein as the percentages by weight of total FA. In case where n-x is not specified for unsaturated FA, all isomers of a given component have been combined. Statistical analyses and comparisons between tissues were performed on only the paired samples for each parameter (i.e., milk versus blubber of individual mothers and blubber of a pup versus blubber or milk of its mother; Table 1). In general, non-parametric tests (Wilcoxon signed-rank test for paired comparisons and the Kruskal-Wallis test for comparisons over time) were used for analysis of percentage data due to small sample sizes. However, in order to compare FA of maternal milk versus maternal blubber by lactation stage, we used a 2-way ANOVA on data after arcsin transformation was performed. We did not use multivariate tests because we were interested in particular FA rather than all FA as a group. However, given the number of FA comparisons tested in Table 3, some of the indicated differences could arise from chance alone. Thus, the comparisons with significance level at $P < 0.01$ are emphasized. Analyses were performed using StatView for the Macintosh. Although we would have preferred to assess changes in maternal milk or in maternal blubber from birth to weaning directly, we were able to obtain only cross-sectional samples from individual animals. Given that the initial FA composition of the lipid stores of mothers at parturition would vary with individual diet (Iverson 1993), we could not be certain whether the subsequent differences in blubber or milk FA at 2 and 4 days were the result of changes over lactation or individual variability. Thus, comparisons of FA in maternal tissues over time were restricted primarily to the ratios of blubber to milk FA within females. Average values are presented throughout as mean ± SEM unless otherwise indicated.

Results

BM and estimated blubber mass (i.e., fat and connective tissue) of our study animals are presented in Table 1. At parturition, mothers averaged 189 kg, approximately 44% of which (82 kg) was blubber. Near weaning 4 days later, the average BM (163 kg) of mothers in this study was 26 kg less and blubber (58 kg, comprising 36% of BM) was 24 kg less than at parturition. Reduction in blubber accounted for about 92% of the decline in BM of mothers. Newborn pups averaged 20.2 kg and blubber mass was 3.7 kg or about 18% of birth mass. At 4 days postpartum, pups had gained on average 25.3 kg. Blubber mass had increased to 19.8 kg (43% BM) and accounted for about 64% of the mass increase of pups (Table 1).

Pups that were considered to be within several hours of birth were used for the <1 day analyses ($n = 3$). However, only Pup 1 had not yet suckled from its mother (Table 1). Pups 2 and 3 had 25 and 365 ml, respectively, of milk in their stomachs and could have been several hours old. Since even small amounts of absorbed milk fat might alter the FA composition of the prenatal blubber, only Pup 1 was considered to be truly representative of the term fetus in assessment of prenatal FA deposition. Thus, FA data for Pup 1 are presented separately from the other two newborn pups.

The blubber mass of Mother 1 at parturition was 102 kg or 44% of maternal BM (232 kg). During gestation, her pup had deposited 3.7 kg of blubber (sculp

Table 1 Hooded seal mother-pup pairs in this study. Values are means ± SEM

Days postpartum	Maternal blubber	Maternal milk	Pup blubber
	Mother 1	Mother 1	Pup 1[a]
< 1 day	Mother 2		Pup 2[b]
	Mother 3	Mother 3	Pup 3[b]
	Mother 4	Mother 4	
Body mass (kg)	189 ± 17.7		20.2 ± 3.03
Blubber mass (kg)[c]	82 ± 8.0		3.7 ± 1.04
	Mother 5	Mother 5	Pup 5
2 days	Mother 6	Mother 6	Pup 6
	Mother 7	Mother 7	Pup 7
Body mass (kg)	179 ± 17.5		35.4 ± 1.27
Blubber mass (kg)	59 ± 6.7		13.1 ± 0.61
	Mother 8		Pup 8
	Mother 9		Pup 9
4 days	Mother 10	Mother 10	Pup 10
	Mother 11	Mother 11	
	Mother 12	Mother 12	
Body mass (kg)	163 ± 10.3		45.5 ± 6.27
Blubber mass (kg)	58 ± 4.5		19.8 ± 3.46

[a] Freshly newborn and empty stomach (prior to first suckling)
[b] Within a few hours of birth, but evidence of prior suckling (milk in stomach)
[c] Blubber mass calculated as sculp mass minus mass of attached skin. For mothers, skin mass assumed to be 10 kg (Bowen et al. 1987); for pups, skin mass assumed to be 3.4 kg at birth (Bowen et al. 1987) and to increase in proportion to body length and mass of organs (e.g., Oftedal et al. 1989) over 4 days

weight 7.2 kg) accounting for 17% of its BM. The composition of the prenatally deposited blubber of the newborn was very different from that of its mother (Table 2). The FA composition of newborn blubber was higher in both SFA and MUFA than was maternal blubber, but this was attributed to only particular FA in each group. Also, a number of components present in maternal blubber were absent in pup blubber. In the pup, 14:0 and 16:0 were largely responsible for the high SFA levels and both occurred at a ratio of 1.5 in pup blubber to that in mother's blubber; 18:0 was reduced compared to that in the mother at a ratio of 0.5. One of the most unique features of the pup blubber was the high content (4.16%) of 14:1n-5 compared to 0.16% in its mother's blubber (Table 2). The other primary MUFA in the newborn, totaling 61.6% of pup blubber lipids, were 16:1n-7, 18:1n-9 and 18:1n-7, with pup to mother blubber ratios of 2.6, 1.4 and 1.1, respectively. The MUFA 18:1n-11, 20:1n-9 and 22:1n-11, which comprised 2.7, 14.9, and 7.0% of the mother's blubber, respectively, were found in very small amounts or were completely absent in newborn blubber. PUFA were reduced (7.2%) in the newborn compared to levels (17.9%) found in the mother; nevertheless, notable amounts of 18:2n-6, 20:5n-3, 22:5n-3 and 22:6n-3 were deposited prenatally in pup blubber at pup to mother ratios of 0.4, 0.5, 0.6 and 0.4, respectively (Table 2).

