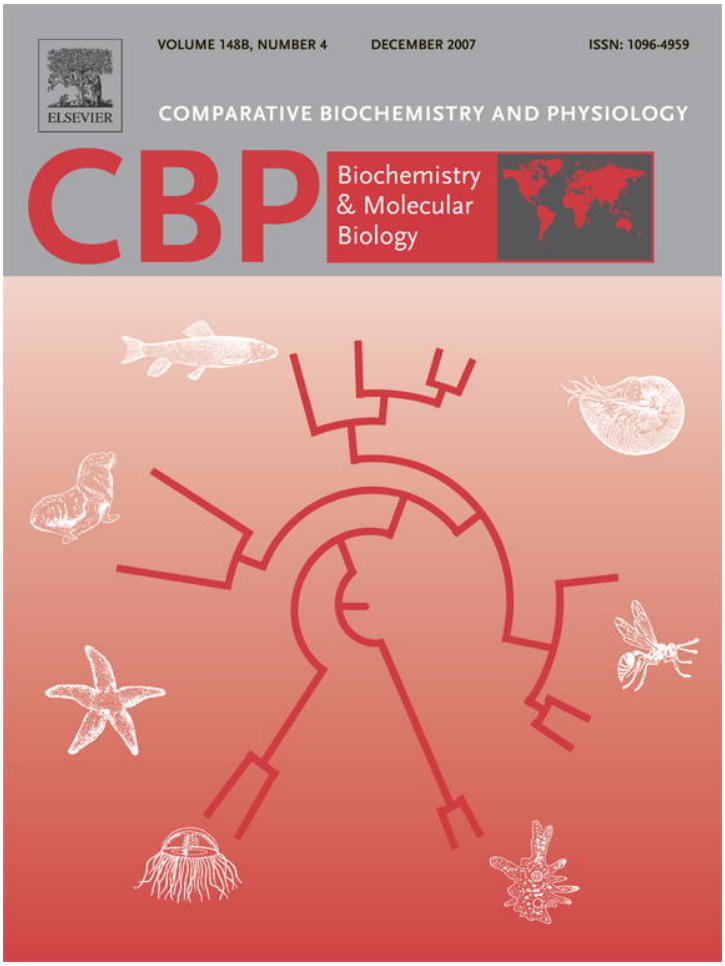

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Vitamin A and E tissue distribution with comparisons to organochlorine concentrations in the serum, blubber and liver of the bowhead whale (*Balaena mysticetus*)

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Abstract

Vitamin A and E concentrations were determined in liver ($n=51$), blubber ($n=23$) and serum ($n=53$) of subsistence-hunted bowhead whales (*Balaena mysticetus*), between 1998 and 2001. Retinol and alpha-tocopherol were the major forms of vitamins A and E detected, respectively. Liver contained the highest mean concentrations of vitamin A, followed by epidermis, blubber, and serum. Liver also contained the highest mean concentration of vitamin E, followed by serum, epidermis, and blubber. Stratification of retinol and tocopherol was examined throughout the blubber cores collected. Retinol concentrations were significantly higher in the epidermis than in the deeper blubber layers. Tocopherol concentrations were similar for epidermis and the intermediate layer of blubber. Both the epidermis and the intermediate layer of blubber had significantly higher tocopherol concentrations than the innermost and outermost blubber layers. Vitamin A and E concentrations were investigated with respect to gender and reproductive status of females (males, non-pregnant females, pregnant females), age groups and season of harvest. Certain persistent organic contaminants are known to have a negative effect on retinol concentration in serum of pinnipeds and cetaceans. Bowhead whales have relatively low concentrations of organochlorines (OCs) in comparison to other mysticete species. The relationships between serum, liver and blubber retinol and serum and blubber OC concentrations were examined with no significant correlations noted.

Keywords: Alpha-tocopherol; *Balaena mysticetus*; Biomarker; Bowhead whale; Cetacean; Organochlorine; Retinol; Vitamin A; Vitamin E

1. Introduction

Vitamin A is necessary to support the growth, health and life of mammals, and a state of deficiency or excess of this vitamin can constitute a serious threat to health (Machlin, 1991; Geraci, 1981; McDowell, 2000). This fat-soluble vitamin plays a direct role in the maintenance of vision and epithelial tissues, reproduction, bone development and immune system function (Machlin, 1991; McDowell, 2000). In most species, there is a strong homeostatic control of serum vitamin A with levels commonly maintained within a normal range for the species until liver stores are exhausted. For this reason, serum levels of vitamin A must be interpreted with caution, with low levels being useful mainly as an indication of deficiency (McDowell, 2000). The livers of terrestrial mammals can contain up to 90% of the total body vitamin A, with the remainder stored in

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kidneys, lungs, adrenals, blood and other organs (Machlin, 1991). In certain pinniped species, this distribution differs with the blubber storing a greater proportion of vitamin A than found in the liver (Schweigert et al., 1987; Mos and Ross, 2002). Zinc deficiency is known to increase vitamin A requirements in domestic animals (McDowell, 2000). There are no published reports of pathological lesions directly attributable to vitamin A excess or deficiency in cetaceans, but one can predict that vitamin A and related compounds are essential but also potentially toxic. Understanding the dynamics of vitamin A mobilization and its distribution in tissues is important in light of recent attempts to employ retinol as a biomarker of organochlorine exposure (Rolland, 2000; Simms and Ross, 2000, Borrell et al., 2002, Tornero et al., 2004b).

