J Comp Physiol B (2013) 183:1065–1074
DOI 10.1007/s00360-013-0773-0

ORIGINAL PAPER

Characterization of blubber fatty acid signatures in northern elephant seals (*Mirounga angustirostris*) over the postweaning fast

Dawn P. Noren · Suzanne M. Budge · Sara J. Iverson · Michael E. Goebel · Daniel P. Costa · Terrie M. Williams

Received: 28 November 2012 / Revised: 24 June 2013 / Accepted: 10 July 2013 / Published online: 8 August 2013

Abstract Phocids routinely fast for extended periods. During these fasts, energetic requirements are met primarily through the catabolism of blubber lipid. To assess whether fatty acid (FA) composition changes during the postweaning fast in northern elephant seals, blubber biopsies were acquired longitudinally from 43 pups at 2.3 ± 1.5 and 55.2 ± 3.7 days postweaning in 1999 and 2000. At weaning, short-chain monounsaturated FA (SC-MUFA, ≤18 carbons) dominated the blubber while saturated FA (SFA) were found in the next highest proportion. The major FA (all ≥1 % by mass) comprised approximately 91 % of total blubber FA. In both years, 18:1*n*-9 and 16:0 were the most prevalent FA. Major FA mobilized during the fast consisted of polyunsaturated FA (PUFA), SFA, and SC-MUFA. Long-chain MUFA (>18 carbons) tended to be conserved. The fractional mobilization value of 20:5*n*-3 was the highest, resulting in significant reductions of this PUFA. Although concentrations of some blubber FA changed significantly during the postweaning fast, the general FA signature of blubber was similar at weaning and near the end of the fast. Changes in some FA differed across years. For example, the concentration of 20:4*n*-6, a minor PUFA, was significantly reduced in 1999 but not in 2000. FA mobilization patterns in northern elephant seal pups are somewhat similar to those reported previously for other fasting phocids and terrestrial mammals, though there are some notable differences. Differences in FA mobilization patterns across mammalian species may be related to differences in diets, geographical distribution, environmental factors, physiological adaptations, and life history stage.

Keywords Blubber · Catabolism · Fasting · Fatty acid · Marine mammal · Mobilization

Communicated by H.V. Carey.

D. P. Noren · D. P. Costa · T. M. Williams
Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, CA 95064, USA

D. P. Noren (✉)
Conservation Biology Division, NOAA NMFS Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Blvd. East, Seattle, WA 98117, USA
e-mail: dawn.noren@noaa.gov

S. M. Budge
Canadian Institute of Fisheries Technology, Dalhousie University, Halifax, NS B3J 2X4, Canada

S. J. Iverson
Department of Biology, Dalhousie University, Halifax, NS B3H 4J1, Canada

M. E. Goebel
Antarctic Ecosystem Research Division, Southwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, La Jolla, CA 92038, USA

Introduction

The marine mammal blubber layer represents a specialized form of adipose tissue that serves many important functions (Iverson 2002). It is the main site of fat storage (Pond 1978) and is important for insulation, buoyancy, and body streamlining in the aquatic environment (Ryg et al. 1988; Castellini et al. 2009). Lipids in this tissue are also mobilized and oxidized in times of energetic need (Ryg et al.1988). Thus, the biochemical composition of blubber can provide insights into the nutritive status, lipid turnover,

and general lipid metabolism of individuals (e.g., Ackman et al. 1975a, b; Aguilar and Borrell 1990; Lockyer 1991; Trumble and Kanatous 2012).

The fatty acid (FA) composition of this tissue can be used to examine patterns of deposition, maintenance, mobilization, and replenishment of body lipid stores (Ackman et al. 1965; Lockyer et al. 1984; Iverson et al. 1995; Kirsch et al. 2000; Pettinger 2000). Particular FA may be critical for blubber layer structure and adequate thermal insulation. For example, unsaturated FA are found in abundance in the blubber of pinnipeds and cetaceans (Sokolov 1962). Unsaturated FA, as well as shorter carbon-chain FA, form fats of lower melting points reducing the temperature at which fat solidifies (Irving et al. 1957). This enables animals, specifically the distal parts of their extremities, to withstand very cold temperatures (Irving et al. 1957; Käkelä and Hyvärinen 1996a, b).

Most phocid species undergo predictable periods of fasting in their annual cycle, during which they rely almost entirely on the mobilization of fat from blubber stores for energy (e.g., Ortiz et al. 1978; Castellini et al. 1987; Worthy and Lavigne 1987; Reilly 1991). Adults fast during their reproductive and molting periods, and pups fast for a period of several weeks to months after being abruptly weaned (Costa 1993). During these fasting periods, blubber must continue to serve as the primary thermal barrier, while FA are concurrently mobilized to provide the main source of energy. In the case of recently weaned pups, FA are also utilized for continued growth and development. It is not known whether mobilization of particular FA in the blubber of fasting phocid pups occurs to support metabolism and growth and/or to ensure that FA critical for blubber structure and thermal insulation are conserved.

Selective mobilization of FA from adipose tissue during fasting has previously been reported (e.g., Phinney et al. 1990; Raclot and Groscolas 1995; Conner et al. 1996; Groscolas and Robin 2001; Mustonen et al. 2007a, b). However, the majority of these studies have been conducted on species such as humans, rats, and rabbits under induced fasting and where essential FA and PUFA may be limiting. In contrast, phocid seals are adapted to routine periods of prolonged fasting and store levels of polyunsaturated fatty acids (PUFA) and other important FA in blubber far in excess of basic metabolic needs. Hence, FA mobilization patterns may differ. A few early studies have reported how specific lipid stores and FA concentrations change in fasting phocid seals (Worthy and Lavigne 1983; Bryden and Stokes 1969; Iverson et al. 1995), but these studies relied on cross-sectional sampling, which provides limited insight. Given the degree of variability found in blubber FA composition within a species as a function of individual diet (e.g., Iverson et al. 1997a, b), individuals must be sampled longitudinally to assess true patterns of mobilization. A more recent study (Wheatley et al. 2008) longitudinally sampled lactating female Weddell seals (*Leptonychotes weddellii*) to determine FA mobilization patterns during fasting. However, FA mobilized during the lactation period are not simply being used to meet the energetic demands of the female but are also being transferred through the female's milk to meet the energetic demands of her pup. Thus, a longitudinal study on FA mobilization in fasting phocids that do not have the additional burden of supplying milk to a pup is warranted.