Table 2 Composition of blubber fatty acids of a hooded seal mother in comparison to that of her pup at birth prior to first suckling. Values are weight % of total fatty acid methyl esters. Blank spaces indicate component not detected

	Weight %		
Fatty acid	Mother 1	Pup 1	Ratio Pup:Mother
12:0	0.06	0.22	3.7
14:0	5.81	8.56	1.5
15:0 anti	0.12	0.13	1.1
15:0	0.24	0.16	0.7
16:0 iso	0.02	0.03	1.6
16:0	10.28	15.21	1.5
17:0			–
18:0 iso			–
18:0	1.90	0.99	0.5
Saturated	18.43	25.32	1.4
14:1n-5	0.16	4.16	26.0
16:1n-11	0.29	0.07	0.2
16:1n-9	0.31	0.79	2.5
16:1n-7	14.14	37.05	2.6
16:1n-5	0.26	0.09	0.3
17:1	0.16		0.0
18:1n-11	2.69		0.0
18:1n-9	14.12	19.16	1.4
18:1n-7	4.69	5.36	1.1
18:1n-5	0.44	0.20	0.5
20:1n-11	2.08	0.11	0.1
20:1n-9	14.95	0.45	0.0
20:1n-7	1.13		0.0
22:1n-11	7.04		0.0
22:1n-9	1.27		0.0
Monounsaturated	63.72	67.44	1.1
16:2n-4	0.61		0.0
16:3n-4	0.33	0.13	0.4
16:4n-1	0.47		0.0
18:2n-6	1.16	0.46	0.4
18:2n-9	0.06		0.0
18:3n-3	0.30		0.0
18:4n-3	1.17	0.09	0.1
18:4n-1		0.06	–
20:2n-6	0.20		0.0
20:4n-6	0.06	0.27	4.5
20:4n-3	0.46		0.0
20:5n-3	4.91	2.50	0.5
22:4n-6	0.27		0.0
22:5n-3	2.12	1.30	0.6
22:6n-3	5.76	2.42	0.4
Polyunsaturated	17.88	7.23	0.4

Blubber and milk of lactating females ($n = 9$ paired samples) compared across all lactation stages contained the same array of FA (Table 3). Although the proportions of FA in milk generally reflected those in blubber, milk was significantly higher than blubber in percentages of the SFA 16:0 and 18:0, but lower in 14:0. Milk was notably lower in major isomers of the MUFA 20:1 and 22:1. Proportions of most PUFA were similar between milk and blubber, with the primary exception of 20:5n-3 which was particularly high in milk (6.8%) compared to blubber (4.3%, Table 3).

The magnitude of differences between milk and maternal blubber FA tended to change with lactation

Table 3 Fatty acid composition of maternal blubber and milk during the 4-day lactation period in hooded seals. Values are mean weight % of total fatty acid methyl esters ± SEM ($n = 9$)

Fatty acid	Maternal blubber	Milk
12:0	0.08 ± 0.003	0.08 ± 0.003
14:0	5.08 ± 0.256	4.24 ± 0.176**
15:0 anti	0.16 ± 0.009	0.12 ± 0.006**
15:0	0.23 ± 0.008	0.24 ± 0.004*
16:0 iso	0.02 ± 0.008	0.03 ± 0.009
16:0	9.23 ± 0.697	11.93 ± 0.272*
17:0	0.00 ± 0.000	0.06 ± 0.017*
18:0 iso	0.00 ± 0.000	0.05 ± 0.042
18:0	1.53 ± 0.130	2.15 ± 0.087**
Saturated	16.32 ± 0.919	18.90 ± 0.328*
14:1n-5	0.48 ± 0.078	0.22 ± 0.015**
16:1n-11	0.36 ± 0.023	0.52 ± 0.014**
16:1n-9	0.35 ± 0.028	0.37 ± 0.013
16:1n-7	11.52 ± 0.472	12.42 ± 0.503
16:1n-5	0.18 ± 0.027	0.23 ± 0.023
17:1	0.14 ± 0.016	0.18 ± 0.016
18:1n-11	3.67 ± 0.322	3.96 ± 0.183
18:1n-9	18.18 ± 1.366	18.37 ± 0.648
18:1n-7	4.14 ± 0.212	4.74 ± 0.173**
18:1n-5	0.40 ± 0.008	0.46 ± 0.008**
20:1n-11	2.30 ± 0.119	2.29 ± 0.076
20:1n-9	13.95 ± 0.513	11.40 ± 0.442**
20:1n-7	0.75 ± 0.057	0.72 ± 0.023
22:1n-11	6.49 ± 0.691	3.76 ± 0.328**
22:1n-9	1.23 ± 0.088	0.85 ± 0.063*
Monounsaturated	64.14 ± 1.083	60.49 ± 0.624**
16:2n-4	0.50 ± 0.035	0.46 ± 0.061
16:3n-4	0.14 ± 0.044	0.16 ± 0.020
16:4n-1	0.39 ± 0.092	0.31 ± 0.047
18:2n-6	1.42 ± 0.056	1.38 ± 0.028
18:2n-9	0.03 ± 0.013	0.10 ± 0.009*
18:3n-3	0.42 ± 0.032	0.43 ± 0.020
18:4n-3	1.23 ± 0.102	1.11 ± 0.080
18:4n-1	0.04 ± 0.026	0.11 ± 0.017*
20:2n-6	0.10 ± 0.030	0.11 ± 0.024
20:4n-6	0.04 ± 0.020	0.27 ± 0.034**
20:4n-3	0.63 ± 0.041	0.65 ± 0.035
20:5n-3	4.31 ± 0.412	6.83 ± 0.397**
22:4n-6	0.25 ± 0.042	0.27 ± 0.032
22:5n-3	2.39 ± 0.122	1.90 ± 0.076**
22:6n-3	7.61 ± 0.395	6.46 ± 0.371*
Polyunsaturated	19.50 ± 0.512	20.55 ± 0.451

