Differential Mobilization of Blubber Fatty Acids in Lactating Weddell Seals: Evidence for Selective Use

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ABSTRACT

A major source of energy during lactation in mammals is provided through the mobilization of blubber fatty acids (FAs). We investigated the extent to which FAs were mobilized to support both maternal metabolic requirements and milk production in the Weddell seal and how this was reflected in the FA composition of the pup's blubber at the end of lactation (EL). FA composition of postpartum female blubber was similar in the 2 yr of study (2002 and 2003) but differed markedly by EL. Pup blubber FAs (at EL) were also different between years and did not match that of the mother's milk or blubber. Milk FA composition changed during lactation, which may have been a reflection of an increase in pup energy demands at different stages of development. In addition, there was evidence of feeding by some females during lactation, with higher levels of some FAs in the milk than in the blubber. Our results indicate that differential mobilization of FAs occurred in lactating Weddell seals and that this was related to total body lipid stores at postpartum. Furthermore, growing pups did not store FAs unmodified, providing evidence that selective use does occur and also that using FA composition to elucidate dietary sources may be problematic in growing individuals.

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Introduction

A major characteristic of mammalian reproductive strategies is the evolution of lactation modalities to deal with extreme energetic costs associated with offspring growth. Producing milk is one of the most energetically expensive activities for female mammals, so its delivery to offspring essentially defines the reproductive strategy a species evolves (Bonnet et al. 1998). The storage of energy represents an important component of life-history variation, and its delivery to the offspring helps define the trade-off between survival and future reproduction (Ferguson 2006). Within reproductive strategies, there has been recognized a capital-income breeding continuum that represents differing tactics of energy utilization (Jönsson 1997; Houston et al. 2007; Wheatley et al. 2008). This continuum ranges from capital breeding, which relies extensively on stored energy for reproduction, to income breeding, where energy used in reproduction is acquired throughout the course of the reproductive period (Stearns 1992; Jönsson 1997). Lactation strategies of pinnipeds show high diversity from extreme capital breeding, with offspring provisioned entirely from stored reserves over just a few days, to income breeding, with prolonged lactation over months or years (Boyd 1998; Trillmich and Weissing 2006; Houston et al. 2007). Females of the family Phocidae ("true seals") generally follow the capital strategy (Oftedal and Iverson 1987; Boness and Bowen 1996), which is typically associated with mobilization of fat stores and higher milk lipid content, reducing the time the pup is dependent on the mother. However, some phocids feed during lactation (Lydersen and Kovacs 1999; Bowen et al. 2001; Eisert et al. 2005), suggesting that late-lactation food intake may at times help offset the energetic costs of lactation.

In response to the high physiological demands of lactation, a major source of energy is provided through the mobilization of fatty acids (FAs) from the breakdown of triacylglycerol (TAG). FAs are stored primarily in the blubber and form an essential part of physiological regulation as precursors to the synthesis of other compounds, as fuels for energy production, and as building blocks for cell membranes (Dalsgaard et al. 2003). FAs may be accumulated directly from the diet, modified once ingested, or formed endogenously. The omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) are essential fatty

acids (EFAs) required for structural growth and brain and normal cell development (Innis 2005). These EFAs cannot be formed de novo by mammalian cells and so must be obtained from the diet. Therefore, FA mobilization and transfer is important not only for the energetic requirements of the fasting mother but also for the development of her offspring.

During lactation, lipid metabolism is intensified and the FA composition of the blubber changes as a consequence of utilization of depot FA (Iverson et al. 1995*a*; Grahl-Nielsen et al. 2000). Specific FAs may be mobilized or sequestered to accommodate the physiological requirements of both mother and pup (Samuel and Worthy 2004). In addition, because FAs can be differentially mobilized according to their molecular structure (Connor et al. 1996; Herzberg and Farrell 2003; Raclot 2003), loss of FAs from adipose tissue is not merely a function of the relative abundance of individual FAs. Metabolism and deposition of FAs may not therefore be predictable, depending on the time of year or physiological state of the individual at the time of measurement. Furthermore, because FA composition of stored fat is primarily a product of diet, FA composition may itself affect energy expenditure (Pierce and McWilliams 2005; Maillet and Weber 2006), and so variation in diet composition too may have an influential role in FA mobilization and transfer patterns during lactation.

FAs have been of interest from both nutritional and trophodynamic perspectives, with FAs used as qualitative markers to trace or confirm predator-prey relationships in the marine environment for more than 30 yr (see Dalsgaard et al. 2003). At higher trophic levels, markers become obscured because FAs originate from a variety of dietary sources. However, research has focused on applying FA signature analysis (FASA) to elucidate the dietary source of lipid reserves in upper-trophic-level predators, such as pinnipeds (Iverson 1993) and cetaceans (Hooker et al. 2001). FASA has been used to provide support to traditional diet analyses (e.g., stomach content and fecal analyses) in determining temporal, physiological, and spatial scales of diet variation. FAs have also been used to identify species and group interactions in food webs, thereby defining trophic exchanges (Iverson et al. 1997*b*; Walton et al. 2000; Bradshaw et al. 2003; Dalsgaard et al. 2003).

The use of FASA in constructing linkages requires that FAs be deposited and mobilized in a predictable way, with little modification throughout the chain of ingestion (Iverson 1993). The temporal dynamics (i.e., turnover rate of individual FAs) can be species specific and are often linked to metabolic condition or reproductive status (e.g., lactation). Consequently, FAs have been used mostly as qualitative food web or trophic markers (Dalsgaard et al. 2003). To quantify relationships using FAs in marine mammals, specific aspects of FA dynamics, including timescales for incorporation of new FAs and differential utilization, are required. Although quantitative methods have been developed recently (Iverson et al. 2004), an improved understanding of FA turnover and deposition is an essential precursor to their application in quantified FASA studies.

Nursing phocid mother-pup pairs offer a good opportunity to study FA mobilization, use, and deposition, given that the extent of lipid depletion is a key factor in the selectivity of FA mobilization (Raclot and Groscolas 1995). We examined the blubber FA composition of adult female Weddell seals at the beginning and end of lactation in relation to the FA composition of their milk to determine the extent to which FAs were used to fuel maternal energy requirements. Our approach also permitted the identification of FAs that were selectively mobilized and transferred to the pup. We compared this with the FA composition of the pups at weaning to determine the proportion of FAs that were used for growth and maintenance and stored in the pup's blubber. Our specific aims were to determine (1) whether particular FAs were selectively mobilized and/or transferred during lactation, (2) whether mobilization was influenced by initial FA composition, and (3) whether particular FAs were selectively deposited or used.