The postweaning fast of northern elephant seal (*Mirounga angustirostris*) pups provides an excellent opportunity to study this energetic role of FA for a fasting-adapted species. Northern elephant seal pups are weaned at about 28 days postpartum and fast for approximately 62 days before they enter the water to forage (Noren et al. 2003). To assess blubber FA mobilization patterns during the postweaning fast, we longitudinally measured blubber FA composition in 43 pups within the first week of weaning and again after 5–8 weeks of fasting. Samples were collected from animals over a 2-year period to also assess inter-year variability.

Materials and methods

Study area and subjects

Forty-three weaned northern elephant seal pups (13 females and 10 males in 1999, 8 females and 12 males in 2000) were longitudinally sampled during the postweaning fast at Año Nuevo, CA, USA (37°06′30″N, 122°20′10″W). Weaned pups that were nursed for the typical duration (at least 28 days) were selected for the study. Initial measurements were taken in the field within 5 days postweaning (mean: 2.3 ± 1.5 SD days) in late January through early March and repeated on the same individuals 50–57 days later (mean: 53.6 ± 2.3 SD days) in late March through late April. Due to the earlier departure of animals with low weaning mass (Noren 2002; Noren et al. 2003), second measurements for two pups (81.5 and 99.0 kg) were taken 36 and 46 days after the first set of measurements, respectively.

Pups were captured on the beach using a plastic-coated nylon bag fitted with adjustable straps (Pernia 1984). Body mass was measured using a metal tripod and 250 kg capacity digital scale (accuracy to 0.1 kg; Measurement Systems International, Seattle, Washington, USA). After weighing, seals were initially immobilized by an intramuscular injection (1 mg kg^{-1}) of tiletamine HCl and zolazepam HCl (Telazol; Fort Dodge Animal Health, Fort Dodge, IA, USA). Immobilization was maintained by intravenous doses of ketamine HCl (Ketaset; Fort Dodge

Animal Health, Fort Dodge, IA, USA) and diazepam (Elkins-Sinn Inc., Cherry Hill, NJ, USA) into the extradural spinal vein. Maintenance dosages depended on the activity level of the animal and time remaining for completion of procedures. Axillary girth, standard length, and curvilinear length (American Society of Mammalogists—Society's Committee on Marine Mammals, Scheffer VB 1967) were measured while seals were chemically immobilized. Pups were given a unique green identification tag (Jumbo Roto, Dalton ID Ltd, Henley on Thames, UK) in the inter-digital webbing of each hind flipper and distinctively marked with blonde hair dye (Procter & Gamble-Clairol, Inc., Stamford, CT, USA) to facilitate re-sighting.

Blubber sampling

Full-depth blubber biopsies (approximately 278–548 mg) for FA analysis were taken from chemically immobilized seals with a sterile 6 mm disposable biopsy punch (Miltex Instrument Company, Inc., Lake Success, NY, USA). Samples were taken from the side of each pup anterior to the pelvic region. Each sample was immediately wrapped in aluminum foil and placed in a cooler containing frozen freezer packs for transport from the field. Upon arrival to the laboratory each blubber biopsy sample was weighed on an electronic balance and placed in a separate Kimax test tube containing 7 ml chloroform with 0.01 % butylated hydroxytoluene (BHT). The tubes were capped with a Teflon-lined cap and frozen at −60 °C until lipid extraction and fatty acid analysis which were completed within 15 months of biopsy sample collection.

Lipid extraction and fatty acid analysis

Lipids were extracted from all blubber biopsies and subsequently analyzed for FA composition. A 3.5-ml volume of methanol was added to each Kimax test tube containing a blubber sample and chloroform with BHT. Samples were mashed manually with a glass rod until thin and transparent, and the remaining connective tissue was discarded. Lipids were then fully extracted according to a modified Folch method (Folch et al. 1957; Iverson et al. 2001). FA methyl esters (FAME) were prepared directly from the pure extracted lipid (filtered and dried over anhydrous sodium sulfate), using 1.5 ml hexane and 1.5 ml 8 % boron trifloride (BF_3) in methanol (v/v), capped under nitrogen, and heated at 100 °C for 1 h. FAMEs were extracted into hexane, concentrated, and brought up to volume (50 mg FAME ml^{-1} hexane) for analysis.

FAME were analyzed in duplicate using temperature-programmed gas liquid chromatography according to Iverson et al. (1992), on a Perkin-Elmer Autosystem II Capillary FID gas chromatograph GC fitted with a 30 m × 0.25 mm i.d. column coated with 50 % cyanopropyl polysiloxane (0.25 μm film thickness; J&W DB-23; Folsom, CA, USA) and linked to a computerized integration system (Turbochrom 4 software, PE Nelson). Identifications of FA and isomers were determined from known standard mixtures (Nu Check Prep., Elysian, Minn., USA), silver-nitrate (argentation) chromatography, and GC-mass spectrometry (Iverson et al. 1997a, b). FA are designated by shorthand nomenclature of carbon chain length: number of double bonds and location (n–x) of the double bond nearest to the terminal methyl group and expressed as mass percent of total FA.

Statistical analysis

Sigma Plot 12.0 Software (Systat Software Inc., San Jose, CA) was used to conduct statistical analyses on body condition data and to construct figures. Repeated measures MANOVA of blubber FA profiles were conducted in SPSS Version 19 (IBM Corporation, Armonk, NY). Due to limitations in sample size and assessing changes in FA with relatively low concentrations in the blubber, FA with mean values <0.1 % were excluded prior to analysis and the remaining FA were normalized to 100 %.

Due to the relatively low number of seals sampled ($n = 23$ in 1999, $n = 20$ in 2000), a maximum of 19 FA could be analyzed by repeated measures MANOVA to compare blubber FA signatures at weaning and at the end of the fast as well as the effect of year; we chose to take a conservative approach and analyzed only 13 FA. These included the 12 major FA (concentrations representing ≥1 % mass of the total FA mass) as well as 20:4n-6 (initial mean concentration in 1999: 0.73 ± 0.084 and in 2000: 0.63 ± 0.056), which is an important precursor of bioactives (Smith and Murphy 2002). Several of these 13 FA are preferentially mobilized in other fasting mammals (e.g., Bryden and Stokes 1969; Raclot and Groscolas 1995; Nieminen et al. 2006; Wheatley et al. 2008). Prior to conducting MANOVA, FA data were transformed using an additive logratio transformation:

$$x_{\text{trans}} = \ln\left(\frac{x_i}{c_r}\right),$$

where x_{trans} is the transformed fatty acid data, x_i is a given fatty acid expressed as percent of total fatty acids, and c_r is the percentage of a reference fatty acid, in this case, 18:0 (Filzmoser et al. 2009). When MANOVA indicated an overall significant difference, univariate ANOVA was carried out to identify the FA responsible for the differences. Because several multivariate comparisons were made, a Bonferroni adjusted critical p value $\left(\frac{0.05}{13} = 0.0038\right)$ was used to determine significant changes in FA before and after the fast. To more easily display results, normalized and

adjusted means of FA with standard deviations are presented in figures, following methods for repeated measures data described in Atkinson (2001).