Organochlorine contaminants (OCs) are ubiquitous in the environment and have been reported in the blubber of marine mammals worldwide (O'Shea, 1999, AMAP, 2002). These compounds are known to bioaccumulate in the lipids of marine mammals and are present in increasing amounts in the Arctic (AMAP, 2002). Previous studies have demonstrated a negative correlation between organochlorine (OC) and retinol concentrations in marine mammal tissues secondary to the binding of OC to transthyretin (Brouwer et al., 1989; de Swart et al., 1994; Simms and Ross, 2000; Jenssen at el., 2003). Monitoring the relationship between these substances is important as OCs possess the potential to adversely affect marine mammal health (de Swart et al., 1994; O'Shea, 1999; AMAP, 2002) and are known to affect metabolism, reproduction, endocrine function and the immune system of marine mammals (Dierauf and Gulland, 2001).

Vitamin E, another fat-soluble vitamin, is essential for integrity and optimal function of reproductive, muscular, circulatory, nervous and immune systems (Hoekstra, 1975; Sheffy and Schultz, 1979; McDowell, 2000). Vitamin E has complex interactions in the body involving selenium and the sulfur-bearing amino acids cystine and methionine and also functions as an important biological antioxidant. Vitamin E is involved in membrane structure, prostaglandin synthesis, blood clotting, disease resistance and regulation of DNA synthesis. Dietary requirements depend upon many factors, including levels of poly-unsaturated fatty acids (PUFA), selenium, sulfur amino acids and other antioxidants in the diet (McDowell, 2000). Marine mammals are highly dependent upon adequate vitamin E in their diet to protect their body tissues against oxidative stress (Debier et al., 2002). Selenium exerts a sparing effect on vitamin E and delays onset of deficiency signs, while vitamin E serves a similar role in protecting against selenium deficiency (Machlin, 1991; McDowell, 2000).

The bowhead whale (*Balaena mysticetus*) is an endangered mysticete species that lives in Arctic and sub-Arctic waters. Although the Bering–Chukchi–Beaufort Sea (BCBS) Stock was commercially exploited to near extinction in the late 1800's, it is currently growing (George et al., 2004) and is an important subsistence species to residents of northern Alaska, Russia and Canada. The BCBS whales undertake a yearly migration from the Bering Sea to the Beaufort Sea to reach the highly productive summer feeding grounds located there, providing the opportunity to obtain high quality biological samples from the Inuit subsistence hunt in the spring and fall.

This study measured the levels of vitamins A and E in serum, liver, epidermis and blubber (at multiple depths) of subsistence-harvested bowhead whales obtained from 1998–2001. We evaluated the relationships between vitamin concentration and gender, season of collection, age class, and reproductive status. We also examined as the associations between vitamin concentrations and percent lipid and concentrations of OC contaminants and selected essential elements.

2. Materials and methods

2.1. Field techniques and blood collection

Blubber, liver and blood samples were collected during the Inuit subsistence hunt in Barrow, Alaska (spring and fall) from 1998–2001. These sample collections were conducted with permission of the Barrow Whaling Captain's Association and the Alaska Eskimo Whaling Commission through the Department of Wildlife Management (North Slope Borough, Alaska) under the purview of a National Oceanic and Atmospheric Administration (NOAA) permit [#932-1489-00 and 932-1489-03 for the Marine Mammals Health and Stranding Response Program]. Blood was collected as soon after death as possible (typically within 2–14 h) from the palatal sinus into untreated red top vacuum tubes (Vacutainer/BD, Franklin Lakes, New Jersey USA). The blood was allowed to clot and centrifuged for 10 min at 3500 g within 4–6 h of collection. The serum was then removed and frozen immediately at −20 °C (Arctic Research Facility, Barrow, Alaska USA) during the remainder of the field season (~1 month) and then at −80 °C in the laboratory in a light-proof container until thawed for analysis (University of Alaska Fairbanks, Fairbanks, Alaska USA). Blubber samples were taken from an area approximately one meter caudal to the blowhole on the dorsal aspect of the whale (BD=blowhole dorsal). Full thickness samples (epidermis to internal muscular layer) were collected. The epidermis was removed at the level of the papillary dermis and the remaining adipose tissue was divided into fifths of equal depth (Fig. 1). The epidermis and the

