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H. N. Koopman · S. J. Iverson · D. E. Gaskin

Stratification and age-related differences in blubber fatty acids of the male harbour porpoise (*Phocoena phocoena*)

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Abstract Fatty acid composition of blubber was determined at four body sites of 19 male harbour porpoises. A total of 65 fatty acids were quantified in each sample. The array of fatty acids contained in harbour porpoise blubber was similar to those found in other marine mammals. While chemical composition of total blubber was uniform over the body, with the exception of the caudal peduncle, vertical stratification was evident between the deep (inner) and superficial (outer) blubber layers. Fatty acids with chain lengths shorter than 18 carbons were present in significantly greater amounts in the outer blubber layer, while the longer-chain unsaturated fatty acids were more prevalent in the inner layer. This distribution suggests that the inner blubber layer is more active metabolically than the outer layer in terms of lipid deposition and mobilization. The degree of stratification between the two layers appears to increase with age, indicating a predictable turnover in the blubber layer of male porpoises. Harbour porpoise blubber contained high levels (2–27%) of isovaleric acid in the outer blubber layer, and these levels were positively correlated with age.

Key words Blubber · Fatty acids · Isovaleric acid · Harbour porpoise, *Phocoena phocoena*

Abbreviations *Caud* caudal dorsal body site · *GC* gas chromatograph · *FA* fatty acid(s) *IUPAC* International Union of Pure and Applied Chemistry · *PUFA* polyunsaturated fatty acid(s) · *II dor* II dorsal body site · *III dor* III Dorsal body site · *II Ven* II ventral body site

H.N. Koopman (✉) · D.E. Gaskin
Department of Zoology, University of Guelph, Guelph, Ontario, Canada N1G 2W1

S.J. Iverson[1]
Canadian Institute of Fisheries Technology, Technical University of Nova Scotia, Halifax, Nova Scotia, Canada B3J 2X4

Present address:
[1]Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1

Introduction

The blubber layer is the most important site of fat storage in marine mammals (Pond 1978). It functions as an insulator, buoyancy adjuster and body streamliner, and lipids in this tissue are mobilized in times of energetic need and replenished when food is in excess (Ryg et al. 1988). The biochemical composition of blubber can provide insights into the nutritive status, feeding habits, lipid turnover and general lipid metabolism of a given species (Ackman et al. 1975b, c; Aguilar and Borrell 1990; Lockyer 1991; Iverson 1993; Käkelä et al. 1993). In particular, the FA composition of this tissue can be used to examine patterns of deposition, maintenance, mobilization and replenishment (Ackman et al. 1965; West et al. 1979; Lockyer et al. 1984; Iverson et al. 1995).

Harbour porpoises (*Phocoena phocoena*) rely on a relatively thick blubber layer to provide insulation in temperate waters where the seasonal average temperatures range from 4 to 16 °C (Worthy and Edwards 1990; Gaskin 1992). The diet of this species has been well documented (Smith and Gaskin 1974; Recchia and Read 1989; Smith and Read 1992) and a few studies have shown a relationship between blubber thickness and condition (Read 1990; Koopman 1994). To date only two studies have examined the FA composition of harbour porpoise blubber. In an early study, Lovern (1934) quantified ten FA in the blubber of one porpoise, and suggested that the composition of head blubber and body blubber differed. Lovern (1934) also noted the presence of isovaleric acid in both depots. Isovaleric acid is an unusual, very short-chain (C5) fatty acid, that has been reported in a few other

odontocete species, although its functional significance is still unknown (Ackman and Lamothe 1989). Litchfield et al. (1975) also found relatively high amounts of isovalerate lipids in harbour porpoise blubber, but no other FA were quantified, although this study was based on only one animal. To date no systematic examination of the FA composition of harbour porpoise blubber has been made.

In mysticetes (*Balaenoptera* spp.) and sperm whales (*Physeter catodon*) the blubber is stratified: the inner layers (adjacent to the muscle) are metabolically active while the outer layer (adjacent to the skin) is relatively inert (Ackman et al. 1965; Ackman et al. 1975b, c; Lockyer et al. 1984; Aguilar and Borrell 1990; Lockyer 1991). In these large cetaceans regional differences in blubber layers are evident both in macroscopic structure and FA composition. Bottlenose dolphins (*Tursiops truncatus*) also have stratified blubber with higher lipid content in the middle layer (Shoda et al. 1993), but the FA composition of these layers have not yet been compared. There is evidence (Koopman 1994) that harbour porpoises may selectively mobilize lipid from blubber in the thoracic/abdominal region of the body, and not utilize the blubber in the caudal peduncle. It is not known whether lipid throughout the depth of the blubber is metabolized nor if the layer is biochemically homogeneous.

The purpose of this study was to compare the FA composition of the blubber of the harbour porpoise at four body sites, and to determine whether this tissue is biochemically stratified in this species. To eliminate the potential variability in blubber specifically associated with lactation and pregnancy in females (Read 1990; Koopman 1994), only males were examined.

Materials and methods

Porpoises

Blubber samples were taken from 19 male harbour porpoises from the Bay of Fundy ($n = 8$) and the Gulf of Maine ($n = 11$). Porpoises from the Bay of Fundy were incidentally caught either in herring weirs ($n = 6$) or demersal gill nets ($n = 2$) during August and September 1993. Animals from the Gulf of Maine were all caught incidentally in the gill net fishery during October–December 1992 and 1993, and April 1992. All porpoise carcasses were robust and in good condition, and were necropsied or frozen within 24 h of death.

Porpoises were classified into three reproductive classes: calves [Gulf of Maine: $n = 2$, Bay of Fundy: $n = 0$; identified by incomplete eruption of teeth and presence of milk in the stomach (Read and Gaskin 1990)]; immature males (Gulf of Maine: $n = 5$, Bay of Fundy: $n = 4$; lack of spermatozoa in testes; testis mass < 100 g); and mature males [Gulf of Maine: $n = 4$, Bay of Fundy: $n = 4$; presence of spermatozoa in the testes; testis mass > 100 g (outlined in Hohn et al. (1985) and Read and Hohn, 1995)]. Ages of all porpoises were estimated from counts of dentinal growth layers in stained decalcified thin sections of teeth as recommended by the Oslo harbour porpoise age determination workshop (Bjorge et al. in press).

Samples

The sites of blubber sampling are shown in Fig. 1a. The complete blubber layer was removed from the carcass at these sites (blubber pieces about 10 cm × 10 cm) and frozen at −20 °C until further analysis.

To examine stratification in blubber, these frozen pieces were subsampled to obtain samples from the deep and superficial layers (see Fig. 1b). Due to the absence of definitive histological information on these layers, we decided to sample only the innermost and outermost layers to test for stratification. The *m. cutaneous trunchii* muscle and subdermal connective tissue sheath were removed and a sample of approximately 0.5 g was trimmed from the deep blubber surface (inner layer sample). The skin (epidermis and dermis) was also removed and a blubber sample of 0.5 g was taken (outer layer sample). The remaining blubber between these layers, corresponding to about 40% of the blubber depth at most sites, was not sampled.

Sample extraction and preparation

Blubber samples (approximately 0.5 g each) were weighed and placed in 9 ml of 2:1 chloroform:methanol with 0.005% butylated hydroxytoluene in Kimax test tubes with Teflon caps. Samples were mashed manually with a glass homogenizer until thin and transparent, then vortexed for 20 s, and allowed to soak for 2–5 days. Next, samples were extracted according to a modified Folch procedure (Folch et al. 1957; Iverson 1988). FA analysis was carried out on total lipid samples because harbour porpoise blubber is almost entirely triglyceride and contains little, if any, wax ester and only trace amounts of phospholipid (H.N. Koopman, unpublished data). FA butyl esters were used in place of methyl esters because many of the FA of interest had short chains (< C6) and hence would be exceptionally vulnerable to volatilization as methyl esters (Shantha and Napolitano 1992). Butyl esters were prepared by placing 50 mg of lipid with 1 ml hexane and 1 ml 10% BF_3 in butanol in a Kimax tube. Tubes were incubated at 100 °C for 1 h, then cooled to room temperature. Water (3 ml) was added, the sample was vortexed and centrifuged at 480 *g* for 5 min. The hexane layer was removed and washed with 5 ml H_2O and dried with sodium sulphate. Samples were flushed with N_2 to prevent oxidation, and were capped tightly.

Fatty acid analysis

Samples were analyzed using temperature-programmed capillary gas-liquid chromatography on a Perkin-Elmer Autosystem GC fitted with a 30 m × 0.25 mm i.d. column, coated with 50% cyanopropyl polysiloxane (0.25 μm, DB-23). The injection and detector temperatures were held at 250 and 270 °C, respectively. The temperature program was developed by trial and error until all components were satisfactorily separated within the shortest time period as follows: 65 °C for 2 min, ramped at 20 °C · min^{-1} to 165 °C, held for 0.4 min, ramped at 2 °C · min^{-1} to 215 °C and held for 6.6 min, ramped at 5 °C · min^{-1} to 240 °C and held for 1.0 min. The entire program took 45 min to complete.

