J Comp Physiol B (2003) 173: 247–261
DOI 10.1007/s00360-003-0329-9

ORIGINAL PAPER

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High concentrations of isovaleric acid in the fats of odontocetes: variation and patterns of accumulation in blubber vs. stability in the melon

Accepted: 6 January 2003 / Published online: 8 March 2003

Abstract Isovaleric acid (iso5:0) is an unusual fatty acid that is important for echolocation and hearing in acoustic tissues of some odontocetes, but its functional significance in blubber is unknown. We examined patterns of accumulation of this compound in blubber in 30 species of odontocetes ($n = 299$). Iso5:0 concentrations in blubber varied with phylogeny, ontogeny and body topography. Iso5:0 accumulated in greater quantities in superficial/outer blubber than in deep/inner blubber. In the outer blubber of northern right whale and Hector's dolphins, iso5:0 accounted for one-third to one-half of all fatty acids. Total blubber burden of iso5:0 in harbour porpoises represented up to 15 times the amount deposited in the melon. The composition of the melon does not change during starvation in harbour porpoises, supporting the hypothesis that lipids in melon are conserved for a specific function. Some odontocetes continually deposit iso5:0 in blubber after levels in melon have reached asymptotic levels, suggesting independent control of iso5:0 synthesis and storage in these compartments. Dolphins and porpoises inhabiting cold waters possess higher concentrations of iso5:0 in their outer blubber layers than species from warmer regions. We propose that this relationship represents an adaptive secondary role for iso5:0 in maintaining blubber flexibility in cold environments.

Communicated by L.C.-H. Wang

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Keywords Isovaleric acid · Blubber · Odontocetes · Fatty acids · Harbour porpoise

Abbreviations iso5:0 isovaleric acid · GC gas chromatograph · GLC gas-liquid chromatography · IUPAC International Union of Pure and Applied Chemistry · IVA isovaleric academia · IVD isovaleryl-CoA dehydrogenase · mol% molar percentage of total fatty acids · PUFA polyunsaturated fatty acid · SE standard error · wt% weight percentage of total fatty acids

Introduction

Isovaleric acid (iso5:0) is an unusual fatty acid because of its structure, and mode of synthesis. Most fatty acids in nature are long, straight chains of even numbers of carbons (Stryer 1988), but iso5:0 is short (containing only five carbon atoms) and has a branched structure. Rather than following the typical pattern exhibited by most fatty acids, of synthesis via the sequential addition of two-carbon units in the cytoplasm (Stryer 1988), iso5:0 is produced in mitochondria (as iso5:0-coA) as one of the intermediate steps in the catabolism of leucine (Tanaka et al. 1966; Morii and Kaneda 1982). There is considerable evidence that iso5:0 can be extremely toxic to mammals. Early experiments with rats (Wretlind 1957) demonstrated that when circulated in the bloodstream, iso5:0 produced convulsions and death at very low doses. In humans, accumulation of iso5:0 in the blood leads to vomiting, convulsions, coma, and death, and may have direct deleterious effects on the function and metabolism of the central nervous system (Tanaka et al. 1966; Budd et al. 1967; Efron, 1967; Nyhan 1984). Consumption of plants that synthesise iso5:0 leads to "Jamaican vomiting sickness" (Malins et al. 1972b).

Despite its unusual structural and pharmacological characteristics, iso5:0 is a major constituent of the fatty tissues of some cetaceans. The first record of iso5:0 in marine mammal tissue was reported by Lovern (1934),

who detected this molecule in the blubber of a harbour porpoise (*Phocoena phocoena*). During the early 1970s, a series of studies conducted by Varanasi and Malins (1971, 1972) and Litchfield and colleagues (Litchfield et al. 1971, 1973, 1975; Litchfield and Greenberg 1974; Wedmid et al. 1973) documented the presence of iso5:0 in the blubber, melon (the fatty "forehead" tissue), and mandibular fat (fat pad lying inside each mandible) in specimens of a selected group of odontocete (toothed whale) species. These authors reported that iso5:0 occurred in appreciable levels in phocoenids (porpoises), delphinids (dolphins), and monodontids (belugas and narwhals), but not in the other odontocetes: the ziphiids (beaked whales), kogiids (pygmy and dwarf sperm whales), or physeterids (sperm whales). Iso5:0 has never been observed in the tissues of mysticetes (baleen whales), pinnipeds (seals, sea lions and walruses), or polar bears (Lockyer et al. 1984; West et al. 1979a, 1979b; Grompone et al. 1990; Pond et al. 1992; Iverson 1993; Borobia et al. 1995; Iverson et al. 1997).

The high concentrations of iso5:0 (ca. 40% of all fatty acids, by weight) in the acoustic fats of some odontocete specimens (Litchfield et al. 1971; Wedmid et al. 1973), combined with the unusual acoustic properties of this fatty acid (see Litchfield et al. 1973), have led to the hypothesis that it is important for modulating the propagation and reception of high frequency sound in these animals (see Malins and Varanasi 1975; Litchfield et al. 1979; Cranford et al. 1996). The melon and mandibular fat play important roles in echolocation and hearing in odontocetes. The melon acts to focus high frequency sound produced in the nasal passages, and the fat body in the lower jaw transmits received sounds to the ear (Wartzok and Ketten 1999; see Cranford 2000). It is generally accepted that the lipid composition of the melon is critical to its function, and that it represents a large, non-recoverable energy investment; lipids in the melon are not believed to be metabolised during starvation (see Cranford et al. 1996), although this hypothesis has not been tested empirically.

The proposed acoustic function of sound modulation for iso5:0 in melon and mandibular fats does not explain the presence of this compound in blubber. Blubber acts to insulate and streamline the body, reduce locomotory expense, adjust buoyancy, and store excess energy in the form of lipid (Pabst 1996; Ryg et al. 1988; Pabst et al. 1999). Its importance for marine mammals is widely recognised, but surprisingly little is known about the patterns of lipid acquisition, deposition and mobilisation in the blubber of most species. To date, the only comparative investigation of iso5:0 in odontocete blubber (18 species; Litchfield et al. 1975) reported relative proportions of triacylglycerol molecules containing one, two or no molecules of iso5:0, and many species were represented by only one specimen, or by commercially produced or rendered oils rather than fresh tissue. More recently, iso5:0 levels in the blubber of male harbour porpoises were shown to be stratified through the depth of the blubber, and to increase with age (Koopman et al. 1996). However, this is the only species in which patterns of iso5:0 deposition have been examined in detail and the significance of these findings remains unclear. The acute toxicity of circulating iso5:0 in other mammals (e.g. Wretlind 1957, Nyhan 1984) suggests that mobilising this fatty acid with other blubber lipids as a source of energy would be detrimental to the health of an individual. Thus the role, if any, of iso5:0 in odontocete blubber is unknown. The variation in iso5:0 levels found in porpoises suggests that more detailed examination in other species might permit inference into possible functions of this compound, and into general patterns of lipid deposition in odontocete blubber.

The main objectives of this study were: (1) to quantify iso5:0 in the blubber of as many odontocete species, and in as many individuals within species, as could be obtained, (2) to use these data to examine possible relationships between iso5:0 in blubber and the factors of age, body size, and thermal habitat, and (3) to use the harbour porpoise as a model to more thoroughly investigate iso5:0 burdens in tissues throughout the body and to assess age-related patterns of accumulations and effects of body condition on iso5:0 in blubber and melon. This study also permitted an update of the earlier work by Litchfield et al. (1975), allowing us to report iso5:0 levels in relative proportion to the other 70 fatty acids in the blubber, and as both weight percentage and molar percentage (i.e. proportion of total fatty acid molecules present), rather than just as fraction of triacylglycerol molecules containing iso5:0.

Materials and methods

Samples analysed

Blubber samples were obtained from 30 species of odontocetes ($n = 299$ individuals), representing all odontocete families except the river dolphins; samples from river dolphins were not available. Species, scientific names and sample sizes are given in Table 1. Samples were collected from the thoracic/abdominal regions of animals that had been killed incidentally in commercial fishing operations or had stranded. Where possible, sex, reproductive class and body size (standard length, cm) were recorded from each specimen. All blubber samples were frozen (−20 °C) prior to analysis. Blubber samples collected from specimens in poor body condition (see Kastelein and van Battum 1990), as well as poor quality samples (i.e. those with obvious rendering, dehydration or other post-mortem/storage effects), were excluded (except for selected juvenile harbour porpoises, see below).

For some of the comparisons, specimens were classified as "adults" (sexually mature animals) or "juveniles" (neonates, calves, sexually immature animals). Depending on the species, assignments to maturity classes were based on a number of definitions: standard length ranges for sexually mature or adult animals given in Leatherwood and Reeves (1983) and Doidge (1990); evidence of pregnancy or lactation; and examination of gonads for evidence of sexual maturity (see Read and Hohn 1995). If a maturity class could not be assigned with confidence, the specimen was labelled "unknown". Age estimates were obtained for 103 harbour porpoises (51 females, 52 males) from counts of dentinal growth layers in stained decalcified thin sections of teeth (Bjørge et al. 1995). Age estimates were also available for the belugas (S.J. Iverson and K.J. Frost, unpublished data).