Material and Methods

Data Collection

This study was done at Hutton Cliffs, Antarctica (77°51′S, 166°45′E) during the austral summers (October to December) of 2002 and 2003. Thirty mother-pup pairs in 2002 and 25 pairs in 2003 were captured 1–6 d (mean ± SEM = 3.8 ± 0.22) postpartum. Individual females were identified by flipper tags attached in previous years, and pups were marked with hind flipper tags soon after birth as part of a long-term tagging study (Hadley et al. 2006). Once captured, each female was immobilized with Telazol (Wheatley et al. 2006*b*) and weighed to the nearest 1 kg, and body length and axial girth measurements were recorded. Each pup was weighed to the nearest 0.5 kg, and length and girth measured.

In 2003, milk was collected at postparturition (PP), midlactation (ML; 21–22 d PP), and end lactation (EL), using a modified 50-mL syringe following an intravenous injection of oxytocin (1 mL, 10 IU mL^{-1}). In both years, blubber biopsies were taken at PP and EL captures (5–6 wk later) for females and at the EL capture for pups. First, a small area on the posterior flank of each animal was shaved and disinfected. A small (~1 cm) incision was made with a scalpel blade in an anterior-posterior direction, a 6-mm biopsy punch was inserted through the incision, and a core was taken from the whole blubber layer (i.e., through until the muscle layer was reached; Bradshaw et al. 2003). Each sample was stored in a preweighed glass vial (with a Teflon-coated lid) containing a solution of 2 : 1 v/v chloroform and methanol and 0.05% (by weight) butylated hydroxytoluene (Sigma, St. Louis). Vials were reweighed, and all samples were stored at −20°C until laboratory analysis. Data were collected under permits from the University of Tasmania Animal Ethics Committee (A6790 and A6711) and the Department of Conservation of New Zealand (Per/22/2002/149 and Per/17/2003/188).

Table 1: Absolute fatty acid composition (kg) of female and pup blubber at postpartum (PP) and end lactation (EL) in 2002

	Female Blubber				Pup Blubber	
	PP ($N = 18$)		EL ($N = 25$)		EL ($N = 26$)	
Fatty Acid	Mean	SEM	Mean	SEM	Mean	SEM
14:1ω5c	3.03	.17	1.73	.10	.51	.02
14:0	16.08	.36	8.26	.37	3.91	.13
i15:0	.66	.02	.37	.02	.12	.00
16:1ω9c	.55	.02	.29	.01	.22	.01
16:1ω7c	23.25	.84	10.68	.54	6.49	.25
16:1ω5c	.66	.02	.30	.01	.19	.01
16:0	14.80	.33	6.61	.32	4.83	.15
i17:0	.33	.01	.18	.01	.08	.00
18:4ω3	1.66	.04	.78	.03	.39	.01
18:2ω6	3.02	.10	1.72	.07	.81	.03
18:1ω9c	52.70	1.81	28.48	1.23	14.75	.55
18:1ω7c	11.72	.36	6.18	.25	3.12	.10
18:1ω5	.94	.03	.50	.02	.23	.01
18:0	1.71	.06	.93	.04	.54	.02
20:4ω6	.68	.02	.35	.02	.20	.01
20:5ω3 EPA	5.76	.15	2.09	.12	1.49	.05
20:4ω3	.43	.02	.23	.01	.12	.01
20:2ω6	1.04	.05	.68	.03	.12	.01
20:1ω9c	8.02	.30	5.19	.25	1.35	.06
20:1ω7c	.81	.03	.52	.02	.12	.01
22:6ω3 DHA	7.47	.26	4.54	.18	1.63	.06
22:5ω3 DPA	2.01	.10	1.35	.07	.49	.02
22:1ω11c[a]	1.28	.06	.80	.05	.05	.00
22:1ω9c	1.08	.04	.75	.04	.09	.01
24:1	.34	.01	.23	.01	.00	.00

[a] Includes 22:1ω13c.

Laboratory and Data Analyses

Body composition, water flux rates, and milk intake were determined using hydrogen isotope dilution (Wheatley et al. 2006*a*, 2008). Blubber and milk lipids were quantitatively extracted using a modified overnight Bligh and Dyer (1959) one-phase methanol/chloroform/water extraction (2 : 1 : 0.8, v/v/v). Following extraction, chloroform and water (0.9% NaCl) were added to make a biphasic system (final solvent ratio, 1 : 1 : 0.9, v/v/v, methanol/chloroform/water). Total lipid was concentrated from the lower chloroform phase by rotary evaporation at 40°C. A subsample of lipid was transmethylated to produce FA methyl esters using a methanol/chloroform/hydrochloric acid reagent (10 : 1 : 1, v/v/v; 80°C; 2 h). After the addition of water, FA methyl esters were extracted into hexane/dichloromethane (4 : 1, v/v, 3 × 1.5 mL). Gas chromatographic (GC) analyses were done with an Agilent 6890N GC (Avondale, PA) equipped with a HP-5 cross-linked methyl silicone–fused silica capillary column (50 m × 0.32 mm i.d.), a flame ionization detector, a split/splitless injector, and an Agilent 7683 autosampler. Helium was the carrier gas. Samples were injected in splitless mode at an oven temperature of 50°C. After 1 min, the oven temperature was raised to 150°C at 30°C min^{-1}, then to 250°C at 2°C min^{-1}, and finally to 300°C at 5°C min^{-1}. FA peaks were quantified by Agilent Technologies GC ChemStation software (Palo Alto, CA). Individual components were identified by mass spectral data and by comparing retention time data with those obtained for authentic and laboratory standards. GC results are typically subject to an error of ±5% of individual component area. GC-mass spectrometric (GC-MS) analyses were performed on representative samples on a Finnigan Thermoquest GCQ GC-mass spectrometer fitted with an on-column injector with Thermoquest Xcalibur software (Austin, TX). The GC was fitted with a capillary column similar to that described above.