Differences between maternal blubber and milk tested by Wilcoxon signed-rank test: $^{**}P < 0.01$, $^{*}P < 0.05$

stage (e.g., see 20:1, 22:1, 22:6n-3, Fig. 1). Most of these changes occurred by day 2 postpartum and did not differ between days 2 and 4. In most cases, differences between milk and blubber in proportions of specific FA appeared to decline with time postpartum (Fig. 1). This is illustrated by plotting the averages of the within-female ratios of milk to blubber FA against time postpartum (Fig. 2). The milk:maternal blubber ratios of some FA of common biosynthetic origin, 16:0, 16:1, 18:0 and 18:1, were generally greater than 1.0 when combined across lactation stages in comparison to FA of dietary origin, major PUFA (except 20:5n-3) and the MUFA 20:1 and 22:1, which were less than 1.0 across stages ($P < 0.05$). However, when separated by stage, most components were significantly different from 1.0 at day 0 but approached a ratio of 1.0 by 2 or 4 days postpartum (Fig. 2). The single component which did not follow these patterns was 20:5n-3. In contrast to other PUFA, the milk:blubber ratio of 20:5n-3 was the highest of any FA throughout lactation, averaging 1.5 at birth and 1.6–1.8 later.

Although it was not possible to precisely test whether changes occurred over lactation within blubber or in milk due to cross-sectional sampling, levels of most FA in both milk and blubber appeared to fluctuate slightly or remain relatively constant among females across stages (e.g., Fig. 1). The largest consistent difference was observed in 20:5n-3, which averaged 6.3 ± 0.91% of blubber in the mothers at <1 day postpartum, but averaged 3.8 ± 0.54% and 3.6 ± 0.36% in the mothers at 2 and 4 days, respectively ($P < 0.05$). In these same mothers, levels of 20:5n-3 in milk were relatively more similar across stages, averaging 7.9 ± 0.05%, 6.5 ± 0.37%, and 6.03 ± 0.85%, at <1, 2 and 4 days, respectively.

The FA composition of blubber of pups changed dramatically after birth. Changes in the FA of blubber stores of pups could be compared with that of the milk consumed. By days 2 and 4 postpartum, the FA composition of pup blubber was almost identical to the FA composition of mothers' milk (Fig. 3). The newborn pup which had not yet suckled (Pup 1) was most different, as described above (see Table 2). The other two pups at <1 day were similar to Pup 1, but may have deposited some milk FA postnatally such that values were intermediate. In the first day of life, pup blubber was characterized by high proportions of 14:0, 14:1n-5, 16:0, 16:1 (mainly n-7) and 18:1 (only n-9 and n-7 isomers). In contrast, by 2 days postpartum the FA of pup blubber were generally reduced in the former components and greatly enriched in the MUFA 20:1 (mainly n-9) and 22:1 (mainly n-11) and PUFA (primarily 20:5n-3 and 22:6n-3, $P < 0.05$), as was found in both maternal blubber and milk (Table 3, Fig. 1).

At 4 days postpartum, when weaning occurs, there were no significant differences in the FA composition of blubber of the three pups compared to that of their mothers, with the exception of 4 components out of the 39 measured. Reflecting the initial pattern from birth (e.g., Table 2, Fig. 3), the proportion of 16:1n-7 was still slightly higher in pup's blubber than in mother's ($P < 0.01$), while 20:1n-9 and 22:1n-11 were still somewhat lower in pup blubber ($P < 0.05$). Only one component, 20:5n-3 which was lower in pup blubber at birth, by weaning had become significantly higher in pup blubber (7.0%) than in that of their mothers (3.7%, $P < 0.05$).