Table 1 Levels of isovaleric acid (iso5:0) in the inner and outer thorax blubber of odontocetes. Values are mean percentage of total fatty acids present (*wt%*; mean ± SE). Scientific names used here follow Rice (1998). Sample sizes (*n*) are given after each common name. *ND* indicates not detected or level was <0.1%. For all species with $n \geq 8$, an *asterisk* indicates that the mean iso5:0 concentration in the outer blubber was significantly higher than that of the inner blubber; inner and outer blubber samples from species with $n < 8$ and kogiids/physeterids/ziphiids were not tested. Superscripts *A* (arctic/cold), *M* (mid/cosmopolitan) and *L* (tropical/warm) indicate thermal regime category for Fig. 4

Species	Common name	*n*	Inner iso 5:0 (wt%)		Outer iso 5:0 (wt%)	
			Mean	Range	Mean	Range
Delphinids						
Lissodelphis borealis	Northern right-whale dolphin[A]	8	4.6 ± 1.0	1.3–9.6	23.5 ± 2.5*	15.0–32.0
Cephalorhynchushectori	Hector's dolphin[A]	8	18.4 ± 2.8	4.2–28.8	35.2 ± 2.0*	24.4–40.4
Lagenorhynchus obscurus	Dusky dolphin	11	2.8 ± 0.2[a]	1.6–4.5[a]	-	-
Feresaattenuata	Pygmy killer whale[T]	3	0.1 ± 0.02	0.05–0.12	0.2 ± 0.01	0.16–0.20
Globicephala melas	Long-finned pilot whale[A]	9	0.8 ± 0.2	0.3–1.8	5.2 ± 0.7*	2.0–8.0
Globicephala macrorhynchus	Short-finned pilot whale	1	0.17	-	0.68	-
Grampusgriseus	Risso's dolphin[T]	15	0.9 ± 0.1	0.1–1.8	3.1 ± 0.4*	0.9–6.4
Orcinus orca	Killer whale[M]	1	2.5	-	3.8	-
Stenellaclymene	Clymene dolphin[T]	5	1.7 ± 0.8	0.2–3.7	3.9 ± 1.2	1.1–7.7
Stenellacoeruleoalba	Striped dolphin[W]	6	0.8 ± 0.3	0.1–2.2	2.5 ± 0.4	1.3–4.1
Stenellafrontalis	Atlantic spotted dolphin[T]	3	0.8 ± 0.4	0.3–1.7	2.2 ± 0.8	1.2–3.9
Delphinus delphis	Common dolphin[M]	13	1.4 ± 0.2	0.3–3.3	5.4 ± 0.7*	1.9–10.9
Tursiops truncatus	Bottlenose dolphin[M]	22	0.8 ± 0.1	0.2–1.5	2.7 ± 0.2*	0.8–4.6
Stenellaattenuata	Pantropical spotted dolphin[T]	1	1.4	-	3.0	-
Lagenodelphis hosei	Fraser's dolphin	1	0.25	-	0.78	-
Steno bredanensis	Rough-toothed dolphin[T]	4	0.2 ± 0.05	0.04–0.3	1.6 ± 0.6	0.4–2.6
Monodontids						
Delphinapterus leucas[B]	Beluga[A]	11	2.1 ± 0.6	0.4–6.8	9.5 ± 1.0*	5.7–17.0
Monodon monoceros	Narwhal[A]	8	3.5 ± 0.9	0.9–6.7	6.8 ± 0.5*	4.8–9.2
Phocoenids						
Phocoenoidesdalli	Dall's porpoise[A]	8	5.6 ± 0.8	2.3–9.6	10.4 ± 2.0*	6.0–23.2
Phocoenaphocoena	Harbour porpoise[A]	111	4.1 ± 0.3	0.9–15.9	8.9 ± 0.5*	3.1–24.0
Phocoenadioptrica	Spectacled porpoise	1	1.2	-	1.4	-
Phocoenasinus	Vaquita[T]	6	2.6 ± 0.5	1.3–4.3	4.6 ± 0.6	2.3–6.4
Phocoena spinipinnis	Burmeister's porpoise[M]	5	5.2 ± 1.0	2.9–8.2	8.0 ± 2.5	3.9–17.4
Neophocaenaphocaenoides	Finless porpoise[T]	4	3.4 ± 1.1	1.8–6.7	6.2 ± 1.6	2.8–9.4
Kogiids/Physeterids						
Kogia breviceps	Pygmy sperm whale	10	0.1 ± 0.1	0–0.4	0.1 ± 0.1	0–0.9
Kogia sima	Dwarf sperm whale	5	ND	-	0.05 ± 0.05	0–0.2
Physeter macrocephalus	Sperm whale	6	ND	-	ND	-
Ziphiids						
Mesoplodon bidens	Sowerby's beaked whale	9	0.03 ± 0.02	0–0.2	ND	-
Mesoplodon europaeus	Gervais' beaked whale	2	ND	-	ND	-
Ziphiuscavirostris	Cuvier's beaked whale	2	ND	-	0.08 ± 0.07	0–0.15

[a]Orientation of dusky dolphin samples could not be established to permit sampling of inner and outer layers, thus values represent the entire blubber layer

[b]Fatty acid data for belugas from S.J. Iverson and K.J. Frost (unpublished data)

Samples of additional tissues, besides thoracic blubber, were collected from harbour porpoises to allow a more detailed examination of iso5:0 distribution throughout the body. Samples of tailstock blubber ($n = 93$), melon ($n = 18$), mandibular fat ($n = 6$), epaxial muscle ($n = 13$), and liver ($n = 13$) samples were obtained from harbour porpoises in robust body condition. Tailstock blubber samples were taken on the dorso-lateral surface of the caudal peduncle, between the anus and the insertion of the flukes. Thorax blubber and melon samples were also collected from a harbour porpoise foetus, estimated to be one month pre-parturition. Data from the foetus were not included in any calculations of mean iso5:0 values in tissues, and were only used to compare with results obtained from a 3-month-old calf in the blubber burden analysis (see below). Prior to sample collection, all blubber (with epidermis attached) was carefully removed from each carcass (see McLellan et al. 2002) and weighed to the nearest gram; this total blubber mass was used in the iso5:0 body burden calculations below.

The blubber of eight harbour porpoises was sampled more extensively for estimation of the total blubber burden of iso5:0. A range of sex (four males, four female), size (standard length 102.5–160 cm), and reproductive (calf to sexually mature) classes were used to capture the potential range of iso5:0 burdens in the blubber of porpoises. From these animals, blubber samples were collected from a total of 12 body locations. Dorsal, lateral and ventral samples were taken at each of three body landmarks: axilla, anterior insertion of dorsal fin, and anus. In addition to these nine sites, three additional sites were analysed: (1) dorsally, directly behind the blowhole, (2) just lateral to the centre of the dorsal fin, and (3) the dorso-lateral aspect of the tailstock.

To examine whether a decline in body condition is associated with changes in the fatty acid composition of the melon, melon samples were collected from an additional five juvenile harbour porpoises in extremely poor body condition (all exhibited evidence of starvation as described by Stegall et al. 1999). Because concentrations of blubber fatty acids can be significantly affected by starvation (Koopman 2001), thorax blubber from the same individuals was also sampled to evaluate changes in iso5:0 levels in this tissue. These represent the only tissues from animals in poor body condition in the study.

All blubber samples were further subdivided into "inner" (adjacent to the muscle) and "outer" (adjacent to the epidermis) samples (see Fig. 1). For the outer blubber, the pigmented epi-

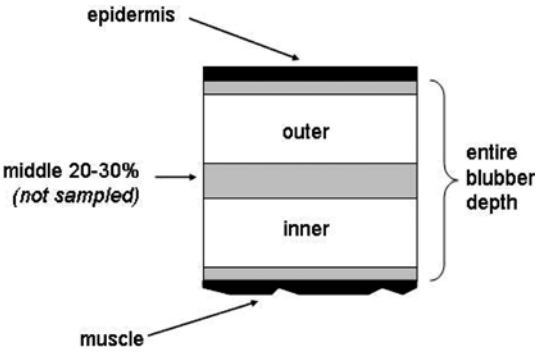

Fig. 1 Schematic of blubber sampling regime (inner/deep and outer/superficial layers) used for all species except the dusky dolphins. The middle of the blubber layer (comprising 20–30% of blubber depth) was not analysed to avoid any overlap between inner and outer layers

dermis and the first 2 mm of superficial blubber were removed before the sample was taken. The inner blubber sample was collected after the panniculus muscle and the first 2 mm of deep blubber were removed. To avoid any possible overlap between the inner and outer samples, the middle layer of the blubber, comprising 20–30% of the total blubber thickness, was not analysed (Fig. 1). The epidermis and inner connective tissue had been removed in the samples from dusky dolphins, preventing identification of orientation and thus prohibiting sampling of the inner and outer layers; thus values reported in this species represent the entire blubber layer.

Fatty acid analysis

Lipids were extracted from tissue samples using a modified Folch method (Folch et al. 1957) as described in Koopman et al. (1996). Lipids were then resuspended in hexane and transesterified for fatty acid analysis. Because short chain fatty acids esterified conventionally as methyl esters are too volatile to allow accurate quantification, fatty acid butyl esters were prepared instead using 10% boron trifluoride in butanol (Koopman et al. 1996). Fatty acid butyl esters were stored under nitrogen at −20 °C prior to injection into the gas chromatograph (GC).

