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Variation in the fatty acid composition of blubber in Cape fur seals (*Arctocephalus pusillus pusillus*) and the implications for dietary interpretation

Abstract Analysis of the fatty acid (FA) composition of blubber is a valuable tool in interpreting the diet of marine mammals. This technique is based on the principle that particular FA present in prey can be incorporated largely untransformed into predator adipose tissue stores, thereby providing biochemical signatures with which to identify prey species. Several studies of phocid seals and cetaceans have documented vertical stratification in the FA composition of blubber such that inferences about diet may vary greatly depending on the layer of the blubber that is analysed. It is not known whether blubber in otariid seals (fur seals and sea lions) also displays vertical stratification in FA composition. Furthermore, it is not known whether the FA composition of blubber is uniform in these species. In the present study, the vertical and regional variation in FA composition of blubber was investigated in seven adult female Cape fur seals (*Arctocephalus pusillus pusillus*). The proportion of monounsaturated fatty acids (MUFA) was greater in the outer ($43.6 \pm 1.3\%$) than inner portion ($40.9 \pm 1.2\%$; $t_{20} = 5.59$, $P < 0.001$) whereas the proportions were greater in the inner than outer portions for saturated fatty acids ($23.6 \pm 0.5\%$ and $21.9 \pm 0.6\%$, respectively, $t_{20} = 5.31$, $P < 0.001$) and polyunsaturated fatty acids (PUFA, $35.5 \pm 0.7\%$ and $34.5 \pm 0.7\%$, respectively, $t_{20} = 3.81$, $P < 0.001$). There was an inverse relationship between MUFA and PUFA in the blubber, independent of sampling location. In addition, with the exception of the inner portion from non-lactating females, blubber from the mammary area had the highest proportions of $18:1\omega9c$ and total MUFA, followed by blubber from the rump and neck, suggesting that the deposition and mobilisation of blubber lipids may not be uniform around the body in otariid seals. These results support the need for blubber tissue to be sampled from the same site on animals, and to the full depth of the blubber layer, to minimise variation in FA profiles that could occur if different sites and depths were sampled. Such standardisation of sampling will further aid in interpreting diet in otariid seals using the FA Signature Analysis approach.

Keywords Blubber · Diet · Fatty acid · Fur seal · Pinniped

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Introduction

Information concerning diet is fundamental to understanding the role of predators in the ecosystem as well as gaining insights into the factors which influence their behaviour, physiology, survival, and reproductive success (e.g. Berruti 1991; Springer et al. 1996; Fuglei and Oritsland 1999; Jones and Barmuta 2000; Connell 2002; Mcdonald 2002). Because in many cases their aquatic

lifestyle prevents direct observations of their feeding behaviour, marine mammals are difficult subjects on which to obtain dietary data. Information on prey species and their proportions in the diet of marine mammals, therefore, usually has been inferred from indirect evidence of feeding events (Iverson 1993; Kelly 2000; Cottrell and Trites 2002).

It has been well established that traditional methods of dietary analysis in marine mammals, primarily stomach and faecal analyses (Laws 1993; Pauly et al. 1998), have numerous inherent biases associated with incomplete consumption of prey items, differential degradation of prey remains, and gut passage rate (Dellinger and Trillmich 1988; Gales and Cheal 1992; Staniland 2002; Arim and Naya 2003). Consequently, several new techniques have been utilised to provide additional insights into the trophic relationships of marine mammals. For example, the use of stable isotope analysis of soft and hard tissues of pinnipeds and cetaceans has assisted in clarifying the trophic level or geographic location of the prey consumed (e.g. Abend and Smith 1997; Hobson et al. 1997; Burton and Koch 1999; Hobson et al. 2004) while molecular genetic analysis of faecal remains has identified their prey species (Jarman et al. 2004; Orr et al. 2004; Purcell et al. 2004).

One technique which has recently attracted attention is fatty acid signature analysis (FASA; Iverson et al. 1997b; Smith et al. 1997; Iverson et al. 2004). This technique is based on the principle that particular fatty acids (FA) present in prey can be incorporated largely untransformed into predator adipose tissue stores and milk, thereby providing biochemical signatures with which to identify prey species of marine mammals (Iverson 1993; Raclot et al. 1998). Because of the relative ease of obtaining large numbers of samples from lactating animals at breeding colonies, FASA of milk has proved a useful tool for investigating diet and the factors affecting it in female pinnipeds (Iverson 1993; Iverson et al. 1997a; Brown et al. 1999; Grahl-Nielsen et al. 2000; Lea et al. 2002a). However, analysis of milk limits FASA to investigating the diet in females associated with milk production. In contrast, analysis of FA in the blubber (the continuous layer of subcutaneous adipose tissue between the epidermis and the fascia of the underlying muscle; Laws 1993), allows all age and sex classes to be sampled (Iverson et al. 1997b; Kirsch et al. 2000; Bradshaw et al. 2003; Grahl-Nielsen et al. 2003; Falk-Petersen et al. 2004; Iverson et al. 2004). In addition, using blubber for FASA lends the technique to remote sampling (e.g. by biopsy darts) to collect dietary information in species where it would otherwise not be possible (Borobia et al. 1995; Hooker et al. 2001).

There has been discussion recently about the need for appropriate sub-sampling of the entire blubber layer depth of marine mammals when conducting FASA (Best et al. 2003; Grahl-Nielsen et al. 2004; Thiemann et al. 2004a, b). Several studies have documented vertical stratification in the FA composition of subcutaneous blubber (e.g. Kakela and Hyvarinen 1996; Koopman et al. 1996; Hooker et al. 2001; Best et al. 2003; Olsen and Grahl-Nielsen 2003). Results indicate that the inner layer (closest to muscle) is more metabolically active than the outer layer (closest to the skin). Inferences about diet, therefore, may vary greatly depending on the layer of the blubber that is analysed (Thiemann et al. 2004b). These studies, however, have generally involved cetaceans or phocid seals. It is not known whether blubber in otariid seals (fur seals and sea lions) also displays vertical stratification in FA composition.

