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ORIGINAL PAPER

Fatty acid mobilization and comparison to milk fatty acid content in northern elephant seals

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Abstract A fundamental feature of the life history of true seals, bears and baleen whales is lactation while fasting. This study examined the mobilization of fatty acids from blubber and their subsequent partitioning into maternal metabolism and milk production in northern elephant seals (*Mirounga angustirostris*). The fatty acid composition of blubber and milk was measured in both early and late lactation. Proportions of fatty acids in milk and blubber were found to display a high degree of similarity both early and late in lactation. Seals mobilized an enormous amount of lipid (~66 kg in 17 days), but thermoregulatory fatty acids, those that remain fluid at low temperatures, were relatively conserved in the outer blubber layer. Despite the stratification, the pattern of mobilization of specific fatty acids conforms to biochemical predictions. Long chain (>20C) monounsaturated fatty acids (MUFAs) were the least mobilized from blubber and the only class of fatty acids that showed a proportional increase in milk in late lactation. Polyunsaturated fatty acids (PUFAs) and saturated fatty acids (SFAs) were more mobilized from the blubber, but neither proportion increased in milk at late lactation. These data suggest that of the long chain MUFA mobilized, the majority is directed to milk synthesis. The mother may preferentially use PUFA and SFA for her own metabolism, decreasing the availability for deposition into milk. The potential impacts of milk fatty acid delivery on pup diving development and thermoregulation are exciting avenues for exploration.

Keywords Marine mammal · Lactation · Fasting · Fatty acids · Lipolysis

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Introduction

The combination of fasting and lactation is a rare life history strategy, involving conflicting metabolic demands. Lactation is a period of high energy expenditure (Gittleman and Thompson 1988), while fasting is normally one of energy conservation (Castellini and Rea 1992). Despite these challenges, the evolution of simultaneous fasting and lactation has occurred in pinnipeds, mysticete whales and bears. While lactation and fasting have conflicting demands, they both rely on lipid as an energy source because it contains more energy per gram than either carbohydrate or protein (Frayn 2010).

In adult northern elephant seals (*Mirounga angustirostris*), lipid stores are built up over two long foraging trips thousands of km out to sea (Robinson et al. 2012). When elephant seals are ashore, they fast for several consecutive weeks or months during breeding, post-weaning development or molt. The lipid stores gained at sea support >90 % of metabolism during their terrestrial, fasting phase (Crocker et al. 2001). Like other phocids, elephant seals have a very short, intense lactation period that is enabled by the rapid transfer of energy in lipid-rich milk. Female

elephant seals give birth to a single offspring, and lactate while fasting for ~26 days while producing lipid-rich milk (20–55 % lipid) (Crocker et al. 2001; Costa et al. 1986). The combination of fasting and lactation is one of the factors believed to have driven the composition of pinniped milk (i.e., high fat, low water, almost no carbohydrate) (Oftedal 1993; Costa 1991).

The utilization of lipid stores to support maintenance metabolism and milk production consists of mobilization from the lipid depot (blubber) and uptake by tissue, either mammary gland or by other tissue for oxidation. In fasting seals, lipids to be directed to milk production can only come from stored lipid or via de novo synthesis in the mammary gland. The mother's diet will impact the fatty acid distribution in her blubber available to be mobilized during fasting (Iverson et al. 2004). The chain length of milk fatty acids can provide some insight into their source. Short and medium chain fatty acids (≤16 carbons) can be synthesized de novo in the mammary gland (Dils 1983). Seal milk studied previously has shown chain lengths of 12 carbons to be the shortest chain fatty acid present, but only trace levels, with small amounts of 14 carbon fatty acids (Iverson et al. 1992; Debier et al. 1999; Riedman and Ortiz 1979; Wheatly et al. 2008). 16:0 is commonly synthesized by the mammary gland in several species, including ruminants, (Grummer 1991). However, previous studies have assumed that de novo synthesis in seal mammary glands is minimal (Iverson 1993).

Fatty acids can be divided into broad classes including saturated fatty acids (SFAs), containing no double bonds, monounsaturated fatty acids (MUFAs), containing one double bond, and polyunsaturated fatty acids (PUFAs), containing more than one double bond. In laboratory studies, for a given number of double bonds, shorter chain fatty acids are more readily mobilized from the storage depot; but for a given chain length, fatty acids are more readily mobilized as the degree of unsaturation increases (Conner et al. 1996; Raclot 2003; Raclot et al. 1995b). In addition, the mobilization of each fatty acid is generally unaffected by the relative prevalence of each fatty acid, until the exhaustion of a particular fatty acid (Raclot and Groscolas 1995; Raclot et al. 1995b).

The lipid composition of milk is critical to the developing neonate. The phocid-weaned pup will subsist mainly on stored lipid for the duration of the post-weaning fast (2–2.5 months) (Noren et al. 2003). In addition to providing energy, fatty acids serve multiple purposes in a developing pup; they are involved in sensory systems, particularly vision, immune function and development, among many other functions (Jump 2002).