The concentrations of individual FAs were converted to a mass percent of total FAs, and FAs present in trace amounts (<0.5%) were excluded from analyses. When examining proportional changes over lactation, some FA values increased, others remained the same, and some decreased. Considering lipids are being mobilized at a relatively high rate (Oftedal 2000), all FAs should decrease to some extent. Therefore, FAs expressed as a percentage of mass composition do not accurately reflect the absolute changes in FAs over lactation. This is due to the overall changes in lipid content of milk and individuals (i.e., body composition; Costa et al. 1986; Hindell et al. 1994; Mellish et al. 1999; Wheatley et al. 2006*a*, 2008). Therefore, proportional FAs were converted to absolute (mg g^{-1}) values to compare changes over time. To do this, representative samples ($n = 24$) were analyzed for lipid class composition by Iatroscan MK V TH10 thin-layer chromatography/flame ionization detection (Phillips et al. 2002). Results indicated that blubber and milk samples were composed virtually entirely of TAG (99.9%). TAG stored in the tissue consists of glycerol esterified with three FA molecules, and the FA moieties represent about 95% of the mass of TAG (Groscolas 1990). Therefore, the mass (kg) of lipid in each animal, as determined from hydrogen isotope dilution techniques (see Wheatley et al. 2006*a*), was multiplied by 95% to obtain the mass (kg) of FAs in each individual at each capture. Several studies have shown that total blubber mass estimates are highly correlated with total body fat estimates using isotope dilution (Gales and Burton 1987; Hall and McConnell 2007), since the predominant site of FA storage is in the blubber of pinnipeds (Kirsch et al. 2000).

The total amount of FAs (kg) lost by females in milk (during lactation) was calculated by using the total milk lipid output (kg) for early lactation (PP to ML) and late lactation (ML to EL; Wheatley et al. 2008), multiplied by 95% (see above), and the average FA composition of milk for those periods. Total milk lipid output also represented total lipid intake by pups, and changes in pup body composition indicated the amount of milk lipid (kg) stored by the pup.

From evidence that females do not feed during the first 3

Table 2: Absolute fatty acid composition (kg) of female blubber, pup blubber, and milk at postpartum (PP), midlactation (ML), and end lactation (EL) in 2003

	Female Blubber				Pup Blubber		Milk			
	PP ($N = 20$)		EL ($N = 10$)		EL ($N = 22$)		PP–ML ($N = 8$)		ML–EL ($N = 8$)	
Fatty Acid	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
14:1ω5c	2.38	.18	1.29	.12	.38	.03	.20	.02	.40	.04
14:0	11.91	.86	5.83	.41	2.62	.21	2.53	.27	3.54	.42
i15:0	.50	.03	.29	.02	.08	.01	.08	.01	.14	.02
16:1ω9c	.48	.02	.25	.02	.18	.01	.16	.01	.22	.02
16:1ω7c	19.19	.75	8.25	.49	4.74	.31	3.31	.31	4.62	.51
16:1ω5c	.55	.02	.23	.01	.13	.01	.11	.01	.15	.02
16:0	11.54	.63	4.90	.32	3.46	.25	3.89	.39	4.70	.55
i17:0	.30	.02	.16	.01	.06	.01	.06	.00	.09	.01
18:4ω3	1.42	.06	.64	.04	.27	.02	.25	.02	.35	.04
18:2ω6	2.58	.11	1.48	.09	.65	.04	.52	.05	.80	.08
18:1ω9c	44.65	1.73	23.84	1.47	11.85	.68	9.00	.76	13.66	1.44
18:1ω7c	10.28	.41	5.25	.34	2.53	.16	2.12	.16	3.12	.32
18:1ω5	.81	.03	.43	.03	.18	.01	.15	.01	.24	.03
18:0	1.41	.06	.79	.05	.44	.03	.55	.04	.78	.08
20:4ω6	.55	.02	.28	.02	.16	.01	.15	.01	.21	.02
20:5ω3 EPA	4.91	.25	1.59	.13	1.01	.08	1.15	.11	1.24	.15
20:4ω3	.47	.05	.24	.04	.10	.01	.09	.01	.13	.02
20:2ω6	.86	.05	.64	.05	.16	.01	.10	.02	.20	.02
20:1ω9c	6.43	.32	4.37	.36	1.09	.07	.79	.07	1.54	.17
20:1ω7c	.71	.03	.50	.03	.10	.01	.10	.01	.19	.02
22:6ω3 DHA	6.76	.34	3.87	.25	1.32	.09	1.10	.08	1.89	.23
22:5ω3 DPA	2.17	.22	1.41	.20	.47	.05	.36	.04	.61	.10
22:1ω11c[a]	.94	.08	.65	.06	.04	.00	.07	.01	.14	.02
22:1ω9c	.83	.06	.65	.06	.07	.01	.11	.01	.22	.03
24:1	.23	.02	.20	.02	.00	.00	.03	.00	.07	.01

[a] Includes 22:1ω13c.

wk of lactation (Eisert et al. 2005), we calculated a "feeding index" for the second half of lactation based on differences in milk energy output values, which represented the number of times that milk energy (%) exceeded that of nonfeeding individuals. This allowed us to rank the relative amount of feeding that occurred among individuals (Wheatley et al. 2008), and these values were used as a covariate in some analyses.

Principal components analysis (PCA) was used on proportional (% of total FAs) and absolute (mg g^{-1}) values to investigate patterns of FA in blubber and milk and over time. Principal component scores were used in a series of generalized linear and mixed-effects models (GLM, GLMM) to examine differences in FA composition and mobilization. Examination of the residuals for all models determined the statistical error distribution (Gaussian) and link function (identity). Model selection was based on Akaike's Information Criterion corrected for small samples (AIC_c; Burnham and Anderson 2002). Models were ranked according to relative AIC_c weights ($wAIC_c$). Specific model comparisons were based on the information-theoretic evidence ratio (ER), which is equivalent to the AIC_c weight (w) of one model divided by the w of the null (or other) model (Burnham and Anderson 2002). Model goodness-of-fit was assessed by calculating the percent deviance explained (% DE). The FA most responsible for the multivariate patterns were identified in SIMPER (similarity percentages) analysis (Clarke 1993). The SIMPER procedure compares the average abundances and examines the contribution of each FA to the average Bray-Curtis dissimilarity between two defined groups of samples (e.g., blubber or milk). All statistical analyses were done using PRIMER (ver. 5.2.9) or the R package (ver. 2.4.1; R Development Core Team 2004). All proportional values were arcsine–square root transformed before analysis. Values are presented as mean ± 1 SEM unless otherwise stated.