Identification of FA was made from known standard mixtures (Nu Check Preparations, Elysian, Minn., USA) and from samples earlier identified using silver nitrate chromatography (Iverson 1988). GC response factors for each FA were used to calculate weight percentage and were based on modified theoretical response factors (Ackman 1991) that were adjusted according to values from known standard mixtures (Iverson 1988).

All FA components were converted to a weight percentage of the total array of FA plus unknowns and are named according to the IUPAC nomenclature of #carbons:#double bonds, where n–x denotes the position of the last double bond relative to the terminal methyl end of the FA.

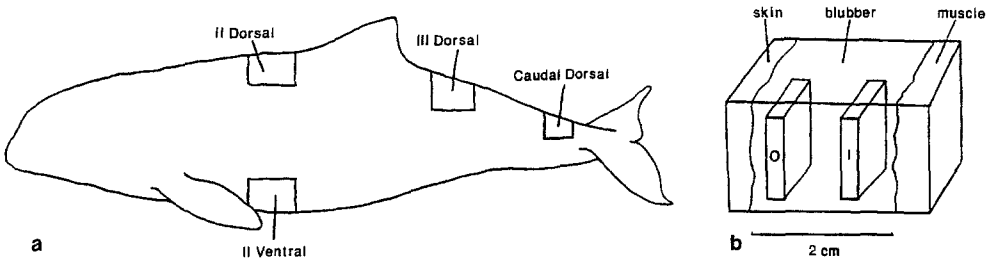

Fig. 1a, b Sampling sites of blubber for fatty acid analysis of 19 male harbour porpoises. Porpoises were caught incidentally in fishing gear (either gill nets or weirs) in the Gulf of Maine and the Bay of Fundy, 1992–1993. (**a**) the four body sites where blubber was removed from carcasses for analysis; (**b**) division of the blubber layer of each site into inner (*I*) and outer (*O*) samples. See Materials and methods for further details

Statistical analyses

It was not possible to examine directly the differences between porpoises from the Gulf of Maine versus the Bay of Fundy, because the two groups of porpoises were not sampled at the same time of year and seasonal diets might confound the comparison. Additionally, given the distribution, genetic similarities, contaminant profiles, and proposed migratory patterns of these groups, they likely are not distinct subpopulations [Wang (1993); Westgate and Johnston (1995); A.J. Read and A.J. Westgate, unpublished data]. Thus, data from all animals were combined.

FA compositions of the four body sites were compared using the Kruskal-Wallis non-parametric test at an alpha level of 0.01. Because of the large number of multiple comparisons being made, this alpha level was used in order to reduce the chances of making a Type I error. Comparisons were made separately for inner and outer blubber layers to eliminate any layer/site interaction. The small size of the data set restricted the number of FA that could be examined specifically, thus comparisons were restricted to major components: iso 5:0, 14:0, 14:1n-5, 16:0, 16:1n-7, 18:0, 18:1n-11, 18:1n-9, 18:2n-6, 20:1n-11, 20:1n-9, 20:4n-6, 20:5n-3, 22:1n-11, 22:5n-3 and 22:6n-3. Additionally, total PUFA and the ratio of total n-3 to total n-6 FA were also compared. Levels of these FA in the inner versus the outer blubber layers were also compared separately for each body site using the Mann-Whitney non-parametric test at an alpha level of 0.01.

The degree of stratification between older (> 4 years old; $n = 7$) and younger (calves and yearlings; $n = 9$) porpoises was also examined. The relative differences between levels in the inner and the outer blubber layer of the II Dor were calculated for the 14 FA that showed significant differences between inner and outer layers: iso 5:0, 14:1n-5, 16:0, 16:1n-7, 18:0, 18:1n-11 18:2n-6, 20:1n-11, 20:1n-9, 20:4n-6, 20:5n-3, 22:1n-11, 22:5n-3, and 22:6n-3. The II Dor was chosen for this comparison because this is closest to the site at which blubber is most often sampled on cetaceans (Geraci and Lounsbury 1993). The "old" and "young" differences were then compared using Mann-Whitney tests.

Because isovaleric acid was the most variable component, and its presence is unusual and not derived from the diet, the relative concentrations were regressed against age at the site where the highest levels occurred (II Ven), as well as at the II Dor. Levels of total PUFA and the w3/w6 ratio in the outer blubber layer of the II Dor were also regressed against age. Again, the II Dor was chosen for its proximity to standard sampling locations. All statistics were performed using Statview 4.01 for Macintosh (Abacus Concepts, Berkeley, Calif., USA 1992).

Results

A total of 65 FA were typically quantified in harbour porpoise blubber. The mean percent of each compound in each layer, at each site, is presented in Table 1.

Body site

The FA composition of the inner blubber layer showed more significant differences with respect to location than the outer layer. Generally, the inner layers of the three anterior-most body sites (II Dor, II Ven and III Dor) were similar in FA composition, while the caudal site was significantly different in levels of iso 5:0, 14:1n-5, 16:0, 16:1n-7, 18:0, 20:1n-9, 22:1n-11, 22:5n-3, 22:6n-3, total PUFA and the n-3/n-6 ratio compared to the other three sites (Table 2). In the outer blubber layer the caudal site and III Dor had significantly higher proportions of 18:2n-6, 20:1n-11, 20:4n-6, 22:6n-3 and total PUFA, and lower levels of 14:0 than the other two sites. There were no significant differences in the levels of the other FA examined in the outer blubber layer among the four body sites.

Inner and outer layers

At most sites FA with chain lengths shorter than 18 carbons were present in significantly larger amounts in the outer layer, while the longer-chain unsaturated fatty acids were more prevalent in the inner layer (Table 3). Relative amounts of 14:0 and 18:1n-9 were not significantly different with respect to layer at any site. Differences between inner and outer layers in levels of iso 5:0, 20:1n-11, 20:1n-9, 22:1n-11, 22:5n-3, 22:6n-3 and total PUFA were significant at all four body sites. Levels of 14:1n-5, 16:1n-7, 18:0, 18:2n-6, 20:4n-6, 20:5n-3, and the n-3/n-6 ratio were significantly different in both layers at the II Dor, II Ven and III Dor. Significant differences in the levels of 18:1n-11 between the layers occurred at the II Dor and II Ven. Levels of 16:0 were significantly different between the two layers only at the Caud.

In addition to the gross differences between layers observed in all animals, our data suggest that the degree of stratification in the blubber layer may increase

Table 1 Fatty acid composition of blubber of male harbour porpoises ($n = 19$). Porpoises were caught incidentally, either in gill nets or in weirs, in the Gulf of Maine and the Bay of Fundy during 1992 and 1993. Levels of each fatty acid are presented separately for inner and outer blubber layers at the four body sites examined in this study (see Figs. 1a, b). Values given are mean percent content of total lipid ± S.D.

