

# Changes in Blubber Distribution and Morphology Associated with Starvation in the Harbor Porpoise (*Phocoena phocoena*): Evidence for Regional Differences in Blubber Structure and Function

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## ABSTRACT

To examine patterns of blubber loss accompanying a decline in body condition, blubber thickness of juvenile harbor porpoises in normal/robust body condition ( $n = 69$ ) was compared with that of starved conspecifics ( $n = 31$ ). Blubber thickness in the thorax of starved porpoises (9–11 mm) was only 50%–60% of that of normal animals (18–20 mm); however, very little tailstock blubber was lost during starvation. Adipocytes in thorax and tailstock blubber were measured in both groups ( $n = 5$ ) to determine whether thickness changes were homogeneous throughout blubber depth. In the thorax of normal porpoises, adipocytes near the epidermis (outer blubber) were smaller (0.11 nL) than inner blubber adipocytes (0.17 nL). Conversely, the size of tailstock adipocytes was uniform. Starved animals had fewer, smaller adipocytes in the inner thorax blubber, suggesting a possible combination of adipocyte shrinkage and loss. Lipids were withdrawn only from the inner layer of thorax blubber during starvation, supporting a hypothesis of regional specialization of function in blubber. Blubber of the thorax serves as the site of lipid deposition and mobilization, while the tailstock is metabolically inert and likely important in locomotion and streamlining. Therefore, some proportion of the blubber of small odontocetes must be considered structural/mechanical rather than an energy reserve.

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## Introduction

Blubber is a dynamic, complex, multifunctional tissue, unique to marine mammals, that serves many roles: defining the hydrodynamic shape of the body; adjusting buoyancy; aiding in locomotion; insulating the body; and serving as a source of stored energy in the form of lipid (reviewed in Ryg et al. 1988; Pabst et al. 1999b). Marine mammals invest heavily in blubber's structure and maintenance: blubber mass can constitute 15%–55% of total body mass (e.g., Ryg et al. 1993; McLellan et al. 2002).

Blubber is a critical component of mammalian adaptation to the aquatic environment, having evolved several times in parallel among cetaceans and pinnipeds, leading to remarkable similarities in structure and function in this tissue. Many marine mammals exhibit physiological and anatomical adaptations that allow them to rely on the lipids stored in blubber as a source of energy during annual fasting periods. For example, over the course of a 36-d molting fast, male southern elephant seals (*Mirounga leonina*) derive over 95% of the energy they require from fat, depleting their blubber masses by 48% (Slip et al. 1992). Similarly, several large baleen whales rely heavily on lipids stored in their blubber during reproduction and lactation. Blubber in the dorsal flank of female fin whales (*Balaenoptera physalus*) thickens by 25%–45% during pregnancy compared with mature males and then is depleted during lactation (Lockyer 1986).

There is significant variation in the patterns of lipid metabolism in fasting-adapted species. Many phocid seals withdraw blubber lipids uniformly over the entire body surface (e.g., Slip et al. 1992; Beck and Smith 1995), but female fin whales mobilize lipids primarily from the dorsal flank blubber (Lockyer 1986). Typically, mammalian adipose cells shrink and swell during periods of fasting and fattening rather than exhibiting a change in the numbers of adipocytes in a given depot (Young 1976; Pond 1998). This phenomenon has been well studied in terrestrial mammals such as rats, mice, and woodchucks (see Young 1976 and Pond 1998 for reviews). Other groups of aquatic animals adapted for routine fasting, such as emperor penguins (*Aptenodytes forsteri*) and polar bears (*Ursus maritimus*), follow the typical mammalian trends of adipocyte size

changes in adipose tissue (Groscolas 1990; Ramsay et al. 1992). There is little information, though, on changes in the number or size of blubber adipocytes during fasting in pinnipeds or cetaceans.

How are blubber lipids used as an energy source in an animal not adapted to seasonal anorexia preceded by fattening? Each year, many juvenile harbor porpoises (*Phocoena phocoena*) strand along the Northeast and mid-Atlantic coasts of the United States. These animals exhibit classic signs of emaciation, with reduced blubber layers, pronounced depressions behind the blowhole, and sunken axial musculature (Kastelein and van Battum 1990). Examination of muscle fiber profiles in the emaciated porpoises indicates that they have starved to death (Stegall et al. 1999). These starved porpoises provide an opportunity to examine morphological and cellular changes in blubber structure in a species that is not adapted for long-term fasting. The distribution, regional variation in thickness, mass, growth, and biochemical composition of blubber in healthy harbor porpoises is reasonably well understood (Read 1990; Koopman et al. 1996; Koopman 1998; McLellan et al. 2002). However, nothing is known about the cellular structure of porpoise blubber beyond an early investigation of one specimen by Parry (1949).

This basic understanding of the structure and variation of blubber in porpoises makes this species a good model for identifying changes in blubber that accompany a decline in body condition in a small odontocete. Here we compare blubber morphology and cellular structure between juvenile porpoises in normal body condition and those that have stranded in extremely emaciated condition. Our objectives for this study were to determine which regions of the body blubber lipids are mobilized, by comparing the topographical distribution of blubber thickness and adipocyte shape, size, and relative number between porpoises in normal and in starved body condition. This study is also the first detailed examination of adipocyte structure in the blubber of an odontocete.

## Material and Methods

### *Porpoises Examined*

All measurements were made on, and samples collected from, 100 porpoise carcasses. Porpoises were divided into two groups on the basis of external appearance at necropsy: those in normal body condition ( $n = 69$  [33 females, 36 males]; exhibiting a robust body shape with no signs of emaciation) and those in emaciated body condition ( $n = 31$  [12 females, 19 males]; exhibiting pronounced depressions behind the blowhole and sunken epaxial musculature; see Kastelein and van Battum 1990). Analysis of muscle morphology on a subset of these animals indicated that the emaciated animals had entered phase III, or terminal starvation (Stegall et al. 1999). All porpoises in the normal category had been killed incidentally in commercial fishing operations. Samples of normal animals were collected from the Bay of Fundy (Canada) and the Gulf of Maine and

along the mid-Atlantic coast of the United States (from New Jersey to North Carolina) during 1993–2000. All porpoises in the starved category had stranded on beaches along the mid-Atlantic coast from 1994 to 2000, exhibited no indications of entanglement in fishing gear (see Cox et al. 1998), and were assumed to have died of natural causes (i.e., ultimate cause of death was starvation, although proximate causes were generally unknown). Porpoise carcasses were examined within 24 h of death, or they were frozen within 24 h of death and subsequent examinations were performed on thawed tissues. Damage to blubber from the freezing process was assumed to be negligible (Pond and Mattacks 1985). All porpoises examined in this study were restricted to those 0–2 yr of age on the basis of age estimates from dentinal growth layers in stained decalcified thin sections of teeth (Bjørge et al. 1995), with standard lengths ranging from 100 to 125 cm.

Two measures of body size were collected from each porpoise: standard length and body mass. Standard length, which is not affected by changes in body condition, was not significantly different (ANOVA,  $P > 0.05$ ) between the two groups (normal mean = 117.0 cm, SE = 0.7,  $n = 69$ ; starved mean = 114.9 cm, SE = 1.2,  $n = 31$ ). Thus, our assumption that the two groups of porpoises represented the same age class was satisfied. However, total body mass does reflect body condition (Cherel et al. 1992). Mean body mass was significantly different (ANOVA,  $P < 0.001$ ) between normal (mean = 30.2 kg, SE = 0.6,  $n = 63$ ) and starved (mean = 19.2 kg, SE = 0.6,  $n = 17$ ) porpoises, providing additional evidence that the two groups represented different body condition states.