In phocid seals, the mobilisation of blubber lipids has been shown to occur uniformly around the body (Nordy and Blix 1985; Slip et al. 1992; Beck and Smith 1995). Consequently, a single standardised sampling location on the body has been deemed adequate for interpreting diet using FASA of blubber in these species (Bradshaw et al. 2003; Grahl-Nielsen et al. 2003; Walton and Pomeroy 2003). Similarly, as the dorsal thorax is the most dynamic region of blubber in cetaceans (Lockyer 1986; Koopman et al. 2002), samples for FASA from this region are considered the most representative of recent diet (e.g. Dahl et al. 2000; Hooker et al. 2001). There is no information, however, on the pattern of lipid mobilisation in otariid seals.

Consequently, in addition to validation through experimental feeding trials (Iverson et al. 2004), information on the degree of vertical stratification and regional body differences in the FA composition of blubber is needed for FASA of this tissue to be a useful tool in assessing the diet of free-ranging otariid seals. The aims of this study, therefore, were: 1) to test for vertical stratification in the FA composition of blubber in the Cape fur seal (*Arctocephalus pusillus pusillus*); and 2) to investigate the variability in FA composition of this tissue around the body in this species. In addition, a preliminary assessment of the influence of nutritional and reproductive status on the FA composition of blubber was made.

Materials and methods

Sample collection

The study was conducted using samples obtained opportunistically from individuals collected as part of ongoing investigations of the demography and foraging ecology of adult female Cape fur seals by Marine and Coastal Management (Department of Environmental Affairs and Tourism, Republic of South Africa). All individuals were killed with a shot to the head from a 0.22 rifle. Pups of females sampled while suckling were also killed. All procedures were approved by the Animal Ethics Committee of Marine and Coastal Management.

Three individuals were sampled from a small breeding colony at Robbesteen (33°63' S, 18°24' E) in the period following weaning, just prior to the commencement of the next pupping season (November 2002). The females were confirmed to be pregnant and non-lactat-

ing from inspection of the uterus and mammary glands, respectively. A further four individuals were sampled during the lactation period at a time when pregnant females are in embryonic diapause (February 2003; Guinet et al. 1998). Of these, two were collected while foraging at sea (33°44'S 17°30'E) 100 km from the nearest colony and were confirmed to be producing milk by inspection of the mammary gland. They were also confirmed to have recently been foraging from inspection of the digestive tract. The remaining two individuals were collected while nursing pups at the breeding colony at Kleinsee (29°34' S, 16°00' E). The mean mass and length of the animals sampled was 49.4 ± 3.0 kg (range: 40.0–57.5 kg) and 138.0 ± 3.8 cm (range: 125.0–143.0 cm), respectively, and all appeared to be in healthy body condition (W.H. Oosthuizen unpublished data).

Studies of terrestrial mammals (including carnivores) have documented the presence of discrete subcutaneous adipose depots which, depending on species and nutritional state, are deposited and mobilised at various rates (Pond and Mattacks 1985b; Pond and Ramsay 1992; Pond et al. 1995). In the absence of information on the dynamics of lipid mobilisation in otariid seals, blubber was sampled at three tissue locations: (1) dorsal region between the pelvic girdle and the tail (Rump); (2) side of the lower neck (Neck); and (3) ventral abdomen between the fore- and hind-teats (Mammary; Fig. 1). These tissue sampling locations were deemed analogous to the superficial depot sites identified in terrestrial mammals by Pond and Mattacks (1985a) as IFS (in front of the shoulder), BOT (posterior back fat), and PAUNCH (external to abdominal wall), respectively. These tissue sampling locations were chosen because they could represent discrete subcutaneous depots and, in view of the postures resting animals adopt on land (Warneke and Shaughnessy 1985; Goldsworthy et al. 1997), could also serve as potential sites for remote biopsy sampling.

Samples were collected from each location by making a 20-cm anterior-posterior incision with a scalpel down to the muscle layer. A cross-section core of approximately 1×1 cm was cut vertically through the adipose tissue layer down to the muscle facia, the sample was wrapped in aluminium foil, and then placed whole inside a screw-cap glass vial sealed with Parafilm (Sigma-Aldrich, St Louis, MO, USA) before being stored at $-20°C$ until analysis in the laboratory.

Lipid extraction and analyses

In the laboratory, the thawed samples were extended to their full length (mean 35.5 mm) and halved to provide the outer (closest to the skin) and inner (closest to the muscle layer) portions. Any muscle or skin left attached to the adipose tissue was removed before the individual sub-samples (mean 300 mg) were quantitatively extracted overnight using a modified Bligh and Dyer (1959) one-phase methanol—chloroform–water extraction (2:1:0.8 v/v/v). After the addition of chloroform–water the following day (final solvent ratio, 1:1:0.9 v/v/v methanol—chloroform–water), the lower chloroform phase was concentrated using rotary evaporation at 40°C to produce the total lipid.

An aliquot of the total lipid was trans-methylated to produce fatty acid methyl esters (FAME) using methanol–chloroform–hydrochloric acid (10:1:1, 80°C, 2 h) (Christie 1982). FAME were extracted into hexane–chloroform (4:1, 3×1.5 ml) and treated with N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA 50 µl, 70°C, 0.5 h) to convert sterols (mainly cholesterol, with no differences in sterol composition seen between samples) and alcohols to their corresponding TMSi ethers. Gas chromatographic (GC) analyses were performed with a Hewlett Packard 5890A GC (Avondale, Pennsylvania, USA) equipped with an HP-5 cross-linked methyl silicone fused silica capillary column (50 m × 0.32 mm i.d.), a flame ionisation detector, a split/splitless injector, and an HP 7673A auto sampler. Helium was the carrier gas. Following addition of methyl nonodecanoate and methyl tricosanoate internal standards, 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}.