Fatty acid	II Dorsal inner	II Dorsal outer	II Ventral inner	II Ventral outer	III Dorsal inner	III Dorsal outer	Caudal inner	Caudal outer
iso 4:0	0.42 ± 0.45	2.29 ± 1.04	0.61 ± 0.67	2.32 ± 1.23	0.56 ± 0.77	1.71 ± 0.90	0.52 ± 0.36	1.42 ± 0.75
iso 5:0	2.16 ± 1.22	11.47 ± 5.97	3.15 ± 2.37	11.61 ± 7.06	3.11 ± 2.09	8.61 ± 4.05	0.23 ± 0.15	8.04 ± 3.84
12:0	0.68 ± 0.22	1.47 ± 0.55	0.86 ± 0.32	1.29 ± 0.50	0.75 ± 0.29	1.00 ± 0.53	0.80 ± 0.23	0.98 ± 0.46
13:0	0.17 ± 0.09	0.78 ± 0.19	0.24 ± 0.14	0.76 ± 0.26	0.25 ± 0.18	0.48 ± 0.17	0.30 ± 0.11	0.57 ± 0.18
iso 14:0	0.01 ± 0.02	0.01 ± 0.02	0.01 ± 0.02	0.02 ± 0.03	0.01 ± 0.02	0.02 ± 0.04	0.01 ± 0.02	0.01 ± 0.03
14:0	7.21 ± 1.90	8.02 ± 0.88	7.68 ± 1.00	7.67 ± 0.78	7.56 ± 0.70	7.19 ± 0.95	7.24 ± 0.80	6.97 ± 0.64
iso 15:0	0.47 ± 0.16	1.46 ± 0.27	0.56 ± 0.24	1.40 ± 0.34	0.61 ± 0.34	1.06 ± 0.21	0.84 ± 0.29	1.38 ± 0.35
anti 15:0	0.21 ± 0.06	0.28 ± 0.09	0.23 ± 0.08	0.29 ± 0.11	0.26 ± 0.10	0.22 ± 0.07	0.23 ± 0.11	0.24 ± 0.06
15:0	0.38 ± 0.11	0.41 ± 0.07	0.38 ± 0.08	0.40 ± 0.07	0.35 ± 0.04	0.37 ± 0.07	0.34 ± 0.05	0.34 ± 0.09
iso 16:0	0.30 ± 0.12	1.45 ± 0.53	0.37 ± 0.15	1.34 ± 0.54	0.41 ± 0.44	1.01 ± 0.43	0.57 ± 0.38	1.30 ± 0.52
16:0	7.83 ± 1.16	7.95 ± 0.96	7.92 ± 1.00	7.61 ± 1.14	7.22 ± 1.04	7.68 ± 0.74	6.54 ± 0.73	7.46 ± 0.69
7-Me-16	0.26 ± 0.04	0.31 ± 0.05	0.27 ± 0.04	0.32 ± 0.05	0.28 ± 0.04	0.33 ± 0.06	0.31 ± 0.04	0.31 ± 0.05
iso 17:0	0.26 ± 0.08	0.24 ± 0.09	0.25 ± 0.04	0.26 ± 0.09	0.25 ± 0.05	0.23 ± 0.06	0.22 ± 0.07	0.20 ± 0.06
17:0	0.17 ± 0.12	0.11 ± 0.09	0.22 ± 0.13	0.13 ± 0.10	0.21 ± 0.13	0.18 ± 0.11	0.20 ± 0.10	0.13 ± 0.10
18:0	1.23 ± 0.28	0.60 ± 0.29	1.14 ± 0.38	0.63 ± 0.32	1.03 ± 0.26	0.74 ± 0.24	0.84 ± 0.14	0.78 ± 0.21
20:0	0.14 ± 0.07	0.11 ± 0.11	0.11 ± 0.04	0.10 ± 0.07	0.13 ± 0.04	0.09 ± 0.07	0.12 ± 0.04	0.10 ± 0.06
Total saturates	22.07 ± 2.57	37.22 ± 5.93	24.23 ± 3.57	36.41 ± 7.19	23.18 ± 3.90	31.16 ± 5.34	23.94 ± 2.04	30.51 ± 4.57
14:1n-9	0.17 ± 0.06	0.58 ± 0.28	0.23 ± 0.10	0.50 ± 0.27	0.22 ± 0.10	0.43 ± 0.29	0.24 ± 0.11	0.58 ± 0.28
14:1n-7	0.30 ± 0.15	0.78 ± 0.34	0.35 ± 0.17	0.68 ± 0.32	0.32 ± 0.16	0.51 ± 0.27	0.35 ± 0.11	0.53 ± 0.27
14:1n-5	0.90 ± 0.51	3.53 ± 0.89	1.10 ± 0.69	3.45 ± 1.18	1.47 ± 0.79	3.05 ± 0.70	2.99 ± 0.39	3.34 ± 0.71
15:1n-8	0.04 ± 0.08	0.07 ± 0.04	0.05 ± 0.05	0.05 ± 0.06	0.05 ± 0.03	0.07 ± 0.08	0.06 ± 0.08	0.08 ± 0.09
15:1n-6	0.04 ± 0.05	0.11 ± 0.04	0.04 ± 0.03	0.11 ± 0.05	0.05 ± 0.03	0.10 ± 0.05	0.09 ± 0.23	0.10 ± 0.04
16:1n-11	0.21 ± 0.05	0.19 ± 0.02	0.22 ± 0.04	0.19 ± 0.03	0.21 ± 0.03	0.20 ± 0.03	0.18 ± 0.02	0.17 ± 0.02
16:1n-9	0.87 ± 0.20	1.47 ± 0.56	1.01 ± 0.26	1.33 ± 0.53	0.91 ± 0.21	1.14 ± 0.53	0.86 ± 0.27	1.06 ± 0.51
16:1n-7	9.29 ± 2.38	19.25 ± 1.72	10.05 ± 2.71	18.61 ± 2.50	11.53 ± 3.65	18.05 ± 1.72	16.09 ± 3.45	18.20 ± 2.19
16:1n-5	0.12 ± 0.01	0.07 ± 0.06	0.13 ± 0.03	0.09 ± 0.07	0.14 ± 0.07	0.11 ± 0.06	0.13 ± 0.04	0.11 ± 0.04
17:1	0.16 ± 0.07	0.20 ± 0.05	0.17 ± 0.05	0.22 ± 0.08	0.19 ± 0.07	0.25 ± 0.07	0.22 ± 0.06	0.29 ± 0.12
18:1n-11	5.69 ± 0.82	4.48 ± 0.90	5.68 ± 0.72	4.49 ± 1.06	5.79 ± 0.84	5.30 ± 1.06	5.64 ± 1.62	4.87 ± 1.16
18:1n-9	12.65 ± 1.62	12.14 ± 1.10	12.68 ± 1.47	12.21 ± 1.54	12.89 ± 1.32	13.27 ± 1.43	13.22 ± 1.29	12.78 ± 1.50
18:1n-7	1.98 ± 0.40	1.54 ± 0.60	1.96 ± 0.40	1.60 ± 0.64	2.00 ± 0.32	1.88 ± 0.44	2.08 ± 0.43	1.91 ± 0.43
18:1n-5	0.36 ± 0.05	0.22 ± 0.11	0.35 ± 0.07	0.24 ± 0.11	0.36 ± 0.04	0.29 ± 0.09	0.34 ± 0.05	0.29 ± 0.08
20:1n-11	3.54 ± 0.89	1.85 ± 0.52	3.27 ± 0.64	1.93 ± 0.63	3.34 ± 0.76	2.53 ± 0.52	3.41 ± 0.93	2.43 ± 0.50
20:1n-9	8.71 ± 1.55	2.70 ± 1.81	8.05 ± 1.40	3.16 ± 2.19	7.97 ± 1.99	4.45 ± 2.09	6.64 ± 0.96	4.54 ± 2.06
20:1n-7	0.21 ± 0.04	0.07 ± 0.08	0.19 ± 0.04	0.08 ± 0.09	0.20 ± 0.05	0.11 ± 0.09	0.20 ± 0.11	0.14 ± 0.11
20:1n-5	0.09 ± 0.10	0.04 ± 0.04	0.10 ± 0.08	0.03 ± 0.03	0.08 ± 0.07	0.08 ± 0.09	0.07 ± 0.05	0.07 ± 0.08
22:1n-11	9.33 ± 2.83	2.14 ± 1.38	8.07 ± 2.09	2.40 ± 1.82	7.73 ± 2.56	3.27 ± 1.70	5.30 ± 1.04	3.29 ± 1.75
22:1n-9	0.57 ± 0.18	0.12 ± 0.09	0.49 ± 0.13	0.14 ± 0.12	0.46 ± 0.14	0.18 ± 0.11	0.32 ± 0.09	0.21 ± 0.12
22:1n-7	0.11 ± 0.06	0.02 ± 0.02	0.08 ± 0.05	0.02 ± 0.03	0.06 ± 0.03	0.01 ± 0.02	0.05 ± 0.04	0.03 ± 0.04
24:1	0.25 ± 0.14	0.08 ± 0.05	0.22 ± 0.09	0.09 ± 0.09	0.19 ± 0.09	0.10 ± 0.06	0.13 ± 0.08	0.08 ± 0.06
Total mono unsaturates	55.58 ± 3.23	51.62 ± 3.39	54.48 ± 2.83	51.61 ± 3.81	56.13 ± 2.82	55.36 ± 2.61	58.61 ± 1.81	54.93 ± 2.13
16:2n-6	0.17 ± 0.03	0.23 ± 0.05	0.18 ± 0.03	0.26 ± 0.09	0.19 ± 0.05	0.21 ± 0.05	0.17 ± 0.04	0.23 ± 0.06
16:2n-4	0.57 ± 0.11	0.52 ± 0.07	0.56 ± 0.06	0.53 ± 0.06	0.56 ± 0.07	0.52 ± 0.06	0.53 ± 0.07	0.50 ± 0.07
16:3n-4	0.30 ± 0.09	0.38 ± 0.12	0.32 ± 0.10	0.38 ± 0.12	0.32 ± 0.08	0.37 ± 0.13	0.36 ± 0.13	0.37 ± 0.12
16:3n-1	0.12 ± 0.05	0.10 ± 0.06	0.12 ± 0.06	0.11 ± 0.08	0.12 ± 0.06	0.13 ± 0.06	0.14 ± 0.04	0.13 ± 0.07
16:4n-1	0.34 ± 0.07	0.20 ± 0.09	0.33 ± 0.08	0.20 ± 0.09	0.36 ± 0.09	0.25 ± 0.08	0.28 ± 0.06	0.25 ± 0.08
18:2d5,7	0.03 ± 0.04	nd	0.03 ± 0.03	nd	0.03 ± 0.04	nd	nd	nd
18:2n-7	0.07 ± 0.07	0.08 ± 0.06	0.06 ± 0.05	0.09 ± 0.07	0.07 ± 0.05	0.08 ± 0.04	0.09 ± 0.03	0.09 ± 0.03
18:2n-6	1.21 ± 0.10	0.92 ± 0.17	1.20 ± 0.14	0.93 ± 0.17	1.22 ± 0.15	1.08 ± 0.19	1.20 ± 0.22	1.03 ± 0.17
18:2n-4	0.08 ± 0.03	0.07 ± 0.03	0.08 ± 0.02	0.08 ± 0.04	0.07 ± 0.03	0.09 ± 0.04	0.07 ± 0.03	0.08 ± 0.04
18:3n-6	0.15 ± 0.02	0.07 ± 0.04	0.14 ± 0.03	0.08 ± 0.04	0.13 ± 0.03	0.10 ± 0.04	0.11 ± 0.02	0.10 ± 0.03
18:3n-4	0.14 ± 0.04	0.07 ± 0.04	0.16 ± 0.08	0.07 ± 0.05	0.13 ± 0.03	0.09 ± 0.04	0.11 ± 0.04	0.08 ± 0.03
18:3n-3	0.72 ± 0.11	0.54 ± 0.14	0.72 ± 0.14	0.56 ± 0.15	0.75 ± 0.14	0.66 ± 0.15	0.71 ± 0.14	0.65 ± 0.15
18:4n-3	1.04 ± 0.21	0.41 ± 0.29	1.03 ± 0.23	0.44 ± 0.27	1.04 ± 0.31	0.60 ± 0.27	0.83 ± 0.15	0.62 ± 0.26
18:4n-1	0.22 ± 0.07	0.12 ± 0.09	0.22 ± 0.06	0.12 ± 0.07	0.21 ± 0.05	0.18 ± 0.11	0.20 ± 0.08	0.16 ± 0.10
20:2n-6	0.16 ± 0.05	0.06 ± 0.06	0.14 ± 0.03	0.07 ± 0.07	0.15 ± 0.09	0.10 ± 0.10	0.13 ± 0.03	0.10 ± 0.06
20:3NMT	0.02 ± 0.05	0.03 ± 0.04	0.01 ± 0.02	0.01 ± 0.02	0.02 ± 0.03	0.03 ± 0.04	0.03 ± 0.03	0.04 ± 0.05