### *Blubber Thickness Measurements*

To evaluate changes in the topographical distribution of blubber associated with body condition, blubber thickness was measured at nine different body landmarks: nuchal crest, axilla, midthorax (midway between the axilla and the cranial insertion of the dorsal fin), the cranial insertion of the dorsal fin, lateral to the dorsal fin, caudal insertion of the dorsal fin, anus, mid-keel (midway between the anus and the insertion of the flukes), and fluke insertion (see Fig. 1). Transverse cuts were made from the dorsal to the ventral midlines through the epidermis to the muscle/blubber interface at each landmark (effectively forming half-girths). Blubber thickness was measured to the nearest millimeter, from the blubber/subdermal sheath interface to the blubber/epidermis interface (i.e., the pigmented epidermis was excluded) at seven equidistant positions (measurements 1, 4, and 7 at each girth are referred to as “aspects” [dorsal, lateral, and ventral, respectively]) at each body landmark (similar to the methods used for belugas [*Delphinapterus leucas*] by Doideg 1990). The presence of the dorsal fin prevented blubber thickness measurements at the dorsal-most position of that half-girth, leaving 62 potential measurements for each porpoise. Blubber thickness was measured on one side of the body only,

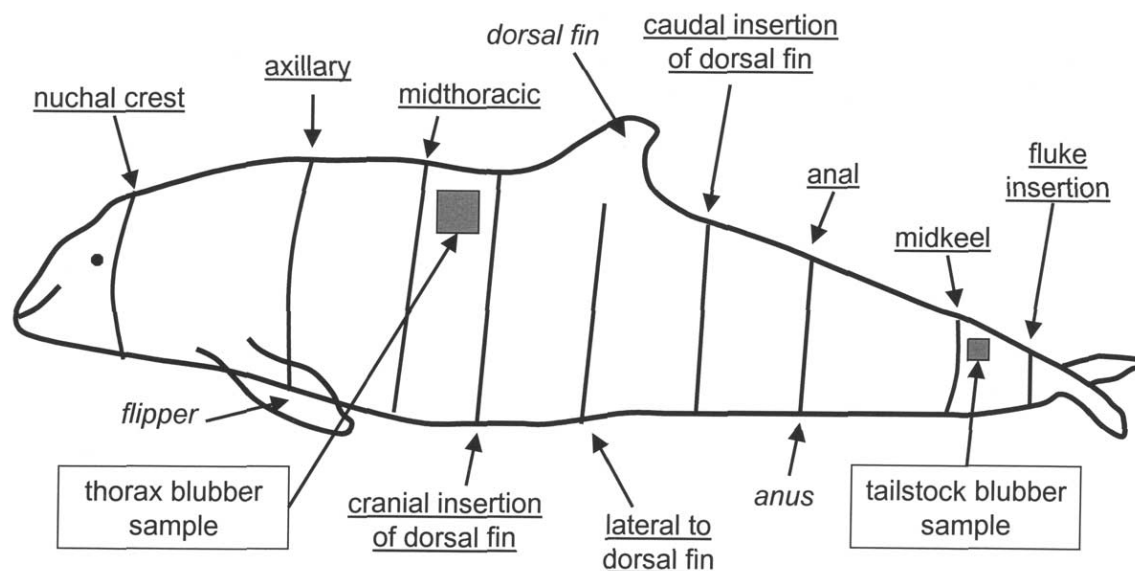


Figure 1. Locations of blubber thickness measurements on harbor porpoises. Underlined locations are referred to as “girths” (see “Material and Methods”). Other features are labeled in italics for orientation. Blubber thickness was measured at seven equidistant sites on one half of each girth, from dorsal (measurement 1) to ventral (measurement 7). Blubber samples for adipocyte measurements were taken from thorax and tailstock (*gray squares*).

since blubber thickness in harbor porpoises is bilaterally symmetrical (see Koopman 1998). Although most porpoise carcasses were in good condition (fresh, defined as Smithsonian Institution code 2; see Geraci and Lounsbury 1993), measurements were not taken at locations where the blubber had been damaged by scavengers or fishing gear; missing data points were not estimated or interpolated.

#### *Assessing Blubber Loss over the Body*

The term “blubber loss” used here refers to the difference in mean blubber thickness (or volume) between normal and starved porpoises and is assumed to represent the amount of blubber that has been mobilized by the emaciated porpoises in their transition from the normal to the starved state.

At each girth (see Fig. 1), the seven positions at which blubber thickness was measured (i.e., 1–7; dorsal to ventral) are referred to as “measurements.” Each combination of girth and measurement is referred to as a “site.” The difference (in millimeters) between the blubber thickness means of the normal and starved porpoises was calculated for each of the 62 sites, and these difference values were then examined to identify the location of blubber loss over the body.

Mean blubber thicknesses at the dorsal and lateral sites of all nine half-girths were compared between normal and starved porpoises using univariate ANOVA. Before statistical analyses, blubber thickness data were log transformed to better approx-

imate normal distributions. Because of the large number of comparisons made, the  $\alpha$  value for evaluating significance of differences between means was dropped to  $0.05/18 = 0.0028$  to reduce the chance of making a Type I error (Bonferroni-type correction; Tabachnick and Fidell 1996). See Figure 2 for variables and calculations used to estimate blubber loss.

#### *Adipocyte Measurements*

Adipocyte measurements were made on blubber collected from a subset of five normal and five starved porpoises. Blubber was sampled from two body sites: thorax and tailstock (see Fig. 1). Samples included epidermis and the entire thickness of the blubber. Samples were frozen at  $-20^{\circ}\text{C}$  until histological processing. Porpoise blubber could not be sectioned using a freezing microtome since it did not freeze solid at the operating temperature of the apparatus ( $-30^{\circ}\text{C}$ ); therefore, the following method was developed to fix blubber. Small ( $0.5\text{ cm} \times 0.5\text{ cm}$ ) subsamples of the entire depth of the blubber layer were fixed overnight in Rossman’s solution (90% ethanol saturated with picric acid, 10% formalin; see Presnell and Schreiber 1997). Samples were then further dehydrated in a series of acetone and toluene washes, followed by overnight infiltration with paraffin. Samples were embedded in paraffin and sectioned ( $9\text{--}11\ \mu\text{m}$ ) on a rotary microtome. Unstained sections were viewed using phase contrast optics on an Olympus BH-2 microscope. Digital images of contiguous fields forming a transect

through each section were captured with a SPOT RT digital camera (Diagnostic Instruments, Sterling Heights, Mich.). Images were imported into Scion Image image analysis software (beta 4.0.2 release [2000], Scion Corporation, Frederick, Md.) for adipocyte measurements.

In each image, 30 adipocytes were selected for measurements, using a uniform random grid (Howard and Reed 1998). Cross-sectional area, perimeter, height, and width were measured for each adipocyte. The height was defined as the length of the major axis of the adipocyte; width was measured perpendicularly to the height. In addition, the angle of the major axis of each adipocyte, relative to the epidermal surface, was measured (i.e., if the major axis was 90°, the cell was longer along the epidermal-muscle axis, and an angle of 0° would indicate that the long axis of the cell ran parallel to the epidermal surface). Adipocyte counts were made by placing a 400- $\mu$ m-wide vertical strip on each image and counting all adipocytes that touched the upper and left boundaries of the strip but not the lower and right boundaries. Counts from all images in each sample were summed to provide an index of the total number of adipocytes. Counts were repeated three times for each image, and the mean was used for analysis.

The aspect ratio (height/width) provided an index of cell shape for each cell. To facilitate comparisons with published values, adipocyte volumes were also estimated. Adipocyte volume is typically estimated using only cell diameter, assuming that cells are spherical (e.g., Goldrick 1967; Pond 1986; Pond and Mattacks 1988). Harbor porpoise blubber adipocytes are columnar (elongate) in shape (see "Blubber Adipocyte Size and Shape in Normal Porpoises"), so cell volume was estimated by multiplying cell area (cross-sectional area as measured using Scion Image software) by cell width and then converting this value to nanoliters. This method assumes that cell width is uniform in longitudinal and transverse sections, an assumption that was supported by preliminary examination of several duplicate sections taken in a 90° rotation from the original section (data not presented here).