Fatty acid samples were analysed in duplicate using temperature-programmed capillary gas-liquid chromatography (GLC) 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) and equipped with a flame ionisation detector. Temperature programs were set according to Koopman et al. (1996) to accurately quantify short- as well as long-chain fatty acids. Identification of fatty acids was made from known standard mixtures (Nu Chek Preparations, Elysian, Minn.), silver nitrate chromatography and GC mass spectrometry (Iverson et al. 2001). GC response factors for each fatty acid were set according to Iverson et al. (1997). All identified fatty acids were converted to weight percentage (wt%) of the total array of fatty acids and named according to the International Union of Pure and Applied Chemistry nomenclature of X:Yn-z, where X is the number of carbons, Y is the number of methylene-interrupted double bonds, and n-z denotes the position of the last double bond relative to the methyl terminus.

To express concentrations of iso5:0 as the relative number of molecules present, rather than its contribution to the total mass of all fatty acids in the sample, wt% values for each fatty acid were converted to molar percentage of total fatty acids (mol%) based on known molecular weights of all fatty acids present. Because iso5:0 concentrations in the blubber of the physeterids, kogiids, ziphiids and pygmy killer whales were either extremely low or non-detectable (see Table 1), they were excluded from these calculations. Also excluded were species represented by only one specimen.

Calculation of total burden of iso5:0 in harbour porpoise blubber

The total blubber burden of iso5:0 (g) was calculated for eight harbour porpoises (see above) using the levels of iso5:0 (wt%) in 24 samples (12 body locations; inner and outer blubber samples at each location) for each porpoise. Because the topographical distribution of blubber over the body is heterogeneous (Koopman 1998), the relative proportion of the total blubber mass represented by each body site was calculated. This was then corrected for epidermal mass (15%, H.N.Koopman unpublished data) and lipid content. Because we did not analyse the middle 20–30% of the blubber layer, we assumed that the total blubber mass was made up of equal weights of inner and outer layers (i.e. 50% of the total blubber mass was "inner" and 50% was "outer"). Since iso5:0 concentrations are graded from inner to outer, this assumption was considered the most valid estimate. The amount of iso5:0 present in the inner and outer layers of each of the 12 sites was calculated by multiplying the total amount of lipid (g) by the wt% of iso5:0 in each layer in that sample, and these values were then summed to generate the total inner and outer blubber iso5:0 burden. For each porpoise, the blubber iso5:0 burden was also expressed as the number of moles of iso5:0 present. The total moles of two other primarily endogenous fatty acids (14:1n-5 and 16:1n-7) were calculated using the same methods. These two fatty acids were present in high levels in the outer blubber and exhibited significant stratification between inner and outer layers, and were presented for comparison with iso5:0.

Data from the single foetus were used to estimate the iso5:0 burden deposited in the blubber in utero. The foetus was a 53-cm male weighing 2.4 kg; its blubber mass was 588 g. For this animal, we assumed that the thorax blubber of the foetus was representative of the composition of the entire blubber (i.e. we used only one body site to represent the entire animal), and that the inner and outer layers each comprised half of the total blubber mass. The total iso5:0 burden in each layer of the blubber was then estimated as above and summed.

Data analyses

All mean values are presented herein as mean ± SE, unless otherwise specified. In all the following analyses, wt% and mol% fatty acid data were arcsine-transformed prior to analysis (Steel and Torrie 1980). To look at overall patterns of iso5:0 in odontocete blubber, the concentrations of iso5:0 (wt%) in the inner vs. outer layers of thorax samples from all specimens combined ($n = 288$) were compared using a paired t-test. Paired t-tests were also conducted separately within several species; because of the wide variation in iso5:0 concentrations in the samples, both among and within species, only species with sample sizes of $n \geq 8$ were included in these tests (see Table 1). Because differences between inner and outer samples within a given species were assumed to be independent of differences in the other species, all t-tests were evaluated at $\alpha = 0.05$. Species with extremely low concentrations of iso5:0 (all kogiids, physeterids, ziphiids) were not included in tests of inner vs. outer concentrations as levels measured were often at the limits of detection. Stratification was defined as the absolute difference between the inner and outer iso5:0 wt% of an individual (outer wt%−inner wt%). Mean stratification values were calculated for all species that were represented by more than one specimen and had mean outer blubber iso5:0 levels > 1 wt%.

Linear regression analysis was used to examine the relationships between standard length and iso5:0 concentrations in blubber in the species represented by at least eight specimens. These relationships were examined separately in both the inner and outer layers, and were thus assessed for significance at $\alpha = 0.025$ (Bonferroni correction; Tabachnick and Fidell 1996). The adjusted R^2 (which corrects the original R^2 for artificial correlational inflations associated with small sample sizes; Tabachnick and Fidell 1996) was reported for significant relationships.

Relationships between age and iso5:0 concentrations were able to be examined in harbour porpoises. Because age and size co-vary, we wanted to determine which of these variables exhibited the strongest

relationship with iso5:0 concentrations. First, the correlation coefficients of both age and length with iso5:0 concentrations were compared, separately for male and female harbour porpoises in each of the inner and outer layers of the blubber of the thorax. Stepwise regression was then used to further assess whether the use of length as a predictor improved the strength of the relationships between iso5:0 and age, and was assessed for significance at $\alpha = 0.01$. Ages were also available for the belugas, and correlation coefficients of length and age with iso5:0 concentrations were examined separately for each blubber layer. Stepwise regression (at $\alpha = 0.025$) was then used to evaluate relationships between age, length and iso5:0 concentrations in the blubber as above. Finally, the relationships between age, length and iso5:0 levels in the melons of harbour porpoises was examined with correlation analysis and then stepwise regression at $\alpha = 0.05$. Because melon iso5:0 levels did not change with body condition (see results below), data from all porpoise melons (normal and emaciated) were included to increase the sample size to 22.

To determine whether a decline in body condition was associated with a change in the fatty acid composition of the melon, levels of iso5:0 in the melons of five emaciated, juvenile harbour porpoises were compared with corresponding values from 13 juvenile porpoises in robust body condition. To better assess the melon's composition in the two groups of porpoises, both lipid content and levels of its eight other major constituents (iso4:0, iso14:0, 14:0, iso15:0, iso16:0, 16:0, 16:1n-7 and 18:1n-9; together accounting for an average of 84% of total fatty acids present), as well as two representative polyunsaturated fatty acids (20:5n-3 and 22:6n-3), were compared. To illustrate relative changes in blubber composition associated with a decline in body condition, concentrations of these 11 fatty acids in the thorax blubber were also compared between the 13 normal and 5 emaciated porpoises using ANOVA with an α level of 0.01. These comparisons were limited to the inner layer of the blubber, as this is the blubber compartment in which composition is most affected by starvation (Koopman 2001).

Results

Presence and heterogeneity of iso5:0 in the thorax blubber of odontocetes

Approximately 70 fatty acids were routinely identified and quantified in the thorax blubber of odontocetes. Of these, iso5:0 was generally one of the more abundant fatty acids present in delphinids, monodontids and phocoenids (Table 1). In contrast, iso5:0 was not detected in the blubber of most of the kogiids, physeterids and ziphiids; mean iso5:0 concentrations in all species from these latter three families were < 0.1 wt% of total fatty acids. Hence we restricted our further analyses of iso5:0 patterns to the delphinids, monodontids and phocoenids.

All phocoenids (except the spectacled porpoise), as well as the monodontids, exhibited mean iso5:0 concentrations of > 2.0 wt% in inner blubber (Table 1). Conversely, delphinids (except northern right whale dolphins and Hector's dolphins) exhibited mean iso5:0 concentrations < 2 wt% in inner blubber. However, there was significant heterogeneity between layers in thorax blubber, with the outer layer containing higher concentrations of iso5:0 than the inner layer across all specimens (paired t-test, $P < 0.001$). In addition, iso5:0 concentrations were greater in outer compared to inner blubber in all ten species in which separate analyses could be performed (i.e. with $n = 8$, all $P < 0.04$; see Table 1). The highest mean concentrations of iso5:0 (> 8.0 wt%) in the outer thorax blubber were spread across all three families and found in northern right whale dolphins and Hector's dolphins (both delphinids), the beluga (monodontid) and Dall's porpoises and harbour porpoises (both phocoenids).