Not only do fatty acids function in fuel and cell signaling, marine mammals utilize fatty acids for thermal insulation. Phocid seals store their fat in a subcutaneous blubber layer, rather than in scattered internal depots. It has been reported in many different species of marine mammals that blubber layers are stratified from inner to outer layers (Best et al. 2003; Strandberg et al. 2008; Wheatley et al. 2007; Koopman et al. 1996). External layers have a higher proportion of medium chain (≤18C) MUFA and are more prevalent in the exterior possibly as a homeoviscous adaptation (Sinensky 1974), i.e., for the purpose of maintaining membrane fluidity at low temperatures encountered at sea. Interior layers in phocid blubber are more enriched in saturated fatty acids, long chain (≥20C) MUFA and are more heavily metabolized (Best et al. 2003; Strandberg et al. 2008). In Weddell seal pups, the distribution of MUFA and PUFA may affect the development of thermoregulatory capabilities and oxidative capacity for diving (Wheatly et al. 2008; Trumble et al. 2010).

For the mother, differing proportions of MUFA, PUFA or SFA mobilized from blubber may affect plasma cholesterol (Kris-Etherton and Yu 1997) and triglycerides (Harris 1997), as well as stimulate lipolysis (Guo et al. 2005). Additionally, females must sequester adequate amounts of the appropriate fatty acid in the outer, "thermoregulatory" layer of blubber for their return to sea. The mobilization and subsequent utilization of specific fatty acids is likely to have consequences for both the mother and pup.

The dynamics of movement of lipid transfer from blubber to milk have been investigated in only a few species, including hooded seals (Iverson et al. 1995), grey seals (Grahl-Nielsen et al. 2000; Arriola-Ortiz 2010) and Weddell seals (Wheatly et al. 2008). Only two of these studies in other pinniped species have quantified the mobilization of specific fatty acids (Arriola-Ortiz 2010; Wheatley et al. 2008). Our goals in this study were (1) to quantify how northern elephant seals mobilize lipid stores relative to other mammals and (2) to understand partitioning of fatty acids between maternal metabolic use and milk production in northern elephant seals under controlled fasting and lactation durations. These questions have not been addressed in northern elephant seals. In order to give the most complete study to date on pinniped lipid mobilization, we combined the quantification of specific fatty acid mobilization in a longitudinal study where, contrary to the above studies, we analyzed the inner and outer blubber layers separately.

Methods

Study site and subjects

This study was carried out at Año Nuevo State Reserve, San Mateo County, CA during the 2005 breeding season (January–February). To facilitate identification, adult

female seals were marked with hair dye (Lady Clairol, Stamford, CT), shortly after arrival on land. Parturition dates were established by daily observations and considered to be the first day a marked female was observed with a pup, provided she had been observed without a pup the previous day. Twenty-two mother/pup pairs were captured early in lactation (day 5 post-partum) and 19 of these recaptured late in lactation (day 22 post-partum). From these animals, blubber was obtained from 15 mothers early in lactation and 17 at late lactation. Fourteen individuals were blubber sampled in both early and late lactation. Milk was obtained from 22 animals in early lactation and 19 in late lactation, with 19 individuals sampled in both. Within early lactation, 15 animals were sampled for both blubber and milk. Within late lactation, 17 individuals were sampled for both blubber and milk.

Body composition estimates were made using the truncated cones method (Crocker et al. 2001; Gales and Burton 1987). This method calculates the proportion of mass due to adipose and lean tissue and has been validated in elephant seals using isotopic dilution (Webb et al. 1998). Dorsal, lateral and ventral blubber depth measurements were made using a portable ultrasound (Ithaca Scanprobe, Ithaca, NY) at each of six locations along the seal. Lengths and girths were taken at these six points, as well as total curved length. Mass was measured using a tripod, canvas sling and scale (±1 kg) MSI, Seattle, WA).

Sample collection and processing

Females were initially immobilized with Telazol (tiletamine/zolazepam HCl, Fort Dodge Labs, Ft. Dodge, IA) at a dosage of ~1 mg/kg, administrated intramuscularly. Continued immobilization was maintained with ~100 mg bolus intravenous injections of ketamine via the extradural vein. At both captures milk and blubber samples were collected from the mothers. A blubber biopsy extending the full depth of the blubber layer was taken laterally, several cm anterior to the pelvis using a 6-mm biopsy punch (Uni-Punch, Premier Medical, Plymouth, PA, USA). Milk was collected from the teat using a clean cut-off syringe after an intramuscular injection of 40 IU of oxytocin (American Pharmaceuticals Partners, Los Angeles, CA, USA) near the mammary gland. Samples were placed on ice for transport to the lab. The blubber core was separated into inner and outer cores and stored and analyzed separately. While some studies of fatty acid stratification in blubber have subsampled more than two sections (Strandberg et al. 2008, 2011; Grahl-Nielsen et al. 2011), others have similarly split the cores into only two sections as we have done (Best et al. 2003; Wheatley et al. 2007). Furthermore, previous studies that related blubber fatty acid and milk fatty acid profiles averaged the entire blubber core and did not split the blubber core into any section (Iverson et al. 1995; Grahl-Nielsen et al. 2000; Wheatley et al. 2008; Arriola-Ortiz 2010). While it would have been ideal to split our samples into three or more sections, given the longitudinal nature of our study, this would have resulted in a significant increase in the number of samples that had to be analyzed and was beyond our means. While further splitting our blubber cores into multiple sections would have provided a more refined delineation of stratification, we were still able to document distinct patterns between just the inner and outer blubber cores and thus our results are robust.

All samples were stored at −80 °C in Nunc tubes until analysis. Work was conducted under NMFS Marine Mammal permit #87-1463 and all procedures were approved by the Sonoma State University Institutional Animal Care and Use Committee.