To illustrate the magnitude of mobilization for each of the FA, relative to initial FA concentrations, fractional mobilization was calculated by the formula (following methods similar to Mustonen et al. 2007a):

$$\frac{(\%\,\text{mass of total FA early in the fast} - \%\,\text{mass of total FA late in the fast})}{\%\,\text{mass of total FA early in the fast}}$$

FA that tend to be mobilized have positive fractional mobilization values while FA that tend to be conserved have negative values.

Results

Body condition

Body condition of weaned northern elephant seals pups varied significantly between 1999 and 2000. Although standard length of pups at the first capture did not differ significantly between years (1999 mean: 145.2 ± 6.5 SD cm, range: 126–155 cm; 2000 mean: 147.9 ± 7.4 SD cm, range: 136–164 cm), body mass in 2000 (mean: 129.5 ± 17.2 SD kg, range: 81.5–159.5 kg) was significantly greater (Rank Sum Test, $T = 532$, $P = 0.026$) than body mass in 1999 (mean: 120.2 ± 13.9 SD kg, range: 78.6–141.2 kg).

Blubber fatty acid profiles

At weaning (within 5 days), short-chain monounsaturated FA (SC-MUFA; ≤18 carbons) dominated the blubber of northern elephant seal pups and saturated FA (SFA) were found in the next highest proportion for both years of the study (Table 1). The least dominant FA classes were PUFA and long-chain MUFA (LC-MUFA, Table 1). For both years, the two most abundant FA in the blubber of recently weaned northern elephant pups were 18:1*n*-9 (~35 % by mass) and 16:0 (~13 % by mass). An additional 11 FA (14:0, 16:1*n*-7, 18:0, 18:1*n*-7, 18:2*n*-6, 20:1*n*-11, 20:1*n*-9, 20:5*n*-3, 22:1*n*-11, 22:5*n*-3, 22:6*n*-3) each accounted for ≥1 % of total blubber FA by mass. Together, these 13 major FA comprised approximately 91 % of total blubber FA by mass. Although there was some variation in the relative concentrations of major (Fig 1) and minor (Fig 2) blubber FA at weaning between the 2 years, the overall FA profiles were similar.

The overall FA profile of blubber near the end of the postweaning fast was similar to that at the beginning of the fast (Table 1; Figs 1, 2). Near the end of the postweaning fast, SC-MUFA continued to dominate the blubber (Table 1). PUFA were the least dominant class of FA in both years. For both years, 18:1*n*-9 (~35 % by mass) and 16:0 (~12 % by mass) remained the most abundant FA in the blubber of northern elephant seals nearing the end of their natural fast. The 11 other FA that accounted for ≥1 % of total blubber FA by mass at the end of the fast were also identical to those at weaning. Together, the 13 major FA comprised approximately 92 % of total blubber FA by mass at the end of the postweaning fast.

Although the overall FA signature of the blubber was similar at the beginning and end of the postweaning fast, the concentrations of some FA did change significantly. FA that demonstrated an overall significant change in concentration with time (in at least 1 year) included 14:0, 16:0, 16:1*n*-7, 18:1*n*-7, 20:1*n*-11, 20:1*n*-9, 20:4*n*-6, and 20:5*n*-3 (Figs. 1a, b and 2a, b). However, all but two FA (20:1*n*-11 and 20:5*n*-3) demonstrated a significant interaction with year. Thus, blubber FA mobilization and conservation patterns varied by year.

Calculated fractional mobilization values differed by individual, by FA, and by year, suggesting some differences in lipid mobilization. Individual variability in FA mobilization patterns is evident, given the range of fractional mobilization values for several FA (Fig. 3a, b). FA with positive fractional mobilization values (Table 2; Fig. 3a, b) included in our analysis comprised PUFA (20:4*n*-6 and 20:5*n*-3), SFA (14:0 and 16:0), and SC-

Table 1 Fatty acid composition of northern elephant seal blubber by class at the beginning and end of the postweaning fast

FA Classes	1999 Initial concentration % ± SD ($n = 23$ pups)	1999 Final concentration % ± SD ($n = 23$ pups)	2000 Initial concentration % ± SD ($n = 20$ pups)	2000 Final concentration % ± SD ($n = 20$ pups)
SC-MUFA	51.98 ± 2.88	50.84 ± 2.88	51.54 ± 2.45	51.50 ± 2.36
SFA	19.28 ± 1.29	18.74 ± 1.22	18.76 ± 1.09	17.78 ± 1.11
PUFA	14.48 ± 2.49	14.03 ± 2.41	12.93 ± 1.06	12.46 ± 0.97
LC-MUFA	14.27 ± 2.57	16.39 ± 3.00	16.77 ± 2.35	18.25 ± 2.20

FA concentrations are provided in mean mass percent ± SD of total fatty acids in the blubber

SC-MUFA short-chain (≤18 carbons) monounsaturated FA, *SFA* saturated FA, *PUFA* polyunsaturated FA, *LC-MUFA* long-chain (>18 carbons) monounsaturated FA