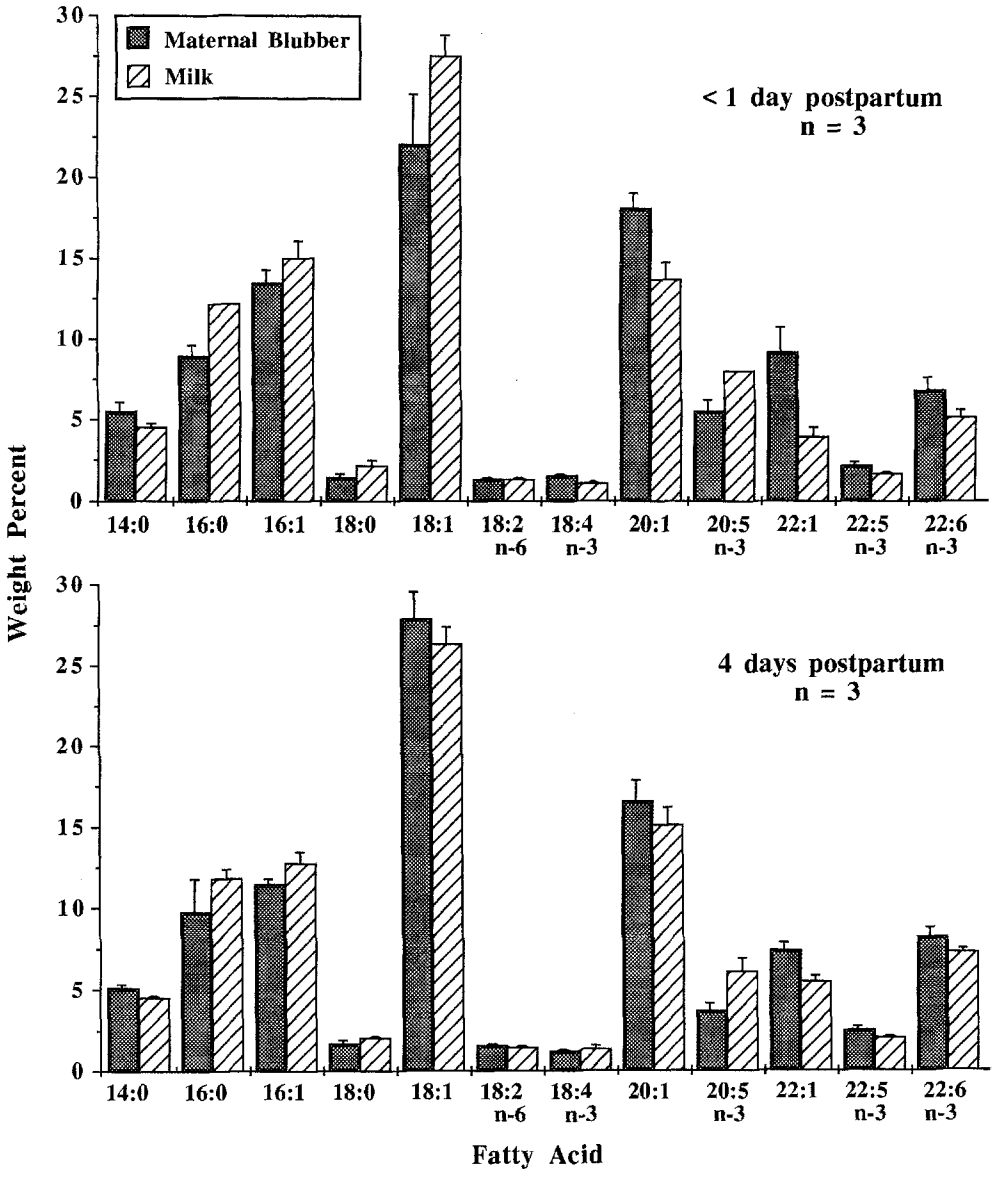

Fig. 1 Fatty acid composition (mean ± 1 SEM) of maternal blubber and milk from lactating hooded seals at <1 day and 4 days postpartum. Results of 2-way ANOVA (tissue × stage) revealed tissue differences for 14:0, 16:0, 18:0, 20:1, 20:5n-3, 22:1, 22:5n-3 and 22:6n-3 ($P < 0.05$) and stage differences for 16:1, 18:1, 18:2n-6, 20:5n-3, 22:5n-3 and 22:6n-3 ($P < 0.05$). Data for day 2 are not presented but did not differ from day 4

Discussion

Sources of fetal lipids

Despite their importance to neonatal physiology, FA transport and metabolism during the fetal period are not well understood. Hooded seal pups are unusual in being born with a blubber layer that accounts for about 18–19% of total BM [Table 1; Bowen et al. (1987)] and a total body fat content of 14% (Oftedal et al. 1993). Given that the newborn pup contains a substantial reservoir of lipid as blubber, which represents 90% of the total fat in the body (O.T. Oftedal, W.D. Bowen and D.J. Boness, unpublished data), we could examine the nature as well as net transfer of FA deposited prenatally in the young. Although we did not directly study FA synthesis or desaturation activity in the hooded seal fetus, the nature of lipid deposition in both mother and fetus allows some inferences to be made about placental FA transfer. During intensive fattening, phocid mothers likely deposit excess dietary FA directly into blubber (Iverson 1993). Maternal circulating FA will reflect either those from diet or those mobilized from blubber during fasting. If lipid deposition in the fetus is largely the result of transfer of circulating FA from the mother, then blubber of the newborn should be similar to that of its mother. This was clearly not the case in hooded seals, as many FA found in maternal blubber were reduced or entirely missing in blubber of the newborn prior to first suckling (Table 2). In contrast, FA such as 16:0, 16:1, and 18:1, which are readily biosynthesized in mammalian systems (Jeffcoat 1979), were quite high in blubber of