Fatty acid quantification

Fatty acid profiles were obtained by gas–liquid chromatography of the fatty acid methyl ester derivatives. Lipids were extracted using the method of Folch modified by Christie (1982) for blubber and using an adaptation of the method of Radin (1981) and Schweigert and Strobo (1994) for milk [see (Debier et al. 1999) for more details]. Fatty acids from the lipid extract were methylated in a solution of KOH in methanol (0.1 mol/L) at 70 °C for 60 min, then in a solution of HCl in methanol (1.2 mol/L) at 70 °C for 20 min. The fatty acid methyl esters (FAME) were then extracted with hexane and separated and quantified with a gas–liquid chromatograph (GC Trace ThermoQuest, ThermoFinnigan, Milan, Italy) equipped with a flame ionization detector, an automatic injector and a fused silica capillary column (100 m × 0.25 mm internal diameter) coated with a 0.2 μm film of biscyanopropyl polysiloxane (Rt-2560, Restek, Bellefonte, PA, USA) (Van Dang et al. 2011). Each peak was identified and quantified by comparison of retention times with pure FAME standards.

Fatty acids are expressed as percent of total fatty acids quantified within an individual sample. The 20 fatty acids quantified here represent around 85 % of the total fatty acids present in the samples (areas ratios), at both early and late lactation. Data for both outer and inner layers are presented here and the differences statistically evaluated, but we focus on the inner layer for the comparisons to milk, as the inner layer is metabolically more active (Best et al. 2003; Koopman et al. 1996; Strandberg et al. 2008).

Mass of each fatty acid was estimated by first using body composition measurements to calculate the mass of blubber stores of the animal. Previous studies have shown that elephant seal adipose tissue is ~90 % lipid both early and late in lactation (Crocker et al. 2001), TAG makes up 99.9 % of the lipid in phocid blubber and 95 % of the mass

of TAG is due to fatty acid (Wheatly et al. 2008). Total kilograms of adipose stores was estimated by multiplying the proportion of adipose tissue by mass of the animal. The adipose mass was multiplied by 0.90 to obtain the lipid mass of adipose tissue. The fatty acid mass was estimated by multiplying lipid mass by 0.95 to get total mass of the fatty acid. This total fatty acid mass was multiplied by 0.85 to obtain the total fatty acid mass quantified. Because blubber samples were split equally into inner and outer portions and the fatty acid composition analyzed separately, the total mass of the fatty acid quantified was multiplied by 0.5 and then by the proportion of the fatty acid in inner and outer, respectively. The total mass of each blubber fatty acid (KG_B) is the sum of the inner and outer blubber compositions.

Mobilization of individual fatty acids was assessed relative to how much was initially available by assessing the amount of fatty acid that remained in late lactation relative to the amount of fatty acid that was available in early lactation. Proportion of each fatty acid mobilized (pmFA) was estimated for matched early and late samples ($n = 14$) as in (Arriola-Ortiz 2010):

$$\text{pmFA} = 1 - \left(\text{FA}_{\text{kgL}}/\text{FA}_{\text{kgE}}\right)$$

where FA_{kgE} represents kilograms of fatty acid in early lactation, and FA_{kgL} represents kilograms of fatty acid in late lactation.

Statistical analyses

Statistical analyses were performed using the software R (version 2.13.1, R Development Core Team, www.R-project.org). Packages AED (Zuur 2009) and nlme (Pinheiro et al. 2009) were used to assess normality, homogeneity of variance and perform mixed effects modeling. Post hoc tests were carried out using the multcomp package (Hothorn et al. 2008).

Due to a mixture of paired and non-paired samples, a linear mixed model fitted using REML was used to evaluate significant differences from early to late lactation. To account for longitudinal sampling, subject was included as a random effect in the models. Variances were found to be unequal among classes for both double bonds and chain length, thus a variance component structure (varIdent) was used in the analysis. Paired t tests were run to investigate differences between inner and outer blubber core fatty acids. Statistical significance was considered at $p < 0.05$; for comparisons between classes of fatty acids, a Bonferroni correction was applied.

The similarity among the fatty acids in the blubber and those in the milk was assessed using two similarity indices. A percent similarity index was calculated as:

$$\%\text{Sim} = \sum_i \text{minimum}\,(p1_i, p2_i).$$

where $p1_i$ represents the proportion of fatty acid i in blubber and $p2_i$ is the proportion of fatty acid i in milk. (Krebs 1999).

A cosine similarity index (Petraitis 1981; Yin et al. 2011) was calculated as:

$$\text{Cosine} = B \cdot M/||B||\,||M||$$

where B equals the vector of the inner blubber fatty acid proportion values and M is vector of the milk fatty acid proportion values. This value varies between 1 and 0, with 1 representing identical vectors and 0 representing completely different vectors.

Results

Mass and body composition

Mass decreased significantly from 446 (S68) kg to 326 (SD = 47) kg ($F_{1,16} = 471.5$; $p < 0.001$) with seals losing 120 ± 23 kg or 27 % of body mass over 16 days. The proportion of adipose tissue decreased significantly from 37.2 (SD = 2.0) to 29.4 (SD = 2.0) % ($F_{1,15} = 193.5$; $p < 0.001$) over the study period losing an average of 70 kg or 42 % of their adipose tissue reserves. The mean total mass of fatty acids lost from the blubber depot was 66 (SD = 15) kg.