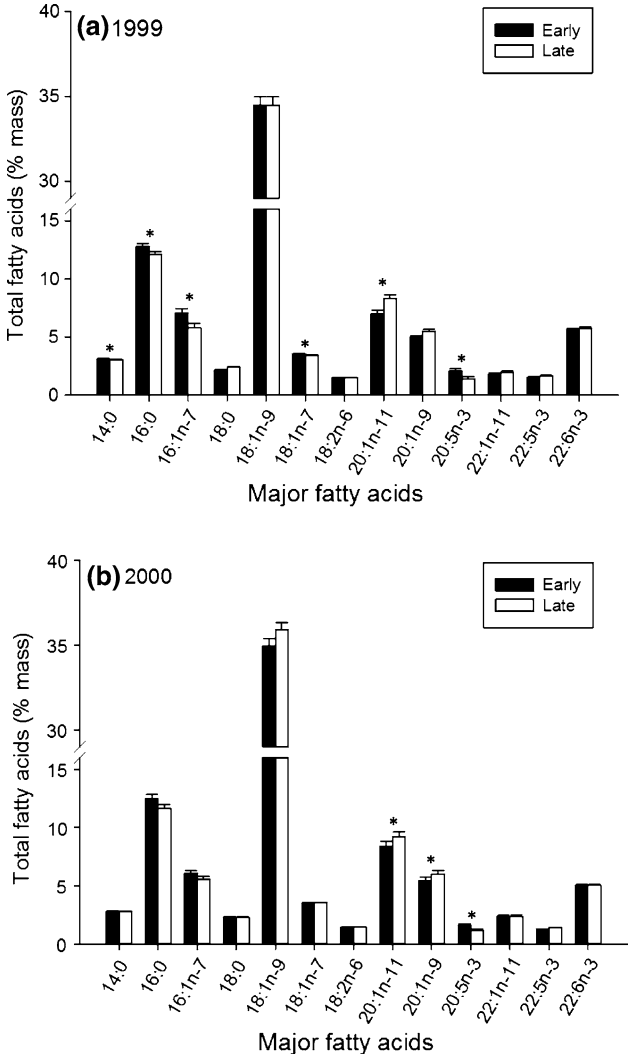

Fig. 1 Comparison of the composition of major fatty acids (concentrations representing ≥1 % mass of the total FA mass) early and late in the northern elephant seal postweaning fast in A) 1999 ($n = 23$ pups) and B) 2000 ($n = 20$ pups). *Bars* are normalized and adjusted means of percent of total FA mass with standard deviations calculated by methods for repeated measures data, as described in Atkinson (2001). *Black bars* represent values from early in the fast and *white bars* represent values from late in the fast. FA noted by an *asterisk* changed significantly (MANOVA, $P < 0.0038$) during the postweaning fast

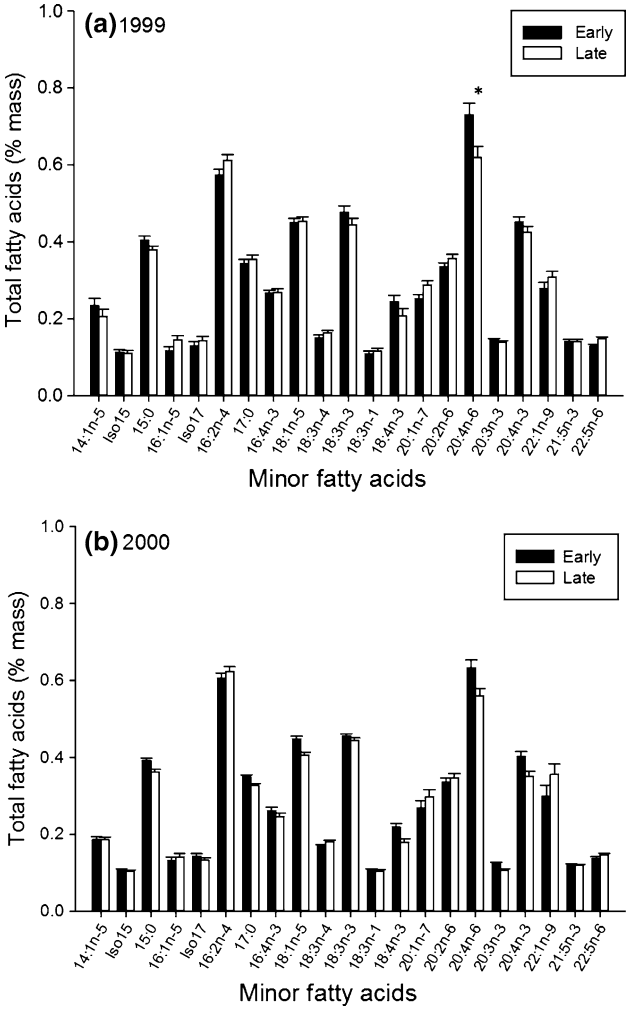

Fig. 2 Comparison of the composition of minor fatty acids (concentrations representing ≥0.1 but <1 % mass of total FA mass) early and late in the northern elephant seal postweaning fast in A) 1999 ($n = 23$ pups) and B) 2000 ($n = 20$ pups). *Bars* are normalized and adjusted means of percent of total FA mass with standard deviations calculated by methods for repeated measures data, as described in Atkinson (2001). *Black bars* represent values from early in the fast and *white bars* represent values from late in the fast. FA noted by an *asterisk* changed significantly (MANOVA, $P < 0.0038$) during the postweaning fast. It is important to note that only one minor FA (20:4*n*-6) was included in the statistical analysis. The remaining FA are presented to show trends only

MUFA (16:1*n*-7, and in 1999, 18:1*n*-7). Although positive fractional mobilization values indicate that FA were likely catabolized during the fast, it is evident that positive fractional mobilization values do not necessarily result in significant reductions in concentrations of some FA (Table 2; Fig. 3a, b). Concentrations of all FA with positive fractional mobilization values that were included in the statistical analysis were significantly reduced over the fast in 1999, while in 2000 only 20:5*n*-3, the FA with the greatest positive fractional mobilization value, was significantly reduced over the fast (Table 2). In general, LC-MUFA were conserved in both years, indicated by negative fractional mobilization values (Table 2; Fig. 3a, b) and increases in concentrations over the fast (Table 2; Fig. 1a, b). Similar to findings related to positive fractional mobilization values, concentrations of FA with negative fractional mobilization values did not always increase significantly over the fast. However, increases in concentrations of FA with negative fractional mobilization values that were included in the statistical analysis were significant for 20:1*n*-11 (both years) and 20:1*n*-9 (2000 only).