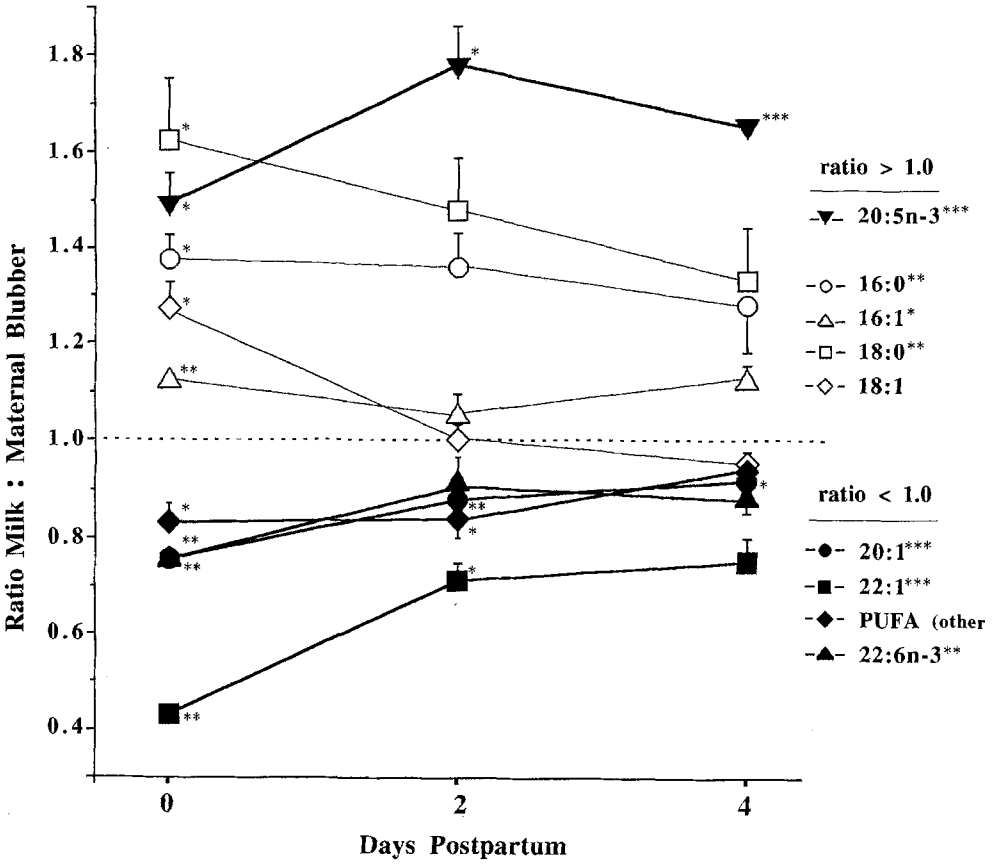

Fig. 2 Within female ratios of levels of fatty acids in milk: maternal blubber. Values for each fatty acid at each lactation stage are mean ratios from 3 females + 0.5 SEM; SEM is presented as 0.5 SEM in either direction in order to avoid overlap. Data were tested by a 1-sample test against a hypothesized value of 1.0. For all stages combined, fatty acids 20:5n-3, 16:0, 16:1, and 18:0 were found to be greater than 1.0, whereas fatty acids 20:1, 22:1, the other major PUFA (18:2n-6, 18:4n-3, and 22:5n-3 combined), and 22:6n-3 were found to be less than 1.0. When data were separated by stage, differences from 1.0 were present for all components at day 0, but in most cases differences were not found at days 2 and 4. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$

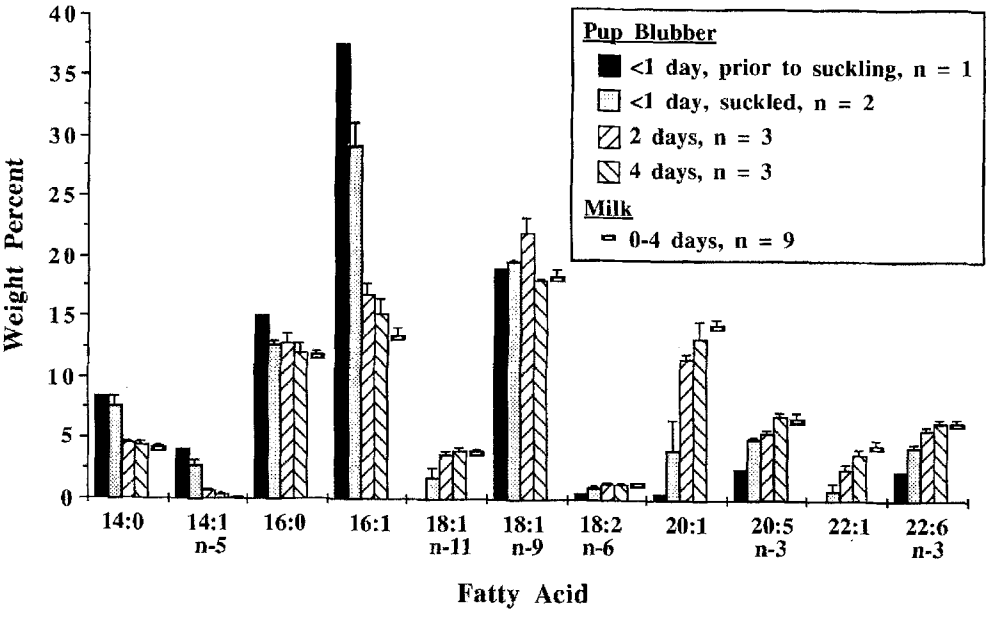

Fig. 3 Fatty acid composition (mean + 1 SEM) of blubber of pups at birth and over the 4-day lactation in comparison to that of the milk fat consumed. Significant differences across stages (all three pups at < 1 day combined) were found for all major components except 16:0 and 22:6n-3 ($P < 0.05$, Kruskal-Wallis Test). In all cases, the fatty acid composition of pup blubber at day 2 did not differ from day 4, and day 2 and day 4 blubber did not differ from milk

the newborn in comparison to its mother. These characteristics were also present, but less pronounced, in the other < 1-day-old pups which had already suckled (e.g., Fig. 3).