Fig. 1 The three tissue sites on the body of adult Cape fur seals sampled for blubber (Rump, Neck, Mammary)
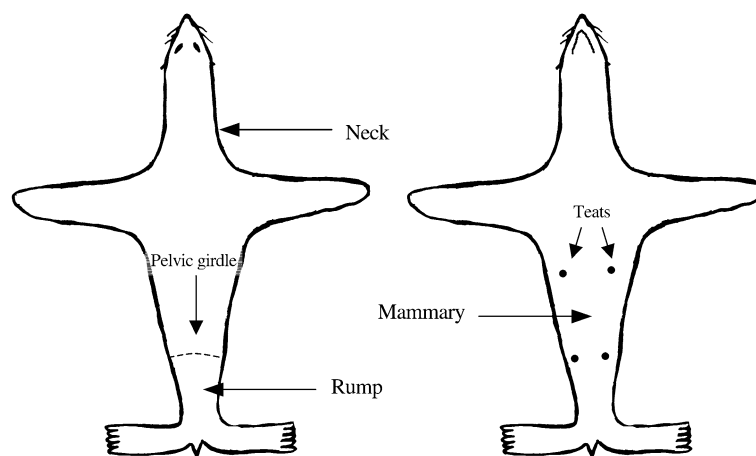
Peaks were integrated and quantified with Waters Millennium software (Milford, Massachusetts, USA). Individual components were identified using mass spectral data and by comparing retention time data with those obtained for authentic and laboratory standards. Gas chromatography results are subject to an error of ± 5% of individual component area. To confirm component identification, GC-mass spectrometric (GC-MS) analyses of representative samples containing all FA present were performed on a Finnigan Thermoquest GCQ GC-mass spectrometer (Austin, TX, USA) fitted with an on-column injector. The GC was fitted with a capillary column similar to that described above.

The concentrations of individual FA in each sample (mg $100g^{-1}$ tissue) were converted to percentage contributions of the total FA. Fatty acids present in trace amounts ($< 0.5\%$) were excluded from the statistical analyses. Due to the multivariate nature of FA profile data, Principal Components Analysis (PCA) was used on the arcsin-transformed proportional data to investigate patterns in FA associations among the different samples (Sokal and Rohlf 1981). Paired t-tests (Sokal and Rohlf 1981) were used to detect significant differences in the proportion of FA groups between the inner and outer portions of the blubber. Results are presented as Means ± SE and statistical analyses were performed using SPSS 11.5 (SPSS Inc., Chicago, IL, USA).

Results

Fatty acid composition and vertical stratification of blubber

In the 42 blubber samples (inner and outer portions of three sampling locations from seven individuals) that were analysed, 16 individual FA (comprising 90–94% of the total FA) were consistently recorded in proportions $> 0.5\%$ (Tables 1, 2, 3). An additional FA, $16:2\omega4$, was present in 58% of samples in proportions $> 0.5\%$. The dominant four FA in all samples were (proportion of total FA): $18:1\omega9c$ (14–33%), $22:6\omega3$ docosahexaenoic acid (DHA, 12–22%), 16:0 (10–16%) and $16:1\omega7c$ (5–12%). Correspondingly, monounsaturated fatty acids (MUFA) comprised the greatest proportion of all blubber samples (35–57%, Fig. 2) followed by polyunsaturated fatty acids (PUFA; 26–39%) and saturated fatty acids (SFA; 15–27%). The proportion of MUFA comprised of short-chain MUFA (SC-MUFA, defined as having chains ≤ 18 carbon), was consistently within the range of 75–90%.

The first two components derived from the PCA accounted for 69% of the variation (PC1 45%, PC2 24%) in FA composition among the samples. Inclusion of PC3 (18%) and PC4 (7%) increased the cumulative variation explained to 94%. Though a bivariate plot of PC1 and PC2 did not show clear separation according to tissue layer, the variation between the portions was largely explained by PC2 with the outer portion samples tending

to have positive values while the inner portion samples were mostly negative. The FA driving these differences were the positive Eigen values for $16:1\omega7c$, $18:2\omega6$, and $20:4\omega3$ and the negative Eigen values for 16:0, 18:0, and $22:1\omega11$ (Table 4).

While the PCA did not reveal clear separation between the inner and outer tissue portions, there were small significant differences (paired t-tests) in gross FA composition between the layers (Fig. 2). The proportion of MUFA was greater in the outer ($43.6 \pm 1.3\%$) than inner portion ($40.9 \pm 1.2\%$; $t_{20} = 5.59$, $P < 0.001$), whereas the proportions were greater in the inner than outer portions for SFA ($23.6 \pm 0.5\%$ and $21.9 \pm 0.6\%$, respectively, $t_{20} = 5.31$, $P < 0.001$) and PUFA ($35.5 \pm 0.7\%$ and $34.5 \pm 0.7\%$, respectively, $t_{20} = 3.81$, $P < 0.001$). The proportion of MUFA composed of SC-MUFA was consistently greater in the outer than inner portion of the blubber.

Variation in fatty acid composition between individuals and tissue sampling locations on the body

In addition to differences in FA composition between the inner and outer portions of the blubber, there were differences between individuals and between the tissue sampling locations on the body. While the sample sizes for each group were small, there was a tendency for tissue from animals foraging at sea during the lactation period to have higher total MUFA (44–53%) and lower total PUFA (28–33%) than either lactating or non-lactating animals on land (37–44% and 35–38%, respectively; Fig. 3a). The comparatively higher total MUFA in tissue from animals at sea was dominated by higher levels of $18:1\omega9c$, whereas the comparatively higher total PUFA in tissue from animals on land was dominated by higher DHA concentrations (Fig. 3b). Interestingly, despite the similarity in the total proportions of PUFA and MUFA in tissue samples from both lactating and non-lactating individuals on land, nursing females had higher DHA and $18:1\omega9c$ levels.