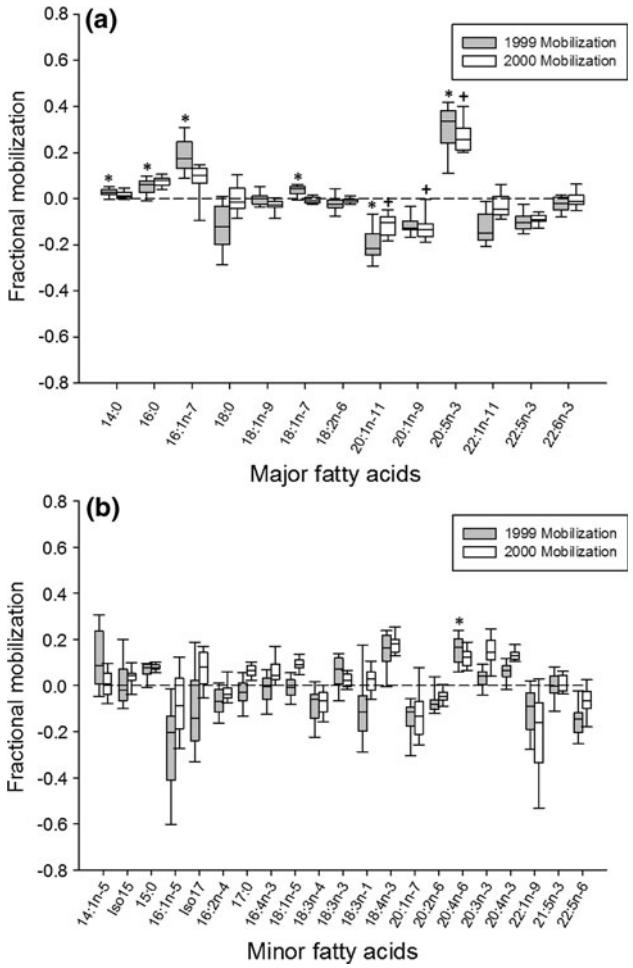

Fig. 3 Fractional mobilization of A) major fatty acids (concentrations representing ≥1 % mass of total FA mass) and B) minor fatty acids (concentrations representing ≥0.1 but <1 % mass of total FA mass) during the northern elephant seal postweaning fast in 1999 and 2000. *Box plots* are presented with *solid lines* within the *boxes* representing the median values. *Box boundaries* indicate the 25th and 75th percentiles and *error bars* indicate the 10th and 90th percentiles. *Gray bars* represent data from 1999 ($n = 23$ pups) and *white bars* represent data from 2000 ($n = 20$ pups). The *dashed lines* designate zero mobilization, such that values *above* the line indicate mobilization while values *below* the line indicate conservation. Concentrations of FA noted by an *asterisk* in 1999 and plus sign in 2000 changed significantly (MANOVA, $P < 0.0038$) during the postweaning fast. It is important to note that all major FA, but only one minor fatty acid (20:4*n*-6), were included in the statistical analysis

Discussion

This is the first longitudinal study to investigate changes in blubber FA over a long-duration fast in a fasting adapted pinniped pup. The results demonstrate that concentrations of some blubber FA change during the northern elephant seal's natural postweaning fast and that FA mobilization patterns can vary annually.

Earlier studies on FA mobilization patterns during natural fasting in phocids have reported results that are similar to those of the present study. A study on southern elephant seals (*Mirounga leonina*, Bryden and Stokes 1969) found that 20:5*n*-3 was significantly reduced in the blubber of fasting pups while 16:0 and 20:5*n*-3 were significantly reduced in the blubber of fasting females. For northern elephant seal pups, we found that both 16:0 and 20:5*n*-3 had positive fractional mobilization during both years of the study, resulting in significant reductions of both 16:0 and 20:5*n*-3 in 1999 and 20:5*n*-3 in 2000. These findings suggest that 16:0 and 20:5*n*-3 are mobilized during fasting periods in elephant seals from both hemispheres. Similar to fasting elephant seals, 20:5*n*-3 had the highest fractional mobilization value, and 14:0, 16:0, and 16:1*n*-7 were also mobilized from blubber in fasting, lactating Weddell seals (Wheatley et al. 2008). Iverson et al. (1995) also reported that 20:5*n*-3 was depleted from maternal blubber during the 4-day lactation period in hooded seals (*Cystophora cristata*). Rea et al. (1997) found that 20:1*n*-9 decreased while 20:5*n*-3 increased markedly in the plasma of fasting Weddell seal pups, which suggests that like northern elephant seal pups, Weddell seal pups conserve 20:1*n*-9 while mobilizing 20:5*n*-3 from blubber during the postweaning fast.

Differences in FA mobilization patterns across phocids may be related to ecological factors or differences in life history stage. For example, lactating female Weddell seals significantly mobilize 18:1*n*-9 while fasting (Wheatley et al. 2008), but northern elephant seal pups do not. Besides inhabiting a different geographic region, lactating female Weddell seals have the additional requirement of providing nourishment to their pups, which would no doubt influence FA mobilization patterns. Indeed, 18:1*n*-9 is a biosynthesized FA (Iverson et al. 2004), which may be important for pup development and thus necessary for females to mobilize from their blubber to milk for transfer to their pup.

Comparisons of FA mobilization patterns across diverse mammals may provide some insight into whether energetic requirements, physiological processes, or ecological factors drive mobilization patterns during food deprivation. Like fasting northern elephant seals, fasting raccoon dogs, (*Nyctereutes procyonoides*, Mustonen et al. 2007a), rats (*Rattus norvegicus*, Raclot and Groscolas 1995), and rabbits (Conner et al. 1996) mobilize 20:5*n*-3, 16:1*n*-7 and 20:4*n*-6. Thus, mobilization of some FA may be related to meeting energetic requirements or maintaining physiological processes. For example, 20:4*n*-6 is a pre-cursor of bioactives (Smith and Murphy 2002) and both *n*-3 and *n*-6 PUFA are important ligands for peroxisome-proliferator-activated receptors (PPAR) that control numerous genes involved in fatty acid metabolism (Desvergne and Wahli 1999; Guglielmo 2010). Ecological factors could also be important. Similar to other marine and freshwater phocids

Table 2 Initial concentrations and fractional mobilization values of 13 blubber FA from northern elephant seals during the postweaning fasts in 1999 and 2000

1999 FA	1999 Initial concentration % ± SD (n = 23 pups)	1999 Fractional mobilization ± SD (n = 23 pups)	2000 FA	2000 Initial concentration % ± SD (n = 20 pups)	2000 Fractional mobilization ± SD (n = 20 pups)
*20:5n-3**	2.09 ± 0.65	0.30 ± 0.12	*20:5n-3**	1.63 ± 0.35	0.26 ± 0.089
*16:1n-7**	7.08 ± 0.68	0.18 ± 0.10	20:4*n*-6	0.63 ± 0.056	0.11 ± 0.62
20:4*n*-6*	0.73 ± 0.084	0.15 ± 0.071	*16:1n-7*	6.06 ± 0.52	0.081 ± 0.082
*16:0**	12.79 ± 0.91	0.053 ± 0.038	*16:0*	12.45 ± 0.79	0.066 ± 0.060
*18:1n-7**	3.57 ± 0.32	0.034 ± 0.024	*14:0*	2.82 ± 0.20	0.015 ± 0.026
*14:0**	3.13 ± 0.46	0.024 ± 0.034	*22:6n-3*	5.01 ± 0.47	−0.003 ± 0.035
18:1n-9	34.47 ± 2.88	−0.00014 ± 0.028	*18:1n-7*	3.51 ± 0.22	−0.006 ± 0.016
18:2n-6	1.49 ± 0.14	−0.016 ± 0.059	*18:2n-6*	1.44 ± 0.061	−0.007 ± 0.019
22:6n-3	5.62 ± 1.27	−0.027 ± 0.035	*22:1n-11*	2.36 ± 0.45	−0.024 ± 0.090
22:5n-3	1.53 ± 0.38	−0.094 ± 0.049	*18:1n-9*	34.93 ± 2.60	−0.028 ± 0.027
20:1n-9	4.97 ± 0.87	−0.11 ± 0.064	*22:5n-3*	1.28 ± 0.18	−0.084 ± 0.034
22:1n-11	1.78 ± 0.46	−0.12 ± 0.11	*20:1n-11**	8.40 ± 1.27	−0.10 ± 0.096
*20:1n-11**	6.98 ± 1.25	−0.20 ± 0.092	*20:1n-9**	5.44 ± 0.68	−0.11 ± 0.095