Blubber fatty acid signatures and mobilization

A total of 20 different fatty acids were quantified in blubber and milk (Table 1), ranging in chain length from 12 to 24 carbons. In both early and late lactation, the majority of the inner blubber core was made up of 18:1n-9. The second and third most abundant fatty acids were 16:0 and 20:1n-11. Similarly, outer blubber was made up of primarily 18:1n-9, 16:0 and 20:1n-11 in both early and late lactation.

The proportions of fatty acids were categorized into saturated (SFA), monounsaturated (MUFA) or polyunsaturated (PUFA) (Table 1), and investigated with respect to outer versus inner blubber (in both early and late lactation), as well as between classes within each layer from early to late lactation.

All classes of fatty acids were different from inner to outer portion of the blubber except for late lactation PUFA (Table 2; Fig. 1). MUFAs were further subdivided into medium chain (≤ 18 carbons) (MC MUFA) or long chain (≥ 20 carbons) (LC MUFA) in keeping with previous studies regarding stratification in marine mammals (Strandberg et al. 2008; Best et al. 2003; Koopman et al.

Table 1 Proportion of fatty acids in inner and outer blubber layers and milk samples in early and lactation in northern elephant seals

	Inner blubber early lactation	SD	Inner blubber late lactation	SD	Outer blubber early lactation	SD	Outer blubber late lactation	SD	Milk early lactation	SD	Milk late lactation	SD
C12:0	0.06	0.01	0.06	0.01	0.08	0.01	0.07	0.01	0.07	0.01	0.06	0.01
C14:0	3.85	0.44	3.91	0.71	2.56	0.18	2.45	0.20	3.06	0.34	3.29	0.51
C14:1n-5	0.11	0.04	0.12	0.04	0.22	0.03	0.22	0.03	0.09	0.02	0.09	0.03
C16:0	14.46	0.81	11.36	1.34	11.10	0.73	10.73	0.90	17.85	0.97	14.64	1.50
C16:1n-7	4.99	0.90	3.44	0.95	7.96	0.89	7.75	0.99	5.74	1.00	4.39	1.06
C18:0	3.73	0.21	3.86	0.24	2.72	0.26	2.62	0.24	3.62	0.35	3.78	0.24
C18:1n-11	2.53	0.53	3.27	0.46	3.88	0.84	4.49	0.62	5.83	0.78	5.51	0.80
C18:1 n-7	3.83	1.01	3.74	1.26	4.73	0.68	4.76	0.56	4.67	0.88	4.52	1.01
C18:1 n-9	30.99	2.07	29.77	3.13	39.91	1.81	40.18	1.87	35.13	2.70	32.87	2.42
C18:2 n-6	1.69	0.10	1.76	0.13	2.21	0.15	2.20	0.13	1.79	0.11	1.75	0.09
C18:3 n-3	0.50	0.05	0.37	0.05	0.50	0.05	0.47	0.06	0.60	0.06	0.57	0.15
C20:0	0.30	0.03	0.40	0.07	0.16	0.03	0.15	0.02	0.10	0.01	0.19	0.03
C20:1n-11	12.53	1.73	16.76	2.91	8.52	1.01	8.79	1.22	6.66	1.26	10.80	2.26
C20:1n-9	8.87	1.28	11.17	2.06	6.86	0.64	7.12	0.71	4.89	0.91	8.02	1.40
C20:3 n-3	0.19	0.06	0.15	0.03	0.12	0.03	0.10	0.03	0.16	0.03	0.22	0.04
C20:4 n-6	0.57	0.07	0.39	0.06	0.63	0.10	0.64	0.10	0.88	0.08	0.73	0.08
C20:5 n-3	2.22	0.88	0.74	0.42	1.30	0.36	1.09	0.39	2.80	0.99	1.40	0.81
C22:5 n-3	1.53	0.36	1.75	0.55	1.57	0.23	1.52	0.29	1.08	0.25	1.26	0.33
C22:6 n-3	6.38	0.96	5.93	1.21	4.76	0.65	4.46	0.75	4.89	0.80	5.64	0.88
C24:1 n-9	0.68	0.09	1.04	0.26	0.20	0.06	0.19	0.05	0.10	0.02	0.27	0.07
∑MC MUFA	42.45	2.40	40.35	3.41	56.71	1.96	57.40	1.50	51.45	1.85	47.37	1.92
∑LC MUFA	22.08	2.56	28.97	4.58	15.59	1.65	16.10	1.92	11.65	3.28	19.10	2.04
∑PUFA	13.09	1.71	11.16	1.98	11.09	1.19	10.48	1.44	12.21	1.87	11.69	1.81
∑SFA	22.39	1.09	19.41	1.55	16.61	0.80	16.02	0.99	24.70	1.38	21.88	1.94

Values are expressed as mean percent (SD are standard deviations)

MC MUFA ≤18C monounsaturated fatty acids, *LC MUFA* ≥20C monounsaturated fatty acids, *PUFA* polyunsaturated fatty acid, *SFA* saturated fatty acid

Table 2 Statistical comparison of fatty acids in inner and outer blubber cores and in milk in early and late lactation