The FA composition of blubber varied among the tissue sampling locations, with differences of up to 7% in the proportion of MUFA between sites (Table 1, 2 and 3, Fig. 3a). With the exception of the inner portion of adipose tissue samples from non-lactating individuals on land, there was a consistent trend in decreasing proportion of MUFA and increasing proportion of PUFA between the Mammary, Rump, and Neck tissue sampling sites within individuals (Fig. 3a). The inner portion of adipose tissue from non-lactating individuals on land had higher proportions of MUFA in the Rump than in the Mammary site samples. This tissue, along with that from females nursing on land (both inner and outer portions), had lower proportions of PUFA in the Rump than in the Mammary site samples. These trends were also evident for the relationship between $18:1\omega9c$ and DHA (Fig. 3b). The greatest variation in FA composition between the tissue sampling locations was evident in

Table 1 Fatty acid profiles (mean ± SE, % total FA) of blubber from the three body locations (rump, neck, mammary) sampled from two adult female Cape fur seals foraging at sea during the lactation period (see text for details)

Fatty acid	Rump		Neck		Mammary	
	Inner	Outer	Inner	Outer	Inner	Outer
14:0	4.2 ± 0.9	3.9 ± 1.1	4.8 ± 0.6	4.9 ± 0.6	4.3 ± 0.6	3.8 ± 0.6
16:0	11.8 ± 1.1	10.6 ± 1.2	13.3 ± 0.4	12.9 ± 0.6	12.1 ± 0.6	11.2 ± 0.5
16:1ωc	8.0 ± 0.6	8.9 ± 0.1	6.0 ± 0.6	7.8 ± 0.2	8.1 ± 0.1	9.1 ± 0.3
16:2ω4	0.5 ± 0.3	0.4 ± 0.3	0.7 ± 0.2	0.7 ± 0.2	0.6 ± 0.2	0.3 ± 0.1
18:0	2.6 ± 0.3	2.2 ± 0.4	3.4 ± 0.1	2.8 ± 0.2	2.7 ± 0.3	2.3 ± 0.3
18:1ω7c	3.8 ± 0.1	4.1 ± 0.1	3.3 ± 0.2	3.6 ± 0.2	3.9 ± 0.1	4.3 ± 0.1
18:1ω9c	23.0 ± 3.6	26.2 ± 4.2	19.7 ± 1.6	20.8 ± 2.1	23.8 ± 2.8	27.7 ± 2.4
18:2ω6	1.4 ± 0.1	1.5 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.5 ± 0.1
18:4ω3	1.1 ± 0.4	1.1 ± 0.3	1.2 ± 0.2	1.2 ± 0.2	1.1 ± 0.2	1.0 ± 0.1
20:1ω9c	6.0 ± 1.5	5.5 ± 0.8	6.3 ± 1.1	5.7 ± 0.9	5.5 ± 0.6	5.1 ± 0.1
20:1ω11c	1.5 ± 0.3	1.7 ± 0.3	1.8 ± 0.3	1.6 ± 0.2	1.6 ± 0.2	1.7 ± 0.1
20:4ω3	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.1
20:4ω6) AA	1.1 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
20:5ω3 EPA	4.9 ± 2.5	4.6 ± 1.8	4.4 ± 1.3	4.5 ± 1.1	5.0 ± 1.3	4.1 ± 0.6
22:1ω11	2.5 ± 0.4	1.9 ± 0.1	3.1 ± 0.4	2.4 ± 0.3	2.2 ± 0.1	1.6 ± 0.3
22:5ω3	5.0 ± 0.2	5.2 ± 0.1	5.4 ± 0.2	5.0 ± 0.3	5.0 ± 0.2	4.9 ± 0.1
22:6ω3 DHA	13.6 ± 1.3	13.1 ± 0.7	15.1 ± 0.7	14.3 ± 0.8	13.0 ± 0.2	12.2 ± 0.2
Other (<0.5%)	8.1	7.3	8.4	8.6	7.4	7.2
Sum SFA	20.8 ± 2.6	18.5 ± 2.7	23.4 ± 1.0	23.1 ± 1.8	21.0 ± 1.4	19.2 ± 1.3
Sum MUFA	48.1 ± 5.2	51.3 ± 4.9	43.7 ± 2.6	45.2 ± 3.0	48.5 ± 3.5	52.6 ± 2.6
Sum PUFA	31.2 ± 2.6	30.3 ± 2.3	33.0 ± 1.6	31.7 ± 1.2	30.6 ± 2.0	28.3 ± 1.2

AA arachidonic acid; *EPA* eicosapentaenoic acid; *DHA* docosahexaenoic acid; *SFA* saturated fatty acids; *MUFA* monounsaturated fatty acids; *SC* short-chain; *LC* long-chain; *PUFA* polyunsaturated fatty acids; Other includes components present at <0.5%: 12:0, i14:0, C14 PUFA, 14:1, br15:0, i15:0, a15:0, 15:1, 15:0, C16 PUFA, i16:0, 16:3ω4, 16:1ω9c, 16:1ω5c, 16:1ω13t, br17:0, a17:0, 17:1, 17:0, C18 PUFA, 18:3ω6, 18:5ω3, i18:0, 18:1ω7t, 18:1ω5c, 19:1, 20:3ω6, 20:4ω3, 20:2ω6, 20:1ω11c, 20:1ω7c, 20:1ω5c, 20:0, C21 PUFA, 21:0, 22:4ω6, 22:1ω11, 22:1ω9, 22:1ω7, 22:0, C24 PUFA, 24:1, 24:0

Table 2 Fatty acid profiles (mean ± SE, % total FA) of blubber from the three body locations (rump, neck, mammary) sampled from two adult female Cape fur seals nursing and fasting on land during the lactation period (see text for details)