The degree to which iso5:0 levels were stratified in blubber varied both among and within families (Fig. 2). Mean stratification was highest in northern right whale dolphins (*Lissodelphis borealis*; 18.9 ± 2.6 wt%) and Hector's dolphins (*Cephalorhynchus hectori*; 16.8 ± 3.6 wt%), followed by belugas (*Delphinapterus leucas*; 7.3 ± 0.7 wt%), Dall's porpoises (*Phocoenides dalli*; 4.9 ± 1.5 wt%), and harbour porpoises (*Phocoena phocoena*; 4.8 ± 0.5 wt%). Adults and juveniles were identified in ten of the species. In six of these species (long finned pilot whales *Globicephala melas*, Risso's dolphins

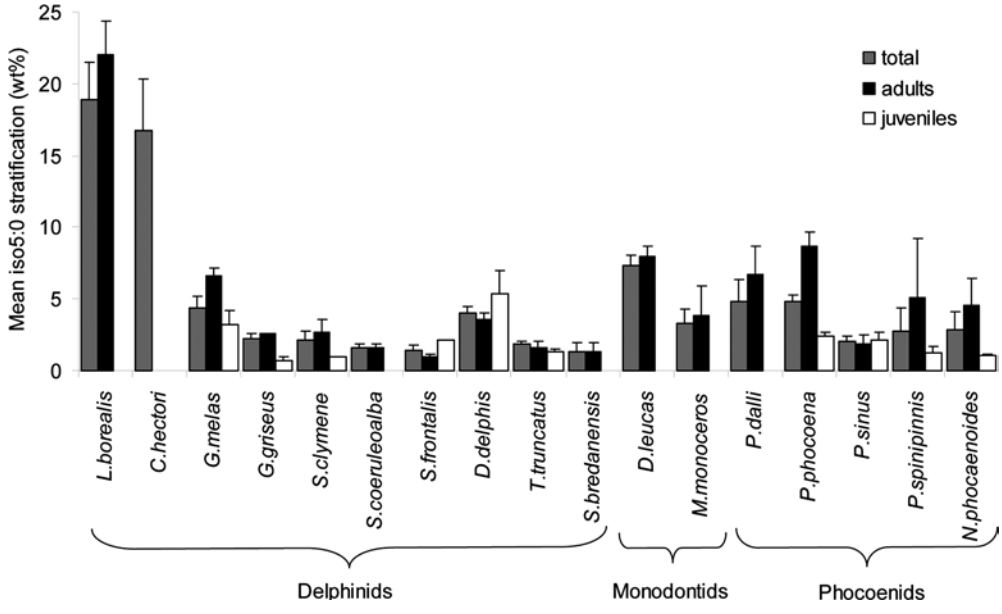

Fig. 2 Stratification of iso5:0 (mean ± SE) in the thorax blubber of odontocetes. Stratification was defined as the absolute difference (in wt%) between the outer and inner iso5:0 levels for individuals of each species. Mean stratification values are presented for all members of the species (*total*, *striped bars*), as well as for those that could be classified as either sexually mature (*adults*, *black bars*) or sexually immature (*juveniles*, *white bars*). See Table 1 for total sample sizes

Grampus griseus, clymene dolphins *Stenella clymene*, harbour porpoises *P. phocoena*, Burmeister's porpoises *Phocoena spinipinnis*, and finless porpoises *Neophocaena phocaenoides*), adults appeared to exhibit greater stratification of iso5:0 in their blubber than did juveniles (Fig. 2).

The expression of iso5:0 concentrations as mol%, rather than wt%, permits an assessment of the relative abundance of this low molecular weight compound as a fraction of total fatty acid molecules present in odontocete blubber (Table 2). For instance, in northern right whale dolphins and Hector's dolphins, iso5:0 accounted for over one-third to one-half of the fatty acids contained in outer blubber. In many phocoenids, as well as in some other delphinids and the monodontids, a mean of >10 mol%, and up to 18 mol%, of all fatty acids in outer blubber were represented by iso5:0 (Table 2). In the inner blubber, there was more variation among families in iso5:0 mol% values. For most delphinids, mean iso5:0 abundance in the inner layer was <5 mol%, except for 9 mol% in northern right whale dolphins and the exceptionally high 30 mol% in Hector's dolphins. However, in all species of phocoenids and in the narwhals, iso5:0 comprised ≥5 mol% of all fatty acid molecules in inner blubber.

Relationships between iso5:0 in blubber, and body size and age in odontocetes

Iso5:0 concentrations increased significantly ($P < 0.01$) with increasing body size in the outer blubber layers of long-finned pilot whales (adjusted $R^2 = 0.67$) and harbour porpoises (adjusted $R^2 = 0.50$). In the inner blubber, harbour porpoises also exhibited a significant increase ($P < 0.01$) in iso5:0 concentrations with increasing body size (adjusted $R^2 = 0.08$), however, larger bottlenose dolphins showed significant decreases in inner blubber iso5:0 (adjusted $R^2 = 0.42$) compared to smaller conspecifics ($P < 0.01$). There were no significant relationships between body size and iso5:0 levels in either the inner or outer blubber for the other seven species tested.

Obviously size co-varies with age up to some point (i.e., physical maturity) in odontocetes and thus, a more appropriate test would be that of age vs. blubber iso5:0 concentrations, using size as a co-variate. For two species, the belugas and harbour porpoises, we were able to obtain age data in addition to size to evaluate this. In all cases in which relationships were significant ($P < 0.01$), iso5:0 concentrations were more strongly positively correlated with age than with length, as indicated by higher correlation coefficients. In harbour porpoises, iso5:0 concentrations showed significant accumulations with age (all $P < 0.001$) in the inner layer of the thorax blubber of females (Fig. 3a), and in the outer layer of the thorax in both males and females (Fig. 3b). When size was used as a covariate, it no longer had any effect beyond its relationship with age in any of the layer/sex combinations (all $P > 0.04$). Thus only age was used as a predictor for iso5:0 concentrations; length was excluded. In the blubber of porpoises, relationships of iso5:0 with age were best described by power functions (in the form $y = axb$). In the outer blubber layer, the slope (b) value for males (0.45 ± 0.03) was greater than that of females (0.37 ± 0.03), suggesting that males accumulate iso5:0 at a faster rate than females (Fig. 3b) in this tissue.

Similarly, in the belugas there was a strong positive relationship between age and iso5:0 levels in the blubber, with size adding no additional effect ($P > 0.5$). In belu-

Table 2 Isovaleric acid (iso5:0) levels expressed as molar percentage of total fatty acids (*mol%*; mean ± SE) in the inner and outer thorax blubber of delphinids, monodontids and phocoenids. Sample sizes for each species are as in Table 1

Species	Inner iso 5:0 (mol%)		Outer iso 5:0 (mol%)	
	Mean	Range	Mean	Range
Delphinids				
Lissodelphis borealis	9.0 ± 1.9	2.7–17.9	37.3 ± 3.3	25.4–48.2
Cephalorhynchushectori	30.2 ± 4.1	8.5–43.9	50.6 ± 2.4	37.8–56.6
Lagenorhynchusobscurus	5.7 ± 0.4[a]	3.3–8.9[a]		
Globicephalamelas	1.7 ± 0.3	0.6–3.6	10.0 ± 1.3	4.0–15.0
Grampusgriseus	1.8 ± 0.2	0.3–3.8	6.2 ± 0.8	1.9–12.6
Stenellaclymene	3.5 ± 1.5	0.4–7.4	7.6 ± 2.3	2.2–14.6
S.coeruleoalba	1.7 ± 0.6	0.3–4.4	5.0 ± 0.8	2.6–8.0
S.frontalis	1.7 ± 0.9	0.6–3.5	4.5 ± 1.5	2.5–7.5
Delphinus delphis	2.9 ± 0.5	0.7–6.8	10.4 ± 1.2	3.9–19.4
Tursiops truncatus	1.7 ± 0.2	0.3–3.0	5.3 ± 0.5	1.6–9.0
Steno bredanensis	0.4 ± 0.1	0.1–0.6	3.2 ± 1.2	0.8–5.3
Monodontids				
Delphinapterus leucas[b]	4.2 ± 1.1	0.9–13.1	17.4 ± 1.7	11.2–29.1
Monodon monoceros	6.9 ± 1.6	1.9–12.8	13.0 ± 0.8	9.5–17.1
Phocoenids				
Phocoenoides dalli	10.7 ± 1.5	4.6–17.9	18.3 ± 2.9	11.1–36.6
Phocoena phocoena	8.0 ± 0.5	1.9–27.0	15.8 ± 0.7	6.1–37.9
P. sinus	5.0 ± 1.0	2.6–8.3	8.7 ± 1.2	4.5–12.0
P. spinipinnis	9.8 ± 1.8	5.7–15.1	14.3 ± 4.0	7.5–29.2
Neophocaenaphocaenoides	6.6 ± 2.1	3.6–12.8	11.7 ± 2.9	5.6–17.3

[a]Orientation of dusky dolphin samples could not be established to permit sampling of inner and outer layers, thus values represent the entire blubber layer

[b]Fatty acid data for belugas from S.J. Iverson and K.J. Frost (unpublished data)