	MC MUFA	LC MUFA	PUFA	SFA
Blubber				
Early vs. late lactation (inner blubber)	$F_{(1,13)} = 14.2$, $p = 0.04$*	$F_{(1,13)} = 69.3$ $p = 0.02$*	$F_{(1,13)} = 42.7$; $p = 0.02$*	$F_{(1,13)} = 54.9$; $p = 0.02$*
Early vs. late lactation (outer blubber)	$F_{(1,13)} = 1.7$, $p = 0.22$	$F_{(1,13)} = 7.9$, p = 0.20	$F_{(1,13)} = 4.8$, $p = 0.045$	$F_{(1,13)} = 8.4$, $p = 0.20$
Inner vs. outer (early lactation)	$t = -23.4$, $df = 14$; $p = 0.02$*	$t = 8.6$, $df = 14$, $p = 0.02$*	$t = 5.1$, $df = 14$, $p = 0.02$*	$t = 16.0$, $df = 14$, $p = 0.02$*
Inner vs. outer (late lactation)	$t = -20.9$, $df = 16$, $p = 0.02$*	$t = 11.0$, $df = 16$, $p = 0.02$*	$t = 1.8$, $df = 16$, $p = 0.09$	$t = 9.0$, $df = 16$, $p = 0.02$*
Milk				
Early vs late lactation	$F_{(1,18)} = 246.2$; $p = 0.02$*	$F_{(1,18)} = 375.0$; $p = 0.02$*	$F_{(1,18)} = 4.9$, $p = 0.80$	$F_{(1,18)} = 135.8$, $p = 0.02$*

MC MUFA medium chain monounsaturated fatty acid, *LC MUFA* long chain monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid, *SFA* saturated fatty acid

* $p < 0.05$ after Bonferroni correction

1996; Wheatley et al. 2007). In early lactation, the proportions of MC MUFA were lower in the inner layer relative to the outer blubber layer; while proportions of LC MUFA, PUFA and SFA were higher in the inner layer than in outer blubber (Fig. 1). In late lactation there were fewer MC MUFA in the inner layer relative to the outer blubber

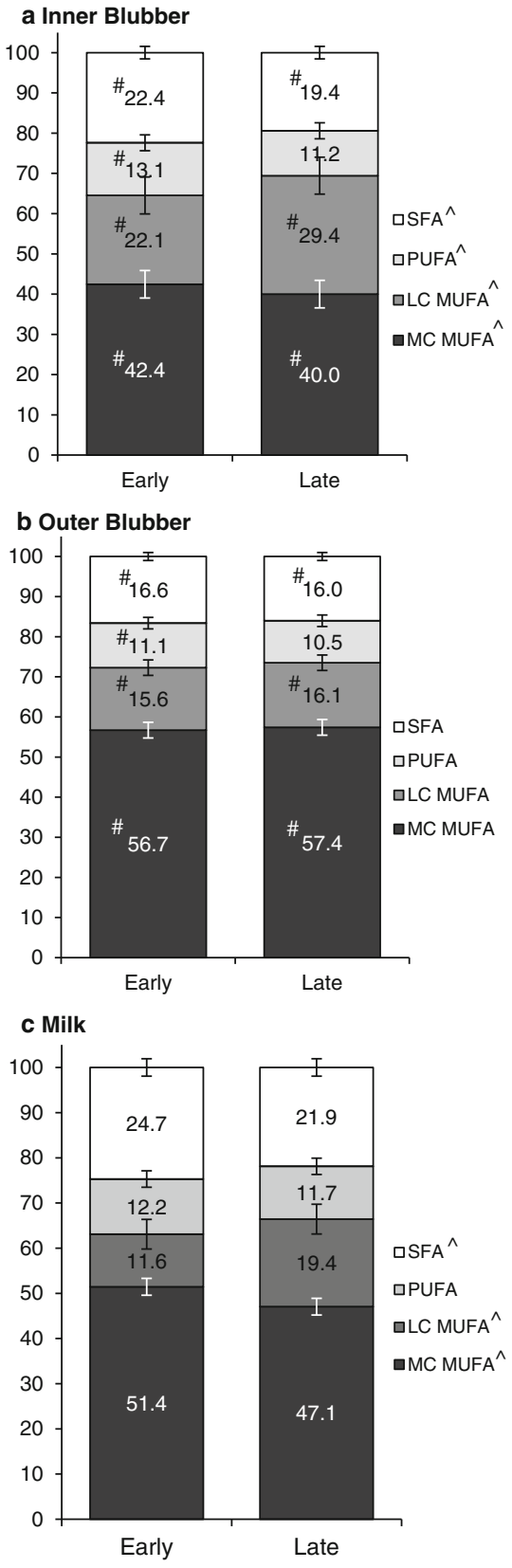

Fig. 1 Change in proportion of fatty acid classes in inner blubber (**a**), outer blubber (**b**), and milk (**c**) across lactation in northern elephant seals. *Bars* are standard deviations. *Wedge symbol* significantly different between stages; *hash symbol* significantly different between blubber layers. See Table 2 for *p* values. *SFA* saturated fatty acid, *PUFA* polyunsaturated fatty acid, *LC MUFA* long chain monounsaturated fatty acid, *MC MUFA* medium chain monounsaturated fatty acid

layer and higher proportions of LC MUFA and SFA in inner blubber compared to outer blubber. The proportion of PUFA remained stable between inner and outer layers in late lactation.