Estimates of adipocyte volume were then used to predict the total number of adipocytes in the entire blubber layer of the porpoises. Total blubber adipocyte calculations were weighted; blubber was assumed to be 7/8 thorax and 1/8 tailstock. Blubber mass (corrected for epidermal mass [15%]; H. N. Koopman, unpublished data) was multiplied by blubber lipid content (Koopman 2001) to generate total blubber lipid mass. Following Pond et al. (1992), a lipid density of 0.9 g/cm<sup>3</sup> (Sjöström 1980) was used to calculate total blubber lipid volume. Mean estimates of adipocyte volume at each body site were then used to predict total blubber adipocyte complement for all five normal porpoises and two of the emaciated specimens.

Overall shrinkage of samples during processing was estimated at 14% (on the basis of measurements of tissue samples before and after processing) and assumed to be uniform in all samples and in all directions. However, to ensure consistency

in comparisons among samples, adipocyte sizes were not adjusted for shrinkage and are presented and analyzed in uncorrected form. Adipocytes in a subset of images were measured three times to allow estimation of measurement error (6%). Estimates of total adipocyte counts in the 400- $\mu$ m strips were compared for body site/body condition effects. Thorax and tailstock adipocyte counts were compared within individuals using paired *t*-tests, with separate tests for normal and starved specimens. Differences in adipocyte counts between normal and starved porpoises were compared separately at each body site using unpaired *t*-tests. An  $\alpha$  value of 0.05/4 = 0.0125 was used to evaluate significance of these tests (Bonferroni-type correction; Tabachnick and Fidell 1996).

## Results

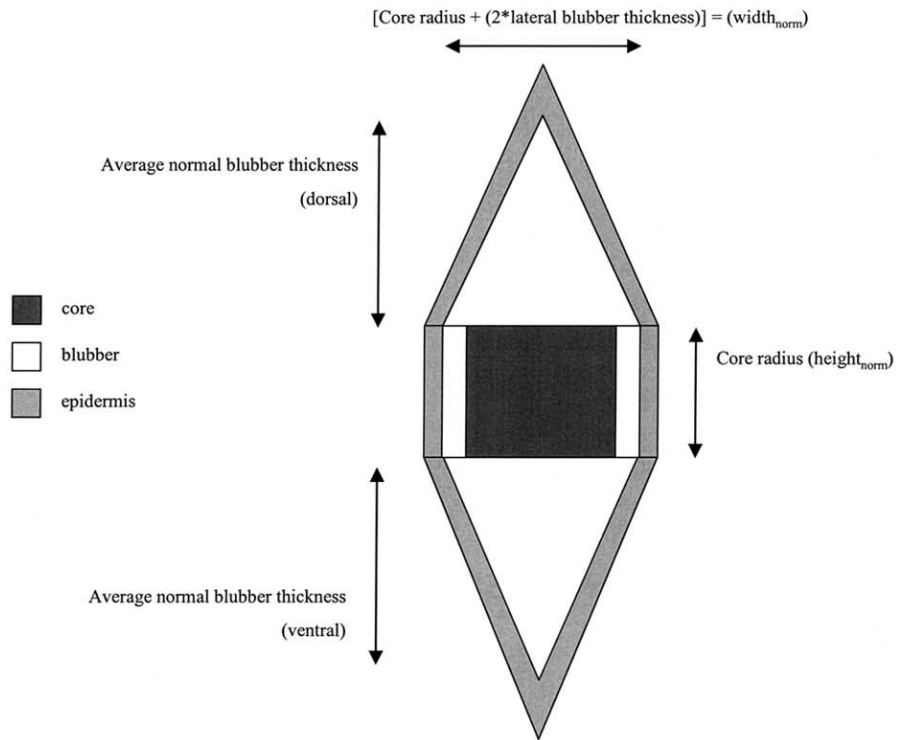
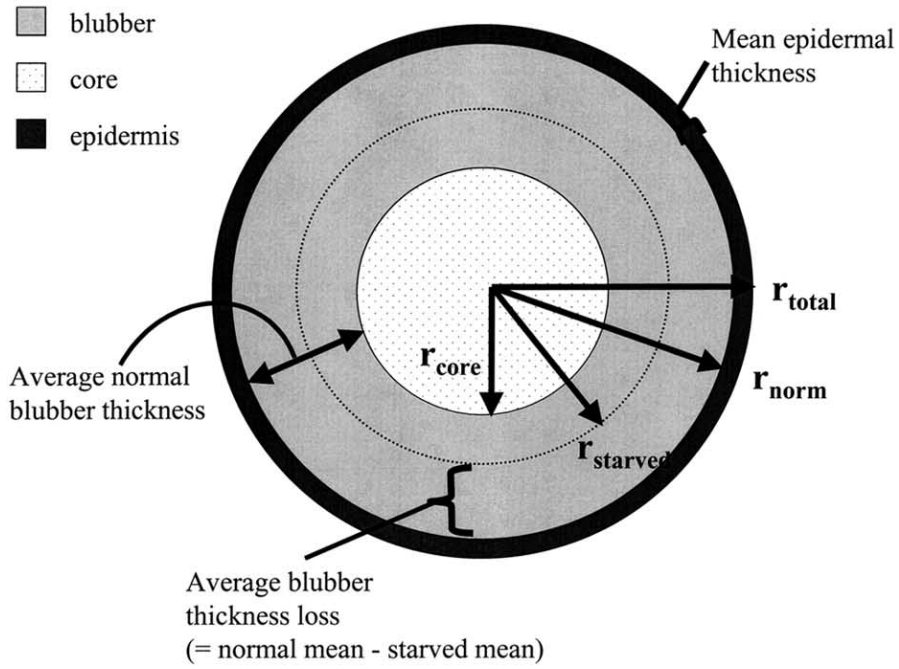
### *Blubber Thickness*

Mean blubber thicknesses of juvenile porpoises varied with both body site and body condition (Table 1; Fig. 3). On the basis of these observed differences, two body regions will be discussed: the thorax (comprising all girths from the axilla to the cranial insertion of dorsal fin) and tailstock (the midkeel and fluke insertion). The greatest differences between porpoises in normal and starved body conditions occurred cranial to the anus (Table 1; Fig. 3, *top*). Starved porpoises had significantly thinner blubber ( $P < 0.001$ ) at all locations cranial to and including the anus, but there were no significant differences ( $P > 0.05$ ) between the two groups caudal to the anus (Table 1; Fig. 3, *bottom*).

The greatest loss of blubber (as percent of normal thickness; relative blubber volume loss [RBVL]) associated with starvation occurred at the midthoracic girth (Table 1), where blubber thickness had decreased by more than 50% in starved porpoises. At most sites cranial to the anal girth, blubber thickness decreased by at least 30% during starvation. Caudal to the anus, the relative losses were much lower (ranging from 2% to 22%), but these losses were not significant (see above paragraph).

### *Loss of Blubber Volume by Starved Porpoises*

As with calculations of relative thickness loss (Table 1), the greatest loss of blubber volume by starved porpoises occurred between the nuchal crest and the anus, and less blubber was lost from the tailstock (Table 2). Starved porpoises had lost more blubber volume at girths where there was more blubber to begin with (i.e., axillary, midthoracic, and cranial insertion of dorsal fin). More than one-fourth (28.1%) of the total blubber volume loss occurred at the midthoracic girth alone, and together the axillary, midthoracic, and cranial insertion of dorsal fin girths combined account for just over 70% of all blubber volume loss. Very little of the overall blubber volume loss came from the tailstock; more than 92% of the cumulative loss oc-



curred cranial to the anus, and less than 1% came from the midkeel girth (Table 2).