Results

Twenty-four FAs (comprising 94%–98% of total FAs) were found in greater than trace amounts (>0.5%; for absolute values, see Tables 1, 2). Short-chain monounsaturated FAs (SCMUFAs; ≤18 carbons) dominated both the blubber and the

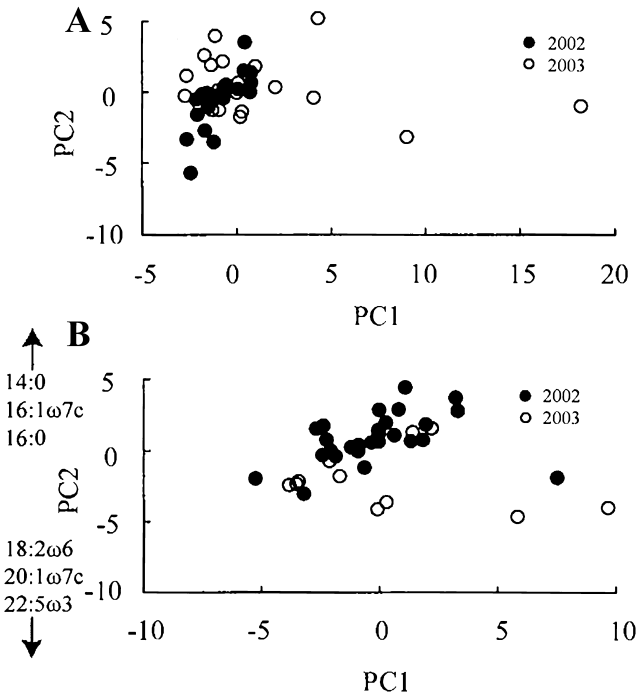

Figure 1. Principal component analysis of female blubber fatty acids at postpartum (*A*) and end lactation (*B*) in 2002 and 2003. The main determinants of the second principal component (*PC2*; eigenvalues) for end lactation are shown along the axis in *B*.

milk, with saturated FAs (SFAs) the next highest in proportion, followed by PUFA and long-chain monounsaturated FAs (>18 carbons; LC-MUFAs). There was a strong positive linear relationship between FA loss (kg) during lactation and total body lipid (TBL; kg) at postparturition (TBL; ER = 2.28×10^3, % DE = 88.5%) but no evidence for a relationship with maternal PP mass (ER = 1.23, % DE = 25.7%). Therefore, TBL was used as a covariate in our analyses.

Female Blubber Fatty Acids

There was little evidence for any difference between years in the proportional FA in female blubber samples at PP, according to PCA (Fig. 1*A*). The terms age and TBL were the only important correlates of FA composition (GLM $w\text{AIC}_c = 0.596$), although the % DE by this model was relatively low (% DE = 14.6). There was evidence for a difference between years in proportional FA at EL along the PC2 axis (Fig. 1*B*). The GLM with the highest weight ($w\text{AIC}_c = 0.986$) included all terms (year, age, and TBL) and explained a relatively high proportion of the deviance in the first principal component for FA composition at EL (% DE = 57.5%). The top five FAs that contributed to 38.9% of the dissimilarity between years were SFAs 14:0 and 16:0 (2002 > 2003) and MUFAs 16:1ω7c (2002 > 2003), 18:1ω9c, and 20:1ω9c (2003 > 2002). In absolute terms of FAs mobilized, 73.5% of the difference between years resulted in more of SFAs 14:0 and 16:0 and in MUFAs 18:1ω9c, 16:1ω7c, and 18:1ω7c being mobilized in 2002 instead of in 2003.

The most parsimonious model testing for the effects of TBL, year, and feeding index on fractional mobilization of FAs (i.e., the fraction of initial mass of the FA that was lost from the blubber during lactation) included both TBL and feeding ($w\text{AIC}_c = 0.533$, % DE = 38.5%). There was a strong negative linear relationship between feeding and fractional mobilization (ER = 1.49×10^4, % DE = 61.7%). In all but three females, EFA 20:5ω3 had the highest fractional mobilization from the blubber (range: 36.9%–81.6%).

Milk Fatty Acids

A plot of the first two principal components divided the milk samples into three distinct groups (PP, ML, EL; Fig. 2). The first component (PC1) accounted for 40.8% of the variation in FA composition among samples, while the second component (PC2) accounted for 26.5%. The GLMM used to examine the influence of stage of lactation only on PC1 scores revealed that stage explained 87.6% of the variation in samples. Between PP and ML, there was an increase in 20:1ω9c, 22:6ω3, and 22:1ω9c and a decrease in 20:5ω3 and 16:0. Between ML and EL, 18:1ω9c and 22:5ω3 increased while 16:0, 14:0, and 20:5ω3 decreased. Overall, there was a gradual decrease in SFAs, a slight decrease in PUFAs between PP and ML, and an increase in SC-MUFAs and LC-MUFAs.

The total mass of FAs (kg) lost by some females during lactation in 2003 differed from the FA mass transferred in milk (Tables 3–5). Because some of the shorter chain FAs can be synthesized de novo, these differences were clear in the PUFAs,

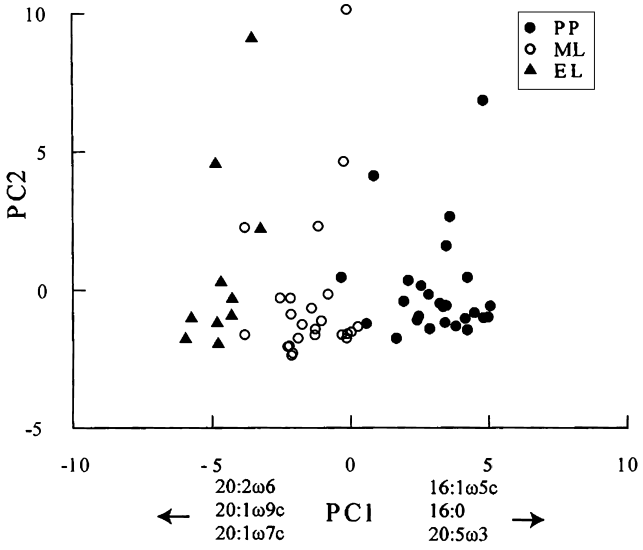

Figure 2. Principal component analysis of milk fatty acids at postpartum (*PP*), midlactation (*ML*), and end lactation (*EL*) in 2003. The three fatty acids with the most extreme positive and negative loadings (eigenvalues) for the first principal component (*PC1*) are shown along the axis.