High levels of 16:0 and 16:1 in newborn adipose tissue compared to that of the mother have also been reported in studies of the human (Hirsch 1965; King et al. 1971), suggesting synthesis by the fetus. However, it

appears that in the hooded seal fetus, FA synthetase produces both 14:0 and 16:0 in large amounts, instead of primarily 16:0 which is the predominant product of most mammalian systems (Jeffcoat 1979; Gurr and James 1980). Oxidation of C16 to C14 may be less likely since the capacity of the fetus to oxidize FA is considered to be limited (Myant 1971; Roux and Myers 1974; Hull 1975; Ramsay and Dunbrack 1986). The hooded seal fetus also appears to have a very active Δ-9 desaturase system as evidenced by the large amounts of 14:1n-5 (up to 4.2%), a FA that is rarely found higher than 0.3% in most mammalian tissue, and 16:1n-7 (up to 37.0%) found in both newborn blubber (Table 2) and in blubber of <1-day-old pups after suckling (Fig. 3). A similar pattern was found in harbor seal pups, which are also born with a substantial blubber layer (11% body fat; Bowen et al. 1992). Blubber biopsies taken from ten pups directly after observed birth and prior to first suckling contained 4.1–4.4% 14:0, 4.0–5.0% 14:1n-5, 10.0–13.1% 16:0 and 43.1–48.5% 16:1n-7, all of which were highly enriched values compared to maternal blubber (S.J. Iverson, W.D. Bowen and H. Heras, unpublished data). The placenta of one of the harbor seal mothers was also analyzed and contained only 0.2% 14:1n-5, as is usually found in tissues and milk [Table 3, Fig. 3; Iverson (1988)] and 5.5% 16:1n-7. Clearly, the high levels of 14:1n-5, and probably 16:1n-7, in the newborn could not have originated from the mother, and contribute substantially to the high degree of unsaturation in newborn blubber (Table 2).

In addition to fetal biosynthesis, some FA were apparently transferred directly from mother to fetus in the hooded seal. As stated previously, one can distinguish between FA which could be readily biosynthesized by the animal versus those that must or most likely originate from the marine diet (Iverson 1993). For instance, FA such as 20:1n-9, 22:1n-11, 20:5n-3 and 22:6n-3, originate almost solely from the diets of marine mammals and are abundant in crustacea and pelagic fish such as capelin, cod, and herring (Ackman 1976, 1986) which are likely prey items of hooded seals (Reeves and Ling 1981). These FA together accounted for about 33% of total blubber FA of the hooded seal mother, and only 5.4% of newborn blubber (Table 2). While the deposition of MUFA 20:1 and 22:1 in the fetus was quite limited (<0.5%), the two n-3 PUFA, which can only come from the mother, appeared in fetal blubber in substantial amounts (4.9%). Similarly, in the ten newly born harbor seal pups, 20:1 and 22:1 were virtually absent and 20:5n-3 and 22:6n-3 accounted for 6–9% of total blubber FA deposited prenatally (S.J. Iverson, W.D. Bowen and H. Heras, unpublished data).

The ratios of FA levels in newborn adipose tissue: maternal adipose tissue can be used to infer transfer from mother to pup and to compare the hooded seal with the human and the guinea pig. These species are also born relatively fat [16% and 10% body fat, respectively; McCance and Widdowson (1977)]. For most FA that can be biosynthesized, the ratios in the human and hooded seal are nearly identical and much greater than 1.0 [calculated from King et al. (1971); Table 2]. However, the ratio for the EFA 18:2n-6 is only 0.1 in humans, whereas ratios for 18:2n-6, 20:5n-3 and 22:6n-3 were each 0.4–0.5 in hooded seals; FA of chain length greater than 18 were not measured in humans. Ratios in the other two <1-day-old pups were all similar to the unsuckled newborn, with the exception that those for n-6 and n-3 FA were slightly higher (0.6–0.7), likely due to some postnatal deposition of milk FA. In contrast, the fetal:maternal ratios in guinea pigs are closer to or greater than 1.0 for all FA measured, including EFA 18:2n-6 and 18:3n-3, which together comprise 23–38% of adipose tissue FA in guinea pigs [calculated from Hershfied and Nemeth (1968)]; longer-chain FA were not measured. These data suggest that direct transfer of FA is more important to total fetal lipids in guinea pigs than is fetal biosynthesis, in contrast to humans and hooded seals (cf. Pavey et al. 1976).

Both hooded and harbor seals appear to be able to transfer EFA and n-3 PUFA directly to the fetus relatively and absolutely more readily than humans. With 3.7 kg total blubber composed of 68% fat (O.T. Oftedal, W.D. Bowen and D.J. Boness, unpublished data), the hooded seal pup is born with about 180 g of n-6 and n-3 PUFA stored in adipose tissue alone. There was no evidence for presence of 20:3n-9 or 22:3n-9, the classical indicators of EFA deficiency.