#### *Blubber Adipocyte Size and Shape in Normal Porpoises*

Most adipocytes in the blubber of normal porpoises were elongate in shape (e.g., Fig. 4A, 4B) as evidenced by aspect ratios (height/width) well above 1.0 (Table 3). Typically, adipocytes appeared to be organized into irregular rows, all oriented with their major axis (adipocyte height) perpendicular to the epidermal surface (mean angles of major axis were approximately 90° in all groups; Table 3). Adipocytes in the tailstock blubber (Fig. 4C) were smaller than those in the thorax because of lower height values (see Table 3). The cross-sectional area of most adipocytes in normal porpoise blubber ranged between 1,000 and 4,000  $\mu\text{m}^2$ . The thorax blubber contained numerous (7% of the entire population) adipocytes larger than 7,000  $\mu\text{m}^2$ , whereas in the tailstock such large cells accounted for only 0.6% of all adipocytes.

Thorax blubber contained significantly more ( $P < 0.001$ ) adipocytes than the tailstock in all five porpoises in normal body condition (Table 4). Qualitatively, the tailstock blubber appeared to contain more collagen than the thorax (cf. Fig. 4C, 4A).

There were also clear patterns in mean adipocyte size and height through the depth of the blubber in the thorax. Mean adipocyte height increased from the epidermal border to the middle of the transects, with a maximum height occurring just before the deepest (innermost) part of the blubber layer. Mean adipocyte width in the thorax of porpoises in normal condition showed a slight increase from the outer to the inner blubber, but these were relatively small changes compared with the ob-

served changes in adipocyte height. In the tailstock, however, mean adipocyte height and width were consistent through the depth of the blubber. Not surprisingly, adipocyte volume increased dramatically from the outer to the innermost blubber in the thorax but not in the tailstock (Fig. 5). The estimate of total blubber adipocyte complement in normal porpoises ranged from  $3.25 \times 10^{10}$  to  $8.60 \times 10^{10}$ , with an average of  $5.7 \times 10^{10}$  (SE =  $9.2 \times 10^9$ ) adipocytes.

#### *Blubber Adipocytes of Starved Porpoises*

The blubber adipocytes of starved porpoises were also elongate (aspect ratios well over 1.0; Table 3), but there were clear differences in adipocyte characteristics of thoracic blubber between normal and starved porpoises. The thoracic blubber of starved porpoises, for example, did not contain any large adipocytes (maximum cross-sectional area was 6,957  $\mu\text{m}^2$ ). Averaged throughout the depth of the blubber layer, the mean adipocyte area of the thorax of starved porpoises (all  $< 2,700 \mu\text{m}^2$ ) was at least 700  $\mu\text{m}^2$  smaller than the area of the thorax adipocytes of normal porpoises (all means  $> 3,400 \mu\text{m}^2$ ). The mean adipocyte areas of the tailstock blubber of both normal and starved porpoises were also  $< 2,700 \mu\text{m}^2$ . The large mean adipocyte size of the thorax of normal porpoises was likely due to greater adipocyte height rather than width in this group compared with all other blubber samples (see Table 3). Contrary to what was observed in the normal porpoises, the mean estimated adipocyte volume in the thorax of starved porpoises was smaller than that of the tailstock adipocyte (Table 3). The estimated total adipocyte complements for the two emaciated porpoises were  $3.7 \times 10^{10}$  and  $3.87 \times 10^{10}$ .

Although there was much variation in the number of adi-

Figure 2. Schematic representation of variables used to calculate blubber loss. To evaluate the relative contributions of various body regions to total blubber loss during starvation, the volume of blubber loss was estimated at each girth. We used body girths measured from normal porpoises, and calculations based on girths for starved animals were estimated. Body girths were not available lateral to the dorsal fin and at the fluke insertion; thus, they were excluded from the volume calculations. *Top*, The cross section of the body was represented as a circle at all girths (nuchal crest, axillary, midthoracic, cranial insertion of the dorsal fin, caudal insertion of the dorsal fin, and anal) used in this part of the analysis except the midkeel (see bottom panel). Total body radius of normal porpoises ( $r_{\text{total}}$ ) at each girth location was determined from mean girths for normal porpoises. Mean epidermal thickness was removed from the total body radius to yield the normal body radius ( $r_{\text{norm}}$ ). Cross-sectional area ( $\text{area}_{\text{normal}}$ ), including both core and blubber, of normal porpoises at each girth was calculated from  $r_{\text{norm}}$ . The body radius (core and blubber) of starved porpoises ( $r_{\text{starved}}$ ) was calculated by subtracting the mean blubber thickness loss at each girth from  $r_{\text{norm}}$ . The area of the cross section (blubber and core) of starved porpoises could then be calculated as  $\text{area}_{\text{starved}}$ . Because the differences in area are only due to changes in blubber thickness, the cross-sectional area of blubber lost by starved porpoises could be calculated as  $\text{area}_{\text{normal}} - \text{area}_{\text{starved}}$ . To calculate relative losses of blubber volume by starved porpoises as a function of the amount of blubber that was originally present in normal porpoises, the total area of the blubber (excluding the core) of normal porpoises was required. The core radius of normal porpoises ( $r_{\text{core}}$ ) was calculated by subtracting the average normal blubber thickness at each girth (sum of normal thickness means/7) from  $r_{\text{norm}}$  and then used to estimate  $\text{area}_{\text{core}}$ . Total blubber area ( $\text{area}_{\text{blubber}}$ ) at each girth location in a normal porpoise was then  $\text{area}_{\text{normal}} - \text{area}_{\text{core}}$ . To convert area to volume, a 1-cm strip at each of the body locations was assumed ( $\text{vol}_{\text{blubber}} = \text{area}_{\text{blubber}} \times 1 \text{ cm}$ ). The index of blubber volume loss (IBVL) at each girth was estimated from  $(\text{area}_{\text{normal}} - \text{area}_{\text{starved}}) \times 1 \text{ cm}$ . All IBVLs were summed for a body total, and the percentage contribution to total body volume loss that occurred at each of the seven girths was then estimated as  $\text{girth IBVL} / \text{total IBVL}$ . Relative blubber volume loss (RBVL; how much of the blubber volume present at each location in normal porpoises that had been lost by the starved porpoises) at each girth was then estimated by  $\text{IBVL} / \text{vol}_{\text{blubber}}$ . *Bottom*, For the midkeel girth, cross-sectional shape was approximated with a rectangle for the core of the tailstock and with two triangles for the dorsal and ventral blubber keels. IBVL, percentage contribution to total IBVL, and RBVL were calculated from these values as for the six other girths listed above.

Table 1: Measurements of blubber thickness ( $\pm$ SE) at dorsal, lateral, and ventral aspects (i.e., measurements 1, 4, and 7, respectively) of nine body girths in normal and starved juvenile porpoises