Table 3: Total fatty acid loss (kg) during lactation for eight females

Fatty Acid	P130	P871	Pu114	Pu517	Pu761	W636	Y536	Y965
14:1ω5c	1.17	1.95	1.72	.43	1.74	1.15	.44	1.42
14:0	6.12	8.18	9.95	2.28	9.48	8.86	3.68	7.40
i15:0	.20	.26	.32	.07	.30	.35	.13	.28
16:1ω9c	.18	.24	.26	.11	.32	.30	.23	.27
16:1ω7c	8.23	11.80	11.91	5.25	17.04	15.14	8.59	13.54
16:1ω5c	.23	.34	.33	.16	.46	.44	.30	.40
16:0	4.85	6.43	7.72	2.71	10.28	10.38	5.34	8.87
i17:0	.08	.11	.18	.06	.19	.19	.22	.18
18:4ω3	.50	.69	.85	.33	1.10	1.06	.70	1.05
18:2ω6	.62	.95	1.32	.44	1.51	1.69	.78	1.58
18:1ω9c	12.43	19.42	25.32	10.32	29.15	31.64	14.38	27.55
18:1ω7c	2.85	4.44	5.91	2.60	6.85	7.26	4.72	6.64
18:1ω5	.26	.35	.41	.16	.51	.56	.34	.48
18:0	.40	.52	.72	.30	.80	.90	.63	.88
20:4ω6	.21	.19	.28	.15	.40	.38	.26	.36
20:5ω3 EPA	2.44	2.71	2.99	1.49	5.56	4.17	3.14	4.33
20:4ω3	.18	.14	.20	.15	.26	.26	.28	.27
20:2ω6	.11	.15	.46	.10	.26	.26	.30	.40
20:1ω9c	.80	1.55	3.68	.49	2.11	3.81	1.71	3.33
20:1ω7c	.09	.14	.35	.06	.25	.41	.19	.36
22:6ω3 DHA	1.79	2.37	3.43	1.40	3.55	3.64	2.44	3.96
22:5ω3 DPA	.38	.52	.96	.39	.73	.88	1.08	1.06
22:1ω11c[a]	.11	.24	.57	.00	.30	.63	.20	.43
22:1ω9c	−.07	−.01	.42	.04	.22	.56	.14	.34
24:1	−.01	.03	.06	.00	.03	.07	−.06	.07
Sum	44.13	63.68	80.32	29.49	93.39	94.98	50.18	85.45

[a] Includes 22:1ω13c.

which for mammals can be acquired only through dietary intake. Female Pu517 was a relatively large animal (430 kg) but had the lowest proportional TBL of the sampled females (25.7%). The discrepancy between FA loss and transfer was the highest for Pu517, indicating that she appeared to feed more than any other female during lactation. This was further supported by data describing milk energy transfer to her pup, where milk energy output (1,735.4 mJ) exceeded that of total energy lost (911.4 mJ) late in lactation (Wheatley et al. 2008). Using this disparity in FA mass loss and transfer as an index of feeding activity, some other females also showed signs of feeding, while others showed virtually none (Table 5).

Pup Blubber Fatty Acids

There was evidence for a difference between years in the FA composition of pup blubber at EL, according to PCA (Fig. 3*A*). This was supported by the top-ranked GLM including both year and TBL terms ($wAIC_c = 0.739$, % DE = 34.4%) from original models of PC1 versus year, TBL, and sex. Forty-three percent of the difference between years resulted from a dissimilarity in SFAs 14:0 and 16:0 (2002 > 2003), PUFA 20:5ω3 (2002 > 2003), and MUFAs 16:1ω7c (2002 > 2003) and 18:1ω9c (2003 > 2002). The FA composition of the pup blubber (EL) was different from that of the milk at all stages of lactation (PP, ML, and EL) but did appear to fall somewhere between PP and ML composition (PCA; Fig. 3*B*). There was also a clear separation between the maternal blubber (PP) and the pup's blubber (PCA; Fig. 4).

Of the FAs received in the milk, 55.9% ± 1.03% (range: 48.6%–61.9%) on average was stored in the pup's blubber (Fig. 5). Although LC-MUFAs appeared to be utilized more by the pup (i.e., less stored), there was no evidence that FA group affected storage rate (GLMM, % DE = 4.3%).

Discussion

Animals obtain energy and nutrients from food ingested, so their diet can be considered a key element affecting all life-history traits (Taylor et al. 2005). FAs represent a large group of molecules that comprise the majority of lipids found in all organisms (Budge et al. 2006) and are mobilized to provide metabolic fuel in situations of negative energy balance (Raclot 2003). During lactation, maternal FAs are mobilized to support maintenance metabolism and milk production, and females may differentially mobilize or modify FAs to suit their particular

Table 4: Total fatty acids in milk (kg) during lactation for eight females

Fatty Acid	P130	P871	Pu114	Pu517	Pu761	W636	Y536	Y965
14:1ω5c	.39	.59	.58	.63	.53	.52	.27	.50
14:0	3.99	6.17	5.61	3.82	5.69	6.04	3.13	5.76
i15:0	.15	.22	.18	.15	.22	.23	.11	.20
16:1ω9c	.25	.35	.27	.34	.43	.33	.25	.31
16:1ω7c	5.36	7.88	7.19	5.57	7.54	7.52	4.67	6.88
16:1ω5c	.17	.26	.23	.19	.29	.24	.18	.22
16:0	5.80	8.55	7.64	4.92	8.22	8.38	5.23	7.93
i17:0	.09	.12	.11	.11	.21	.11	.12	.11
18:4ω3	.40	.55	.49	.39	.64	.54	.38	.53
18:2ω6	.92	1.23	1.10	1.07	1.36	1.22	.72	1.18
18:1ω9c	15.82	21.84	19.33	18.66	22.25	21.03	12.16	19.49
18:1ω7c	3.32	4.80	4.46	4.02	5.77	4.66	3.52	4.40
18:1ω5	.28	.37	.31	.29	.44	.38	.26	.32
18:0	.94	1.21	.96	.99	1.48	1.17	.95	1.12
20:4ω6	.24	.29	.27	.28	.46	.29	.26	.31
20:5ω3 EPA	1.56	2.03	1.95	1.56	2.81	2.10	1.60	2.21
20:4ω3	.13	.16	.16	.21	.32	.17	.21	.14
20:2ω6	.30	.37	.33	.34	.38	.33	.25	.31
20:1ω9c	1.71	2.34	1.87	1.61	2.34	2.30	1.33	2.00
20:1ω7c	.21	.27	.22	.23	.33	.27	.19	.24
22:6ω3 DHA	2.07	2.44	2.11	2.83	3.87	2.47	1.87	2.36
22:5ω3 DPA	.58	.65	.58	1.15	1.52	.64	.77	.63
22:1ω11c[a]	.15	.21	.20	.07	.17	.27	.11	.21
22:1ω9c	.26	.41	.21	.23	.20	.41	.20	.26
24:1	.07	.09	.08	.05	.09	.11	.06	.09
Sum	45.15	63.41	56.44	49.73	67.57	61.72	38.79	57.72