Initial FA concentrations are provided in mean mass percent ± SD of FA in the blubber. FA are listed in order of the highest to lowest fractional mobilization value. FA that tend to be mobilized have positive fractional mobilization values while FA that tend to be conserved have negative values

Italicized text indicates major FA (≥1 % of total blubber FA by mass)

Asterisks (*) indicate a significant difference ($P < 0.0038$) in FA concentrations at the beginning and at the end of the fast within each year of the study

(Bryden and Stokes 1969; Best et al. 2003; Bradshaw et al. 2003; Strandberg et al. 2008; Wheatley et al. 2008; Strandberg et al. 2011), the blubber of weaned northern elephant seal pups is dominated by MUFA, particularly SC-MUFA. These classes of FA form fats with lower melting points, which are critical to the function of the blubber layer, enabling seals to withstand very cold temperatures (Irving et al. 1957). Fasting northern elephant seal pups appear to conserve many shorter carbon-chain FA and unsaturated FA that form fats with low melting points. This FA mobilization pattern could be attributed to preserving the low melting point and fluidity of subcutaneous fat depots, similar to that of fasting minks, which are semi-aquatic mammals (Nieminen et al. 2006). However, the PUFA 20:5*n*-3, which also forms fats with low melting points, was significantly reduced during the northern elephant seal postweaning fast. Given the consistent result that 20:5*n*-3 is mobilized in southern and northern hemisphere phocids as well as terrestrial animals during fasting, it is likely that the mobilization of this particular FA is related to meeting energetic requirements or maintaining physiological homeostasis. Indeed, PUFA, especially *n*-3s, may be functionally related to oxygen management and locomotor performance (Trumble et al. 2010; Trumble and Kanatous 2012), and as mentioned previously, are important ligands involved in fatty acid metabolism (Desvergne and Wahli 1999; Guglielmo 2010).

It is important to note that although the concentrations of some blubber FA changed significantly during the northern elephant seal postweaning fast, the general FA signature of blubber was similar at weaning and near the end of the postweaning fast. Thus, changes in blubber FA composition during fasting appear to be more subtle than those observed following changes in diet (Iverson et al. 1997a; Kirsch et al. 2000).

The low fractional mobilization values of many major FA suggest that FA mobilization in fasting northern elephant seal pups is not simply proportional to initial FA concentrations in the blubber. This is comparable to findings from studies on fasting terrestrial mammals (e.g., Conner et al. 1996), penguins (Groscolas and Robin 2001), and other birds (Price et al. 2008). Similar to fasting-adapted raccoon dogs (Mustonen et al. 2007a) and Weddell seals (Wheatley et al. 2008), northern elephant seals primarily mobilized SC-SFA, SC-MUFA, and the PUFA, 20:5*n*-3, during the postweaning fast. Previous studies on mammals and birds have reported that generalized FA mobilization patterns are related to molecular structure (e.g., Raclot and Groscolas 1995; Conner et al. 1996; Raclot 2003; Nieminen et al. 2006; Mustonen et al. 2007a, b; Price et al. 2008). However, FA mobilization patterns in fasting northern elephant seals do not strictly follow those described previously (e.g., for a given chain length, mobilization increases with increasing unsaturation; for a

given number of double bonds, mobilization decreases with chain length; Raclot and Groscolas 1995; Conner et al. 1996; Raclot 2003). Similarly, some FA mobilized by fasting, lactating Weddell seals do not conform to the molecular structure of highly mobilized FA (Wheatley et al. 2008), as determined by Raclot (2003).

The inter-year differences in northern elephant seal pup body mass and blubber FA mobilization patterns may be due to a change in prey availability caused by a fluctuation in oceanic conditions. The first year of this study, 1999, was a post El Niño year; the second year, 2000, was considered a "normal" year (Noren 2002; Noren et al. 2003). During the 1998 post-breeding season foraging trip under El Niño conditions, adult female northern elephant seals took longer trips, gained less mass, and returned with lower adipose tissue proportions, compared with previous years (Crocker et al. 2006). Significant changes in adult female diving patterns suggest that prey may have been difficult to locate during El Niño conditions (Crocker et al. 2006), and 1999 milk FA signatures indicate that a shift in diet may have occurred (Pettinger 2000). Because pup mass is directly related to maternal body mass and composition (Arnbom et al. 1993; Deutsch et al. 1994; Crocker et al. 2001), it is not surprising that pups born in 1999 weighed less than pups born in 2000. Likewise, dietary effects on FA composition of maternal milk (Pettinger 2000) may explain differences in pup blubber FA mobilization patterns during the 1999 and 2000 postweaning fasts. In hooded seals, many FA in maternal milk appear to be deposited directly and without modification into blubber of pups (Iverson et al. 1995). Thus, the FA composition of female northern elephant seals' milk likely influences the FA composition of their pups' blubber at weaning. Variability in pup blubber FA signatures at weaning could lead to variability in FA mobilization patterns during the postweaning fast. The only other study that has investigated annual variability in phocid blubber FA mobilization patterns during fasting was conducted on lactating female Weddell seals (Wheatley et al. 2008). Similar to the present study, annual variability in mobilization patterns of a few FA (5 FA, Wheatley et al. 2008; 6 FA, present study) was observed in Weddell seals.

In conclusion, we find that northern elephant seal pups appear to mobilize a few specific FA from their blubber during the postweaning fast. Although the concentrations of certain FA changed significantly during the fast, the general FA signature of northern elephant seal pup blubber was maintained. Inter-annual differences in blubber FA mobilization patterns may be related to variability in oceanic conditions that influenced pre-parturition maternal foraging strategies. Blubber FA mobilization patterns during the postweaning fast may ensure that northern elephant seal pups meet their energetic and physiological demands while maintaining the integrity of the major thermoregulatory structure required for foraging at sea.