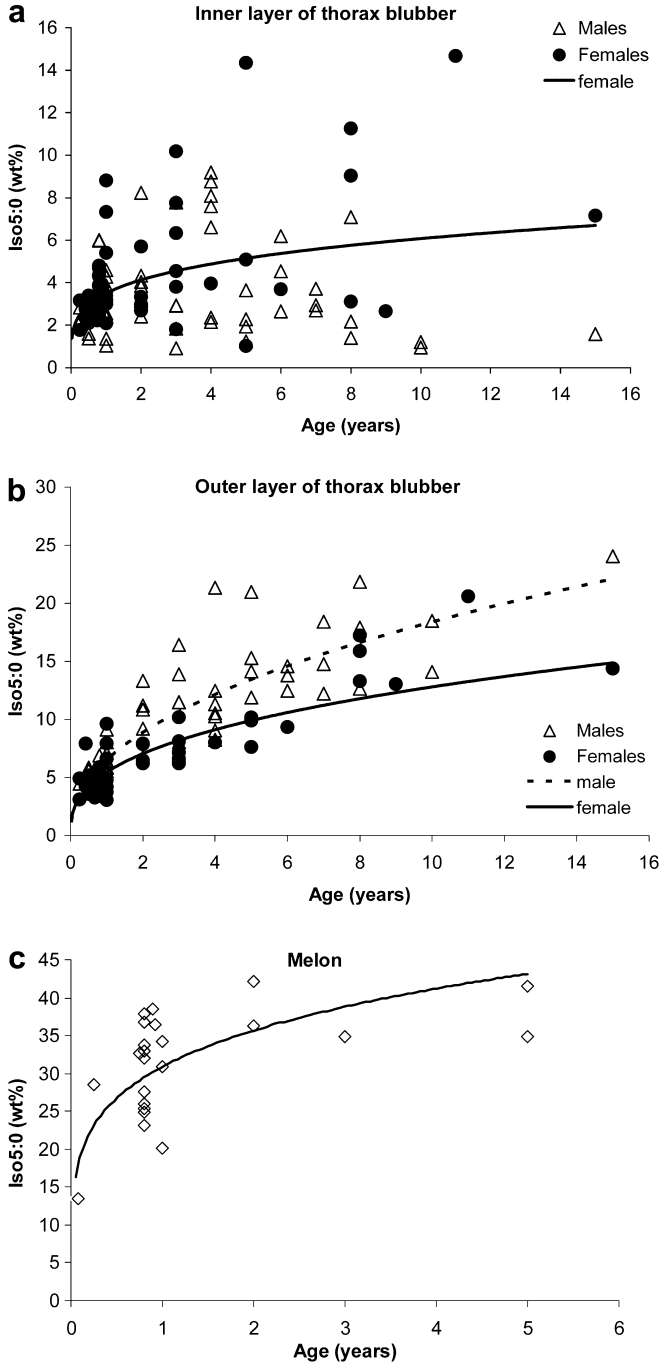

Fig. 3 Age-related patterns of iso5:0 levels (wt%) in tissues of male (*open triangles*) and female (*closed circles*) harbour porpoises. **a** Inner layer of thorax blubber (females, $y = 0.04 * (age * *0.240)$, adjusted $R^2 = 0.20$, $P < 0.001$; males NS, $P = 0.86$). **b** Outer layer of thorax blubber (females, $y = 0.05 * (age * *0.371)$, adjusted $R^2 = 0.70$, $P < 0.001$; males, $y = 0.07 * (age * *0.454)$, adjusted $R^2 = 0.77$, $P < 0.001$). **c** Melon (all porpoises are *open diamonds*, y = 0.31*(age**0.214), *adjusted R2 = 0.44, P < 0.003*)

gas, iso5:0 levels in outer blubber increased linearly with age (adjusted $R^2 = 0.67$; $P < 0.01$), but there was no relationship between age and iso5:0 in the inner blubber of the thorax of this species.

The harbour porpoise: detailed investigations of iso5:0 in blubber and other tissues

Variation in iso5:0 levels among tissues and individuals

In harbour porpoises, iso5:0 makes up a substantial portion of the fatty acids in blubber as well as other tissues. The mean concentration of iso5:0 in the outer thorax blubber of adults averaged 22.6 ± 0.9 mol%, making it the most abundant fatty acid present in this layer. In outer blubber, iso5:0 was followed, in order of decreasing abundance, by 16:1n-7, 18:1n-9, 14:0 and 16:0. In juveniles, the most abundant fatty acid was 16:1n-7 (20.3 ± 0.2 mol%), followed by 18:1n-9 and iso5:0, both with mean levels around 11 mol%. Thus even in juvenile harbour porpoises, iso5:0 was one of the three most abundant molecules in the outer blubber of the thorax.

The melon and mandibular fat of harbour porpoises contained the highest concentrations of iso5:0, followed by blubber, muscle and liver (Table 3). The group of porpoises used for this analysis was represented by a disproportionately high number of juveniles. However, iso5:0 levels in melon varied significantly ($P < 0.001$) with age (Fig. 3c); this relationship was best described by a power function in the form $y = 0.31 * (age * *0.214)$. As in blubber, the relationship between iso5:0 concentrations and age was stronger than that with length; length offered no significant effect ($P > 0.6$) beyond its correlation with age. Melon iso5:0 concentrations increased with age, reaching an asymptote of 35–40% at 3–4 years (Fig. 3c). The melon, inner thorax blubber and outer thorax blubber of the foetus contained 4.4 wt%, 0.5 wt% and 1.3 wt% iso5:0, respectively.

Total blubber burden of iso5:0 in harbour porpoises

Estimates of the total amount of iso5:0 in the blubber of porpoises ranged from 161–781 g (Table 4). Generally, total blubber iso5:0 burden increased with increasing age. In all animals, most of the iso5:0 was found in the outer blubber layer and the stratification of iso5:0 bur-

Table 3 Mean concentrations (wt% ± SE) of iso5:0 in lipids of various tissues from individual harbour porpoises used in the correlation analysis. Mean body size (standard length ± SE) of the 18 porpoises sampled was 120.4 ± 3.2 cm (range: 101.0–158.0 cm)

Tissue	Mean iso5:0 ± SE	*n*	Range
Melon	32.7 ± 1.5	18	20.1–42.8
Mandibular fat	27.3 ± 2.8	6	16.4–34.7
Epaxial muscle	3.0 ± 1.0	13	0.2–10.7
Liver	1.4 ± 0.4	13	0.2–5.9
Inner thorax blubber	3.5 ± 0.4	18	1.8–8.2
Outer thorax blubber	5.5 ± 0.5	18	3.1–10.2
Inner tailstock blubber	3.1 ± 0.3	15	1.7–5.1
Outer tailstock blubber	4.9 ± 0.3	15	3.0–6.8

Table 4 Total burden of iso5:0 in the blubber of harbour porpoises, based on fatty acid analysis of inner and outer blubber at 12 body sites

Length (cm)	Sex/Reproductive class	Age (years)	Body mass (kg)	Blubber mass (kg)	Inner blubber iso5:0 (g)	Outer blubber iso5:0 (g)	Total blubber iso5:0 burden (g)
102.5	F/calf	0.25	21.8	9.2	61	101	161
118.0	F/immature	1	28.0	8.9	110	145	255
121.0	M/immature	0.9	26.9	9.0	113	128	241
121.0	M/immature	1	29.6	9.0	132	173	305
140.0	M/mature	6	47.0	12.2	210	552	762
148.5	M/mature	7	51.0	10.4	191	445	636
158.0	F/pregnant	5	56.4	15.2	274	507	781
160.0	F/lactating	5	65.0	13.3	158	612	770

den became more pronounced as porpoises grew larger and older (Table 4). The blubber of younger, smaller animals contained a greater number of 16:1n-7 molecules than iso5:0, but in older, larger animals the iso5:0 levels had increased to become the dominant fatty acid present.

In the foetus, blubber lipid content (inner layer 65.4%, outer layer 54.2%) and iso5:0 concentrations (0.5 and 1.3 wt% in inner and outer, respectively) resulted in estimates of total iso5:0 blubber burden in the inner and outer layers of 1 g and 2 g respectively, for a total of 3 g in the foetal blubber. Body mass at birth is approximately 5 kg (see Read 1999). Thus, if there were linear deposition of iso5:0 in the last month in utero), then doubling the burden from the 2.4 kg foetus would result in an estimated blubber burden of about 6 g of iso5:0 at birth.

This estimated blubber burden of iso5:0 at parturition can be used to estimate a possible rate of deposition of iso5:0 into the blubber in the first few months of life. The smallest porpoise listed in Table 4 was sampled in late August, making this animal about 100 days old, based on a mean estimated birth date of mid-May (see Read 1999). This calf possessed a blubber burden of 161 g iso5:0. If the estimated burden (6 g) at parturition is subtracted from this, then this porpoise calf would have deposited iso5:0 into its blubber at the rate of about 1.6 g/day.

Effects of starvation on fatty acids in the melon and blubber of juvenile harbour porpoises

Starvation appears to affect the composition of the blubber, but not that of the melon. There were no significant differences between the melons of normal and emaciated porpoises in concentrations of any of the fatty acids tested (all $P > 0.05$; see Table 5). Together, these compounds accounted for ~80 wt% of all melon fatty acids. In all juvenile porpoises, iso5:0 accounted for 23–58 mol% of all fatty acid molecules present in the melon, with means of 47 ± 1.9 mol% and 41 ± 5.3 mol% in normal and emaciated porpoises, respectively. In addition, the mean lipid content of melons of emaciated porpoises was not significantly different from that of normal porpoises ($P > 0.05$). In contrast, the inner blubber layer of emaciated porpoises exhibited significantly higher ($P < 0.01$) concentrations of iso-acids (except iso5:0) and 14:0 than that of normal animals (Table 5). This was balanced by significantly lower ($P < 0.001$) concentrations of 20:5n-3, and

Table 5 Lipid content and major fatty acids (mean wt% of total fatty acids ± SE) in the melons and inner layers of the blubber layer of 13 juvenile harbour porpoises in good (robust) and 5 in poor (emaciated) body condition. In melon, none of these fatty acids exhibited significant differences between normal and emaciated porpoises in mean concentration (all $P > 0.05$). In blubber, variables exhibiting significant differences ($P < 0.01$) between emaciated and normal porpoises are indicated by an *asterisk* in the normal column; *two asterisks* indicate a difference of $P < 0.05$