(continued overleaf)

Table 1 (continued)

20:3n-6	0.07 ± 0.03	0.02 ± 0.02	0.06 ± 0.02	0.02 ± 0.02	0.07 ± 0.04	0.03 ± 0.02	0.06 ± 0.05	0.04 ± 0.02
20:4n-6	0.30 ± 0.13	0.12 ± 0.08	0.27 ± 0.13	0.15 ± 0.12	0.30 ± 0.13	0.19 ± 0.09	0.29 ± 0.16	0.23 ± 0.12
20:3n-3	0.09 ± 0.03	0.04 ± 0.06	0.09 ± 0.09	0.06 ± 0.09	0.07 ± 0.05	0.09 ± 0.13	0.10 ± 0.10	0.09 ± 0.09
20:4n-3	0.70 ± 0.17	0.30 ± 0.21	0.67 ± 0.19	0.35 ± 0.19	0.70 ± 0.20	0.45 ± 0.20	0.59 ± 0.11	0.46 ± 0.20
20:5n-3	3.62 ± 1.27	1.39 ± 1.21	3.48 ± 1.15	1.58 ± 1.41	3.54 ± 1.18	2.13 ± 1.31	3.18 ± 0.86	2.43 ± 1.40
21:5n-3	0.27 ± 0.06	0.08 ± 0.09	0.25 ± 0.08	0.08 ± 0.07	0.23 ± 0.08	0.10 ± 0.06	0.21 ± 0.12	0.12 ± 0.08
22:4n-6	0.06 ± 0.05	0.01 ± 0.02	0.05 ± 0.08	0.01 ± 0.02	0.05 ± 0.09	0.03 ± 0.08	0.05 ± 0.08	0.05 ± 0.10
22:5n-6	0.15 ± 0.09	0.02 ± 0.02	0.15 ± 0.11	0.02 ± 0.03	0.12 ± 0.07	0.08 ± 0.12	0.09 ± 0.05	0.03 ± 0.03
22:4n-3	0.10 ± 0.03	0.01 ± 0.02	0.08 ± 0.02	0.02 ± 0.03	0.08 ± 0.03	0.03 ± 0.04	0.06 ± 0.04	0.10 ± 0.19
22:5n-3	2.92 ± 0.79	0.48 ± 0.44	2.66 ± 1.05	0.60 ± 0.78	2.43 ± 0.97	0.82 ± 0.56	1.63 ± 0.45	0.96 ± 0.63
22:6n-3	6.76 ± 1.58	1.01 ± 1.02	6.35 ± 2.30	1.40 ± 2.15	5.77 ± 2.17	1.80 ± 1.43	4.03 ± 0.94	2.39 ± 1.65
Total poly unsaturates	20.36 ± 3.50	7.27 ± 3.73	19.38 ± 4.92	8.18 ± 5.21	18.73 ± 4.71	10.23 ± 4.11	15.23 ± 2.28	11.33 ± 4.30
Total n-3	16.22 ± 3.31	4.27 ± 3.28	15.31 ± 4.53	5.07 ± 4.77	14.62 ± 4.44	6.70 ± 3.81	11.32 ± 2.16	7.81 ± 4.07
Total n-6	2.30 ± 0.22	1.56 ± 0.27	2.23 ± 0.36	1.64 ± 0.32	2.27 ± 0.35	1.92 ± 0.24	2.18 ± 0.38	1.91 ± 0.25
n-3/n-6	7.03 ± 1.20	2.59 ± 1.81	6.84 ± 1.72	2.85 ± 2.20	6.37 ± 1.68	3.45 ± 1.96	5.38 ± 1.65	4.14 ± 2.26
Others and unknowns	1.16 ± 1.41	1.08 ± 0.20	0.86 ± 0.16	1.06 ± 0.25	0.86 ± 0.12	1.10 ± 0.19	0.91 ± 0.17	1.14 ± 0.22

Table 2 Comparison of levels of fatty acids in the blubber at four body sites of the male harbour porpoise, based on Kruskal-Wallis tests with alpha = 0.01. Inner and outer blubber layers were compared separately. Body sites with significantly higher or lower levels of specific fatty acid components than the other sites are listed in the inner and outer blubber columns (*II Dor* II Dorsal site, *II Ven* II Ventral site, *III Dor* III Dorsal site, *Caud* Caudal Dorsal site). ns = no significant differences among the four sites

Fatty acid	Inner blubber	Outer blubber
iso 5:0	Caud higher	ns
14:0	ns	Caud and III Dor lower
14:1n-5	Caud higher	ns
16:0	Caud lower	ns
16:1n-7	Caud higher	ns
18:0	Caud lower	ns
18:1n-11	ns	ns
18:1n-9	ns	ns
18:2n-6	ns	Caud and III Dor higher
20:1n-11	ns	Caud and III Dor higher
20:1n-9	Caud lower	ns
20:4n-6	ns	Caud and III Dor higher
20:5n-3	ns	ns
22:1n-11	Caud lower	ns
22:5n-3	Caud lower	ns
22:6n-3	Caud lower	Caud and III Dor higher
n-3/n-6	Caud lower	ns
Total PUFA	Caud lower	Caud and III Dor higher

Table 3 Sites of significant differences in relative fatty acid concentration between inner and outer blubber layers of male harbour porpoises; based on Mann-Whitney tests with alpha = 0.01. The layer in which the relative concentration of each fatty acid was highest is given in the Higher Level column; ns = not significant. Sites are labeled as follows: II Dorsal: II Dor; II Ventral: II Ven; III Dorsal: III Dor; Caudal dorsal: Caud. For sampling sites see Fig. 1

Fatty acid	Higher Level	Sites at which difference is significant
iso 5:0	outer	II Dor, II Ven, III Dor, Caud
14:0	ns	ns
14:1n-5	outer	II Dor, II Ven, III Dor
16:0	outer	Caud
16:1n-7	outer	II Dor, II Ven, III Dor
18:0	inner	II Dor, II Ven, III Dor
18:1n-11	inner	II Dor, II Ven
18:1n-9	ns	ns
18:2n-6	inner	II Dor, II Ven, III Dor
20:1n-11	inner	II Dor, II Ven, III Dor, Caud
20:1n-9	inner	II Dor, II Ven, III Dor, Caud
20:4n-6	inner	II Dor, II Ven, III Dor
20:5n-3	inner	II Dor, II Ven, III Dor
22:1n-11	inner	II Dor, II Ven, III Dor, Caud
22:5n-3	inner	II Dor, II Ven, III Dor, Caud
22:6n-3	inner	II Dor, II Ven, III Dor, Caud
n-3/n-6	inner	II Dor, II Ven, III Dor
Total PUFA	inner	II Dor, II Ven, III Dor, Caud

with age. In 10 of the 14 FA examined the degree of stratification was significantly greater in the blubber of older animals (Fig. 2). No significant differences in the degree of stratification within the blubber layer at the II Dor between older and younger animals were detected for levels of 16:1n-7, 18:1n-11, 18:2n-6 or 20:4n-6. Age-related differences in the degree of stratification were most pronounced in levels of iso 5:0, 14:1n-5, 20:1n-9, 22:1n-11, 22:5n-3 and 22:6n-3. In the older animals, mean levels of these components in the inner versus outer blubber varied by factors of 9.7, 8.4, 7.0, 9.0, 34 and 38, respectively, while in the younger porpoises, these factors were 2.5, 2.6, 2.0, 2.5, 3.0 and 3.3, respectively. The mean level of iso 5:0 in the outer blubber of older porpoises (17.5 ± 3.9%) was 2.9 times higher than that of younger animals (6.1 ± 1.4%),