When investigating within the outer blubber layer, all classes of fatty acids remained stable from early to late lactation. There were significant changes in the proportions of all classes within the inner blubber layer from early to late lactation (Fig. 1; Table 2). Proportions of LC MUFA increased in the inner blubber layer across lactation. SFA, MC MUFA and PUFA proportions decreased in the inner layer from early to late lactation (Table 2; Fig. 1).

The mobilization of fatty acids from the blubber (pmFA) was estimated using the change in mass of each fatty acid in the entire blubber core from early to late lactation. This calculation indicated that the three most mobilized fatty acids were PUFAs 20:5n-3, 20:3n-3 and 18:3n-3 (Fig. 3). This mobilization index takes into account how much the mass of a particular fatty acid decreased, relative to how much was initially present. For example, although 18:1n-9 is present in high proportions in both blubber and milk in early and late lactation, when viewed from the mass lost in the blubber, it is only the 9th most mobilized fatty acid.

The proportion of fatty acids mobilized from the blubber was analyzed according to class (MC MUFA, LC MUFA, PUFA, SFA). Different classes were mobilized at different rates ($F_{3,\ 261} = 43.0$; $p < 0.001$; Fig. 2). PUFAs were the most mobilized class, (0.50; SD = 0.1), followed by SFA (0.41; SD = 0.07) and MC MUFA (0.42; SD = 0.11) and then by LC MUFA (0.28; SD = 0.09). Additionally, the number of double bonds ($F_{6,\ 252} = 104.7$, $p < 0.001$) and chain length ($F_{6,252} = 29.9$, $p < 0.001$) were significant predictors of proportion mobilized, with greater mobilization as double bonds increased and chain length decreased.

Milk fatty acids

Milk fatty acids from early and late lactation were composed primarily of 18:1n-9, 16:0 and 20:1n-11, similar to blubber (Table 1). When fatty acids were divided into the classes of saturated, monounsaturated or polyunsaturated, the proportion of all categories except PUFA changed significantly from early to late lactation (Table 2; Fig. 1).

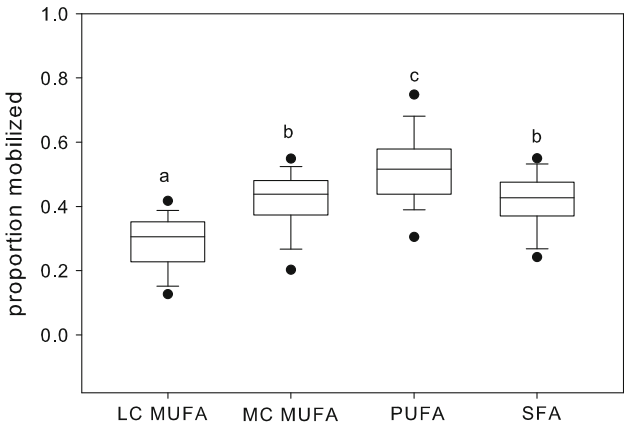

Fig. 2 Boxplot of proportion mobilized in different classes of lipid across lactation. *Box* boundaries are 25th and 75th percentiles, the *line* indicates the median and the whiskers denote 5th and 95th percentiles. *Different letters* indicate significantly different mobilization ($p < 0.05$). *MC MUFA* medium chain monounsaturated fatty, *LC MUFA* long chain monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid, *SFA* saturated fatty acid

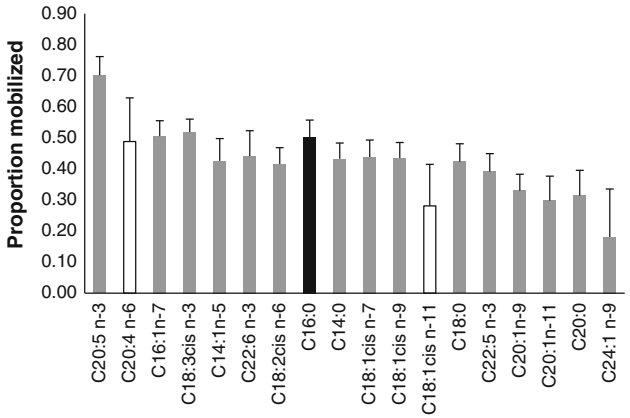

Fig. 3 The proportional mobilization of fatty acids across lactation in northern elephant seals. The fatty acids are ordered from *left* to *right* by decreasing expected order of mobilization (Raclot 03). *Light bars* reflect fatty acids that are slightly less mobilized than expected and *dark bars* are fatty acids that are somewhat more mobilized than expected. *Error bars* represent standard deviations. Estimates for C12:0 and C20:3n-3 were not available based on Raclot 03 and are not included in the figure

Proportions of milk PUFA were stable and milk SFA decreased across lactation as did the proportion of MC MUFA, while LC MUFA was the only class to significantly increase in milk.

Fatty acid signatures between blubber and milk

Inner layer blubber fatty acid patterns relative to milk fatty acid were compared by looking at the difference between each specific fatty acid. The greatest difference between milk and blubber ($\%FA_{milk} - \%FA_{blubber}$) was for 20:1n-11, with a difference of 5.9 % between milk and inner blubber in early lactation, with levels of 20:1n-11 making up a larger proportion of inner blubber than milk. Both 20:1n-9 and 20:1n-11 comprised higher proportions of inner blubber than milk; however, these two LC MUFA exhibited the largest proportional increases in milk from early to late lactation. As shown in Fig. 3, these are among the least mobilized of FA.