Body Girth and Aspect	Normal Mean (mm)	Starved Mean (mm)	Difference (mm)	% Reduction after Starvation
Nuchal crest:				
Dorsal	15.0 $\pm$ .3 (66)	9.8 $\pm$ .4 (25)	5.2*	34.7
Lateral	14.2 $\pm$ .4 (65)	9.6 $\pm$ .4 (27)	4.6*	32.4
Ventral	18.0 $\pm$ .3 (67)	11.5 $\pm$ .5 (25)	6.5	36.1
Axillary:				
Dorsal	20.5 $\pm$ .5 (67)	11.3 $\pm$ .6 (31)	9.2*	44.9
Lateral	19.8 $\pm$ .4 (68)	10.8 $\pm$ .6 (30)	9.0*	45.4
Ventral	20.7 $\pm$ .5 (67)	11.6 $\pm$ .7 (30)	9.1	44.0
Midthoracic:				
Dorsal	20.3 $\pm$ 1.2 (10)	9.6 $\pm$ .6 (8)	10.7*	52.7
Lateral	20.4 $\pm$ 1.9 (9)	9.7 $\pm$ .6 (8)	10.7*	52.5
Ventral	20.2 $\pm$ 1.7 (10)	9.4 $\pm$ .6 (8)	10.8	53.5
Cranial insertion of dorsal fin:				
Dorsal	20.3 $\pm$ .6 (69)	11.3 $\pm$ .5 (31)	9.0*	44.3
Lateral	18.9 $\pm$ .5 (68)	11.2 $\pm$ .7 (31)	7.7*	40.7
Ventral	19.4 $\pm$ .5 (69)	11.5 $\pm$ .6 (31)	7.9	40.7
Lateral to dorsal fin:				
Dorsal <sup>a</sup>	20.0 $\pm$ .6 (68)	11.0 $\pm$ .6 (31)	9.0*	45.0
Lateral	18.9 $\pm$ .5 (69)	10.8 $\pm$ .6 (31)	8.1*	42.8
Ventral	17.9 $\pm$ .5 (65)	11.8 $\pm$ .7 (26)	6.1	34.1
Caudal insertion of dorsal fin:				
Dorsal	30.4 $\pm$ 1.0 (53)	24.2 $\pm$ 1.1 (25)	6.2*	20.4
Lateral	18.3 $\pm$ .5 (69)	11.6 $\pm$ .6 (31)	6.7*	36.6
Ventral	15.9 $\pm$ .5 (65)	12.0 $\pm$ .5 (26)	3.9	24.5
Anal:				
Dorsal	38.4 $\pm$ 1.0 (61)	29.7 $\pm$ 1.0 (30)	8.7*	22.7
Lateral	15.9 $\pm$ .4 (63)	12.0 $\pm$ .5 (31)	3.9*	24.5
Ventral	18.4 $\pm$ .6 (61)	15.5 $\pm$ .6 (30)	2.9	15.8
Midkeel:				
Dorsal	34.3 $\pm$ .8 (61)	31.3 $\pm$ 1.0 (30)	3.0 NS	8.7
Lateral	3.5 $\pm$ .3 (61)	2.8 $\pm$ .2 (28)	.7 NS	20.0
Ventral	21.0 $\pm$ .6 (61)	19.1 $\pm$ .7 (29)	1.9	9.0
Fluke insertion:				
Dorsal	18.8 $\pm$ 1.9 (9)	19.8 $\pm$ 1.3 (8)	-1.0 NS	...
Lateral	1.8 $\pm$ .1 (9)	1.4 $\pm$ .2 (8)	.4 NS	22.2
Ventral	19.0 $\pm$ 1.5 (9)	18.6 $\pm$ 1.3 (8)	.4	2.1

Note. See "Material and Methods" and Figure 1. Numbers in parentheses indicate sample size for each group at each site. The difference ( $d_{ij}$ ) between mean normal and mean starved thicknesses is also reported for each site. NS indicates that the difference between the means was not significant (ventral aspects were not tested in order to reduce the total number of comparisons made).

<sup>a</sup> Dorsal-most recorded measurement, due to presence of dorsal fin.

\* Indicates a significant difference ( $P < 0.01$ ) in mean thickness between normal and starved porpoises at either the dorsal or the lateral aspect of that site.

pocytes present in each of the samples (Table 4), two clear patterns emerged. First, unlike the normal animals, in starved porpoises there was no significant difference ( $P = 0.147$ ) between adipocyte counts in thorax blubber and tailstock blubber. Second, in the thorax blubber, normal porpoises had signifi-

cantly more adipocytes than did starved porpoises ( $P < 0.005$ ), but the tailstock adipocyte counts of normal and starved porpoises were not significantly different ( $P = 0.342$ ).

Compared with the blubber of normal porpoises, starved animals exhibited far less stratification in adipocyte character-

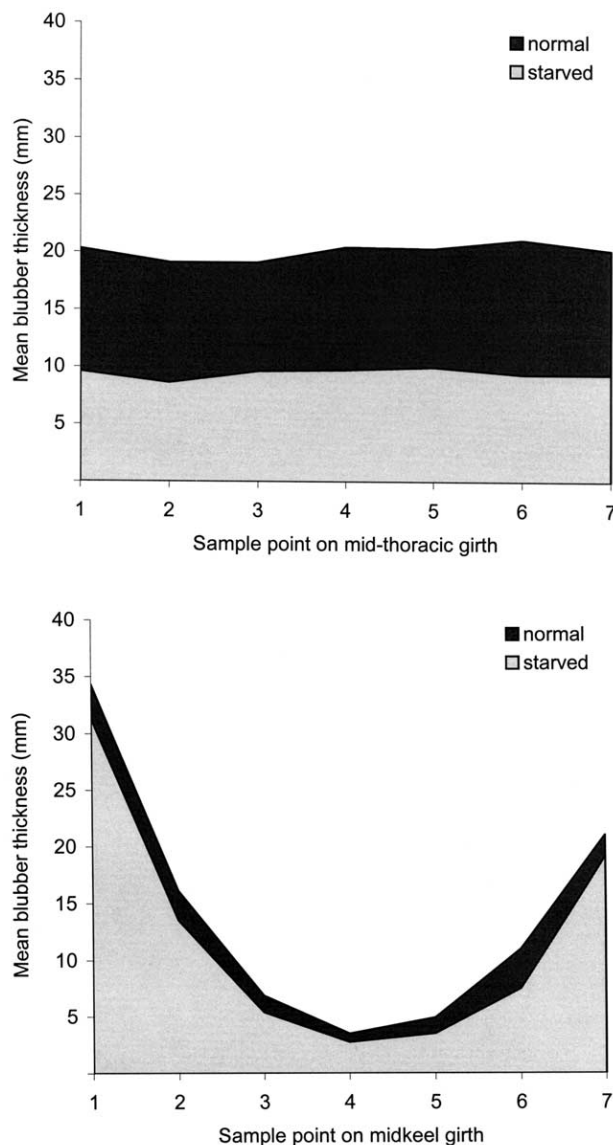


Figure 3. Mean blubber thickness at two representative body girths in normal and starved juvenile harbor porpoises. Measurements were taken at seven equidistant sites, from dorsal (1) to ventral (7). See Figure 1 for positions of girths on body of porpoises and Table 1 for sample sizes. *Top*, Midthoracic (representing the thorax); *bottom*, midkeel (representing the tailstock).

istics throughout the depth of the blubber layer (Fig. 5). In the thorax of starved animals, mean adipocyte volume showed a slight dependence on depth; adipocytes in the outermost blubber were all small (0.03–0.05 nL), increasing to 0.1 nL in the middle of the layer and then decreasing again to 0.03–0.05 nL in the inner blubber (Fig. 5, *top*). The pattern in the tailstock blubber of starved animals was less clear (Fig. 5, *bottom*).

The overall size of the adipocytes in the outer layer of the

thorax blubber of the starved porpoises followed the same pattern as those found in normal animals, from the epidermal border to about 7 mm inward. At this point, the sizes diverged; the adipocytes of the normal porpoises increased in volume by almost a factor of 2, and those of the starved animals decreased (Fig. 5, *top*). Correcting the 7-mm value for shrinkage (14%) and using mean blubber thicknesses from Table 1, this distance represents about 71% of the total thorax blubber thickness of starved porpoises and 39% of that of normal animals.

Adipocyte loss in the thorax blubber by starved porpoises could be traced to the inner layer. Mean cumulative adipocyte counts for the outermost 7 mm of the thorax blubber were similar in normal ( $1,153 \pm 23$ ) and starved ( $1,191 \pm 70$ ) porpoises, but internal to this, adipocyte counts for starved porpoises began to level off, while those for normal animals continued to increase.