Fatty acid	Rump		Neck		Mammary	
	Inner	Outer	Inner	Outer	Inner	Outer
14:0	4.9 ± 0.3	4.8 ± 0.3	4.8 ± 0.2	4.8 ± 0.1	4.7 ± 0.5	4.4 ± 0.3
16:0	13.8 ± 0.6	13.5 ± 0.6	13.8 ± 0.2	13.3 ± 0.2	13.5 ± 0.8	11.8 ± 0.5
16:1ωc	6.3 ± 0.4	6.9 ± 0.5	6.1 ± 0.1	6.5 ± 0.2	6.5 ± 0.5	8.2 ± 0.2
16:2ω4	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
18:0	3.5 ± 0.1	3.2 ± 0.2	3.4 ± 0.1	3.1 ± 0.1	3.3 ± 0.1	2.6 ± 0.1
18:1ω7c	3.7 ± 0.3	3.8 ± 0.3	3.5 ± 0.1	3.6 ± 0.1	3.8 ± 0.4	4.0 ± 0.3
18:1ω9c	20.6 ± 1.4	21.1 ± 1.6	20.1 ± 0.5	20.3 ± 0.6	20.9 ± 2.2	22.7 ± 1.5
18:2ω6	1.5 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.6 ± 0.1
18:4ω3	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.2	1.1 ± 0.1
20:1ω9c	3.1 ± 0.2	3.1 ± 0.2	3.1 ± 0.1	3.0 ± 0.1	3.1 ± 0.2	3.0 ± 0.4
20:1ω11c	1.3 ± 0.1	1.2 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
20:4ω3	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
20:4ω6) AA	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
20:5ω3 EPA	3.4 ± 0.1	3.4 ± 0.2	3.7 ± 0.1	3.6 ± 0.1	3.6 ± 0.4	3.8 ± 0.4
22:1ω11	1.7 ± 0.4	1.6 ± 0.3	1.5 ± 0.2	1.5 ± 0.2	1.6 ± 0.4	1.2 ± 0.4
22:5ω3	6.3 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.3 ± 0.2	6.0 ± 0.4
22:6ω3 DHA	18.7 ± 0.6	18.3 ± 0.6	19.8 ± 0.3	19.7 ± 0.4	18.9 ± 0.6	18.6 ± 0.5
Other (<0.5%)	8.2	8.4	8.2	8.7	8.0	7.8
Sum SFA	24.2 ± 0.9	23.8 ± 1.0	24.1 ± 0.3	23.7 ± 0.2	23.5 ± 1.4	21.0 ± 0.9
Sum MUFA	39.9 ± 1.7	41.0 ± 1.9	39.1 ± 0.5	39.4 ± 0.6	40.4 ± 2.3	43.5 ± 1.3
Sum PUFA	35.9 ± 0.8	35.2 ± 0.9	36.8 ± 0.2	36.9 ± 0.5	36.1 ± 1.0	35.5 ± 0.4

AA arachidonic acid; *EPA* eicosapentaenoic acid; *DHA* docosahexaenoic acid; *SFA* saturated fatty acids; *MUFA* monounsaturated fatty acids; *SC* short-chain; *LC* long-chain; *PUFA* polyunsaturated fatty acids; Other includes components present at <0.5%: 12:0, i14:0, C14 PUFA, 14:1, br15:0, i15:0, a15:0, 15:1, 15:0, C16 PUFA, i16:0, 16:3ω4, 16:1ω9c, 16:1ω5c, 16:1ω13t, br17:0, a17:0, 17:1, 17:0, C18 PUFA, 18:3ω6, 18:5ω3, i18:0, 18:1ω7t, 18:1ω5c, 19:1, 20:3ω6, 20:4ω3, 20:2ω6, 20:1ω11c, 20:1ω7c, 20:1ω5c, 20:0, C21 PUFA, 21:0, 22:4ω6, 22:1ω11, 22:1ω9, 22:1ω7, 22:0, C24 PUFA, 24:1, 24:0

Table 3 Fatty acid profiles (mean ± SE, % total FA) of blubber from the three body locations (rump, neck, mammary) sampled from three adult female Cape fur seals fasting on land following the end of the lactation period (see text for details)

Fatty acid	Rump		Neck		Mammary	
	Inner	Outer	Inner	Outer	Inner	Outer
14:0	5.6 ± 0.3	5.4 ± 0.4	5.3 ± 0.1	5.6 ± 0.1	5.3 ± 0.1	5.1 ± 0.4
16:0	14.3 ± 1.0	13.2 ± 1.0	14.2 ± 0.1	14.1 ± 0.2	14.5 ± 0.7	12.1 ± 0.8
16:1ωc	8.7 ± 0.3	9.8 ± 0.4	7.8 ± 0.3	8.9 ± 0.6	7.4 ± 0.3	11.2 ± 0.1
16:2ω4	0.9 ± 0.1	0.9 ± 0.2	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.2
18:0	3.2 ± 0.1	2.8 ± 0.1	3.4 ± 0.2	3.2 ± 0.4	3.7 ± 0.1	2.4 ± 0.1
18:1ω7c	3.9 ± 0.3	3.9 ± 0.3	3.6 ± 0.1	3.8 ± 0.2	3.6 ± 0.3	4.1 ± 0.3
18:1ω9c	17.1 ± 1.5	18.0 ± 1.5	16.0 ± 0.1	16.8 ± 0.2	15.1 ± 1.4	19.4 ± 1.9
18:2ω6	1.6 ± 0.1	1.7 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.7 ± 0.1
18:4ω3	2.0 ± 0.2	2.1 ± 0.3	2.0 ± 0.1	2.1 ± 0.1	1.9 ± 0.1	2.1 ± 0.2
20:1ω9c	3.3 ± 0.1	3.0 ± 0.1	3.4 ± 0.2	3.1 ± 0.1	3.5 ± 0.2	2.8 ± 0.1
20:1ω11c	1.0 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
20:4ω3	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.2 ± 0.1
20:4ω6) AA	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
20:5ω3 EPA	5.3 ± 0.5	5.5 ± 0.5	5.5 ± 0.2	5.5 ± 0.3	5.7 ± 0.5	6.0 ± 0.4
22:1ω11	1.9 ± 0.3	1.5 ± 0.2	2.1 ± 0.2	1.7 ± 0.1	2.6 ± 0.5	1.2 ± 0.2
22:5ω3	5.2 ± 0.5	5.3 ± 0.7	5.4 ± 0.3	5.0 ± 0.3	5.4 ± 0.4	5.1 ± 0.4
22:6ω3 DHA	15.8 ± 0.2	15.7 ± 0.1	17.5 ± 0.3	16.6 ± 0.2	17.2 ± 0.3	14.7 ± 0.4
Other (<0.5%)	8.5	8.2	8.5	8.4	8.8	8.2
Sum SFA	25.0 ± 1.2	23.2 ± 1.3	24.6 ± 0.2	24.6 ± 0.6	25.4 ± 0.9	21.3 ± 1.2
Sum MUFA	39.5 ± 1.6	41.0 ± 1.4	37.7 ± 0.7	38.9 ± 0.6	37.1 ± 1.2	43.5 ± 1.9
Sum PUFA	35.5 ± 0.4	35.9 ± 0.2	37.7 ± 0.5	36.4 ± 0.2	37.5 ± 0.4	35.3 ± 0.7