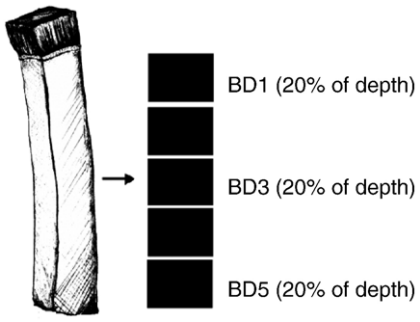

Fig. 1. Blubber core sampling scheme in the bowhead whale. Each core had the epidermis removed (this was analyzed separately) and the remaining blubber was measured and divided into 5 equal sections, each representing 20% of the core. The first (BD1), third (BD3) and fifth (BD5) section (BD1 being closest to the epidermis and BD5 being closest to the internal muscular layer underlying the blubber) were analyzed.

first (BD1), third (BD3) and fifth (BD5) section (BD1 being closest to the epidermis and BD5 being closest to the internal muscular layer underlying the blubber) were analyzed. Liver samples were taken from a central portion of the organ that was most immediately available during butchering.

2.2. Serum analysis

Vitamins A and E were extracted and analyzed using a high-performance liquid chromatography (HPLC)/ultraviolet detection method described in Mazzaro et al., 2003, with some modifications. The volumes of bowhead serum and extraction solvent (methanol) were doubled due to the relatively low concentrations of vitamins in this sample matrix. The internal standard (IS), delta-tocopherol, was added to each sample tube and the residue was dissolved in 100 µl propanol. Additionally, a Microsorb C18 resolve column (Varian, Inc., Palo Alto, California USA) protected with an Upchurch C18 guard/column (Upchurch Scientific, Oak Harbor, Washington USA) was used to separate the vitamins, and detection was 325 nm for retinol and 290 nm for tocopherols.

2.3. Liver samples

Liver vitamins A and E were extracted by adding 1 ml sodium ascorbate and approximately 0.1 g liver to 16×100 disposable, borosilicate glass test tubes (VWR Scientific, West Chester, Pennsylvania USA). This mixture was then homogenized using a polytron (Brinkman Instruments, Westbury, New York USA). During homogenization, 2 ml ethylenediaminetetraacetic acid (EDTA) was added to the mixture, along with 1 ml potassium hydroxide (KOH) (50% potassium hydroxide—50 g KOH in 100 ml methanol) and 500 µl IS (delta-tocopherol). This total mixture was then mixed using a vortex, capped and incubated in water bath for 15 min at 70 °C to saponify the sample. After saponification, the liver mixtures were cooled on ice and 1–2 crystals of butylated hydroxyanisole (BHA) were added to each tube followed by 3 extractions with 2 ml hexane. The mixture was then completely dried under a gentle stream of nitrogen. The final product was reconstituted with 250 µl propanol/dichloroethane (80/20) and analyzed by high-performance liquid chromatography (HPLC). Ten µl of sample was injected onto a Varian C18 column. Methanol/dichloromethane 80/20 was used as the mobile phase at a flow rate of 1.5 ml/min. All samples were analyzed in triplicate.

2.4. Epidermal/blubber vitamin analysis

Epidermis: approximately 0.25 g epidermis, 1 crystal of BHA, 2.5 ml KOH (100 ml ethanol/16 g KOH) and 100 µl of IS (delta-tocopherol) were added to a 20 ml screw top glass test tube.

Blubber: 0.25–0.30 g of blubber, 1 crystal of BHA, 2.5–3.0 ml KOH (100 ml ethanol/16 g KOH) were added to a 20 ml screw top glass test tube.

The mixtures were saponified in a water bath at 80 °C for 30 min and then cooled in cold water for 5 min. Distilled water (2.5–3.0 ml) was added to each sample mixture and the hexane layer was removed. Two extractions were then performed by adding 2 ml hexane. The remaining material was vortexed for 3 min and then centrifuged for 5 min at 2500 rpm. The mixture was evaporated under a gentle stream of nitrogen and reconstituted with 100 µl methanol/dichloromethane (9:1). The sample (50 µl) was then injected into HPLC column (5 um long column) with guard column (5 um 150×4.6 mm×1/4″ Varian C18 resolve column), at a flow rate of 1.5 mL/minute using a mobile phase of methanol/water (98/2). Samples were analyzed in triplicate using the following standards: Retinol (325 nm), delta-tocopherol (298 nm), alpha-tocopherol (292 nm). Detector was set at 325 nm and 290 nm, respectively. An external standard was used for all samples.

2.5. Organochlorine analyses

The OC dataset used in this study was previously reported in Hoekstra et al. (2002).