The two similarity metrics (cosine and percent) were calculated using the proportion of FA in inner blubber and milk FA proportions in early and late lactation, as well as between milk samples, early and late. Data are used from individuals for whom matched samples are available for early and late blubber and milk. The cosine similarity metric comparing the proportions of all fatty acids in early lactation blubber and milk was 0.967 (SD = 0.01), and the comparison of late lactation blubber and milk was 0.972 (SD = 0.01). When comparing milk from early to late lactation, the cosine similarity metric was 0.980 (SD = 0.0). The percent similarity for comparing blubber and milk in early lactation was 86.05 % (SD = 1.36) and for late lactation 87.91 % (SD = 3.1). Comparing the proportions of fatty acids in milk from early to late lactation resulted in a percent similarity of 89.98 % (SD = 1.66).

Discussion

Fatty acid mobilization from blubber

The patterns of fatty acid mobilization from blubber in northern elephant seals are very similar to other studies in rabbits, rats, mink and birds (Raclot et al. 1995a, b; Conner et al. 1996; Price et al. 2008; Raclot and Groscolas 1995; Nieminen et al. 2006) (Fig. 3). In vivo and in vitro studies have shown that the pattern of fatty acid mobilization primarily depends on the structure of the fatty acid, and in particular, on its chain length and number of double bonds (Raclot et al. 1995a; Conner et al. 1996; Raclot et al. 1995b; Price et al. 2008). Mobilization of a fatty acid for a given chain length increases with unsaturation and for a given unsaturation, shorter chain length fatty acids are mobilized faster (Raclot and Groscolas 1993). This relationship is largely independent of the relative amounts of fatty acid present until extreme depletion (Raclot et al. 1995b; Raclot and Groscolas 1995). Chain length and number of double bonds were significant predictors of mobilization in the current study, in agreement with previous studies. 20:5n-3 was the most mobilized fatty acid, which is consistent with laboratory studies with rats (Conner et al. 1996; Raclot and Groscolas 1995). However, there were a few small deviations from the typical pattern

of mammalian fatty acid mobilization. The few fatty acids that diverged slightly from expected patterns were: 16:0 that was slightly more mobilized than expected and 18:1n-11 and 20:4n-6, which were slightly less mobilized than expected (Fig. 3).

When the current fatty acid data are compared to other seal fatty acid patterns, there are many similarities. Although seals with different diets will have different fatty acid in their blubber (Iverson et al. 2004), the proportion mobilized can be used to compare with Weddell and grey seals. The blubber fatty acids of Weddell (Wheatly et al. 2008) and grey seals (Arriola-Ortiz 2010) are mobilized in a similar manner, with 20:5n-3 showing high mobilization in fasting and lactation, similar to this study. Hooded seals also show high depletion of 20:5n-3 over lactation (Iverson et al. 1995). Additionally, during the post-weaning fast, 20:5n-3 is the most mobilized fatty acid in elephant seal pups (Noren et al. 2013).

Fatty acids exhibited some differences of pattern between inner and outer blubber. In the outer layer, the proportion of MC MUFA was higher and remained stable from early to late lactation. MC MUFA has been hypothesized to contribute the most to insulative thermoregulatory capacity, because of the ability to remain fluid at lower temperatures (termed homeoviscous adaptation) (Sinensky 1974). In support of this, Strandberg et al. (2008) found a higher proportion of MUFA with lower melting points in the outer blubber of ringed seals. It appears that these proportions are maintained at a stable level in the outer layer of female blubber, despite high levels of overall mobilization.

Fatty acids in blubber compared to milk

Subsequent to mobilization, fatty acids are utilized by other tissues, including the mammary gland for milk synthesis. When the fatty acid proportions of inner blubber and milk were compared, the metrics used in this study indicate a high degree of similarity. Although there is a high degree of similarity, the data show that overall, LC MUFAs are mobilized least from the blubber while LC MUFA proportions increase in the milk. When the %Sim are compared, there is approximately 10 % difference between proportions in blubber and milk. Other studies do not report the %Sim metric used here, which incorporates all the differences between milk and blubber. In grey seals, Grahl-Nielsen et al. (2000) and Arriola-Ortiz (2010) found that percentages of 18:1n-9 differed by ~6 to 8 % between milk and blubber and ~6 % in 16:0. Proportions calculated from data reported in Wheatley et al. (2008) showed a difference of up to 16 % between percentages of blubber and milk 18:1n-9 in Weddell seals. Hooded seal milk and blubber showed more similarity with regard to 18:1n-9 and 16:0, but an ~2.5 % difference in 20:5n-3 percentages in blubber and milk (Iverson et al. 1995).

In the current study, the differences in milk distribution of FA across lactation seem to be driven by the decrease in proportions of MC MUFA and SFA and increase of LC MUFA in the milk. The differences in fatty acid distribution, albeit small, could be driven by differences in utilization by the mother for energy use versus milk production and variations in mammary gland uptake. Fatty acids mobilized from the blubber may be oxidized by the mother and not available to the mammary gland, thus affecting the distribution in the milk. The fact that the proportion of LC MUFA increase in milk between early and late lactation suggests that the majority of LC MUFA mobilized from the blubber and taken up by the mammary gland is directed to milk production. The oxidation of fatty acids has been shown to be affected by structure, where oxidation increases with unsaturation and decreases with chain length in mammals and birds (DeLany et al. 2000; Price et al. 2011). Long chain fatty acids with only one double bond (20:1 or 24:1) have the potential to be oxidized by maternal tissue slower than other shorter chain or more unsaturated fatty acids. Thus, maternal tissue may not utilize and oxidize each type of fatty acid equally, affecting the availability of mobilized fatty acid for milk production.