Fatty acid	Normal Melon	Emaciated Melon	Normal inner blubber layer	Emaciated inner blubber layer
Percentage lipid (wet wt)	81.5 ± 2.4	76.5 ± 2.3	*84.5 ± 0.8	75.0 ± 2.7
iso4:0	1.8 ± 0.2	2.3 ± 0.3	*0.7 ± 0.1	1.37 ± 0.3
iso5:0	31.9 ± 1.6	27.5 ± 4.2	2.9 ± 0.3	4.9 ± 1.0
iso14:0	2.6 ± 0.1	2.7 ± 0.2	*0.3 ± 0.4	0.8 ± 0.1
14:0	9.6 ± 0.3	10.0 ± 0.6	*6.4 ± 0.2	8.6 ± 0.4
iso15:0	4.8 ± 0.5	4.2 ± 0.4	*0.7 ± 0.1	2.0 ± 0.3
iso16:0	4.7 ± 0.4	5.1 ± 0.5	*0.6 ± 0.1	1.8 ± 0.2
16:0	8.7 ± 0.3	9.2 ± 0.5	7.4 ± 0.2	8.0 ± 0.5
16:1n-7	14.5 ± 0.5	15.5 ± 1.3	16.2 ± 1.1	19.9 ± 1.0
18:1n-9	5.7 ± 0.6	6.0 ± 1.0	14.2 ± 0.5	12.8 ± 1.1
20:5n-3	0.34 ± 0.12	0.47 ± 0.14	*3.6 ± 0.3	1.5 ± 0.4
22:6n-3	0.22 ± 0.08	0.42 ± 0.18	**6.6 ± 0.8	3.0 ± 1.2

lipid content was also lower ($P < 0.001$) in the inner blubber of emaciated animals (Table 5). Concentrations of iso5:0 in the inner blubber layer were higher in the emaciated porpoises, but this difference was not significant ($P > 0.02$).

Discussion

Patterns of iso5:0 distribution in the tissues of odontocetes are extremely complex, varying with phylogeny, body size, age, maturity status, and body site. In the 30 species we examined, iso5:0 levels ranged from being absent to 40.4 wt% in outer blubber and 42.8 wt% in melon. This is also the first study to demonstrate that stratification is a characteristic feature of iso5:0 distribution in odontocete blubber. In almost all specimens examined, iso5:0 concentrations were higher in the superficial (outer) blubber than they were in the deep (inner) blubber (see below), consistent with earlier documentation in harbour porpoises (Koopman et al. 1996).

In general, our results agree with the earlier findings of Litchfield et al. (1975), although there were differences in the absolute levels of iso5:0 measured. This was likely due to a number of factors, including improved analytical techniques and differential data reporting, but especially to the greater range and confirmed condition of the samples we obtained, as iso5:0 levels can vary as much within species as they do among species (e.g., Table 1) and can rapidly deteriorate in poor storage conditions.

Phylogenetic patterns in iso5:0

Patterns of iso5:0 distribution in blubber among the six odontocete families were apparent, but variation within families was complex. The presence of iso5:0 was restricted to delphinids, phocoenids and monodontids. We did observe iso5:0 in the blubber of five kogiids and two ziphiids, although at barely detectable levels. Litchfield et al. (1975) reported similar observations, and suggested that "the capacity for biosynthesis of isovalerate lipids is apparently present in all six odontocete families" but that only the delphinids, monodontids and phocoenids make significant use of such a pathway. Our results support this conjecture. Morris (1973, 1975) also identified iso5:0 in the spermaceti organs of sperm whales, though it was present at very low (< 2 wt%) concentrations.

The species listed in Tables 1 and 2 are arranged according to the most recently reported phylogenetic relationships among the delphinids (LeDuc et al. 1999) and phocoenids (Rosel et al. 1995) to facilitate interpretation of the data. It should be noted that the single spectacled porpoise in the dataset was a dependent calf, estimated to be ca. 6 months old (Evans et al. 2001). Given the clear relationships between age/maturity status and iso5:0 levels in other porpoises (Table 4; Figs. 2, 3), the levels of iso5:0 in the blubber of this porpoise should be considered minimum values for this species.

Based on the species we sampled, we can suggest that odontocetes possessing appreciable quantities of iso5:0 in their thorax blubber fall into three general categories: (1) those with extreme values of iso5:0 [Lissodelphininae (northern right whale dolphins and Hector's dolphins)]; (2) those with consistently high concentrations in both inner and outer layers (phocoenids, monodontids, and perhaps the common dolphin and long-finned pilot whale); and (3) those with generally low levels (all other delphinids). Although not examined here, a riverine delphinid (an adult tucuxi *Sotalia fluviatilis*) was shown to possess high concentrations (18.9 wt%) of iso5:0 in its blubber (Ackman et al. 1975). Although these levels are not consistent with the low concentrations (< 3 wt%) in the blubber of adults of another member of its subfamily (the Stenoninae), the rough-toothed dolphin (Table 1), it may be that specific relationships between iso5:0 levels and taxonomy will be resolved at levels below the subfamily (i.e. genus level).

Iso5:0 in different tissues of harbour porpoises

In harbour porpoises, iso5:0 was found in much higher concentrations in acoustic tissues (i.e. melon and mandibular fat) than in other parts of the body (Table 3; Fig. 3). This agrees with the early analysis of single specimens from a variety of species (Litchfield and Greenberg 1974; Litchfield et al. 1975). Nevertheless, the vast majority of the body's iso5:0 is not stored in the melon, but in the blubber. Melons in harbour porpoises weigh ca. 200 g (W.A. McLellan, personal communication), but blubber in this species comprises at least 8–15 kg (Table 4; McLellan et al. 2002). Thus the iso5:0 burden (˜33 wt%) in the melon (˜80% lipid; Table 5), is estimated to be about 53 g. This estimate, although coarse, allows a preliminary comparison with that in blubber (the amount of iso5:0 stored in the mandibular fat is probably similar to, if not lower than, that of the melon). At about 240–780 g (Table 4), the blubber is estimated to contain up to15-fold that in the acoustic tissues, or almost 90% of the iso5:0 stored in the body. Iso5:0 was also found in liver and muscle, suggesting that some synthesis/deposition does occur in these tissues as observed in striped dolphins by Morii and Kaneda (1982). However the overall burdens of iso5:0 in these tissues were low, because of a combination of low concentrations of iso5:0 (see Table 3) and low lipid content (mean percentage lipid: muscle $2.9 \pm 0.3\%$, liver $4.2 \pm 0.2\%$) compared to blubber and acoustic tissues. Thus the iso5:0 in muscle and liver does not represent a major component of the total body iso5:0 burden.

Our study demonstrated that iso5:0 in melon increases with age in porpoises (Fig. 3c), more rapidly than it does in the outer thorax blubber (Fig. 3b). Our results also suggest that iso5:0 levels build up rapidly at

an early age in the melon, but that those in the blubber increase more gradually, but continuously, over the life of the animal. Melon iso5:0 concentrations increased from 12% to ˜35% in the first year of life, while levels in the outer blubber only increased from ~3% to 9–10% (Fig. 3c, b). By the time these animals reach sexually maturity (ages 3–4), iso5:0 concentrations in the melon will have increased only slightly to 35–40 wt%, but those in the outer blubber will have reached >10 wt% and will continue to rise to >20 wt% beyond this age. Based on these observations, we hypothesise that the rate of storage of iso5:0 by harbour porpoises varies both ontogenetically and among tissues, and that iso5:0 accumulations may be limited to adipose depots. We further suggest that the rapid accumulation of iso5:0 in the acoustic tissues, followed by a levelling off by the age of 1 or 2 years, implies that the lipid composition of these tissues must be established early in life, probably for proper echolocation and hearing function. These data also raise the possibility that full echolocation ability might not be attained until porpoises reach one or two years of age. Based on the foetal data, differential accumulation of iso5:0 in melon and in the inner and outer layers of the blubber appears to be initiated in utero.

Patterns of accumulation and stratification of iso5:0 in odontocete blubber

The stratification of iso5:0 in blubber was present in all species, but appeared to vary with age (using sexual maturity status as a marker). Stratification was not merely a function of the thickness of the blubber (e.g. perhaps a thicker blubber layer provides more "room" for stratification, or thinner blubber is by nature more stratified). The blubber of the northern right whale dolphins was less than half the thickness of the narwhal blubber, for example, but exhibited far greater stratification in iso5:0 levels (Fig. 2). The thinnest blubber examined was from the clymene dolphin, and this species had higher stratification than several other species in the sample (Fig. 2). Further, porpoise calves have significantly thicker blubber than do adult conspecifics (Koopman 1998) but far less iso5:0 stratification (Fig. 2), but porpoise calves also have thicker and more stratified blubber than some delphinids, such as members of the genus *Stenella*.

Not surprisingly, concentrations of iso5:0 in the outer blubbers of both harbour porpoises and belugas exhibited stronger relationships with age than with length. This points to a continuous rate of iso5:0 accumulation in blubber over time, rather than a pattern associated with investment in body size. Given our results from harbour porpoises and belugas, and the fact that length is not a good predictor of age (see Read and Tolley 1996), it is likely that we would see a greater number of significant relationships among other species if blubber iso5:0 was regressed against age rather than length.