The twenty PCB metabolites and OC congeners occurring in highest mean concentrations in serum, blubber and liver were selected for multivariate analysis (canonical correlation)(see Appendix). These organochlorines were detected at low levels in most whales and many are known to have a relatively high affinity for the T4 receptor on the transthyretin (TTR) carrier (Ishihara et al., 2003).

2.6. Age determination

Stable isotopes of carbon signature analyses of baleen and aspartic acid racemization of eye lens nuclei were used independently to determine age (George et al., 1999, Rosa et al., 2004, Lubetkin et al., 2004) in the majority of whales (36/58). The remaining whales in this study were designated juvenile, subadult, or adult using a combination of body length, baleen length and gonadal size/development (Table 1). Adults were considered to be sexually mature via histologic and morphologic assessment of reproductive tissues.

2.7. Essential element analysis

Selenium and zinc were analyzed according to methods detailed in Dehn et al., 2005.

2.8. Blubber lipid quantification

Analyses of lipid percentage for each blubber layer (except epidermis) were conducted according to methods described in Krahn et al., 2001 and Ylitalo et al., 2005.

Table 1
Bowhead whale age group definitions as determined via aspartic acid racemization, carbon baleen isotope ($\delta^{13}C$)measurement and histological analyses

Juvenile	1–3 year old whales that experience an accelerated period of growth
Subadult	3 years of age to ~22 years of age in males and ~25 years of age in females (sexually immature)
Adult	Greater than ~22 years of age in males and ~25 years of age in females (sexually mature)

(George et al., 1999, Lubetkin et al., 2004, Rosa et al., 2004).

Table 2
Retinol concentrations in the serum, liver, epidermis and blubber of the bowhead whale

	Serum (μg/ml)	Liver[a] (μg/g)	Epidermis (μg/g)	BD-1 (μg/g)	BD-3 (μg/g)	BD-5 (μg/g)
Adult						
Female						
Non-pregnant	0.10 (*n*=1)	7568.67±3801.36 (*n*=3)	–	1.87 (*n*=1)	0.06 (*n*=1)	0.09 (*n*=1)
Pregnant	0.09±0.02 (*n*=4)	4385.50±3105.92 (*n*=4)	2.93 (*n*=1)	–	–	–
Male	0.09±0.04 (*n*=6)	7261.33±3134.62 (*n*=6)	1.47±0.81 (*n*=2)	3.09±0.49 (*n*=2)	1.79±2.31 (*n*=2)	0.99±1.15 (*n*=2)
Subadult						
Female	0.10±0.03 (*n*=6)	5349.80±4151.83 (*n*=5)	1.96±0.40 (*n*=2)	1.98±0.54 (*n*=4)	1.51±1.67 (*n*=4)	1.12±0.96 (*n*=4)
Male	0.10±0.03 (*n*=5)	5132.40±4971.06 (*n*=5)	1.67±1.17 (*n*=2)	0.64±0.92 (*n*=3)	0.16±0.06 (*n*=3)	0.09±0.13 (*n*=2)
Juvenile						
Female	0.09±0.02 (*n*=14)	3040.06±1830.55 (*n*=18)	1.50±0.59 (*n*=10)	1.44±0.74 (*n*=9)	0.18±0.11 (*n*=10)	0.17±0.12 (*n*=10)
Male	0.09±0.03 (*n*=17)	4362.80±2747.15 (*n*=10)	2.89±2.89 (*n*=5)	1.49±0.23 (*n*=3)	1.07±0.88 (*n*=3)	1.09±1.21 (*n*=2)

Blubber samples include BD1 (outermost depth), BD3 (middle depth) and BD5 (innermost depth). Mean±standard deviation followed by sample size in class in parentheses below the value.

[a] Denotes a significant difference for this variable between non-pregnant adult, subadult and juvenile age classes with adults>subadults>juveniles=pregnant females.

2.9. Statistical analyses

Data are presented as the mean and standard deviation (SD). All data were analyzed by a three-way analysis of variance (GLM, general linear model) using the SAS® system (SAS Institute Inc, Cary, North Carolina USA) with interaction terms (age class * sex * season). Multivariate relationships were investigated via cannonical correlation, also using SAS. Wilks' lambda was used to test the significance of the first canonical correlation and a likelihood ratio test was used to test the linear relationship between the canonical variables. For the few samples with undetectable levels of OCs (n=16, 4%), the mean

Table 3
Alpha-tocopherol concentrations in the serum, liver, epidermis and blubber of the bowhead whale