Mobilization of maternal blubber fatty acids into milk

Depot FA of marine mammals are thought to be derived in large part from dietary FA and milk FA are thought to derive from depot FA but may differ somewhat (Meara 1952; Ackman and Burgher 1963; Ackman and Jangaard 1965; Ackman and Eaton 1966; Jangaard and Ke 1968; Ackman et al. 1971; Ackman and Hooper 1974). However, these studies have usually analyzed milk and blubber samples from different individuals or from collections of oil obtained by the pooling of blubber from many animals during processing. Given that the FA composition of pinniped milk varies substantially among individuals of a species, with diet and with stage of lactation (Iverson 1993), comparisons should be made on samples from the same individuals and stage of lactation.

Hooded seal females may secrete up to 10 kg of milk daily that contains 60% fat (Oftedal et al. 1993). Thus, about 6 kg of lipid is transferred from body stores into milk daily. Direct incorporation of blubber FA into milk with minimal processing would be the most likely way to achieve such phenomenal rates of milk fat secretion. The similarity of FA composition of blubber and milk in the hooded seal is compatible with this

hypothesis (Table 3). Nevertheless, some consistent differences in the proportions of specific FA are found between blubber and milk (Fig. 1). In early lactation, it appears that the longer-chain unsaturated FA which originate ultimately from dietary sources of the female, and thus from blubber during fasting, are reduced in milk compared to blubber (ratio less than 1.0) except for 20:5n-3 (Fig. 2). In contrast, FA that are commonly synthesized by mammalian systems tend to be enriched in milk compared to blubber (ratio greater than 1.0, Figs. 1, 2). As lactation progresses, these differences are reduced and the ratios of both groups of FA approach unity. These data suggest that direct incorporation of blubber FA into milk is lower at parturition than at later stages and may indicate a greater reliance on some *de novo* synthesis of FA in the mammary gland. It is not known whether *de novo* synthesis of milk FA occurs in pinnipeds, however.

FA in circulating triglycerides are probably the major source of long-chain FA in milk even when FFA are present during fasting (Annison et al. 1967). In lactating grey seals, activity levels of LPL, the enzyme responsible for uptake by the mammary gland of circulating triglyceride FA, are reduced at parturition but increase during lactation. This increase is correlated with an increase in both milk fat concentration and milk fat output (Iverson et al. 1993b). If a similar change in LPL activity occurs in hooded seals, this might in part explain the observation of increased output in milk of FA mobilized from blubber as lactation progresses (Fig. 2).

Selective mobilization of FA from maternal blubber, selective catabolism of mobilized FA to provide for maternal energy needs, or selective uptake of FA by the mammary gland might also explain the observed differences between milk and blubber. In hooded seals, enrichment of 20:5n-3 (and reduction of 22:1) in milk over other FA from blubber was pronounced throughout lactation (Figs. 1, 2). If we assume the milk:blubber ratio represents a measure of mobilization, then our results compare well with differential mobilization measured *in vivo* in fat cells of rats fed a fish-oil diet. In both cases, ratios for the MUFA 20:1n-9 and 22:1n-11 (0.5–0.8) indicate reduced mobilization, whereas ratios for n-3 and n-6 PUFA (approximately 1.0) indicate non-differential mobilization [Table 3; Raclot and Groscolas (1993)]. In contrast, the ratio for 20:5n-3 was higher than all other FA at 1.6 in hooded seals and 2.8 for rat cells. It has been suggested that hormone-sensitive lipase, the enzyme responsible for releasing FA from stored triglycerides, may exhibit preference for types of triglycerides or FA (Soma et al. 1992; Raclot and Groscolas 1993), or for the α-position ($sn-1$ and $sn-3$) on the glycerol backbone (Fredrikson and Belfrage 1983). Interestingly, in hooded seal milk (and blubber) the n-3 PUFA are all located at the *sn*-3 position (Brockerhoff et al. 1966; Brockerhoff et al. 1968; Iverson et al. 1992), but gastric lipase which is specific for the *sn*-3 position appears to preferentially release 20:5n-3 over all other FA from the milk triglyceride (Iverson et al. 1992).

Evidence of relative depletion from maternal blubber of selected FA of dietary origin over the course of lactation would support either selective removal or selective catabolism. We could not directly assess changes within the blubber of an individual female over time, but while most components were found at relatively comparable levels in all blubber samples examined, 20:5n-3 appeared to decline in blubber by greater than 50% over the course of lactation. In a longitudinal study of lactating grey seals, which lose 40% of their BM, 20:5n-3 was also consistently higher in milk than in blubber and declined in blubber over lactation (Iverson et al. 1993b). Depletion may be less pronounced in hooded seal females, since they lose only 16% of their BM over lactation (Bowen et al. 1987; Kovacs and Lavigne 1992).