Acknowledgments D. Crocker, P. Webb, S. Hayes, J. Burns, and many graduate and undergraduate students provided assistance in the field. The rangers and staff at Año Nuevo State Reserve, California State Park were invaluable to the completion of the project. J. Pettinger and A. Banks provided assistance in the laboratory at the University of California, Santa Cruz; and S. Lang, C. Beck and others provided assistance in the laboratory at Dalhousie University, Halifax, Nova Scotia. This project was supported by grants to D.P. Noren from the American Museum of Natural History (Lerner-Gray Fund for Marine Research), Friends of Long Marine Laboratory, and University of California Natural Reserve System (Mildred E. Mathias Graduate Student Research Grant). Further support was provided by National Science Foundation grants to T.M. Williams (OPP-9909862) and to D.P. Costa and M.E. Goebel (OPP-9500072). Grants to S.J. Iverson through the Natural Sciences and Engineering Research council (NSERC) of Canada provided additional support. D.P. Noren was supported in part by NSF grant 9730462 to H. Kibak and D.P. Costa and by a Graduate Assistance in Areas of National Need Fellowship, Dept. of Ecology and Evolutionary Biology, University of California, Santa Cruz (UCSC). A portion of this work was performed while D.P. Noren was a National Research Council Postdoctoral Research Associate at the National Marine Mammal Laboratory, Alaska Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Seattle, Washington. All procedures comply with the current laws of the United States of America. Research was approved by the Chancellor's Animal Research Committee (UCSC) and conducted under U.S. National Marine Fisheries Service permit 836. Two anonymous reviewers provided constructive comments on the manuscript.

References

Ackman RG, Eaton CA, Jangaard PM (1965) Lipids of the fin whale (*Balaenoptera physalus*) from north Atlantic waters. I. Fatty acid composition of whole blubber and blubber sections. Can J Biochem 43:1513–1520

Ackman RG, Hingley JH, Eaton CA, Sipos JC, Mitchell ED (1975a) Blubber fat deposition in mysticeti whales. Can J Zool 53:1332–1339

Ackman RG, Hingley JH, Eaton CA, Logan VH, Odense PH (1975b) Layering and tissue composition in the blubber of the northwest Atlantic sei whale (*Balaenoptera borealis*). Can J Zool 53:1340–1344

Aguilar A, Borrell A (1990) Patterns of lipid content and stratification in the blubber of fin whales (*Balaenoptera physalus*). J Mammal 71:544–554

American Society of Mammalogists—Society's Committee on Marine Mammals, Scheffer VB (ed) (1967) Standard measurements of seals. J Mammal 48: 459–462

Arnbom T, Fedak MA, Boyd IL, McConnell BJ (1993) Variation in weaning mass of pups in relation to maternal mass, postweaning fast duration, and weaned pup behaviour in southern elephant seals (*Mirounga leonina*) at South Georgia. Can J Zool 71:1772–1781

Atkinson G (2001) Analysis of repeated measurements in physical therapy research. Phys Ther Sport 2:194–208

Best NJ, Bradshaw CJA, Hindell MA, Nichols PD (2003) Vertical stratification of fatty acids in the blubber of southern elephant seals (*Mirounga leonina*): implications for diet analysis. Comp Biochem Physiol B 134:253–263

Bradshaw CJA, Hindell MA, Best NJ, Phillips KL, Wilson G, Nichols PD (2003) You are what you eat: describing the foraging ecology of southern elephant seals (*Mirounga leonina*): using blubber fatty acids. Proc R Soc Lond B 270:1283–1292

Bryden MM, Stokes GB (1969) Metabolism of fatty acids in the southern elephant seal *Mirounga leonina* (L.). Can J Biochem 47:757–760

Castellini MA, Costa DP, Huntley AC (1987) Fatty acid metabolism in fasting elephant seal pups. J Comp Physiol B 157:445–449

Castellini MA, Trumble SJ, Mau TL, Yochem PK, Stewart BS, Koski MA (2009) Body and blubber relationships in Antarctic pack ice seals: implications for blubber depth patterns. Physiol Biochem Zool 82:113–120

Conner WE, Lin DS, Colvis C (1996) Differential mobilization of fatty acids from adipose tissue. J Lipid Res 37:290–298

Costa DP (1993) The relationship between reproductive and foraging energetics and the evolution of the Pinnipedia. In: Boyd IL (ed) Marine mammals: advances in behavioural and population biology, vol 66. Symposium Zoological Society of London, Oxford University Press, New York, pp 293–314

Crocker DE, Williams JD, Costa DP, Le Boeuf BJ (2001) Maternal traits and reproductive effort in northern elephant seals. Ecol 82:3541–3555

Crocker DE, Costa DP, Le Boeuf BJ, Webb PM, Houser DS (2006) Impact of El Niño on the foraging behavior of female northern elephant seals. Mar Ecol Prog Ser 309:1–10

Desvergne B, Wahli W (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocrine Rev 20:649–688

Deutsch CJ, Crocker DE, Costa DP, Le Boeuf BJ (1994) Sex- and age-related variation in reproductive effort of northern elephant seals. In: Le Boeuf BJ, Laws RM (eds) Elephant seals: population ecology, behavior and physiology. University of California Press, Berkeley, pp 169–210

Filzmoser P, Hron K, Reimann C (2009) Univariate statistical analysis of environmental (compositional) data: problems and possibilities. Sci Total Environ 407:6100–6108

Folch J, Lees M, Sloan-Stnaley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226:497–509

Groscolas R, Robin J-P (2001) Long-term fasting and re-feeding in penguins. Comp Biochem Physiol A 128:645–655

Guglielmo CG (2010) Move that fatty acid: fuel selection and transport in migratory birds and bats. Integr Comp Biol 50:336–345

Irving L, Schmidt-Nielsen K, Abrahamsen NSB (1957) On the melting points of animal fats in cold climates. Physiol Zool 30:93–105

Iverson SJ (2002) Blubber. In: Perrin WF, Wursig B, Thewissen HGM (eds) Encyclopedia of marine mammals. Academic Press, San Diego, pp 107–112

Iverson SJ, Sampugna J, Oftedal OT (1992) Positional specificity of gastric hydrolysis of long- chain n-3 polyunsaturated fatty acids of seal milk triglycerides. Lipids 27:870–878

Iverson SJ, Oftedal OT, Bowen WD, Boness DJ, Sampugna J (1995) Prenatal and postnatal transfer of fatty acids from mother to pup in the hooded seal. J Comp Physiol B 156:1–12