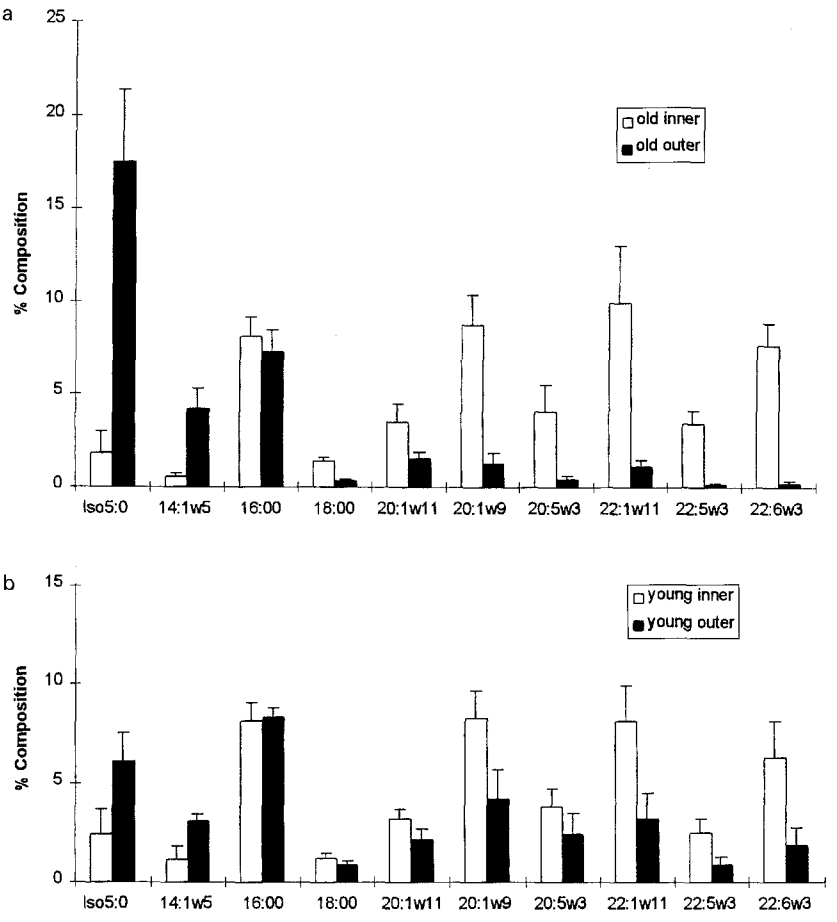

Fig. 2a, b Percent composition of 10 fatty acids in the inner and outer blubber layers of harbour porpoises at the II Dorsal site. Standard deviations are indicated by error bars. (**a**) values for porpoises >4 years of age ($n = 7$). (**b**) values for all calves and yearlings ($n = 9$). For all 10 fatty acids, the degree of stratification (the difference between levels in the inner and outer blubber layers) is significantly greater in the older animals than in the younger sample ($P < 0.05$)

whereas FA of 20 carbons in length or longer generally occurred in very low levels in the outer blubber of the older porpoises. Similar tests showed that levels of these FA followed the same pattern at the II Ven.

Isovaleric acid

In all outer samples isovaleric acid was one of the three most abundant FA, and in a few samples (mainly mature animals) it was the most prevalent FA. The highest relative amounts of this acid occurred in the outer layer at all sites. Isovaleric acid also exhibited the largest range of relative concentrations by layer (Table 1), with the most extensive range observed at the II Ven. The amounts of isovaleric acid in the inner layer of this site did not vary greatly among individuals, and were not correlated with age (Fig. 3a). With one exception, all II Ven inner samples contained less than 7%. However, relative quantities in the outer layer of the II Ven were highly variable and were positively correlated with age ($r^2 = 0.764$, $P < 0.0001$) (Fig. 3b). Mature and immature male porpoises could be distinguished from each other by the percentage of isovaleric acid in the II Ven outer sample: mature porpoises ($n = 8$) had at least 10% isovaleric acid at this spot while immature porpoise ($n = 9$) and calves ($n = 2$) had less than 10%, with the exception of one 2-year-old with an isovaleric acid content of 16%. Isovaleric acid levels at the II Dor showed similar trends as those found at the II Ven.

Total PUFA and the n-3/n-6 ratio

The highest levels of both total PUFA and the n-3/n-6 ratio were observed in the inner blubber of the II Dor (Table 1). In the outer blubber of this site, levels of total PUFA decreased significantly with age, perhaps non-linearly ($r^2 = 0.444$, $P = 0.002$), whereas levels in the inner blubber increased with age but this relationship was not as strong ($r^2 = 0.208$, $P = 0.049$; Fig. 4a). The value of the n-3/n-6 ratio also decreased with age in the outer blubber ($r^2 = 0.403$, $P = 0.004$; Fig. 4b), while the ratio increased with age in the inner blubber ($r^2 = 0.340$, $P = 0.009$). Like total PUFA, the relationship in the outer blubber layer for n-3/n-6 was stronger than in the inner layer and was likely non-linear (Fig. 4b). Similar patterns of age-related increases in

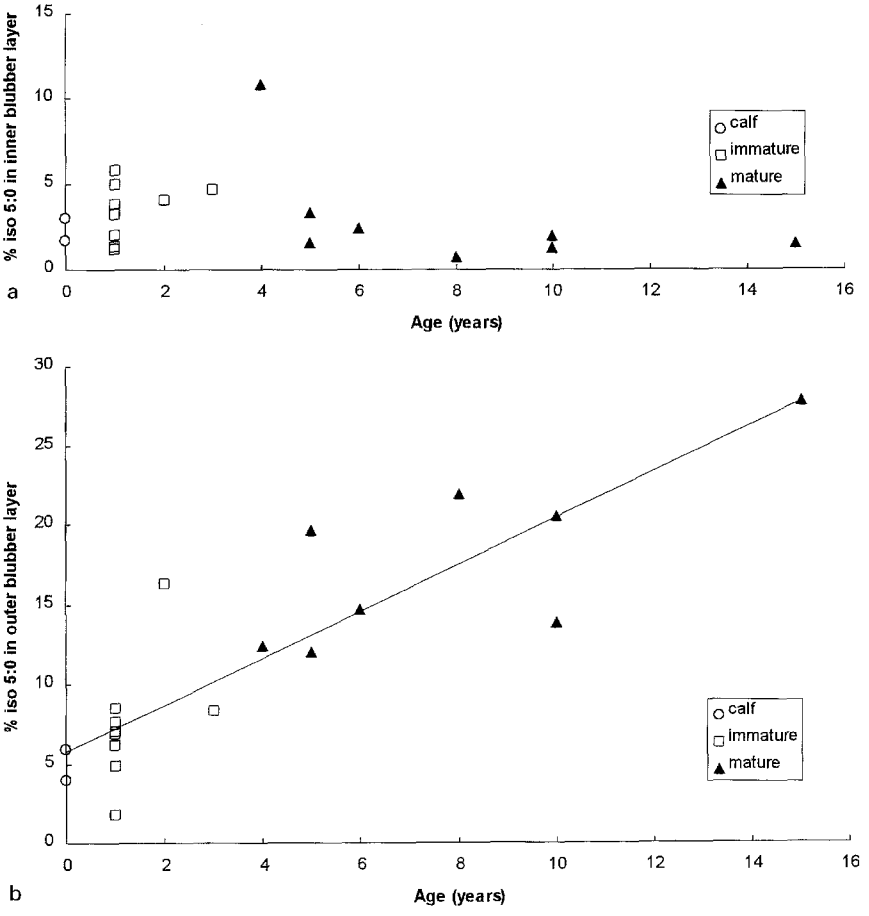

Fig. 3a, b Relationship between the levels (percent) of isovaleric acid in the inner and outer blubber layers of the II Ventral site of 19 male harbour porpoises, and the age of each porpoise. Symbols indicate reproductive status: calves ($n = 2$), immature ($n = 9$) and mature ($n = 8$) males. (**a**) inner blubber ($r^2 = 0.072$, ns); (**b**) outer blubber (isovaleric acid level (%) = 5.790 + 1.477*age, $r^2 = 0.764$, $P < 0.0001$)

levels of total PUFA and n-3/n-6 in the inner blubber and decreases of these levels in the outer blubber were also observed at the other three body sites.

Discussion

Blubber fatty acids of the harbour porpoise

The blubber of harbour porpoises was found to contain the same array of FA present in other marine mammals, including pinnipeds (West et al. 1979; Käkelä et al. 1993; Iverson et al. 1995), mysticetes (Bottino 1978; Lockyer et al. 1984) and other odontocetes (Tsuyuki and Itoh 1969, 1972; Ackman et al. 1971, 1975a), with the primary exception of the presence and high levels of isovaleric acid (Table 1). Our results are consistent with the early observations of harbour porpoise blubber (Lovern 1934), where saturated 5:0, 12:0, 14:0, 16:0, 18:0, and unsaturated total C14, C16, C18, C20, and C22 were quantified. Lovern, however, examined only one porpoise, a pregnant female, and the analytical techniques used were less sophisticated, so it is difficult to compare these results further.