There appeared to be a major shift in iso5:0 blubber burdens, particularly in the outer layer, as harbour porpoises made the transition from juveniles to adults (Table 4). While porpoises grew larger and older, the outer blubber burden of iso5:0 increased significantly, but those of 14:1n-5 and 16:1n-7 (other endogenous fatty acids) rose only slightly. Increases in iso5:0 concentrations with age in the outer blubber were balanced by corresponding decreases in major polyunsaturated fatty acids (PUFAs, 20:4n-3, 20:5n-3, 22:5n-3, 22:6n-3; data not shown). However the continued increase in iso5:0 concentrations in the blubber (Fig. 3a, b) even after the average age at which porpoises become sexually mature (3–4 years in harbour porpoises; Read and Hohn 1995), supports the notion that absolute age, rather than investment in somatic tissue or attainment of sexual maturity, is the most important factor modulating iso5:0 accumulations in blubber, at least in harbour porpoises. It should be noted that there is turnover in the blubber, even in the outermost layers, as adipocytes have a finite lifespan. The patterns of iso5:0 accumulation observed over time in this study indicate that some amount of lipid turnover does occur in the outer layer of the blubber. However, this is difficult to evaluate further because tissue turnover rates have not yet been established for the blubber, and we do not fully understand lipid metabolism in small odontocetes.

Expressing iso5:0 concentrations as mol% even better demonstrates its contribution to blubber fatty acids. In Hector's dolphin blubber, as well as in the outer layer of northern right whale dolphins, iso5:0 accounted for one-third to one-half of all fatty acid molecules present. In adult harbour porpoises, iso5:0 was the dominant fatty acid in the outer blubber. From a molecular point of view, this suggests that in animals with high iso5:0 concentrations, most triacylglycerols in the outer blubber must contain at least one, if not two, iso5:0 molecules (alternatively, there could be many triacylglycerols with two molecules of iso5:0). Litchfield et al. (1975) reported higher proportions of triacylglycerols containing one iso5:0 molecule than triacylglycerols with two. There are no reports of any triacylglycerols with three iso5:0 molecules in blubber, melon or mandibular fat (Litchfield and Greenberg 1974; Litchfield et al. 1975), and it is not known whether odontocetes are capable of assembling such compounds. It would be interesting to determine whether the configuration of iso5:0-containing triacylglycerols, and the enzyme systems responsible for their synthesis, are consistent in odontocetes that deposit these molecules to such varying degrees in their adipose tissues. Further studies comparing the synthesis and molecular structure of iso5:0-containing triacylglycerols in blubber and acoustic tissues, both across species and ontogenetically within species, would be very useful for identifying the factors controlling iso5:0 deposition in these animals.

The development of the stratification of iso5:0 in blubber suggests that there is some type of differential metabolism in the inner and outer blubber layers. The

simplest explanation is that, once synthesised (see below), iso5:0 is selectively deposited in the outer blubber. Alternatively, other fatty acids may be mobilised from the inner blubber during lipolysis, while iso5:0 is left behind, and eventually this results in an accumulation of iso5:0 in the outer blubber as new fatty acids are deposited into the inner layer. Regardless of which process is occurring, these animals must be continuously depositing more iso5:0 in their blubber through life, i.e. the stratification is not just the result of a gradual repositioning over time of an initial deposit of iso5:0 (Table 4).

Possible mechanisms of accumulation, synthesis and relationship to health

How can the remarkably high levels of iso5:0 in odontocetes be reconciled with the apparent toxicity of this compound? Either: (1) odontocetes are immune to, or more resistant to, the toxic effects of iso5:0 found in other mammals, or (2) odontocetes are extremely effective at locally synthesising and sequestering iso5:0 in tissues from which lipids are less likely to be mobilised, reducing the chance that it will be introduced into circulation. The latter option would certainly apply to the melon, the composition of which is not altered by starvation even when considerable portions of blubber lipid have been mobilised (this study; Koopman 2001). Melon fatty acids are probably not utilised during starvation because these compounds offer relatively little in terms of energy value (longer chain fatty acids provide more energy than short- and medium chain acids) or metabolic significance (e.g. acting as precursors for prostaglandins and other molecular messengers), or because acoustic function must be preserved at all costs. However, blubber also appears to be effective in isolating iso5:0. Although porpoises and other small odontocetes are not adapted for routine fasting, there is nevertheless some blubber turnover and short-term withdrawal of fatty acids for daily energy requirements (Koopman et al. 1996); however, iso5:0 does not appear to be mobilised from blubber. Further support for local synthesis and sequestering comes from foetal data. Our results indicate that iso5:0 is stored in utero despite previous evidence that iso5:0 does not cross the placenta in porpoises (Lovern 1934). This implies that all iso5:0 deposited in utero is produced by the foetus itself and that it is likely synthesised locally in the blubber, as the introduction into the circulation of a potentially highly toxic compound at such a vulnerable life stage would be surprising.

The toxic effects of accumulations of iso5:0 for humans afflicted with isovaleric acidemia (IVA) are severe: vomiting, neurological damage, coma, and even death (Tanaka et al. 1966; Budd et al. 1967; Nyhan 1984). IVA is a fairly rare disorder in which one of several mutant forms of isovaleryl-CoA dehydrogenase (IVD, the enzyme that normally catalyses the further transformation of iso5:0-CoA), prevents further conversion of iso5:0-CoA in the degradation pathway of the amino acid leucine (Tanaka et al. 1988). In humans, IVD is located primarily in organs (heart, liver and kidney) and skeletal muscle (Rhead et al. 1981). The only investigation of iso5:0 synthesis in odontocete tissue strongly supports the idea that melon and blubber generate iso5:0 via the conversion of leucine (Malins and Varanasi 1975; Morii and Kaneda 1982), which should be plentiful in their high-protein fish diet. This suggests that porpoises, and other odontocetes, might be utilising the IVA pathway as part of their normal physiology. It also appears that they are able to do this without suffering the effects of this disease, as high concentrations continue to accumulate with age. This process would represent an effective adaptation of an enzymatic disorder, which perhaps confers some advantage for odontocetes. A possible association between IVA and iso5:0 in the melons of odontocetes was also proposed by Malins and Varanasi (1975), but this is the first time such a relationship has been proposed for blubber.

It is possible that the potential toxic effects of iso5:0 are avoided in odontocetes because they are able to isolate the synthesis and storage of iso5:0 within tissue compartments, especially blubber, preventing entry of this molecule into the general circulation. In contrast, other mammals (i.e. humans and rats) degrade leucine to iso5:0 in organs and muscles (see Rhead et al. 1981), leading to the release of iso5:0 into the bloodstream. In these mammals (except those afflicted with IVA, see above), iso5:0-CoA is either conjugated with glycine and excreted, or further degraded to yield acetoacetate and acetyl-CoA (Tanaka and Isselbacher 1967; Ando et al. 1973; Tanaka 1973; Malins and Varanasi 1975; Stryer 1988). Other processes must be occurring in dolphins, porpoises and monodontids to allow such immense accumulations of iso5:0 in their adipose tissues. All iso5:0 accumulations in these animals must be from endogenous sources, as short chain fatty acids in the diet are oxidized before they can be deposited into adipose tissue (Patton 1981). Even if this were not the case, there are no known dietary sources of iso5:0 for odontocetes, and it is absent from their milk (H.N. Koopman, unpublished data). Thus in addition to the questions regarding iso5:0 synthesis (see above), lies the issue of what facilitates its accumulation in tissues. Possibly IVD is operating at extremely low levels, resulting in iso5:0 accumulations. Alternatively, the capacity for glycine-N-acylase to conjugate iso5:0 with glycine might be reduced, limiting the rate of excretion of iso5:0. There is also another possibility: the triacylglycerol synthetase complex (containing the enzymes responsible for assembling triacylglycerols) may have an extremely high affinity for iso5:0 (K. Tanaka, personal communication). The first two options represent a relationship with human disease, and the third an independent enzymatic adaptation in specific families of the toothed whales. The actual mechanism responsible may in fact be a combination of these modifications of typical mammalian physiology.

Hypotheses for roles of iso5:0 in the blubber of odontocetes

From this dataset we can construct a series of testable hypotheses that may help achieve a better understanding of the presence of this unusual compound in odontocete blubber. It is clear that that iso5:0 is a critical component of the lipid matrix that modulates sound transmission and reception in the melon and mandibular fat of delphinids, phocoenids and monodontids (Litchfield et al. 1971, 1973, 1979; Varanasi and Malins 1972; Malins and Varanasi 1975; Varanasi et al. 1982; Cranford et al. 1996). Work by Varanasi et al. (1973, 1975) has shown that in the melon and mandibular fats, the arrangement of iso5:0 molecules on the glycerol backbone (almost exclusively in the 1,3 isomeric form, putting iso5:0 on the outer positions of the triacylglycerol) is critical for modulating density and compressibility and for the formation of an acoustic lens. Thus both the presence and spatial arrangement of iso5:0-containing triacylglycerols are associated with acoustic function. Our data are consistent with the hypothesis that the composition of the melon lipids is maintained for a specific function; the fatty acid composition of the melons of porpoises that have undergone starvation is the same as that of robust porpoises in good body condition.