[a] Includes 22:1ω13c.

physiological needs. A core idea in life-history physiology is that differential allocation of limited maternal resources has a central role in the cost of reproduction, maintenance, growth, and storage (Harshman and Zera 2006). Within the suborder Pinnipedia (order Carnivora), marked behavioral differences in provisioning young have been exhibited (Costa 1991), where most females from the family Phocidae ("true seals") generally follow a capital-based (fasting-based) strategy, while females from the family Otariidae (fur seals and sea lions) generally follow a more income-based strategy (Boyd 2000). For capital breeders, the diet composition before parturition will influence lipid and FA dynamics, while for income breeders, a combination of diet before and during lactation will influence dynamics.

Understanding the influence of diet and FA mobilization on milk production and transfer is important for interpreting the foraging ecology, trophic dynamics, and life-history strategies of mammals (Iverson 1993). We found no evidence for a difference between the PP blubber FA composition of females in 2002 and 2003, suggesting that overall, the diet of study females did not differ substantially between those years. However, by the end of lactation, the FA composition of female blubber was notably different between years. In addition, the FA composition of the pup blubber (at EL) was also different, suggesting that there were differences in milk FA transfer. Proximate milk composition changes over lactation for many species (Mellish et al. 1999; Arnould and Hindell 2002). For Weddell seals, lipid content at PP (39.9% ± 1.29%) increased by ML (50.0% ± 1.64%) and then decreased by the end of lactation (41.7% ± 2.34%; Wheatley et al. 2008). In contrast, milk protein levels begin low and increase throughout lactation (Wheatley et al. 2008). This may indicate that early in lactation, mothers devote resources to producing lipid-rich milk for the pup's thermoregulatory needs, followed by a later increase in protein required for tissue growth. Accordingly, this may be why pup FA signatures track more closely to milk early to ML (Fig. 3*B*).

We determined that more SFAs (14:0 and 16:0) and SC-MUFAs (16:1ω7c, 18:1ω9c, and 18:1ω7c) were mobilized from the female blubber in 2002, which corresponded to more 14:0, 16:0, and 16:1ω7c in the pup blubber of that year. However, we could not examine variation in milk FA transfer between years because milk was collected only during the second season (2003). Several studies suggest that other factors may exert a greater influence on lipid dynamics than diet (Egeler et al. 2003; Pierce and McWilliams 2005). Thus, it appears that differences in FA mobilization were related to the dissimilarity in overall

Table 5: Difference in fatty acids (kg) lost from the female blubber and those present in the milk

Fatty Acid	P130	P871	Pu114	Pu517	Pu761	W636	Y536	Y965
14:1ω5c	.79	1.36	1.14	−.20	1.20	.63	.18	.92
14:0	2.13	2.01	4.35	−1.54	3.79	2.82	.55	1.64
i15:0	.04	.03	.14	−.08	.09	.12	.02	.08
16:1ω9c	−.07	−.11	−.01	−.23	−.12	−.03	−.01	−.04
16:1ω7c	2.87	3.92	4.72	−.32	9.49	7.62	3.92	6.66
16:1ω5c	.06	.08	.10	−.03	.18	.20	.12	.18
16:0	−.95	−2.12	.09	−2.21	2.06	2.00	.11	.93
i17:0	−.01	.00	.07	−.05	−.02	.07	.10	.07
18:4ω3	.09	.14	.35	−.07	.45	.53	.32	.51
18:2ω6	−.30	−.28	.23	−.63	.15	.48	.06	.39
18:1ω9c	−3.39	−2.41	5.98	−8.34	6.90	10.61	2.23	8.06
18:1ω7c	−.46	−.36	1.45	−1.42	1.08	2.60	1.21	2.24
18:1ω5	−.01	−.02	.10	−.13	.06	.19	.08	.16
18:0	−.53	−.70	−.24	−.69	−.68	−.27	−.32	−.23
20:4ω6	−.03	−.10	.02	−.13	−.05	.09	.00	.05
20:5ω3 EPA	.88	.68	1.03	−.07	2.75	2.07	1.54	2.12
20:4ω3	.04	−.02	.04	−.05	−.06	.09	.07	.13
20:2ω6	−.20	−.22	.13	−.24	−.11	−.07	.05	.09
20:1ω9c	−.91	−.79	1.81	−1.13	−.22	1.51	.38	1.33
20:1ω7c	−.13	−.13	.13	−.17	−.08	.15	.00	.12
22:6ω3 DHA	−.27	−.07	1.32	−1.43	−.32	1.17	.57	1.60
22:5ω3 DPA	−.21	−.14	.38	−.76	−.79	.24	.31	.43
22:1ω11c[a]	−.05	.03	.37	−.07	.12	.36	.09	.23
22:1ω9c	−.32	−.42	.20	−.19	.01	.14	−.06	.08
24:1	−.08	−.07	−.02	−.05	−.06	−.04	−.11	−.02
Sum	−1.01	.27	23.87	−20.24	25.81	33.26	11.39	27.73

Note. Negative values indicate that there were more fatty acids (kg) in the milk than was lost from the blubber, indicating a possibility of feeding. Small negative values may represent inherent measurement error in calculation methods.

[a] Includes 22:1ω13c.

condition (TBL; kg) of females at parturition. Females in 2002 had higher TBL stores than those in 2003. This affected not only FA mobilization but also lactation length, maternal expenditure, and pup mass gain: larger females had higher transfer efficiency rates and weaned larger pups (Wheatley et al. 2006*a*). This suggests that the effects of environmental variability (and female condition) can be seen in fine-scale physiological responses such as FA mobilization.