The primary prey species of Bay of Fundy harbour porpoises in the summer is Atlantic herring *Clupea harengus*, followed by silver hake *Merluccius bilinearis* and Atlantic cod *Gadus morhua* (Recchia and Read 1989). There is evidence that the diet of harbour porpoises varies seasonally, becoming more dominated by small mesopelagic fishes in the fall and winter (Read et al. 1994). Levels of the major FA components and n-3/n-6 ratios in adult porpoise blubber appear to reflect a varied diet of marine teleosts (Ackman 1980). The FA composition of marine mammal blubber may directly resemble that of its diet (Iverson 1993; Iverson et al. 1995), however, we did not examine this question specifically. In addition to the need to obtain representative prey samples for analysis, it is clear from the present study that the issue of blubber stratification and unusual biosynthesis in some layers must be considered in attempting to trace dietary FA in the harbour porpoise.

Isovaleric acid and 14:1n-5 are two unusual FA that appeared in relatively high quantities in harbour porpoise blubber (Table 1); the presence and possible functions of isovaleric acid in relation to age are discussed separately. The FA 14:1n-5 is rarely observed at appreciable levels (>2%) in other marine mammals

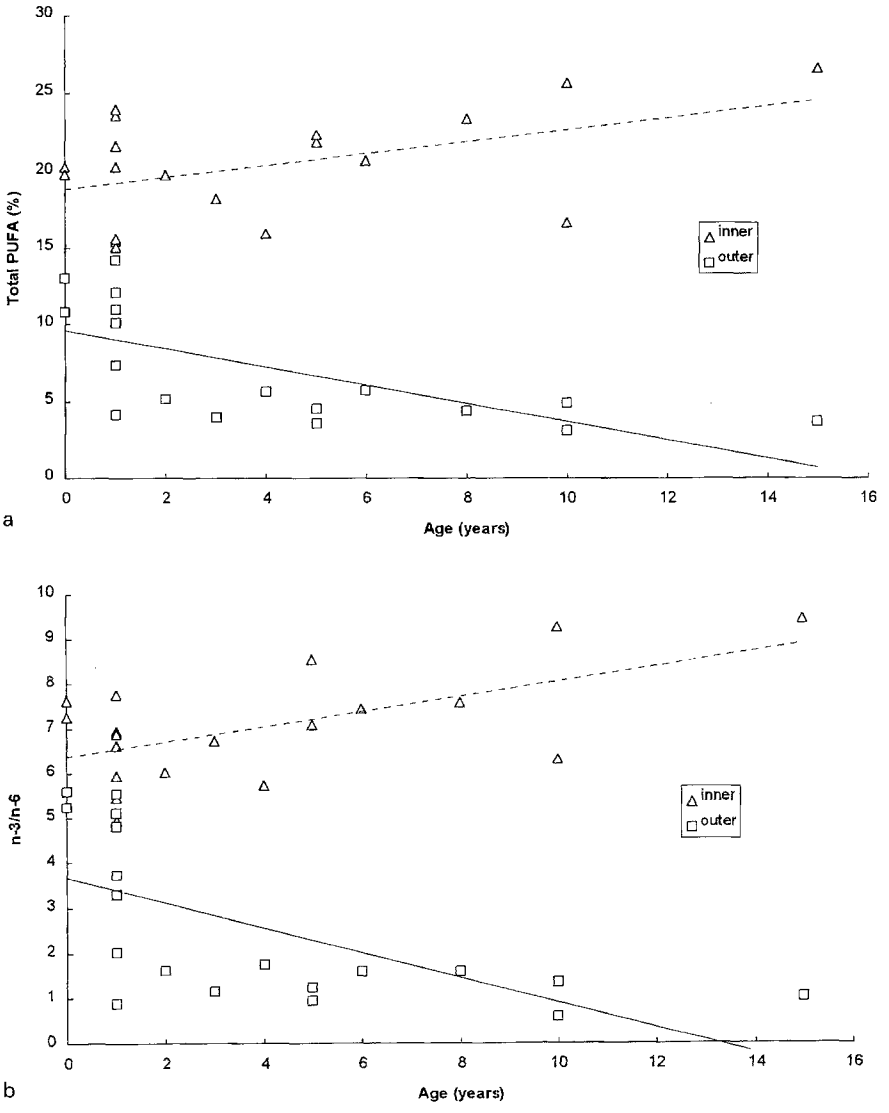

Fig. 4a, b Relationship between (**a**) the levels of total PUFA and (**b**) the n-3/n-6 ratio in the inner and outer blubber layers of the II Dorsal site of 19 male harbour porpoises, and the age of each porpoise. (**a**) Outer blubber ($r^2 = 0.444$, $P = 0.002$); inner blubber ($r^2 = 0.209$, $P = 0.049$). (**b**) Outer blubber ($r^2 = 0.403$, $P = 0.0004$); inner blubber ($r^2 = 0.340$, $P = 0.009$)

(Ackman and Lamothe 1989; Iverson et al. 1995), although high levels (5%) have been reported in the blubber of fetal or newborn phocid seals (Iverson et al. 1995). In harbour porpoises, percentages of 14:1n-5 in blubber ranged from 0.71 to 3.10% in calves, from 0.44 to 4.11% in immature males, and from 0.37 to 6.17% in mature males. Observations at the high end of the ranges were all from the outer layer. The high levels of 14:1n-5, together with high levels of 16:1n-7, may have been the result of increased activity in the outer layer of the Δ9 desaturase system, which adds double bonds at the ninth carbon from the terminal carboxyl end of FA (Gurr and James 1975). High levels of 14:1n-5 were also found to be accompanied by high levels of 16:1n-7 in fetal seals, likely due to Δ9 desaturase activity (Iverson et al. 1995). Conversely, it is possible that 14:1n-5 is not readily mobilized, and accumulates in the less metabolically active outer layer. The significance of the high levels of this FA in harbour porpoises and other marine mammals remains unknown.

Body site

The blubber of emaciated harbour porpoises is thinner at all body sites except those posterior to the anus, which implies that porpoises can selectively mobilize fat from the anterior and thoracic/abdominal area (Koopman 1994). Nevertheless, the FA composition of all sites in the present study was generally uniform (Table 1). The exclusion of the caudal blubber as a source of stored energy, therefore, appears not to be related to its lipid composition and perhaps instead reflects the need to maintain a hydrodynamic shape in the caudal peduncle. Preliminary evidence (H. Koopman, unpublished data) suggests that blubber in the

caudal peduncle may have lower total lipid content than other body sites, perhaps making it less economical as an energy source. Mobilizing lipid uniformly from all other areas of the body would presumably be the best means of maintaining insulation while using lipid stores.

Variation in FA composition of blubber between different body sites has been reported in other cetaceans (Ackman and Lamothe 1989), but unfortunately most of these studies were limited to samples from only one or two individuals of various genders and ages, making it difficult to draw general conclusions. Many terrestrial mammals, especially ungulates, exhibit site-specific variation in the FA composition of stored fat (Garton et al. 1971; Ringberg et al. 1980); however, this usually refers to differences between internal and subcutaneous storage sites.

Our finding that the FA composition is uniform in anterior and mid-region body sites in the harbour porpoise has significance for potential future studies involving blubber biopsies of live porpoises. As long as the entire blubber core can be collected, representative samples can be obtained from virtually any site on the body, perhaps with the exception of the caudal peduncle. The II Dor (Fig. 1) would perhaps be the best position for consistency, as it exhibits the same variability as the II Ven, but is easier to locate using the dorsal fin as a landmark. While this technique can be performed safely on seals (Grahl-Nielsen and Mjaavatten 1991), it has not yet been attempted on harbour porpoises, and should be tested thoroughly prior to any investigation of the composition of the blubber of live animals.

Inner and outer layers

The relatively high levels of PUFA and other dietary FA found in the inner layer of harbour porpoise blubber suggest that it is more metabolically active than the layer adjacent to the skin. These findings are consistent with studies of mysticete whales, where the inner blubber layer is regarded as the site of deposition and mobilization of lipids, while the outer layer plays a relatively minor role in lipid metabolism (Lockyer et al. 1984; Aguilar and Borrell 1990). In the present study, FA such as 18:2n-6, 20:1n-9, 20:5n-3, 22:1n-11 and 22:6n-3, all of which are derived primarily from the diet (Iverson 1993), appear to be deposited initially in the inner blubber of the harbour porpoise (Table 1). However, whether the distribution of FA between the blubber layers of mysticetes mirrors the patterns found in the harbour porpoise is unclear. In fin whales, for example, higher levels of 20:1 and 22:1 were reported in the outer layer in a study by Ackman et al. (1965), while Lockyer et al. (1984) documented higher levels of 20:1 in the inner layer, as we found in our study. Such results may relate to differences in sampling methods, collection, analytical techniques, or to individual variation in stratification.