With the assumption that iso5:0 is important for acoustic function, it is possible that iso5:0 in odontocetes is purely acoustic in nature, and accumulations in all other tissues represent "excess production" and sequestering of this potentially toxic compound. Litchfield et al. (1971) suggested that the presence of iso5:0 in blubber oils might be due to a "spillover" from synthesis in the head for echolocation. However this hypothesis requires the assumption that iso5:0 is produced in some central location(s) (either the melon or elsewhere), and any iso5:0 not deposited in the melon will be transported to another tissue (e.g. outer thorax blubber) for storage. This does not seem likely for several reasons. Malins et al. (1972a) have demonstrated that diisovaleroyl glycerides in melons are synthesised and degraded at the site of deposition, and in vitro studies with striped dolphin tissue (Morii and Kaneda 1982) suggest that iso5:0 can be produced from leucine in a variety of locations, but particularly in melon and blubber. Therefore, it appears that synthesised iso5:0 does not arise from a central pool to be transported around the body. In addition, this hypothesis cannot be reconciled with the immense variability found across species in blubber iso5:0 levels (this study; Litchfield et al. 1975), while those in melons of the same species tend to be quite consistent (Litchfield and Greenberg 1974). This agrees with Malins and Varanasi's (1975) conclusion that the acoustic tissues of odontocetes are "encapsulated" or isolated with regard to synthesis and storage of specialised fatty acids.

Thus, on the basis of all evidence available, we reject the hypothesis above, and propose an alternate hypothesis: that iso5:0 plays an important role in odontocete acoustics, but in some species it has been exploited to serve an additional, secondary function in the blubber. This hypothesis is the simplest explanation for the observed variation in and accumulation of iso5:0 found in the blubber of different odontocetes. This is further supported if data from the other iso-acids are also considered. For example, iso4:0, iso14:0 and iso16:0 are also synthesised from amino acids, but these are generated from isoleucine and valine via specific enzymes distinct from IVD (Morii and Kaneda 1982; Tanaka et al. 1988). In juvenile porpoises, these three iso-acids together represented ~10 wt% of the total fatty acid complement in melons (Table 5), but only 1.5 and 3.5 wt% in the inner and outer blubber layers, respectively (Table 5; H.N. Koopman, unpublished data). The other iso-acids, particularly iso4:0, can constitute significant components of the acoustic fats of some species (e.g. *Tursiops truncatus*; see Ackman et al. 1973), but in blubber, iso5:0 is the only branched fatty acid that reaches considerable concentrations. Generally, iso4:0 concentrations in the blubber were <2 wt%; those of most species were <1% (H.N. Koopman, unpublished data). Although iso5:0 levels in blubber increase substantially with age (Figs. 2, 3), the relative proportions of these other iso-acids remain constant (H.N. Koopman, unpublished data). This pattern suggests that the branched structure of the iso-acids is important in general for the acoustic tissues, but that in the blubber iso5:0 production is independent and functional.

We present one possible functional advantage for iso5:0 in the blubber of odontocetes. Iso5:0 has an extremely low melting point (−37.6 °C; Fasman 1975) which could be important for blubber plasticity for some species, specifically those inhabiting colder waters. There is clear evidence for a thermal gradient across the depth of the blubber, such that the superficial tissue is usually cooler than the deep blubber and activities of individual enzymes in these locations may be adapted to function at the different respective temperatures (see Elsner 1999, Iverson 2002). The colder temperatures of the outer layer would render it more vulnerable to pliability/flexibility problems. The low melting point of iso5:0 could act to buffer the effects of low temperature on tissue flexibility, which would be especially important in the outer blubber for a number of physiological reasons, including that compliance of the body surface reduces energy-dissipating turbulent flow during locomotion. The higher concentrations of iso5:0 in the outer blubber layer might also be explained by differing enzyme activities, and thus possibly greater synthesis, in that area. To examine whether this hypothesis could be supported by our data, we assigned all delphinid, phocoenid and monodontid species represented by more than one individual to one of three thermal habitats, based on the species distributions given in Leatherwood and Reeves (1983): arctic/cold temperate, tropical/warm temperate, and mid-temperate/cosmopolitan (see superscript notations in Table 1). We then calculated a mean iso5:0 concentration in the outer layer of the thorax blubber

for each family occupying each of these thermal regimes. This analysis revealed a clear trend within the phocoenids and delphinids. Iso5:0 levels in the outer blubber layer were lowest in the tropical/warm temperate species, intermediate in the mid-temperate/cosmopolitan species, and highest in the arctic/cold temperate species (Fig. 4). This trend did not appear to be a function of body size; there was no suggestion from the data that smaller species inhabiting colder water possess higher concentrations of iso5:0 in their blubber, for example.

This preliminary examination suggests that iso5:0 accumulations in the outer blubber could be associated with thermal regime. Such an arrangement would also make sense from a nutritional and energetic point of view. The other fatty acids with melting points below 0 °C (e.g. 18:2n-6, 18:3n-3, 20:4n-6, 20:5n-3) are long chain, PUFAs exclusively of dietary origin (Fasman 1975; Iverson 1993). These compounds are therefore essential, and many of them have important roles in metabolism and health (Stryer 1988). Although these compounds are plentiful in the marine environment, if there was another, endogenously produced compound available that could be used for this purpose it might be particularly useful for species living in cold environments. As iso5:0 has little nutritional value compared to PUFAs, and no known metabolic or structural function other than its role in acoustics, sequestering it in the outer, less metabolically active blubber (Koopman et al. 1996) would make energetic sense. As iso5:0 concentrations rise in the outer blubber layer of harbour porpoises, levels of PUFAs decline, which could be interpreted as support for the cold water/pliability hypothesis: iso5:0 could serve to counteract the "decrease" in flexibility resulting from lower PUFA concentrations. The blubber of the harbour porpoise foetus already exhibited signs of stratification in iso5:0, an observation that could be interpreted as preparation for maintenance of blubber's pliability at birth.

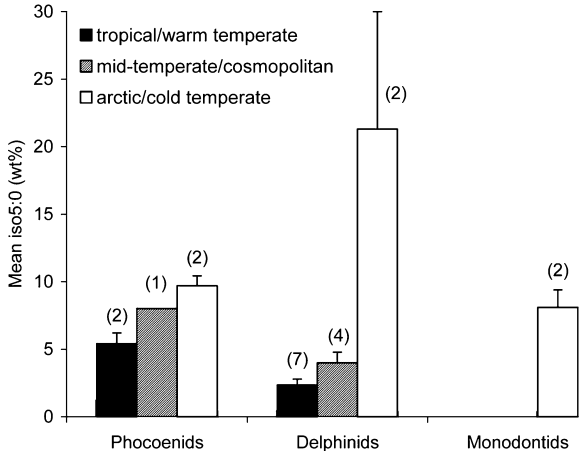

Fig. 4 Mean ± SE wt% iso5:0 concentrations in the outer thorax blubber of odontocetes inhabiting three different thermal regimes. Regimes were assigned according to Leatherwood and Reeves (1983) and are shown in Table 1. Family means were generated from the individual species means within each group, so that each species was weighted equally (to avoid confounding effects of variation in sample size). *Numbers in parentheses* above *bars* indicate number of species in each family mean

In conclusion, the significance of iso5:0 in blubber represents a unique characteristic found only in a few groups of odontocetes. Cranford et al. (1996) stated that its presence in the acoustic tissues of these animals was "evidently a unique biosynthetic process" that "has arisen to create these substances in situ". However, given the available evidence, it is probably not the pathway of production of iso5:0, but rather the apparent ability of delphinids, phocoenids and monodontids to deliberately and locally biosynthesise, retain and deposit this unusual compound in selected tissues in such substantial amounts, that is unique. Although the presence of iso5:0 in odontocetes has long been associated with the evolution of echolocation ability, it appears that perhaps this unusual molecule is also being exploited to serve an additional, secondary function in the blubber of some species. It will be important to design future studies that can elucidate the biochemical and physiological pathways of this compound in odontocetes.

Acknowledgements We wish to thank many individuals and organisations for their generosity in contributing samples and assisting with sample collection: Bill McLellan and Ann Pabst (University of North Carolina at Wilmington; John Nicolas (National Marine Fisheries Service, Northeast Fisheries Science Center); Charley Potter (National Museum of Natural History, Smithsonian Institution); Thomas Jefferson and Susan Chivers (NMFS, Southwest Fisheries Science Center); Ellie Dickson and Elizabeth Slooten (University of Otago); Koen van Waerebeek and Julio Reyes; and many people from: state stranding networks from Massachusetts to Florida; Virginia Marine Science Museum; Hubbs-Sea World, Orlando, Florida; Mote Marine Laboratory, Sarasota, Florida; the Beaufort, North Carolina and Woods Hole, Massachusetts NMFS offices; the Marine Mammal Stranding Program at UNCW; the Duke University Marine Laboratory, Beaufort, North Carolina; the Canadian Department of Fisheries and Oceans; the Grand Manan Whale and Seabird Research Station, New Brunswick, Canada; and the fishermen of the Bay of Fundy and Gulf of Maine. This manuscript was improved by the comments of Ann Pabst and Kay Tanaka, and we also thank Dr. Tanaka for useful insights into, and suggestions regarding, iso5:0 metabolism. Samples were imported into Canada and the United States of America under appropriate CITES and US Department of Fish and Wildlife permits, and all analytical methods complied with current US and Duke University regulations. This study was supported by postgraduate fellowships from the Canadian Natural Sciences and Engineering Research Council (NSERC) and the Duke University Marine Laboratory to H.N.K., a postdoctoral scholarship at Woods Hole Oceanographic Institution to H.N.K., a Duke University Marine/Freshwater Biomedical Center Feasibility Study grant to H.N.K. and A.J.R., and NSERC Research and Equipment Grants to S.J.I. This represents Woods Hole Oceanographic Institution contribution number 10683.

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