AA arachidonic acid; *EPA* eicosapentaenoic acid; *DHA* docosahexaenoic acid; *SFA* saturated fatty acids; *MUFA* monounsaturated fatty acids; *SC* short-chain; *LC* long-chain; *PUFA* polyunsaturated fatty acids; Other includes components present at <0.5%: 12:0, i14:0, C14 PUFA, 14:1, br15:0, i15:0, a15:0, 15:1, 15:0, C16 PUFA, i16:0, 16:3ω4, 16:1ω9c, 16:1ω5c, 16:1ω13t, br17:0, a17:0, 17:1, 17:0, C18 PUFA, 18:3ω6, 18:5ω3, i18:0, 18:1ω7t, 18:1ω5c, 19:1, 20:3ω6, 20:4ω3, 20:2ω6, 20:1ω11c, 20:1ω7c, 20:1ω5c, 20:0, C21 PUFA, 21:0, 22:4ω6, 22:1ω11, 22:1ω9, 22:1ω7, 22:0, C24 PUFA, 24:1, 24:0

the tissues from individuals foraging at sea while the least variation was evident in the individuals nursing on land (Fig. 3).
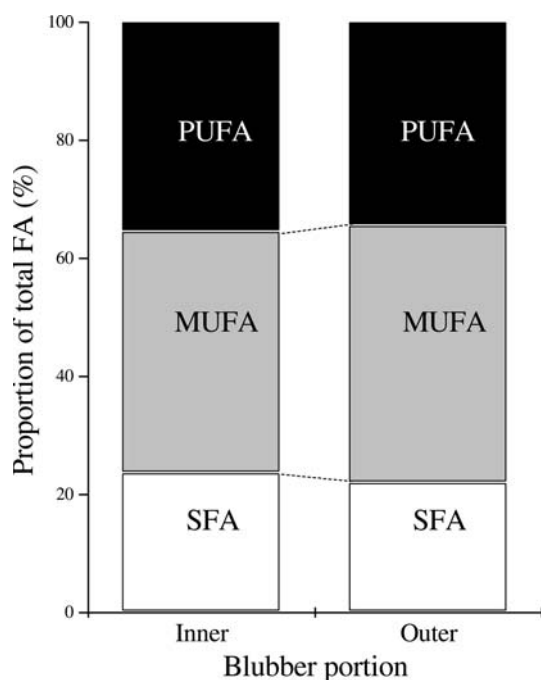
Fig. 2 Mean proportion of monounsaturated (MUFA), polyunsaturated (PUFA), and saturated (SFA) fatty acids in the inner and outer portions of blubber of Cape fur seals (see text for details)

Discussion

Fatty acid composition of blubber

The four most abundant FA recorded in the present study (16:0, 16:1ω7c, 18:1ω9c, DHA) are the same as those recorded as the most abundant in the blubber of the South American fur seal (*A. australis*, Grompone et al. 1990) and numerous phocid seals (e.g. harbour seal *Phoca vitulina*, Iverson et al. 1997b; grey seal *Halichoerus grypus*; Walton et al. 2000; southern elephant seal *Mirounga leonina*, Best et al. 2003). As in all these species, the FA recorded in the highest proportion in the present study was 18:1ω9c and the range of FA observed was similar to that reported in previous studies of pinnipeds and cetaceans (e.g. Lockyer et al. 1984; Grompone et al. 1990; Kakela et al. 1993; Walton et al. 2000; Hooker et al. 2001; Best et al. 2003). The total MUFA (41–44%), PUFA (35–36%), and SFA (22–24%) observed in the present study were also similar to those reported in the blubber of the South American fur seal (43%, 31%, and 26%, respectively) (Grompone et al. 1990). In contrast, the blubber of grey seals and southern elephant seals had higher proportions of MUFA (48–73%) and lower SFA (15–20%), while the proportion of PUFA was similar in grey seals (32–34%) but lower in southern elephant seals (13–16%) (Walton et al. 2000; Best et al. 2003). This variation may be indicative of differences in prey

Table 4 Eigen values for the first two derived components of a Principal Components Analysis of the fatty acid composition of blubber samples from Cape fur seals