	Serum (μg/ml)	Liver (μg/g)	Epidermis (μg/g)	BD-1 (μg/g)	BD-3[a] (μg/g)	BD-5 (μg/g)
Adult						
Female						
Non-pregnant	20.26 (*n*=1)	678.67±275.62 (*n*=3)	–	8.17 (*n*=1)	12.42 (*n*=1)	15.55 (*n*=1)
Pregnant	25.37±14.55 (*n*=4)	587.25±715.89 (*n*=4)	19.09 (*n*=1)	–	–	–
Male	21.21±14.65 (*n*=6)	954.83±690.62 (*n*=6)	20.84±10.54 (*n*=2)	1.75±2.47 (*n*=2)	34.61±14.97 (*n*=2)	15.58±5.20 (*n*=2)
Subadult						
Female	19.63±14.39 (*n*=6)	920.40±444.20 (*n*=5)	15.99±0.11 (*n*=2)	8.05±7.48 (*n*=4)	10.15±7.53 (*n*=4)	6.41±4.32 (*n*=4)
Male	19.49±12.16 (*n*=5)	546.00±351.71 (*n*=5)	15.67±6.60 (*n*=2)	11.77±4.95 (*n*=3)	14.19±7.48 (*n*=3)	21.91±15.70 (*n*=2)
Juvenile						
Female	17.97±10.38 (*n*=14)	396.16±335.98 (*n*=18)	16.15±4.67 (*n*=10)	11.71±11.43 (*n*=10)	16.08±10.24 (*n*=10)	10.86±7.56 (*n*=10)
Male	16.50±9.81 (*n*=17)	708.00±512.90 (*n*=10)	19.12±10.01 (*n*=5)	8.21±4.06 (*n*=3)	13.46±2.71 (*n*=3)	15.66±18.02 (*n*=2)

Blubber samples include BD1 (outermost depth), BD3 (middle depth) and BD5 (innermost depth). Mean±standard deviation followed by sample size in class in parentheses below the value.

[a] Adults had a significantly higher alpha-tocopherol concentrations than subadults or juveniles in the BD3 blubber layer.

was calculated using a value one-half the minimum detectable level for those undetected values (Gilbert, 1987). A probability of <0.05 was considered significant.

3. Results

Tables 2 and 3 summarize the retinol and tocopherol results from samples collected in the spring and fall during the four years. The form of vitamin A recovered was retinol [no Vitamin A_2 (didehydroretinol) was detected]. No retinyl esters were found in the serum. The only form of vitamin E recovered was alpha-tocopherol (γ-and δ-tocopherol were not detected). In general, males had significantly higher mean concentrations of hepatic retinol than females [5374.20 μg/g ($n=21$), 4057.30 μg/g ($n=30$), respectively, $P=0.04$]: however, interactions for sex * season and sex * age class were not significant ($P=0.88$, 0.99, respectively). Liver retinol was significantly higher in the spring-landed versus fall-landed whales [6192.2 μg/g ($n=22$), 3391.3($n=29$) μg/g, respectively, $P=<0.0001$]. Non-pregnant female and male adults had the highest mean concentration of liver retinol, with the subadults of both sexes, pregnant females and juveniles of both sexes following in decreasing order (sample sizes did not allow statistical testing of pregnant versus non-pregnant females). The age classes (non-pregnant adults > subadults > juveniles = pregnant females) had significantly different liver retinol levels (Table 2). There were no significant differences between age class, sex or seasonal groupings with respect to vitamin E concentrations, with the exception of the intermediate layer of blubber described below. Mean tocopherol concentration in the intermediate blubber layer (BD3) was significantly higher in adults than in subadult or juvenile animals.