There are several possible explanations for the stratification observed in harbour porpoise blubber. Increased biosynthesis or deposition of 14:1 and 16:1 may occur in the outer layer, accounting for the higher levels of these FA. Alternatively, these medium chain FA may accumulate in the outer layer because they are not readily mobilized. In addition, if dietary FA are deposited initially in the inner blubber, then these will dilute synthesized or stored FA in the inner layer. If the inner layer is in fact the site of deposition and selective mobilization of FA, then the latter alternative is the most likely.

The difference in the degree of stratification between the youngest and oldest porpoises is striking (Fig. 2), particularly the virtual absence of PUFA and prevalence of isovaleric acid (Fig. 3) in the outer blubber layer of the older males. PUFA are dietary in origin and some are important precursors of other FA and prostaglandins (Gurr and James 1975). The scarcity of PUFA, and in particular of the n-3 PUFA so prevalent in the marine diet, not only supports the idea that the outer layer is less active, but also suggests that there is some transition during the life of the animal. The relationships between age and levels of total PUFA (Fig. 4a) and n-3/n-6 ratio (Fig. 4b) provide further evidence that stratification in the blubber layer increases with age and that the outer layer becomes increasingly less important in metabolism.

The pattern of stratification also provides some insight into the overall metabolism of lipids in harbour porpoises. The finding that the degree of stratification increases with age (Fig. 2) suggests that male harbour porpoises may experience some depletion and replenishment of their blubber lipid stores, perhaps in an annual cycle. Such a cycle could be linked to a number of biological or environmental events: (1) an increased energy requirement associated with seasonality in testicular activity (Fisher and Harrison 1970; Gaskin et al. 1984); (2) the mobilization of stored lipid during the mating season, when males may spend less time foraging and expend more energy trying to mate with as many females as possible (Gaskin 1992); or (3) the need to mobilize stored energy in times of limited prey availability (e.g., during winter months). Evidence from harbour porpoises sampled in the United Kingdom suggests that blubber lipid content does not vary seasonally (Lockyer, in press), but the relationship between lipid levels and FA composition of porpoise blubber is not known. Samples would have to be collected over the entire year to establish patterns of lipid metabolism in the blubber of the harbour porpoise. Unfortunately, as most porpoise carcasses from the Bay of Fundy and Gulf of Maine are obtained from the seasonal gill net fishery, the opportunity to collect and analyze samples is presently restricted to late summer, fall and early winter. It is also important to recognize

that the composition of the blubber could be more complex than our data suggest; there may in fact be a continuous gradient in composition from the deep to the superficial blubber that we were unable to detect from our sampling methods.

Isovaleric acid

The presence of isovaleric acid has previously been reported in harbour porpoise blubber (Lovern 1934; Litchfield et al. 1975), and also in the blubber of the beluga *Delphinapterus leucas* (9%), Dall's porpoise *Phocoenoides dalli* (15%), white beaked dolphin *Lagenorhynchus albirostris* (22%), bottlenose dolphin (10%), and tucuxi (14%); it is absent from the blubber of beaked whales, sperm whales, and Amazon river dolphins *Inia geoffrensis* (Litchfield et al. 1975). The levels of isovaleric acid previously reported by others are lower than the 25% observed in some of the older harbour porpoises in this study.

Varanasi and Malins (1971) suggested that isovaleric acid has unique acoustical properties, which might account for its presence in melon and mandibular fats. Litchfield et al. (1975), however, noted that the absence of this FA in the acoustic tissues of some odontocetes suggests that it is not necessary for echolocation, but that phocoenids and delphinids may use isovaleric acid to focus sound. Other odontocete species were presumed to use different compounds for this purpose, such as wax esters that do not contain isovaleric acid. Unfortunately, neither group of authors offers a functional explanation for the presence of isovaleric acid in blubber. Possibly isovaleric acid has been overlooked or underestimated previously, because of difficulties in recovering short-chain volatile FA. If care is not taken to butylate these compounds and to run the GC at a lower temperature regime, they become buried in the solvent peak and assumed to be absent.

While the role of isovaleric acid in the physiology of harbour porpoise blubber is not known, its relative levels are higher in outer blubber and the degree of stratification increases with age (Fig. 3). It is unlikely that isovaleric acid is stored preferentially as a source of potential expendable energy, given that the outer layer is probably not a major site of lipid mobilization. Tissue cultures of the subcutaneous adipose tissue of striped dolphins (*Stenella coeruleoalba*) have the capacity to biosynthesize isovaleric acid from the amino acid leucine (Morii and Kaneda 1982). Porpoises could manufacture isovaleric acid to replace lipid that has been mobilized in times of limited prey availability, from either dietary leucine (i.e., fish muscle) or from the breakdown of internal muscle proteins. Unfortunately, the conversion of branched-chain amino acids to branched-chain FA has been studied only in tissue culture and the link between amino acids in the blood and the outer blubber layer has not been established. Furthermore, because leucine is an essential amino acid that might be limited during periods of low food supply, it would seem nutritionally uneconomical to convert leucine to a FA. Isovaleric acid might be used to maintain fluidity in the blubber layer closest to the cold environment, but that function could be carried out by long chain PUFA which also maintain fluidity under a wide range of temperatures (Enser 1984; CRC 1989; Pond et al. 1992).

Perhaps the simplest hypothesis for the stratification of isovaleric acid in harbour porpoise blubber is that it accumulates in the outer layer because it is preferentially avoided when lipid is mobilized. In contrast, PUFA are nutritionally important, and sequestering them in the less active outer layer would not be advantageous. The reasons why isovalerate would not be metabolized are unknown. Relatively high levels of isovaleric and other short-chain FA have been observed in the digestive tracts of ruminants, pigs, porcupines, rabbits, rats and humans (Bugault 1987), making it unlikely that isovaleric acid is toxic to mammals as suggested by Wretland (1957). The short-chain FA are produced by microbial activity in the gut, and can comprise 2 –72% of total basal metabolic requirements in the intestine of these mammals (Bugault 1987).

Increased levels of isovaleric acid in older animals suggest that porpoises may continually synthesize isovaleric acid throughout their lives, but the reason is unknown. The melon and jaw fats of the harbour porpoise, Dall's porpoise, and many delphinids contain $>50\%$ isovaleric acid (Litchfield and Greenberg 1974; Litchfield et al. 1975; H. Koopman, unpublished data), suggesting a possible requirement for isovaleric acid synthesis. Rates of isovaleric acid turnover in the acoustical tissues and the regulation of isovaleric acid biosynthesis need to be investigated to establish whether isovaleric acid storage in the blubber is merely a reflection of excess production in the acoustic tissues. Similarly, the presence of what appeared to be an isobutyric compound in porpoises with high levels of isovaleric acid (Table 1), may indicate either a byproduct during synthesis or breakdown of isovalerate.

Again, it must be emphasized that the source and role of isovaleric acid in harbour porpoises and other odontocetes remain unknown. Ingested short-chain FA are completely broken down during digestion (Pethick et al. 1984; Bugault 1987), so even if isovaleric acid was present in the diet it would not be deposited in adipose tissue. Because Litchfield et al. (1975) compared the presence of isovaleric acid among odontocetes based on single samples, the general conclusions drawn by these authors may not be representative of the true distribution of isovaleric acid in toothed whales; for example, the authors recognized levels of 0.8% C5 acids in several ziphiid, physeterid and platanistid species from several other studies, but dismissed them as "too small to be of acoustic significance". It appears

therefore, that the capacity of isovaleric biosynthesis exists in a wider range of odontocete species than is generally accepted.

From the presence and variability of layers in the blubber with apparently different metabolic roles, it appears there is some range and flexibility in the way this tissue is utilized: a healthy porpoise can mobilize some of its blubber and then replenish it. Male harbour porpoises probably deplete and replace a portion of their blubber lipid often, perhaps regularly. Harbour porpoises do not have unusually high metabolic rates (Yasui and Gaskin 1986) as suggested by Kanwisher and Sundnes (1965), and are adequately insulated against their environment (Worthy and Edwards 1990). Yet during periods of limited food availability when it becomes necessary to draw on lipid stores, porpoises may be vulnerable to cold, especially if food remains scarce and the blubber is drawn on heavily. The extent to which the blubber can be mobilized, however, is limited by thermoregulatory and hydrodynamic considerations (Koopman 1994).