Fatty acid	Principal component	
	1	2
14:0	0.123	−0.029
16:2ω4	0.120	0.007
16:1ω7c	0.034	0.210
16:0	0.105	−0.132
18:4ω3	0.125	0.042
18:2ω6	0.051	0.186
18:1ω9c	−0.125	0.071
18:1ω7c	−0.033	0.211
18:0	0.072	−0.135
20:4ω6 AA	−0.097	0.065
20:5ω3 EPA	0.106	0.066
20:4ω3	0.094	0.110
20:1ω11c	−0.110	−0.069
20:1ω9c	−0.084	−0.079
22:6ω3 DHA	0.025	−0.129
22:5ω3	−0.011	−0.007
22:1ω11	0.010	−.181

type or patterns of lipid mobilisation and deposition between the species (Iverson et al. 1997b; Iverson et al. 2004).
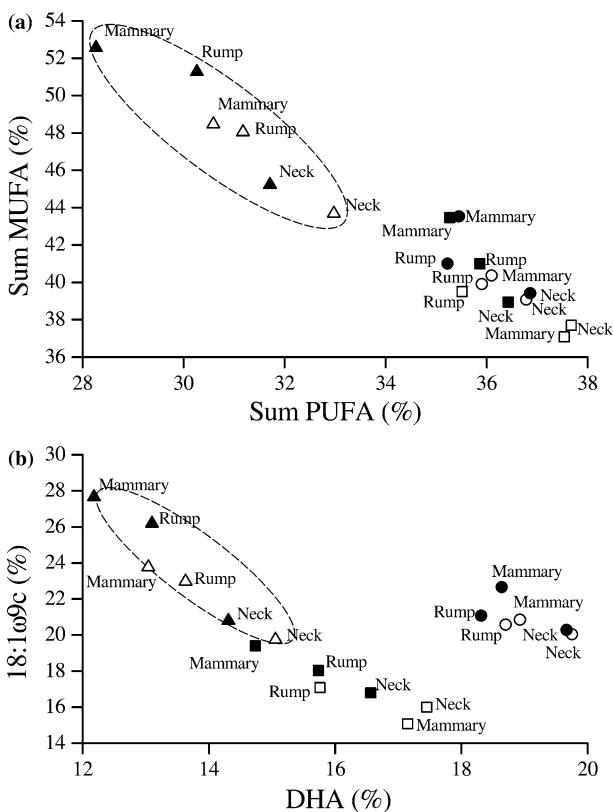

Fig. 3 Relationships between total proportion of **a** MUFA(%) and PUFA(%) and **b** 18:1ω9c(%) and DHA(%) in blubber samples collected from three tissue locations in Cape fur seals. *Triangles* (group in *dashed line*) At sea (lactation), *Circles* On land (lactation), and *Squares* On land (post-lactation). *Open* and *solid* symbols represent inner and outer portions of the blubber, respectively

Strong similarities between the FA composition of blubber and milk have been observed in numerous phocid species (Iverson 1993; Brown et al. 1999; Best et al. 2003; but see also Grahl-Nielsen et al. 2000) due to the lack of substantial *de novo* synthesis of milk FA in carnivores (Iverson et al. 2004). There is no information available on the composition of Cape fur seal milk although the FA profile of blubber observed in the present study was similar to that of Antarctic fur seal milk (Iverson et al. 1997a; Lea et al. 2002a). There were, however, differences in the proportions of individual FA, most notably the lower proportions of 16:0 (11–15%) and higher proportions of DHA (12–20%) in Cape fur seal blubber than Antarctic fur seal milk (16–19% and 5–8%, respectively) (Iverson et al. 1997a; Lea et al. 2002a). These differences would likely reflect differences in the diet of the two species (Iverson et al. 1997b). The diet of female Antarctic fur seals is dominated by krill (*Euphausia superba*) and myctophid fish (Myctophyidae) (Reid and Arnould 1996; Lea et al. 2002a). The FA profile of krill contains (in decreasing order of abundance): 16:0, 20:5ω3 (EPA), 18:1ω9c, DHA, 18:1ω7c, 14:0, and 16:1ω7c (Virtue et al. 1993), with myctophids generally containing lower levels of PUFA and higher levels of MUFA. In contrast, the Cape fur seal diet in South Africa is primarily pelagic schooling fish such as pilchard (*Sardinops sagax* Clupeidae), sardines (*Sardinella* spp. Clupeidae), anchovies (*Engraulis japonicus.* Engraulidae), hake (*Merluccius* spp. Merlucciidae), and cephalopods (particularly the *Loligo* species) (David 1987; Punt et al. 1995; de Bruyn et al. 2003; W.H. Oosthuizen, unpublished data). We have not been able to examine the FA profiles of these species but most would be expected to be PUFA-rich in comparison to myctophid fishes (Lea et al. 2002b). Furthermore, in contrast to Antarctic krill, based on analyses of similar species from Australian waters (Nichols et al. 1998), DHA would generally be in higher proportions than EPA in most of these pelagic species. The differences between the observed FA composition of blubber in Cape fur seals and Antarctic fur seal milk could also reflect differences in the selective mobilisation of fatty acids for milk production (Bryden and Stokes 1969; Grahl-Nielsen et al. 2000). Comparison of FA composition of blubber and milk within these species is needed to clarify this question.

Nutritional state and lactation have been shown to influence the composition of adipose tissue due to the differential mobilisation and deposition of particular FA under different physiological regimes (Bryden and Stokes 1969; Groscolas 1986; Nielsen and Jakobsen 1994). In the present study, differences were observed in adipose tissue FA composition between individuals that were foraging or nursing during the lactation period as well as the non-lactating individuals fasting on land. Individuals foraging at sea had higher proportions of MUFA in portions than the individuals sampled on land. This would suggest that the prey of Cape fur seals is rich in MUFA, in particular 18:1ω9c. The rate of

adipose tissue deposition can be substantial during maternal foraging trips in fur seals (Costa 1991; Arnould et al. 1996) such that nutritionally surplus dietary lipids are likely to influence the whole blubber layer. The higher ratio of 18:1ω9c to DHA in individuals is consistent with the selective mobilisation of DHA for milk production in this species. The sample sizes for the three categories of individuals, however, were small due to the opportunistic nature of their collection and the trends observed here must, therefore, be interpreted with caution. More extensive sampling is required to investigate fully the influence of nutritional state, reproductive status, and potential seasonal variation in diet on the FA composition of blubber in this species.