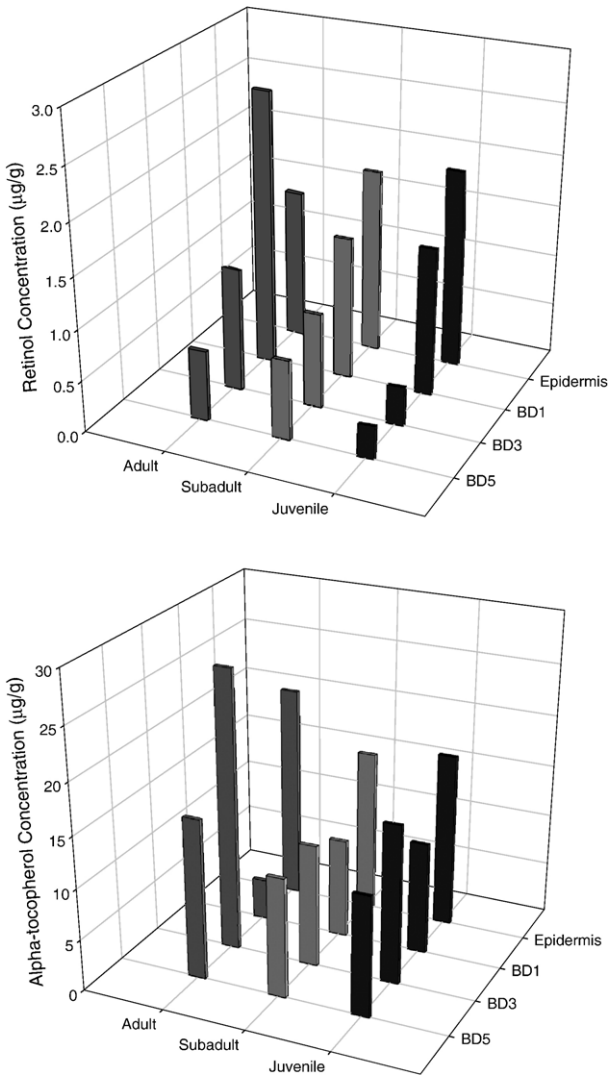

Fig. 2. Distribution of retinol and alpha-tocopherol in blubber layers of juvenile, subadult and adult bowhead whales. Samples include epidermis and blubber: BD1 (outermost depth), BD3 (middle depth) and BD5 (innermost depth). Mean retinol concentration (across all age/sex/seasonal groups) was significantly higher in the epidermis than in the deeper blubber layers. Tocopherol was significantly higher in epidermis (17.24 ± 5.98 μg/g) and the intermediate layer of blubber (BD3: 15.91±10.30 μg/g) than in the innermost and outermost blubber layers.

Vitamin concentrations by blubber depth are summarized in Fig. 2. Mean retinol concentration (across all age/sex/seasonal groups) was significantly higher in the epidermis (1.65± 0.64 μg/g) than in the deeper blubber layers (BD1: 1.60± 0.9 μg/g, BD3: 0.69±1.06 μg/g, BD5: 0.50±0.69 μg/g). Tocopherol was significantly higher in epidermis (17.24±5.98 μg/g) and the intermediate layer of blubber (BD3: 15.91±10.30 μg/g) than in the innermost and outermost blubber layers (BD1: 9.60±8.60 μg/g, BD5: 12.19±8.63 μg/g).

No correlation was found between hepatic Zn and serum vitamin A concentrations ($r=-0.26$, $P=0.23$). A strong positive correlation was found between liver tocopherol and renal Se concentration ($r=0.76$, $P=0.0003$). Positive correlations were also noted between hepatic Zn and hepatic retinol ($r=0.64$, $P=0.0003$) and tocopherol levels ($r=0.58$, $P=0.0012$). There was a positive correlation found between retinol and tocopherol in both the liver and serum samples ($r=0.65$, $P=<0.0001$, $r=0.64$, $P=<0.0001$, respectively). There was no correlation between these two vitamins in the blubber at any depth (BD1: $r=0.08$, $P=0.69$, BD3: $r=-0.05$, $P=0.83$, BD5: $r=0.07$, $P=0.74$). There was no correlation between blubber percent lipid and the retinol or tocopherol concentrations in the corresponding layers (Table 4). There was no correlation found between vitamin A (serum, liver and blubber) and any of the OC metabolites/congeners examined (serum, liver and blubber).

4. Discussion

Liver oils of fish and marine mammals contain some of the richest sources of vitamin A known (McDowell, 2000). Much of the published research investigating vitamin A concentrations in marine mammals has focused on pinnipeds (Schweigert et al., 1987, Ball et al., 1992, Mazzaro et al., 2003) with published liver retinol concentrations up to 100 times the levels found in terrestrial mammals (McDowell, 2000; LeBlanc et al., 2004; Yang et al., 1992). The diet of the bowhead whale is composed mainly of euphausids, amphipods, copepods, and mysid shrimp (Burns et al., 1993, Richardson and Thomson, 2002). These prey items contain relatively high levels of

Table 4
Spearman rank correlation values (noted as *R*) found between blubber retinol, blubber tocopherol and blubber % lipid in the bowhead whale (significance $P<0.05$.)

		Blubber Retinol Depth 1	Blubber Tocopherol Depth 1	Blubber Retinol Depth 3	Blubber Tocopherol Depth 3	Blubber Retinol Depth 5	Blubber Tocopherol Depth 5
BD1	*R*	0.178	0.01	0.22	−0.11	−0.02	−0.22
Lipid%	*P*	0.43	0.97	0.34	0.61	0.94	0.40
	N	22	20	21	23	15	16
BD3	*R*	−0.15	0.10	0.22	−0.22	−0.14	−0.17
Lipid%	*P*	0.49	0.67	0.34	0.30	0.61	0.52
	N	22	20	21	23	15	16
BD5	*R*	0.03	0.09	0.25	−0.18	−0.20	−0.29
Lipid%	*P*	0.88	0.72	0.28	0.41	0.46	0.28
	N	22	20	21	23	15	16