## Discussion

### *Blubber Loss Associated with Starvation*

In the thorax of starved animals, blubber thickness decreased to half of that found in healthy porpoises, and blubber volume loss in this body region was considerable. However, blubber loss caudal to the anus was almost negligible (Tables 1, 2; Fig. 3, *top*). The nonuniform decrease in blubber thickness observed may be unusual for small- or medium-sized marine mammals. Like harbor porpoises, the blubber thickness of southern elephant seals is also distributed nonuniformly over the body, with maximum thickness occurring in the middle regions (Slip et al. 1992). During the molting fast of elephant seals, greater blubber thickness loss (10–15 mm) occurs in this middle region, while less loss (5 mm) occurs at either end of the body. Because there is less blubber to begin with at the head and tail, the overall rate of loss is the same at all body regions in these seals. This pattern is quite different from the harbor porpoise, in which blubber is conserved on the tailstock. Like porpoises, fin and sei (*Balaenoptera borealis*) whales store and mobilize blubber differentially from various body regions. However, these large mysticetes use the dorsal flank blubber as their main lipid storage depot, relying on this as an energy source during fasting (Lockyer et al. 1985).

### *Blubber Adipocytes in Normal Porpoises*

The adipocytes in the blubber of harbor porpoises exhibit unusual morphology by being elongate, a characteristic that differs from fat cells in all other animals, which are typically spherical (Pond 1986). Presumably, this columnar shape is imposed and maintained by the highly organized matrix of collagen and elastin fibers that serves as the structural framework of blubber in cetaceans (e.g., Hamilton et al., in press). In wild mammals, adipocytes tend to be relatively large, ranging from 0.1 to 2 nL (Pond 1998). We observed cells as small as 0.001 nL and few



Table 2: Estimates of blubber volume loss in juvenile harbor porpoises during starvation at seven body locations

Girth	Mean Normal Girth (cm)	Area <sub>normal</sub> (cm <sup>2</sup> )	Area <sub>emac</sub> (cm <sup>2</sup> )	IBVL (cm <sup>3</sup> )	% Total Body Volume Loss	Vol <sub>blubber</sub> (cm <sup>3</sup> )	RBVL (IBVL/Vol <sub>blubber</sub> ) × 100
Nuchal crest	52.7	206.1	181.0	25.1	8.8	73.0	34.5
Axillary	75.1	422.7	362.4	60.3	21.2	132.6	45.5
Midthoracic	80.8	492.5	412.6	79.9	28.1	145.5	54.9
Cranial insertion of dorsal fin	79.8	478.4	417.7	60.7	21.4	138.6	43.8
Caudal insertion of dorsal fin	59.7	261.9	224.3	37.6	13.3	104.0	36.1
Anal	41.9	125.5	107.2	18.3	6.5	68.8	26.7
Midkeel <sup>a</sup>	23.7	31.7	29.6	2.1	.7	19.1	11.0

Note. See Figure 1 for sites of blubber thickness measurements and Figure 2 for calculations of index of blubber volume loss (IBVL), relative blubber volume loss (RBVL), and associated calculations.

<sup>a</sup> Blubber cross-sectional area approximated by two triangles and a rectangle rather than a circle as for the other six locations (see Fig. 2, *bottom*).

cells over 1 nL in volume, making porpoise blubber adipocytes comparatively small for a relatively large animal (Pond 1998). Adipocyte volume is believed to vary within the same individual by a factor of 10 in some species (see Pond 1998); porpoises may exhibit even greater variation. Porpoise blubber adipocytes are similar in size to those found in the subcutaneous adipose tissue of polar bears (0.3–1.3 nL; Pond et al. 1992) but are smaller than those of fin whales, which range up to 4 nL (Pond 1998).

The average estimated adipocyte complement in the blubber of juvenile porpoises was  $5.7 \times 10^{10}$ , an order of magnitude greater than the  $4.11 \times 10^9$  value predicted (using a body mass of 28 kg) by the equation given by Pond and Mattacks (1985). Juvenile gray seals possess similar adipocyte complements ( $1.1$ – $2.4 \times 10^{10}$ ) to those observed here for porpoises, but these pinnipeds are more than five times larger, weighing 130 kg. Pond and Mattacks (1988) estimated the adipocyte complement of fin whales to be  $2.2$ – $4.3 \times 10^{12}$ , or two to five times more cells than predicted. Carnivores in general tend to have more numerous, small adipocytes (e.g., Pond and Mattacks 1985), possibly as an adaptation to facilitate mobilization and fattening; cetaceans may represent an extreme example of this strategy, though further research will be necessary to confirm such a hypothesis, considering the limited capacity of the blubber of small cetaceans to be used as an energy source (see “Implications for Harbor Porpoise Energetics”).

#### *Changes in Blubber Adipocytes Associated with a Decline in Body Condition*

It is generally accepted that the adipocytes of weaned mammals (Miller et al. 1983; Pond 1998) and birds (Groscolas 1990) shrink and expand with fasting and fattening rather than changing in number. However, we found fewer adipocytes in the thorax blubber of starved porpoises than in the other blubber samples examined (Table 4). There are four possible explana-

tions for our observations: (1) adipocytes in starved thorax blubber have shrunk so dramatically that they were undetectable at  $\times 10$  magnification; (2) the starved group represents juvenile porpoises possessing inherently low numbers of adipocytes in their blubber; (3) porpoises may exhibit huge variation in adipocyte number both intra- and interindividually, and our small sample of five starved porpoises has captured only animals at the low end of the range; or (4) starved porpoises have lost adipocytes from their thorax blubber during starvation.

It is possible that the adipocytes have shrunk below detectable size. The adipocytes of starved frogs, for example, have been observed to be almost devoid of lipid and in regression to a “near-mesenchymal” state (Zancanaro et al. 1999). If this occurred, however, one would expect to be able to see qualitative increases in the size of the cell membranes, which are very easily seen at  $\times 10$  magnification. No such thickening was observed, suggesting that these counts are representative of the true number.

The other three options are all plausible explanations for our observations, and without further evidence it is impossible to determine which one is occurring in porpoises undergoing starvation. The second explanation cannot be discounted, since our current knowledge of marine mammal adipocyte physiology prevents us from understanding how, when, and under which conditions these cells proliferate in juvenile marine mammals. Postnatal development of adipocyte complement is influenced by many factors, including early nutrition and litter size (Pond et al. 1995). Although there were no significant differences in the cell counts between normal and starved porpoises in the tailstock, suggesting that most juvenile porpoises possess similar adipocyte complements in this region of the body, this generalization may not apply to the thoracic blubber. Currently, we are unable to assess whether the starved sample is comprised of individuals possessing fewer adipocytes, for whatever reason, before the onset of starvation.