Iverson SJ, Arnould JPY, Boyd IL (1997a) Milk fatty acid signatures indicate both major and minor shifts in the diet of lactating Antarctic fur seals. Can J Zool 75:188–197

Iverson SJ, Frost KJ, Lowry LF (1997b) Fatty acid signatures reveal fine scale structure of foraging distribution of harbor seals and their prey in Prince William Sound, Alaska. Mar Ecol Prog Ser 151:255–271

Iverson SJ, Lang SLC, Cooper M (2001) Comparison of the Bligh and Dyer and Folch methods for total lipid determination in a broad range of marine tissue. Lipids 36:1283–1287

Iverson SJ, Field C, Bowen WD, Blanchard W (2004) Quantitative fatty acid signature analysis: a new method of estimating predator diets. Ecol Monogr 74:211–235

Käkelä R, Hyvärinen H (1996a) Fatty acids in extremity tissues of Finnish beavers (*Castor canadensis* and *Castor fiber*) and muskrats (*Ondatra zibethicus*). Comp Biochem Physiol B 113:113–124

Käkelä R, Hyvärinen H (1996b) Site-specific fatty acid composition in adipose tissues of several northern aquatic and terrestrial mammals. Comp Biochem Physiol B 115:501–514

Kirsch PE, Iverson SJ, Bowen WD (2000) Effect of a low-fat diet on body composition and blubber fatty acids of captive juvenile harp seals (*Phoca groenlandica*). Physiol Biochem Zool 73:45–59

Lockyer C (1991) Body composition of the sperm whale, *Physeter catodon*, with special reference to the possible functions of depot fats. Rit Fisk 12:1–24

Lockyer CH, McConnell LC, Waters TD (1984) The biochemical composition of fin whale blubber. Can J Zool 62:2553–2562

Mustonen A-M, Käkelä R, Käkelä A, Pyykönen T, Aho J, Nieminen P (2007a) Lipid metabolism in the adipose tissue of a carnivore, the raccoon dog, during prolonged fasting. Exp Biol Med 232:58–69

Mustonen A-M, Asikainen J, Aho J, Nieminen P (2007b) Selective seasonal fatty acid accumulation and mobilization in the wild raccoon dog (*Nyctereutes procyonoides*). Lipids 42:1155–1167

Nieminen P, Käkelä R, Pyykönen T, Mustonen A-M (2006) Selective fatty acid mobilization in the American mink (*Mustela vison*) during food deprivation. Comp Biochem Physiol B 145:81–93

Noren DP (2002) Body energy reserve utilization during the postweaning fast of northern elephant seals (*Mirounga angustirostris*): implications for survival dissertation. University of California, Santa Cruz

Noren DP, Crocker DE, Williams TM, Costa DP (2003) Energy reserve utilization of northern elephant seal (*Mirounga angustirostris*) pups during the postweaning fast: size does matter. J Comp Physiol B 173:443–454

Ortiz CL, Costa D, Le Boeuf BJ (1978) Water and energy flux in elephant seal pups fasting under natural conditions. Physiol Zool 51:166–178

Pernia SD (1984) Protein turnover and nitrogen metabolism during long term fasting in northern elephant seal pups dissertation. University of California, Santa Cruz

Pettinger JD (2000) Fatty acid signatures as indicators of foraging ecology of northern elephant seals (*Mirounga angustirostris*). MSc Thesis, University of California, Santa Cruz

Phinney SD, Tang AB, Johnson SB, Holman RT (1990) Reduced adipose tissue 18:3*n*-3 with weight loss by very low calorie dieting. Lipids 25:798–806

Pond CM (1978) Morphological aspects and the ecological and mechanical consequences of fat deposition in wild vertebrates. Annu Rev Ecol Syst 9:519–570

Price ER, Krokfors A, Guglielmo CG (2008) Selective mobilization of fatty acids from adipose tissue in migratory birds. J Exp Biol 211:29–34

Raclot T (2003) Selective mobilization of fatty acids from adipose tissue triacylglycerols. Prog Lipid Res 42:257–288

Raclot T, Groscolas R (1995) Selective mobilization of adipose tissue fatty acids during energy depletion in the rat. J Lipid Res 36:2164–2173

Rea L, Groscolas R, Mioskowski E, Castellini M (1997) Changes in plasma fatty acids indicate change in nutritional status in developing Weddell seal pups. Polar Biol 18:351–357

Reilly JJ (1991) Adaptations to prolonged fasting in free-living weaned gray seal pups. Am J Physiol 260:R267–R272

Ryg M, Smith TG, Øritsland NA (1988) Thermal significance of the topographical distribution of blubber in ringed seals (*Phoca hispidia*). Can J Fish Aquat Sci 45:985–992

Smith WL, Murphy RC (2002) The eicosanoids: cyclooxygenase, lipoxygenase and epoxygenase pathways. In: Biochemistry of lipids, lipoproteins and membranes. Elsevier, Oxford, pp 341–371

Sokolov W (1962) Adaptations of the mammalian skin to the aquatic mode of life. Nature 195:464–466

Strandberg U, Käkelä A, Lydersen C, Kovacs KM, Grahl-Nielsen O, Hyvärinen H, Käkelä R (2008) Stratification, composition, and function of marine mammal blubber: the ecology of fatty acids in marine mammals. Physiol Biochem Zool 81:473–485

Strandberg U, Sipilä T, Koskela J, Kunnasranta M, Käkelä R (2011) Vertical fatty acid profiles in blubber of a freshwater ringed seal—Comparison to a marine relative. J Exp Mar Biol Ecol 407:256–265

Trumble SJ, Kanatous SB (2012) Fatty acid use in diving mammals: more than merely fuel. Front Physiol 3:184. doi:10.3389/fphys.2012.00184

Trumble SJ, Noren SR, Cornick LA, Hawke TJ, Kanatous SB (2010) Age-related differences in skeletal muscle lipid profiles of Weddell seals: clues to developmental changes. J Exp Biol 213:1676–1684

Wheatley KE, Nichols PD, Hindell MA, Harcourt RG, Bradshaw CJA (2008) Differential mobilization of blubber fatty acids in lactating Weddell seals: evidence of selective use. Physiol Biochem Zool 81:651–662

Worthy GAJ, Lavigne DM (1983) Energetics of fasting and subsequent growth in weaned harp seal pups, *Phoca groenlandica*. Can J Zool 61:447–456

Worthy GAJ, Lavigne DM (1987) Mass loss, metabolic rate, and energy utilization by harp and gray seal pups during the postweaning fast. Physiol Zool 60:352–364