vitamins A and E (Krinsky, 1965, Kon and Thompson, 1949), which is important, as retinol cannot be produced endogenously in most mammals and must be provided in the diet. Vitamin A is stored primarily in the liver in the majority of mammals, with pinnipeds appearing to be an exception (McDowell, 2000, Schweigert et al., 1987; Mos and Ross, 2002). In seals and sea lions, blubber is of greater significance than liver as a storage depot for vitamin A with between 40 and 60 percent of total body stores being found in this tissue in grey (*Halichoerus grypus*) and harbor seals (*Phoca vitulina*) (Schweigert et al., 1987, Mos and Ross, 2002). Cetaceans also store vitamin A in their blubber but not at the comparatively high concentrations found in pinnipeds (Tornero et al., 2004a, 2004b). This is consistent with our findings in the bowhead whale. However, because blubber comprises 40–50% of total body mass in the bowhead (J.C. George, personal communication) this is a more significant storage site in this species than in many other cetaceans. Previous research has shown a correlation between retinol and percent lipid in blubber of common dolphins (*Tursiops truncatus*) (Tornero et al., 2004b). However, this relationship was not found in hooded seals (*Cystophora cristata*), harbor porpoises (*Phocoena phocoena*) or harbor seals (Rodahl and Davies, 1949, Borrell et al., 1999, Mos and Ross, 2002). Similar to these findings, no correlations were found between retinol or alpha-tocopherol and blubber lipid percentage in the bowhead whale (Table 4).

Vitamin A and E concentrations in the bowhead whale varied widely with some variability explained by sex, season, age class and reproductive state. Hepatic retinol had the most significant associations present and these related to sex, season and age group. Serum retinol and tocopherol concentrations were the least variable of the measured values with reported ranges similar to those found in terrestrial mammals (LeBlanc et al., 2004; Yang et al., 1992). Serum retinol is homeostatically regulated with respect to fluctuations in food intake and life history events in many mammalian species (Machlin, 1991; Borrell et al., 2002). The hepatic retinol concentrations listed in Table 2 are relatively high in comparison to values reported in domestic mammals, pinnipeds and other cetaceans (Machlin, 1991; McDowell, 2000; Tornero et al., 2004a; Borrell et al., 2002; Borrell et al., 1999; Crissey and Wells, 1999). However, published "reference ranges" must be interpreted with caution due to likely differences among populations and individuals. In addition, inter-laboratory analytical variations make it difficult to adequately compare published values (Ullrey et al., 1995).

Male bowhead whales had significantly higher mean concentrations of liver retinol than females. Similar results have been noted in pinnipeds (Rodahl and Davies, 1949; Schweigert et al., 1987). Lactational loss of retinol may be responsible for this difference (Simms and Ross, 2000). Reproductively mature female marine mammals are known to apportion large amounts of fat and fat-soluble vitamins to offspring via the milk during lactation (Debier et al., 1999, 2002, 2004). This may result in a cyclical and possibly cumulative decrease in maternal levels of fat-soluble vitamins that is dependent upon life history traits including the length of lactation, calving interval and the number of total lactational periods experienced.

Liver retinol concentration was also significantly higher in whales harvested in spring than in fall. Migration requires a high level of energy expenditure and draws heavily upon body lipid reserves (Burns et al., 1993). Decreased hepatic retinol concentrations in the fall may be related to fat mobilization during the spring/summer migration resulting in a redistribution of blubber and liver associated retinoids (Borrell et al., 1999). Restoration of blubber stores of vitamin A during feeding throughout summer and early fall, prior to the fall harvest is likely. However, the dynamics of the distribution of retinol to organs in cetaceans are largely unknown. Blubber seems to be more stable, with respect to concentration, as there were no seasonal differences in vitamin A and E distribution in this tissue. The liver may take longer than blubber to restore its pre-migration retinol levels as a specific strategy for dealing with a large influx of vitamins during a relatively short period of feeding.

Adult male and non-pregnant female bowhead whales have the highest concentrations of hepatic retinol, followed by subadults, pregnant females and juveniles. The effects of age on retinol concentration have been researched in humans and laboratory animals, with a general trend towards an increase in hepatic concentrations with age (van der Loo et al., 2004). The majority of pinniped research supports these findings (Kakela et al., 1997; Southcott et al., 1974; Schweigert et al., 1987; Rodahl and Davies, 1949) and indicates a cumulative increase in liver retinol

concentrations with feeding over a lifetime (Southcott et al., 1974, Schweigert et al., 1987, Borrell et al., 1999). This may be especially important to bowhead whales, due to their extreme longevity (George et al., 1999; Rosa et al., 2004). Additionally, there may be decreases in circulatory clearance of retinoids with increasing age. Higher tissue concentrations of retinol might also be a defensive adaptation to protect against oxidative tissue damage that becomes more prevalent with age (van der Loo et al., 2004). Juvenile bowhead whales had the lowest levels of hepatic retinol of the age groups examined. Mammalian neonates are born with very low liver stores of vitamin A because the transfer of retinol across the placenta is minimal (McDowell, 2000). As these animals develop, they receive appreciable amounts of vitamin A via milk during the nursing period (Debier et al., 2002): Thus, time is needed to build up a cumulative body store of vitamin A via nursing and foraging following weaning.