Variation in the fatty acid composition of blubber and its implications for dietary interpretation

Previous studies in phocid seals and cetaceans have documented vertical differences in the FA composition of blubber and emphasised the potential implications of this on interpretation of diet based on FA analysis (Koopman et al. 1996; Best et al. 2003). In the present study, significant differences in FA composition were observed between the inner and outer regions of the subcutaneous adipose tissue, suggesting that vertical stratification of blubber also occurs in Cape fur seals. As has been found in phocid seals and cetaceans (Lockyer et al. 1984; Koopman et al. 1996; Best et al. 2003; Thiemann et al. 2004b), the outer portion of the blubber in the present study was higher in MUFA, and in particular SC-MUFA, than the inner portion. Similarly in agreement with findings reported for southern elephant seals (Best et al. 2003), significantly greater proportions of SFA and PUFA in the inner than outer portions of the blubber were observed in the present study.

The cause of stratification in blubber FA composition has been discussed in previous studies of phocid seals and cetaceans (e.g. Lockyer et al. 1984; Koopman et al. 1996; Best et al. 2003) and is considered to be primarily due to the inner region of blubber being more metabolically active than the outer region. Nutritionally surplus FA are deposited in the inner blubber region and, consequently, represent the most recent dietary input (Lockyer et al. 1984; Koopman et al. 1996). The inner region is also the first to be mobilised during nutritional stress (Pond and Mattacks 1988). Accordingly, studies using the FA composition of blubber for the analysis of diet in Cape fur seals (and other otariid seals) should apply appropriate sub-sampling of the blubber depth and consider temporal aspects of deposition and mobilisation when interpreting the results (Best et al. 2003; Bradshaw et al. 2003; Thiemann et al. 2004b).

In phocid seals, blubber is considered a continuous subcutaneous layer covering the body core and the mobilisation of blubber lipids has been shown to occur uniformly around the body (Nordy and Blix 1985; Slip

et al. 1992; Beck and Smith 1995). In contrast, studies of the anatomical distribution and biochemical properties of adipose tissue in terrestrial carnivores have indicated that they have distinct subcutaneous depots and that these are mobilised at different rates (Pond and Mattacks 1985b; Pond and Ramsay 1992; Pond et al. 1995). There is little information on the anatomical distribution and structure of adipose tissue in otariid seals and it is not known whether subcutaneous adipose tissue acts as a uniform blubber depot or is mobilised at different rates in these species. In the present study, the FA composition of blubber varied considerably between the three tissue sampling locations on the body. In general, the Mammary tissue site had the highest proportions of 18:1ω9c and total MUFA, followed by tissue from the Rump, and Neck sites. This suggests that the deposition and mobilisation of blubber lipids may not be uniform around the body in Cape fur seals (and potentially all otariid seals). In addition, the proportion of DHA was generally highest in the Neck, Rump and Mammary with the exception of the inner portion of blubber in females that were not lactating and both portions of females nursing on land where the Mammary tissue site had higher DHA levels than the Rump (Fig. 3b). This suggests that the pattern of lipid deposition and mobilisation around the body in otariid seals may be influenced, as is observed in terrestrial mammals (Pond 1988), by nutritional and/or reproductive status.

Because of the regional uniformity of blubber in phocid seals, studies of the FA composition in these species have used a single tissue sampling location on the body for obtaining information on the diet (Bradshaw et al. 2003; Grahl-Nielsen et al. 2003; Walton and Pomeroy 2003). In contrast, the differences observed in the FA composition between the three tissue sites sampled in the present study suggest the potential for error in interpreting diet in otariid seals to arise from sampling an inappropriate site on the body. Due to the small sample size, however, more data are required to assess fully the degree of variation in the three tissue sites sampled as well as other locations of the body. There is currently no information available on the pattern of lipid deposition and mobilisation in otariid seals, making it problematic at present to determine the most appropriate tissue sampling sites to enable dietary comparison between individuals, species, sex, or age for the temporal scales of interest.

Lactation in otariid seals is characterised by females alternating periods of foraging at sea with short durations ashore nursing their pups (Oftedal et al. 1987; Costa 1991). Females have four mammae with an extensive gland structure which can spread over the whole of the ventral abdomen, half way up the lateral abdomen and can even stretch up to the ventral thorax (Oftedal et al. 1987; personal observation). Subcutaneous adipose tissue surrounds the complex mammary alveoli structure, which may facilitate the efficient uptake of lipid into milk, and appears to decrease in thickness during nursing periods ashore (personal

observation). Consequently, this blubber region is likely to experience the most rapid deposition and mobilisation of lipids in females and would, therefore, reflect a combination of the most recent nutrition and milk production. Other body regions are likely to have less dynamic blubber and, therefore, may represent longer intervals for dietary analysis (Pond 1988; Thiemann et al. 2004b).

In conclusion, this study has demonstrated stratification in the FA composition of subcutaneous adipose tissue in female Cape fur seals. This primary result highlights the need, as previously shown in phocid seals and cetaceans (Lockyer et al. 1984; Koopman et al. 1996; Best et al. 2003; Thiemann et al. 2004b), for the sampling of the whole blubber depth and its appropriate sub-sampling for dietary analyses. In addition, differences were observed with tissue location in the FA composition of blubber which highlight the need for standardised sampling techniques for all age and sex classes in otariid seals. Detailed information on the regional pattern of lipid deposition and mobilisation in these species is needed to determine the most appropriate sampling sites for interpreting diet over various temporal scales.

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