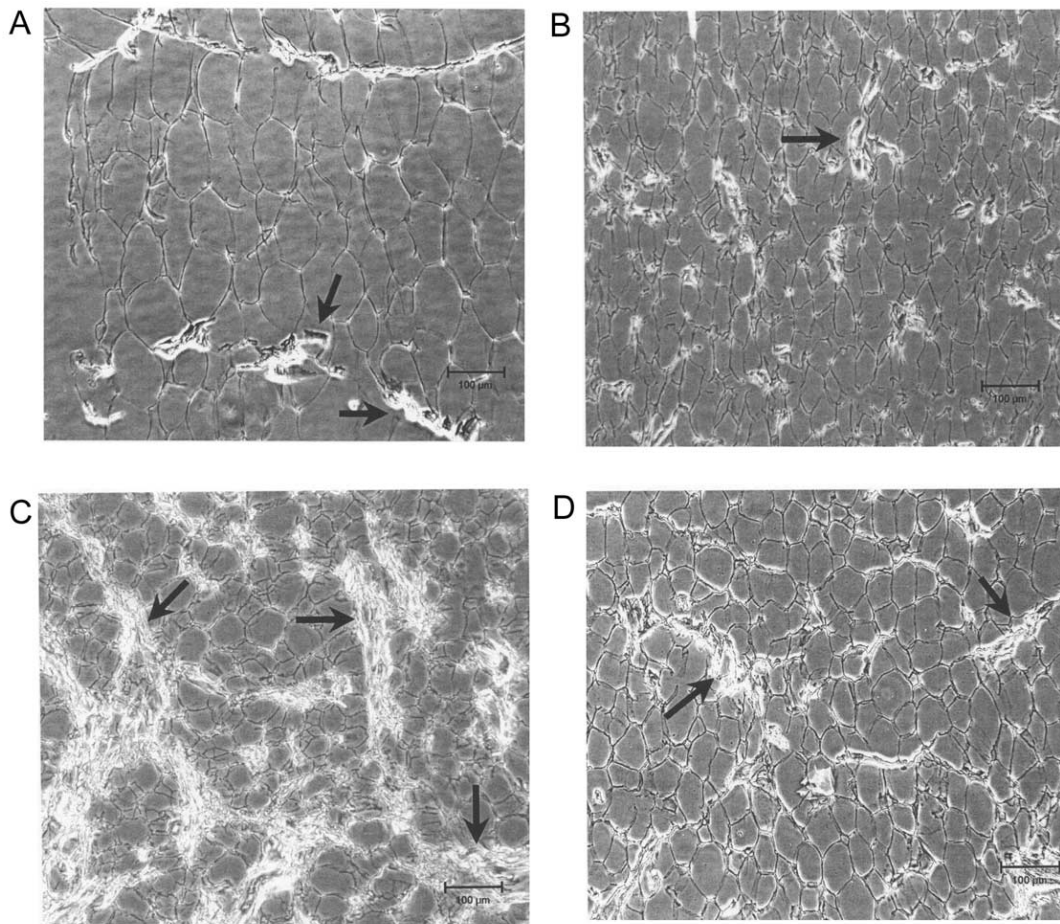


Figure 4. Representative images of blubber adipocytes from harbor porpoises. In all images, the superficial surface is toward the top. Black bars represent 100  $\mu\text{m}$ . Arrows indicate collagen fibers. *A*, Inner thorax blubber of a porpoise in normal body condition. *B*, Outer thorax blubber of a porpoise in normal condition. *C*, Inner tailstock blubber of a porpoise in normal condition. *D*, Inner thorax blubber of a starved porpoise.

The third possibility cannot be ruled out without further examination of a greater number of starved porpoises to establish what the true range of adipocyte complement is in this species.

The last possibility contradicts our conventional understanding of adipocyte metabolism in most mammals, but it is an interesting idea to consider and one that has been postulated previously (Pond 1998). A decrease in the number of adipocytes can occur through reduced production of differentiated adipose cells, or apoptosis (cell death) in preadipocytes (undifferentiated cells) or mature adipocytes (Niesler et al. 1998). Adipocyte proliferation, dedifferentiation of adipocytes, and adipose cell apoptosis can be induced in an artificial setting (see Prins and O'Rahilly 1997), but the specific mechanisms by which mature adipocytes can be eliminated once formed have not been identified (Sjöström 1980; Miller et al. 1983). In hu-

mans, a decrease in the number of adipose cells has been observed to accompany significant weight loss from obese patients (Sjöström 1981). Evidence for whether and how mature adipocytes of wild animals undergo apoptosis is lacking, and apoptosis in adipose cells generally remains poorly understood (Prins and O'Rahilly 1997). One interpretation of our data is that porpoises undergoing starvation may not follow the normal mammalian pattern of maintaining adipocyte cell numbers during periods of natural fasting; rather, porpoises may experience a decline in the adipocyte complement of the inner layers of their thorax blubber in addition to shrinkage of some of these cells. Further investigation, perhaps with other species of small odontocetes, would allow more rigorous assessment of this hypothesis.

There are few comparative data describing changes in adipose cells during fasting or starvation in other animals. At the onset

Table 3: Adipocyte characteristics in normal and starved juvenile harbor porpoises

Body Condition and Site	Mean Cross-Sectional Area ( $\mu\text{m}^2$ )	Mean Perimeter ( $\mu\text{m}$ )	Mean Height ( $\mu\text{m}$ )	Mean Width ( $\mu\text{m}$ )	Mean Aspect Ratio	Mean Angle of Cell Height	Mean Estimated Volume (nL)
Normal:							
Thorax	3,421.7 $\pm$ 42.6	227.5 $\pm$ 1.4	89.9 $\pm$ .6	41.9 $\pm$ .3	2.3 $\pm$ .02	89.5 $\pm$ .3	.173 $\pm$ .004
Tailstock	2,331.3 $\pm$ 40.1	176.7 $\pm$ 1.5	63.9 $\pm$ .5	39.2 $\pm$ .4	1.7 $\pm$ .01	87.8 $\pm$ 1.2	.110 $\pm$ .003
Starved:							
Thorax	2,211.8 $\pm$ 29.7	176.8 $\pm$ 1.2	65.9 $\pm$ .5	36.9 $\pm$ .3	1.9 $\pm$ .02	88.6 $\pm$ .8	.094 $\pm$ .002
Tailstock	2,635.2 $\pm$ 40.7	191.2 $\pm$ 1.5	69.9 $\pm$ .6	40.8 $\pm$ .4	1.8 $\pm$ .02	93.7 $\pm$ 1.0	.127 $\pm$ .003

Note. Values presented are means  $\pm$  SE; each mean represents five samples. Angle of cell height is orientation of longest axis of cell relative to epidermal surface (i.e., 90° is perpendicular to outer body surface so that cells are perfectly columnar).

of the breeding fast, the distribution of adipocyte sizes in emperor penguins is bimodal, split between a small-size population (average cell diameter 25  $\mu\text{m}$ ) and a large one (average diameter 85  $\mu\text{m}$ ; see Groscolas 1990). Over the course of the 3–4-mo fast, the average cell size decreases, and there is a progressive decrease in the proportion of large adipocytes. However, in emperor penguins the decrease in adipose tissue during fasting is attributed mainly, if not entirely, to decreases in cell size rather than cell number (Groscolas 1990).

It is clear from the adipocyte data that the reduction in thorax blubber thickness observed in starved porpoises does not occur uniformly across blubber depth. This leads to a hypothesis of a metabolically “inert” outer blubber and “dynamic” inner blubber, which is supported by two lines of evidence: the maintenance of adipocyte size and shape in the outer 7 mm of blubber (Fig. 5, *top*) and estimates of adipocyte number in this outer tissue. In the outermost layer of the thorax, the blubber of starved porpoises is virtually indistinguishable from that of normal animals. Nearer the body core, however, blubber adipocytes shrink and disappear during starvation. In contrast, starvation appears to have had little or no effect on the adipocytes of the tailstock blubber of starved porpoises.

This “blubber layer” hypothesis is supported by previous fatty acid analyses of the blubber of male harbor porpoises showing that the inner blubber of the thorax is the site of deposition and mobilization, while the outer blubber of the thorax and both layers of the tailstock are more metabolically inert (Koopman et al. 1996). In addition, starvation in juvenile porpoises is associated with significant changes in fatty acid composition of the inner, but not the outer, thorax blubber or the blubber on the tailstock (Koopman 2001). Thus, the outer layer of the thorax blubber appears analogous to the adipose in the dermis of the skin of other mammals, which is not depleted during fasting or starvation (Pond 1998). The inner layer of the thorax blubber is therefore similar to the readily mobilizable adipose layer overlying the superficial muscles in terrestrial mammals (Pond 1998). In harbor porpoises and perhaps other small cetaceans, we can postulate that blubber has differentiated from its adipose tissue precursor into several compartments that appear to operate independently.

#### Implications for Harbor Porpoise Energetics

Changes in blubber’s structure and its thickness distribution accompanying a decline in body condition help to determine the function of this complex feature of marine mammals. On the basis of the data presented here, we conclude that the primary energy store in porpoise blubber is located in the inner blubber of the thorax. Mobilization of lipids from the inner layer of the thorax may be facilitated by the temperature gradient that exists through the depth of the blubber (see Castellini 2002). The inner layer is warmer, experiencing higher blood perfusion. In contrast, the outer layer of the thorax and the tailstock are colder because of reduced blood flow (to conserve heat; see Pond 1998; Pabst et al. 1999b), which would act as an impediment to lipid mobilization. The thorax is also the location of internal organs and most of the axial muscle. Blubber in this region, therefore, must play a role in insulation. Yet the importance of the structural collagen framework in the thorax blubber cannot be overlooked: adipocytes in the inner blubber shrink in such a way that body girth decreases even as the smooth, streamlined contour of the blubber is maintained, suggesting a balance between various functions.