The high proportions of milk MUFA found in this study agree with previous work in fasting and lactating pinnipeds. Proportions of LC MUFA were seen to increase in milk across lactation in a previous elephant seal study (Riedman and Ortiz 1979). In hooded and harp seal milk, the proportion of 20:1 was the only FA to clearly increase in milk across lactation (Debier et al. 1999). Similar to the current study, LC MUFA proportions tend to increase in milk across lactation in Weddell and grey seals (Wheatley et al. 2008; Arriola-Ortiz 2010). However, the proportional increase is higher in elephant seals and Weddell seals than in grey seals, which also exhibit a slight increase in milk MC MUFA proportions. Comparisons with Weddell seal milk should be made cautiously, as the seals were likely foraging late in lactation (Wheatly et al. 2008).

Previous studies have assumed that there is minimal de novo synthesis in pinnipeds, but mammary gland fatty acid synthase has never been quantified in pinnipeds. There is no reason to suspect that elephant seals lack the biochemical mechanisms to synthesize fatty acids in the mammary gland. In ruminants, about half of 16:0 can come from de novo synthesis in the mammary gland (Grummer 1991). In fact, the proportion of 16:0 is higher in milk than in inner blubber in this study, a pattern also seen in hooded seals (Iverson et al. 1995) and grey seals (Grahl-Nielsen et al. 2000; Arriola-Ortiz 2010). However, the magnitude of difference is quite small and there is a high degree of similarity between proportions of fatty acids between inner

blubber and milk. Thus, while seals may be capable of de novo synthesis, it is likely minimal, if occurring at all.

Health consequences for the pup

Changes in milk fatty acid distribution raise the question: is there a benefit to the pup? Fuel for post-weaning fasting, thermoregulation and diving capability are all potential benefits that could be impacted by fatty acid distribution. Proportions of LC MUFA were the only class to increase in milk. It is unlikely that the increase in proportions of LC MUFA in the milk in this study would provide a preferential fuel for oxidation during the post-weaning fast, given the slower oxidation rate of that type of fatty acids (DeLany et al. 2000; Astrup et al. 2010; Price et al. 2011). Additionally, it is unlikely that LC MUFAs are utilized for insulative thermoregulatory benefits unless there is modification prior to deposition, given that MC MUFA is the class that contributes most to homeoviscous adaptation (Koopman et al. 1996, 2002; Strandberg et al. 2008).

Work with Weddell seal pups has examined how different classes of fatty acids in the muscle impact developing pups. PUFA availability to the muscle tissue contributes to the development of oxidative capabilities for diving (Trumble et al. 2010) and provides non-shivering thermoregulatory benefits (Noren et al. 2008; Kanatous et al. 2008). As milk is the source of these fatty acids, the proportions provided by the mother may affect the health and development of the pup. In northern elephant seal pups, MC MUFA makes up the majority of blubber stores, and PUFA and SFA are the most mobilized during the post-weaning fast (Noren et al. 2013). However, inner and outer layers were not delineated in that study, therefore it is unclear how the distribution of fatty acids may affect the thermoregulation or diving development in northern elephant seals.

Long chain (>20 C) PUFA have been shown to be very important in neonatal development for humans (Makrides et al. 1995; Larque et al. 2002). Relative to human milk, seal milk has an excess of two particularly important long chain PUFA (22:6n-3 and 20:5n-3) (Larque et al. 2002; Fleith and Clandinin 2005) which is unsurprising given the marine source of seal diets. Other pinniped studies have postulated selective mobilization of 20:5n-3 (Iverson et al. 1995) and preferential deposition into milk (Wheatley et al. 2008). However, in this study, high mobilization of 20:5n-3 is expected from its biochemical structure and the inner blubber proportions matched milk proportions closely. Given that 20:5n-3 is already present in such excess to recommended levels for human neonates (Fleith and Clandinin 2005), it is difficult to make a confident assertion that there is preferential deposition in the milk for the benefit of the pup.

Conclusion

In the northern elephant seal, an animal that mobilizes an enormous proportion of its lipid stores over a very short period, lipid mobilization conforms to previously described patterns attributed to biochemical properties that govern the mobilization of fatty acids. This agreement with other animals demonstrates a conservation of lipid mobilization patterns across several genera in the face of very different energy demands and life history patterns. Fatty acids conferring a homeoviscous advantage were preserved in the outer layer, despite heavy mobilization of lipid stores. Blubber and milk fatty acid distributions were found to be very similar, implying very little de novo synthesis by the mammary gland. The pattern of fatty acid mobilization compared with the distribution in the milk did elucidate a few discrepancies, however. LC MUFAs were least mobilized from the blubber, but the proportions increased in milk across the fast. LC MUFA may be more available for milk production late in lactation due to differential partitioning among maternal tissues. The way in which high MUFA content affects the metabolism and development of the pup are avenues for future investigation to understand the potential adaptive significance of the observed patterns.

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