Few other studies have examined milk FAs over lactation in marine mammals and instead have sampled on only one occasion (Grahl-Nielsen et al. 2000; Birkeland et al. 2005). Considering that provisioning offspring is energetically the costliest component of reproduction in mammals (Costa et al. 1986; Clutton-Brock 1991; Boness and Bowen 1996; Mellish et al. 1999), this approach may not give a representative description of FA mobilization and transfer. Of those studies that have sampled milk more frequently, Iverson et al. (1995*b*) found changes in some milk FAs between the beginning and end of lactation in hooded seals on the basis of cross-sectional sampling, although ML (i.e., day 2) composition was similar to EL (i.e., day 4) composition. Conversely, Debier et al. (1999) found no evidence that FA profiles in harp and hooded seals underwent large changes over the course of lactation. Both studies do not include data on all FAs, which makes it difficult to generate broad comparisons to Weddell seals. However, changes in milk lipid, protein, and FA composition support the notion of a shift in the function of milk and the physiological priorities of the pups during lactation. This may be related to the relatively long lactation and early introduction to swimming and diving for the pups.

When nutrients or reserves become severely limited in phocid seals, the proximate composition of milk will not be altered; rather, milk output will be reduced (Iverson 1993). We would expect that this may also apply to FAs so that processes other than lipid reserve depletion would affect mobilization rates. We found that milk FAs differed markedly between each stage of lactation (PP, ML, and EL), with the proportion of each FA group either decreasing or increasing over lactation. These changes in proportion may be related to the biochemical properties of the FAs and/or to an evolutionary adaptation for lac-

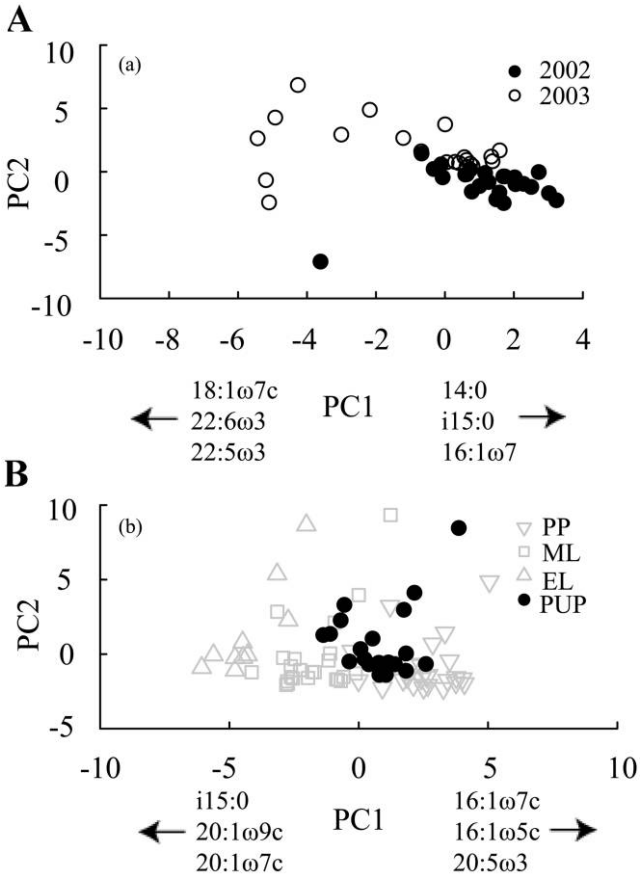

Figure 3. Principal component plot of fatty acid composition for pup blubber at end lactation in 2002 and 2003 (*A*) and fatty acid composition of pups in 2003 compared with milk fatty acids in 2003 (*B*). The three fatty acids with the most extreme positive and negative eigenvalues for the first principal component (*PC1*) are shown on each axis.

tation where FAs may be selectively sequestered to support growth and development of the offspring. For example, SFAs may have been higher in milk at PP because they store more chemical energy useful to blubber-poor neonates (Wheatley et al. 2006*a*). Increasing the amounts of SFAs delivered via milk early in the lactation period may therefore maximize the catalyzable energy the pup receives. Later in lactation, the proportion of MUFAs increased in milk because the latter offers optimal characteristics for energy storage by providing higher-energy density than do PUFAs and higher mobilization and oxidation rates than do SFAs (Maillet and Weber 2006).

Selective mobilization of FAs appears to be a general metabolic feature of adipose tissue (Raclot 2003; Nieminen et al. 2006) and is governed by molecular structure (Raclot and Groscolas 1993; Raclot 2003). Previous studies have shown that FAs are more readily mobilized when they are short and unsaturated and when their double bonds are closer to the methyl end of the chain (Raclot 2003). In Weddell seals, there was evidence of selective mobilization of particular FAs during lactation (e.g., 20:5ω3, 18:1ω9, 16:1ω7, 16:0, and 14:0); however, some of these did not conform to the molecular structure of highly mobilized FAs, as previously cited (i.e., SFAs; Raclot 2003).

Similar to hooded seals (Iverson et al. 1995*b*), the EFA 20:5ω3 had the highest fractional mobilization from Weddell seals blubber during lactation. The proportion of 20:5ω3 was also highest in milk immediately postparturition, indicating that mobilization of this FA most likely occurred early in lactation when the females were fasting and that there is a temporal component to FA mobilization (i.e., some FAs may be selectively mobilized at different times, depending on energetic or growth requirements of the mothers and pups). Both 20:5ω3 and 22:6ω3 are associated with phospholipids of hormone precursors and biomembranes and are thus involved in many physiological processes, including neurological function (Innis 2005). Higher proportions of 20:5ω3 delivered immediately postparturition may be required for early development of various offspring functions. As 20:5ω3 decreased in milk, 22:6ω3 increased, further demonstrating selective mobilization that was most likely related to the physiological requirements of the developing pup.