The tailstock blubber contains less lipid and more collagen than the thorax blubber (Fig. 4A, 4C; see also Koopman 2001). This extra protein provides additional evidence of one of the

Table 4: Cell counts in a 400- $\mu\text{m}$ -wide strip transect through the depth of the blubber layer of normal and starved juvenile harbor porpoises

Body Condition and Site	Mean Cell Count	Cell Count Range
Normal:		
Thorax	2,348.8 $\pm$ 198.2	1,661–2,894
Tailstock	950.6 $\pm$ 142.3	605–1,430
Starved:		
Thorax	1,486.2 $\pm$ 106.3	1,247–1,880
Tailstock	1,206.0 $\pm$ 209.3	686–1,825

Note. Values presented are means  $\pm$  SE; each mean represents five samples.

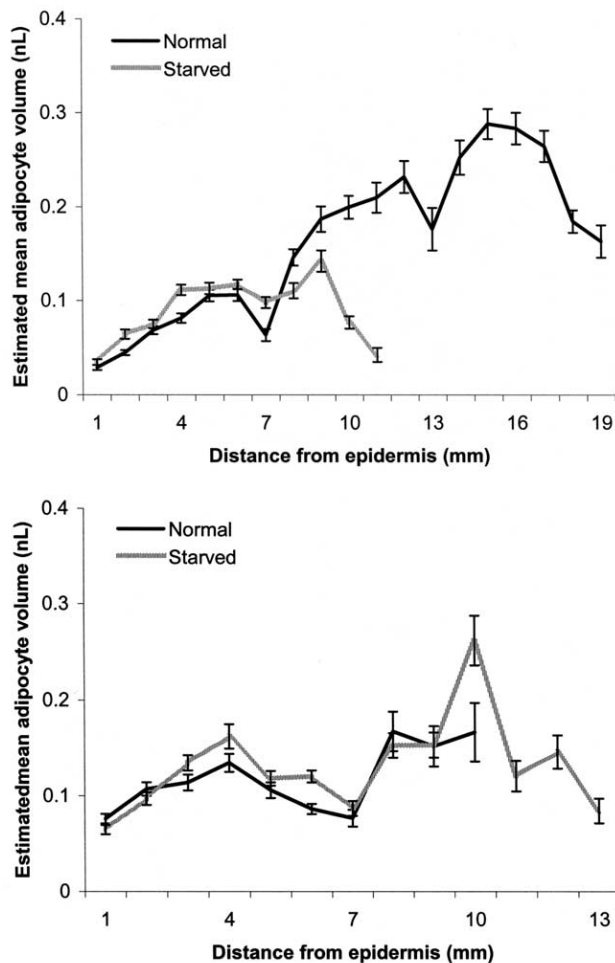


Figure 5. Variation in mean adipocyte volume (estimated; see “Material and Methods”) throughout the depth of the thorax and tailstock blubber of normal ( $n = 5$ ) and starved ( $n = 5$ ) juvenile harbor porpoises. The epidermis is located at 0 mm for reference; all other distances are measured from the epidermis moving inward to the blubber/muscle interface. Total distances (i.e., blubber depths) of the samples were not uniform; thus, lines from different conditions/sites are of different lengths. Bars indicate standard error of the mean. *Top*, Thorax; *bottom*, tailstock.

biomechanical functions of the tailstock blubber, that of streamlining the caudal body (Pabst et al. 1999b; Hamilton et al., in press). Altering the structure of the tailstock blubber by mobilizing its lipids may also have serious effects on locomotory efficiency, since the structure of this region of the blubber serves to reduce the cost of locomotion by acting as a biological spring in small odontocetes (Pabst 1996; Pabst et al. 1999a). Such compartmentalization of function and metabolism is yet another example of the evolution of blubber into a specialized, multifaceted tissue in marine mammals.

In all likelihood, starved porpoises have depleted significant amounts of lean tissue (e.g., skeletal muscle; see Koopman 2001; Stegall 2001), which would, as in other animals, bring about death before all blubber lipids available for mobilization were used. Like other small marine mammals (Pond 1998), porpoises possess few and small internal adipose depots. Stored energy in the form of lipid must come almost exclusively from what is available in the blubber. On the basis of the characteristics of the outer layer of the thorax blubber and the blubber of the tailstock, we hypothesize that the tailstock and outer layer of the thorax blubber represent structural tissue (see below) rather than energy storage depots and that the blubber remaining on starved porpoises was not destined for lipid mobilization. If this is true for porpoises, then the blubber lipid potentially available for mobilization is far less than that contained in the total mass of blubber. It is possible to estimate the proportion of potentially dynamic blubber in an average 28-kg juvenile porpoise, which possesses on average 11 kg of blubber. Correcting this value by 15% to account for the contribution of the epidermis (H. N. Koopman, unpublished data) yields 9.35 kg. Weighting this mass for relative proportions of thorax and tailstock blubber (Table 2) and assuming that the tailstock is inert, as is the outer 39% of the thorax blubber (see “Blubber Adipocytes of Starved Porpoises”) leaves 4.9 kg of blubber for potential use as mobilizable energy, or less than half of the original 11 kg blubber mass. Consequently, the blubber mass of a harbor porpoise is not equivalent to its total fat stores, and the “inert” components of the blubber should be considered to be structural/mechanical rather than energetic/metabolic.

Structural adipose tissue is characterized by smaller cells as compared with storage depots, with different arrangements and proportions of collagen and adipocytes (Pond and Mattacks 1986; Pond 1998). The adipose tissue around the eye socket, in the joints of limbs, and in the paws of mammals contains relatively large quantities of collagen and is recognized as being structural in nature (Pond 1987). This component of adipose tissue is never depleted even in severe starvation and does not expand as a consequence of obesity (Pond 1978, 1987, 1998). The outer thorax blubber and the tailstock blubber of porpoises exhibit these characteristics, indicating that they are indeed structural in nature and should not be thought of as storage depots.

This means, however, that conventional definitions of body condition (i.e., using blubber mass in marine mammals; e.g., Read 1990; Beck et al. 1993) may not be appropriate for all animals. Yet even in animals adapted for routine fasting, such as phocid seals, the blubber layer is never depleted entirely (e.g., Nordøy et al. 1992). If body condition is defined as “a measure of an animal’s energy reserves” (Hanks 1981), then it might be more accurate to estimate body condition in these animals on the basis of the energy that is actually used, for example, the maximum blubber loss during fasting, in phocids (or starva-

tion, in small odontocetes). Measured this way, the 4.9 kg of “mobilizable” body fat in the average 28-kg juvenile porpoise represents only 17.5% of body mass rather than the 39% if total blubber mass is used.

Blubber’s polyphyletic origin suggests that the adipose tissues of mammalian ancestors may have been extremely complex, in turn laying the groundwork for complex functional organization at the cellular level. Yet even with such constraints, all blubber is not constructed or used the same way. The fundamental structure and metabolism of blubber exhibit functional adaptations, perhaps in response to environmental, energetic, and morphological pressures imposed on different groups of marine mammals above and beyond the influence of phylogenetic lineage. The blubber of harbor porpoises exhibits regional specialization of function, with significant portions of the blubber representing nonmobilizable, structural adipose, so that there are trade-offs between blubber’s roles in insulation, energy storage, streamlining, locomotion, and buoyancy. These limitations are likely applicable to other small odontocetes sharing similar body size, habitat, and thermoregulatory constraints.

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