In the present study, retinol and tocopherol concentrations were found to be significantly higher in the epidermis than in the deeper blubber layers. This is similar to findings in several other mammalian species, which tend to concentrate these substances in the epidermis (Vahlquist et al., 1987). The bowhead whale has among the thickest epidermal tissue of all mammals (Haldiman et al., 1985), making this tissue a sizeable depot for these vitamins. Both substances are likely to be important for growth, maintenance of skin, and wound healing (Machlin, 1991; McDowell, 2000). The concentration of retinol in the outermost blubber layer (BD1) was significantly higher in adults than in subadult and juvenile bowhead whales. This outermost region of blubber is thought to serve as a potential long-term storage or highly lipid stable region (Mau, 2004; Ackman et al., 1975) and may be higher in retinol secondary to age-dependent accumulation. The significantly higher concentration of tocopherol found in the blubber layer three (BD3) of adults may be an age-related finding, as well, as tocopherol has been found to accumulate with age in the blubber of marine mammals (Kakela et al., 1997; Schweigert et al., 1990). More information is needed on the mobilization of blubber lipids in order to fully characterize these processes.

In pinnipeds, hepatic vitamin E is thought to increase transiently after ingestion of tocopherol-rich food. Subsequently, hepatic levels drop and blubber levels rise, with the blubber acting as a long-term storage depot for the vitamin (Engelhardt and Geraci, 1977; Käkelä et al., 1997; Schweigert et al., 2002). We found that the blubber of bowhead whales contained much lower concentrations of vitamin E than those reported in seal blubber (Crissey and Wells, 1999; Schweigert et al., 1990; Engelhardt and Geraci, 1977; Schweigert et al., 2002). In contrast, these whales had up to ten times the hepatic concentration of tocopherol reported in seals both during the spring (fasting/transient feeding) and fall (feeding) periods. Available literature contains no vitamin E data for cetacean tissues except serum.

Vitamin E and selenium interact in tissues and are thought to be mutually protective of each other with respect to deficiency (McDowell, 2000). In the current study, correlations were found between vitamin A and E levels and selenium and zinc levels in bowhead tissues. Reasons for these correlations are unknown though one may speculate that these substances may occur in specific ratios in prey and are distributed in similar ways throughout the body of the predator (bowhead whale). Alternatively, an age-related accumulation of these elements may occur (Burns et al., 1993; Bratton et al., 1997).

Levels of hepatic tocopherol were high in bowhead whales compared to those of terrestrial mammals (Hoekstra, 1975; McDowell, 2000). The most important determinant of vitamin E requirements is the dietary concentration of unsaturated fatty acids as PUFAs are highly susceptible to auto-oxidation (Nacka al et., 2001). Animals ingesting high levels of PUFAs require high concentrations of vitamin E to protect tissue lipids from free radical attack (Debier et al., 2002; Lammi-Keefe and Jensen, 1984; Machlin, 1991). Tocopherol levels in the bowhead whale were found to be stable over sex, seasonal, age and reproductive groups.

In our analyses, retinol concentration in the serum, liver and blubber of bowhead whales did not correlate with any of the 20 OCs or PCB congeners measured in serum and blubber. This lack of correlation is likely to be related to the low levels of OCs found in this sample. An alternative explanation is that retinol levels in the bowhead whale are not affected by organochlorines; this is unlikely since it is contrary to most of the marine mammal literature. Further investigation into the significance of the transthyretin (TTR) carrier specificity for retinol in marine mammals is needed. Longitudinal, long-term studies monitoring OC concentrations in tissues (through the subsistence hunt and skin biopsy collection), with additional work investigating the status of retinol transport proteins and distribution patterns will help identify these relationships and ultimately the usefulness of retinol as a biomarker in this species. Adverse health effects may occur in marine mammals secondary to abnormalities in vitamin A and E status. Understanding of the dynamics, tissue distribution and baseline levels of vitamin A and E in mysticetes is critical to interpretation of these changes. The relationship of these findings to organochlorine concentrations helps to assess their utility as biomarkers of exposure to OCs and indicators of health in cetaceans.

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Appendix A

List of organochlorine congeners and PCB metabolites (liver, blubber and serum) analyzed by cannonical correlation analyses in the bowhead whale liver, serum and blubber. HCH = hexachlorocyclohexane, HCB = hexachlorobenzene, DCB = dichlorobenzene

o, *p*′-DDD
p, *p*′-DDE
α-HCH
HCB
β-HCH
Oxychlordane
cis-Heptachlorepoxide
Dieldrin
p, *p*′-DDD
trans-Nonachlor
γ-Chlordane
o, *p*′-DDT
1-4-DCB
c95&c66
γ-HCH (lindane)
c52c49
p, *p*′-DDT
o, *p*′-DDE
c101
Methoxyclor

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