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References

Ackman RG (1980) Fish lipids, part 1. In: Connell JJ (ed) Advances in fish science and technology, Fishing News Books, Surrey, England pp 86–103

Ackman RG (1991) Application of gas-liquid chromatography to lipid separation and analysis: qualitative and quantitative analysis. In: Perkins EG (ed) Analyses of fats, oils and lipoproteins, American Oil Chemists' Society, Champaign, Illinois pp 270–300

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, Eaton CA, Litchfield C (1971) Composition of wax esters, triglycerides and diacyl glyceryl ethers in the jaw and blubber fats of the Amazon river dolphin (*Inia geoffrensis*). Lipids 6: 69–77

Ackman RG, Eaton CA, Kinneman J, Litchfield C (1975a) Lipids of freshwater dolphin *Sotalia fluviatilis*: comparison of odontocete bioacoustic lipids and habitat. Lipids 10: 44–49

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

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

Ackman RG, Lamothe F (1989) Marine mammals. In: Ackman RG (ed) Marine biogenic lipids, fats and oils. Volume II: distribution of marine lipids, CRC Press, Boca Raton, pp 179–381

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

Bjorge A, Hohn AA, Lockyer C, Schweder T Summary report from the harbour porpoise age determination workshop, Oslo, 21–23 May 1990. Rep Int Whal Commn, Special Issue (In press)

Bottino NR (1978) Lipids of the Antarctic sei whale, *Balaenoptera borealis*. Lipids 13: 18–23

Bugault M (1987) Occurrence, absorption and metabolism of short chain fatty acids in the digestive tract of mammals. Comp Biochem Physiol B 86: 439–472

CRC (1989) Handbook of chemistry and physics. 69th edn. Weast RC (ed) CRC Press, Boca Raton

Enser M (1984) The chemistry, biochemistry and nutritional importance of animal fats. In: Wiseman J (ed) Fats in animal nutrition. Butterworths, London, pp 23–51

Fisher HD, Harrison RJ (1970) Reproduction in the common porpoise (*Phocoena phocoena*) of the north Atlantic. J Zool (London) 161: 471–486

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

Garton GA, Duncan WRH, McEwan EH(1971) Composition of adipose tissue triglycerides of the elk (*Cervus canadensis*), caribou (*Rangifer tarandus groenlandicus*), moose (*Alces alces*), and white-tailed deer (*Odocoileus virginianus*). Can J Zool 49: 1159–1162

Gaskin DE (1992) Status of the harbour porpoise, *Phocoena phocoena*, in Canada. Can Field-Nat 106: 36–54

Gaskin DE, Smith GJD, Watson AP, Yasui WY, Yurick DB(1984) Reproduction in the porpoises (Phocoenidae): implications for management. Rep Int Whaling Comm (Special Issue 6): 135–148

Geraci JR, Lounsbury VJ (1993) Marine mammals ashore: a field guide for strandings. Texas A&M Sea Grant Publ, Galveston

Grahl-Neilsen O, Mjaavatten O (1991) Dietary influence on fatty acid composition of blubber fat of seals as determined by biopsy: a multivariate approach. Mar Biol 110: 59–64

Gurr MI, James AT (1975) Lipid biochemistry: an introduction. Chapman and Hall, London

Hohn AA, Chivers SJ, Barlow J (1985) Reproductive maturity and seasonality of male spotted dolphins, *Stenella attenuata*, in the eastern tropical Pacific. Mar Mammal Sci 1: 273–293

Iverson SJ (1988) Composition, intake, and gastric digestion of milk lipids in pinnipeds. Ph. D. Thesis, University of Maryland, College Park, USA

Iverson SJ (1993) Milk secretion in marine mammals in relation to foraging: can milk fatty acids predict diet? Symp Zool Soc Lond 66: 263–291

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 165, 1–12 (1995)

Käkelä R, Hyvärinen H, Vainiotalo P (1993) Fatty acid composition in liver and blubber of the saimaa ringed seal (*Phoca hispida saimensis*) compared with that of the ringed seal (*Phoca hispida botnica*) and grey seal (*Halichoerus grypus*) from the Baltic. Comp Biochem Physiol 105 B: 553–565

Kanwisher J, Sundnes G (1965) Physiology of a small cetacean. Essays Mar Physiol 48: 45–53

Koopman HN (1994) Topographical distribution and fatty acid composition of blubber in the harbour porpoise, *Phocoena*

phocoena. M. Sc. Thesis, Department of Zoology, University of Guelph, Guelph, Ontario, Canada
Litchfield C, Greenberg AJ (1974) Comparative lipid patterns in the melon fats of dolphins, porpoises and toothed whales. Comp Biochem Physiol 47 B: 401–407
Litchfield C, Greenberg AJ, Caldwell DK, Caldwell MC, Sipos JC, Ackman RG (1975) Comparative lipid patterns in acoustical and nonacoustical fatty tissues of dolphins, porpoises and toothed whales. Comp Biochem Physiol 50 B: 591–597
Lockyer C, Aspects of the morphology, body fat condition and biology of the harbour porpoise, *Phocoena phocoena*, in British waters. Rep Int Whaling Comm (Special Issue 16) (in press)
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
Lovern JA (1934) Liver fat metabolism in fishes. III. Selective formation of fat deposits. Biochem J 28: 394–402
Morii H, Kaneda T (1982) Biosynthesis of branched-chain fatty acids from branched-chain amino acids in subcutaneous tissue of the marine little toothed whale, *Stenella caeruleoalba*. Comp Biochem Physiol 71 B: 357–365
Pethick DW, Bell AW, Annison EF (1984) Fats as energy sources in animal tissues. In: Wiseman J (ed) Fats in animal nutrition. Butterworths, London, pp 225–248
Pond CM (1978) Morphological aspects and the ecological and mechanical consequences of fat deposition in wild vertebrates. Annu Rev Ecol Syst 9: 519–570
Pond CM, Mattacks CA, Colby RH, Ramsay MA (1992) The anatomy, chemical composition, and metabolism of adipose tissue in wild polar bears (*Ursus maritimus*). Can J Zool 70: 326–341
Read AJ (1990) Estimation of body condition in harbour porpoises, *Phocoena phocoena*. Can J Zool 68: 1962–1966
Read AJ, Hohn AA (1995) Life in the fast lane: the life history of harbour porpoises from the Gulf of Maine. Mar Mammal Sci 11: 423–440
Read AJ, Gaskin DE (1990) Changes in growth and reproduction of harbour porpoises, *Phocoena phocoena*, from the Bay of Fundy. Can J Fish Aquat Sci 47: 2158–2163
Read AJ, Craddock JE, Gannon D (1994) Life history of harbour porpoises and pilot whales taken in commercial fishing operations off the northeastern United States. NMFS Contract Report, NEFSC, Woods Hole, Mass, USA
Recchia CA, Read AJ (1989) Stomach contents of harbour porpoises, *Phocoena phocoena* (L.), from the Bay of Fundy. Can J Zool 67: 2140–2146
Ringberg T, Nilssen K, Strøm E (1980) Do Svalbard reindeer use their subcutaneous fat as insulation? In: Reimers E et al. (eds) Proceedings of the Second International Reindeer/Caribou Symposium. Direktoratet for Vilt og Fervannsfisk, Trondheim, Norway, pp 392–395
Ryg M, Smith TG, Oritsland NA (1988) Thermal significance of the topographical distribution of blubber in ringed seals (*Phoca hispida*). Can J Fish Aquat Sci 45: 985–992
Shantha NC, Napolitano GE (1992) Gas chromatography of fatty acids. J Chrom 624: 37–51
Shoda LKM, Worthy GAJ, Wade TL (1993) Lipid stratification and contaminant distribution in the blubber of bottlenose dolphins. Abstracts of the Tenth Biennial Conference on the Biology of Marine Mammals, Soc Mar Mammal Galveston, Texas, USA, p 99
Smith GJD, Gaskin DE (1974) The diet of harbor porpoises (*Phocoena phocoena* L.) in coastal waters of Eastern Canada, with special reference to the Bay of Fundy. Can J Zool 52: 777–782
Smith RJ, Read AJ (1992) Consumption of euphausiids by harbour porpoise (*Phocoena phocoena*) calves in the Bay of Fundy. Can J Zool 70: 1629–1632
Tsuyuki H, Itoh S (1969) Fatty acid composition of many toothed pilot whale oil. Sci Rep Whale Res Inst 21: 137–141
Tsuyuki H, Itoh S (1972) Fatty acid component of various blubber oil of Ganges river dolphin. Sci Rep Whale Res Inst 24: 117–125
Varanasi U, Malins DC (1971) Unique lipids of the porpoise (*Tursiops gilli*): differences in triacyl glycerols and wax esters of acoustic (mandibular canal and melon) and blubber tissues. Biochim Biophys Acta 231: 415–418
Wang JY (1993) Mitochondrial DNA analysis of the harbour porpoise, *Phocoena phocoena* (L.). M. Sc. Thesis, Department of Zoology, University of Guelph, Guelph, Ontario, Canada
West GC, Burns JJ, Modafferi M (1979) Fatty acid composition of Pacific walrus skin and blubber fats. Can J Zool 57: 1249–1255
Westgate AJ, Johnston DW (1995) Regional differences in organochlorine and heavy metal contaminants in Western North Atlantic harbour porpoises (*Phocoena phocoena*). Working paper SC/47/SM20 for the International Whaling Commission, Small Cetacean Sub-Committee, Dublin, 1995
Worthy GAJ, Edwards EF (1990) Morphometric and biochemical factors affecting heat loss in a small temperate cetacean (*Phocoena phocoena*) and a small tropical cetacean (*Stenella attenuata*). Physiol Zool 63:432–442
Wretland A (1957) The toxicity of low-molecular triglycerides. Acta Physiol Scand 40:338–343
Yasui WY, Gaskin DE (1986) Energy budget of a small cetacean, the harbour porpoise, *Phocoena phocoena* (L.). Ophelia 25:183–197

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