Postnatal deposition of milk fatty acids during fattening

The average pup in this study contained about 3.7 kg blubber at birth (Table 1), which is similar to the value (4.1 kg) from a larger data set (Bowen et al. 1987). At the average growth rate of 7 kg · day^{-1}, within the first day postpartum a pup would consume about 10 kg milk or 6 kg fat (Oftedal et al. 1993), 4–5 kg of which is deposited in blubber. Thus, the FA composition of blubber may change substantially within just a few hours after birth. This likely accounts for the differences observed between the newborn pup obtained prior to first suckling and the other two <1-day pups which had milk in their stomachs. Although results from these two pups do not change conclusions about the nature of fetal lipids and pup:mother ratios (see *Sources of fetal lipids*), the values are in fact intermediate between those of the unsuckled newborn and pups at 2 days (Fig. 3). By 2 days postpartum, the blubber mass of pups would have increased threefold. The greatest changes in FA composition of pup blubber occurred between 0 and 2 days postpartum. FA that could only originate from the diet of the pup (and originally from the diet of the mother), such as 20:1, 22:1, and n-3 and n-6 PUFA, appear in large proportions. By weaning at 4 days, the proportion of FA in pup blubber were either identical to or approached values of the milk fat consumed (Fig. 3).

Ingested FA appear to be deposited directly and without modification into blubber of pups. In contrast, studies with rabbits and rats fed fish oil have demonstrated that deposition of 22:6n-3, and especially 20:5n-3, are reduced (Lin and Connor 1990; Jandacek et al. 1991). These differences may reflect adaptations of neonatal seals to diets high in n-3 and n-6 PUFA or they may be a function of milk lipid digestion. In

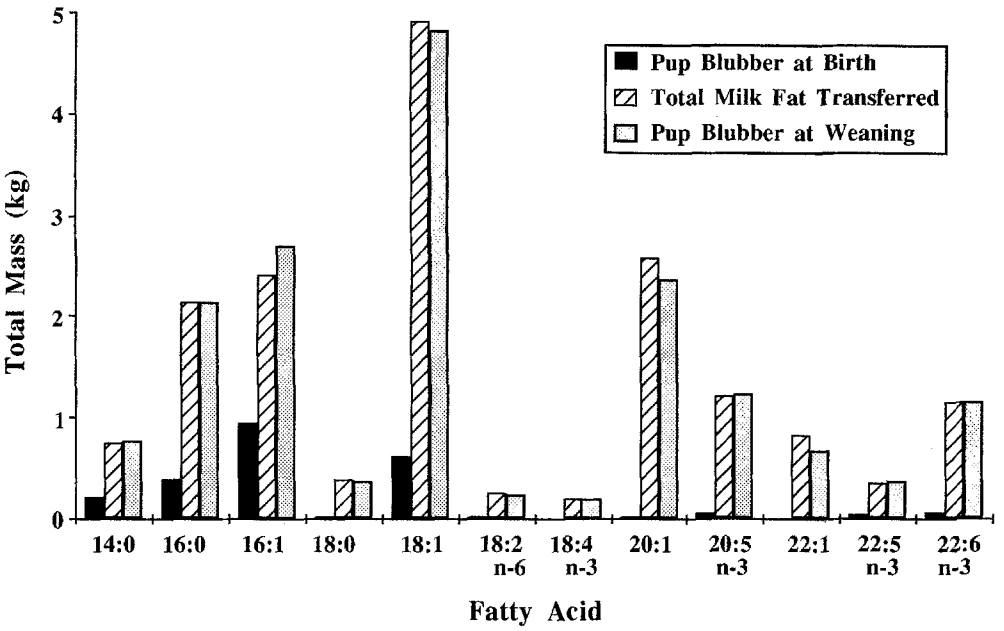

Fig. 4 Calculated total prenatal and postnatal deposition of fatty acids in the blubber of a hooded seal pup in comparison to the total mass of milk fatty acids consumed over the 4-day suckling period. Fatty acid composition data was from Tables 1 and 2 and Fig. 3. We assumed that blubber of pups (Table 1) was composed of 68.1% fat and 89.6% fat at birth and 4 days, respectively (O.T. Oftedal, W.D. Bowen and D.J. Boness, unpublished data). Total milk fatty acid intake was calculated from the average 4-day milk intake of hooded seal pups (approximately 1.46 kg · kg mass gain^{-1}) × average milk fat content (59.5%) from Oftedal et al. (1993)

hooded seal pups, both 20:5n-3 and 22:6n-3 were directly deposited in adipose tissue at the same ratio (0.9) which occurred in their diet (0.9; Fig. 3). In fact 20:5n-3 in pup blubber "overshoots" the values found in maternal blubber by the end of lactation, presumably because 20:5n-3 was enriched in milk and reduced over time in maternal blubber.

Milk FA clearly determine the nature of pup depot lipid. This can be illustrated by comparing the total mass of each FA deposited in blubber prenatally to that contained in pup blubber at weaning (Fig. 4). At weaning, the total FA in pup blubber mirror the total FA which have been transferred in milk over the 4-day lactation period. The ability of the hooded seal pup to digest and deposit such phenomenal quantities of FA is remarkable. Digestion of milk lipids in pinniped pups is facilitated in part due to gastric lipase, which results in extensive gastric lipolysis, and to milk lipase, which presumably aids intenstinal lipolysis (Iverson et al. 1992). If patterns of LPL activity in hooded seal pups are similar to those of grey seal pups, high rates of lipid deposition may be facilitated by high LPL activity of adipose tissue and by increasing total LPL activity with increasing total fat content of blubber (Iverson et al. 1993b). Additionally, since LPL has been found to correlate to total fat content in grey seal pups, perhaps hooded seal pups have an improved ability to deposit fat at birth since they are born with a substantial blubber layer.

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