There was evidence that some females fed during lactation, as indicated by the presence of higher quantities of PUFAs in the milk than were mobilized from the blubber. In addition, less fractional mobilization of blubber FA occurred in response to higher feeding rates. Of all the females that appeared to feed, all but one (P130) had a higher maternal PP mass than the average for that year (393 kg; Wheatley et al. 2006*a*). This is consistent with the finding that larger females tended to feed more during lactation (Wheatley et al. 2008). Female P130 also had a longer lactation and lost a higher proportion of her body

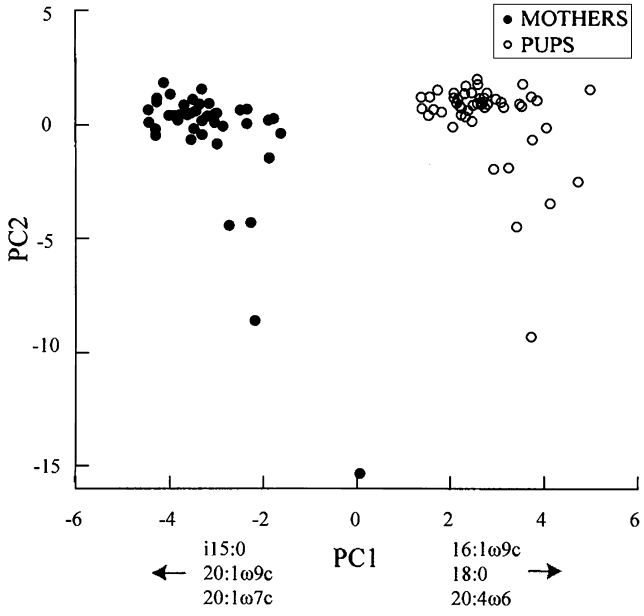

Figure 4. Principal component analysis of female blubber at postparturition and pup blubber at end lactation.

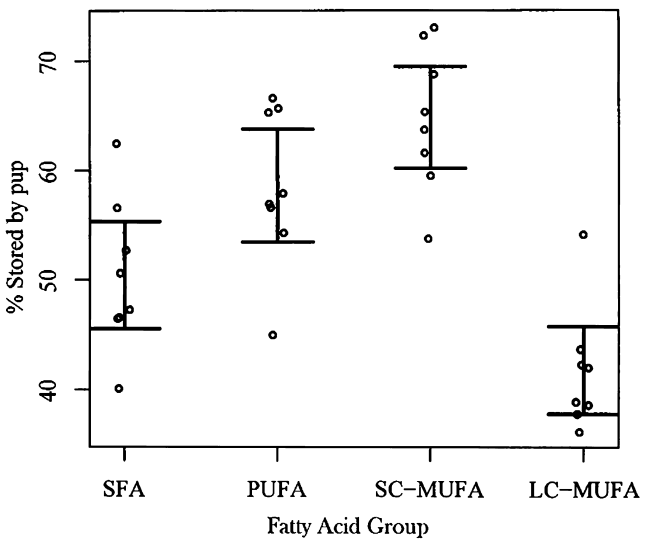

Figure 5. Percent fatty acid storage by pups in 2003 (±SEM). This was determined by dividing the amount of fatty acids (kg) in the pup blubber by the amount of fatty acids (kg) received in the milk, then averaging this for each fatty acid group for each pup. Groups are as follows: *SFA*, saturated fatty acids; *PUFA*, polyunsaturated fatty acids; *SC-MUFA*, short-chain (≤C18) monounsaturated fatty acids; *LC-MUFA*, long-chain (>C18) monounsaturated fatty acids.

mass than the average female (see Wheatley et al. 2006*a*), indicating that although she was feeding, it did not seem to facilitate higher energy delivery to her pup.

Feeding in Weddell seals appears to occur late in lactation (Eisert et al. 2005), so we believe that the milk FAs measured between PP and ML were those of nonfeeding individuals. Although feeding generally occurred later, it was not apparent for all females, and the overall trends in changes to milk FAs between ML and EL (Fig. 2) were consistent (i.e., no large outliers). This suggests either that food intake was insufficient to change overall milk FA composition and fueled the females' energy requirements or that it was from a source similar to that of the blubber. Therefore, we conclude that changes in the FA composition represented differential mobilization of fat reserves, a mechanism that likely evolved in response to the variation in the energetic demands of the pup over the course of its development (Costa et al. 1986; Iverson et al. 1993; Mellish et al. 1999). Of the FAs received from the milk, an average of 55.9% was stored in the pup's blubber, while the rest was used for the pup's growth and maintenance. Although some feeding has been observed by pups from the breeding colony (K. E. Wheatley, unpublished data), Weddell seal pups typically undergo a postweaning fast of 1–3 wk (Rea et al. 1997). Therefore, it is likely that negligible feeding is occurring during lactation, and FA values would be representative of milk ingestion only.

Iverson et al. (1995*b*) found that ingested FAs were deposited directly and without modification into the blubber of hooded seal pups. However, this was not the case for Weddell seals and most likely reflects the longer lactation period (5–6 wk) compared with the brief one of hooded seals (4 d). In addition, differences may also be the result of Weddell seal pups swimming during lactation (Stirling 1969), influencing the relationship between energy intake and growth similar to some otariid species (Costa and Gentry 1986; Arnould et al. 1996).

Milk has been suggested to be a source of FAs with which to study diet in breeding mammals (Iverson 1993). In species where a mother fasts throughout lactation, such as with most phocid seals or during the perinatal period in otariid seals, milk FAs are thought to reflect the diet during the prebreeding foraging trip (Iverson 1993; Iverson et al. 1995*b*). This has prompted a number of studies investigating the applicability of using milk FAs to estimate maternal diet, often producing conflicting results (Iverson et al. 1997*a*; Staniland and Pond 2004). Although feeding did occur with some study females, FA changes were still evident in the milk of those that did not feed. Therefore, these changes in milk FA throughout lactation support previous results (Grahl-Nielsen et al. 2000; Staniland and Pond 2004; Staniland and Pond 2005), demonstrating that the use of milk to estimate diet can be problematic. Given that milk was the only energy source for suckling pups, its FA composition was not well replicated in the blubber. This is most likely a reflection of the differential use of FAs for growth and storage by the pup, as well as changes in milk FA that occurred during lactation. That the pup's blubber did not match the mother's further illustrates that unmodified FA transfer did not occur. It is unlikely that blubber would ever exactly match diet signatures, especially in an animal that is still growing. Therefore, using FASA as a biomarker of dietary intake should be used cautiously because individual FAs are mobilized and stored with differential selectivity, depending on the physiological state of the measured individual. Nevertheless, we have demonstrated that the analysis of FAs can provide other insights into the functions of living systems besides the assessment of diet composition and trophodynamics.

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