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Plasma Vitamin Concentrations (α- and γ-Tocopherols, Retinol, Retinyl Palmitate, and Ascorbic Acid) in Two Free-Ranging Dolphin (*Tursiops truncatus*) Populations

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Abstract

Vitamins are essential for normal growth and development, metabolism, and immune health, yet there is limited knowledge on baseline values in marine mammals. Plasma samples were collected during 2003-2004 from two free-ranging dolphin populations (Charleston [CHS], South Carolina, $n = 72$; Indian River Lagoon [IRL], Florida, $n = 79$). Circulating levels of the following vitamins were analyzed: α- and γ-tocopherols, retinol, retinyl palmitate, and ascorbic acid. Site-specific differences were observed for α-tocopherol, retinyl palmitate, and ascorbic acid, with higher levels occurring in CHS dolphins. Reference intervals (50th, 75th, and 90th percentiles) were calculated for each dolphin population. Higher levels of α-tocopherol were observed in CHS female dolphins compared to males ($p = 0.05$), with a similar trend observed in IRL. This study provides reference intervals for estuarine bottlenose dolphin (*Tursiops truncatus*) populations at two southeastern U.S. sites. Such data on baseline vitamin concentrations in wild populations are necessary prerequisites for understanding the impacts of contaminants or other disruptions of vitamin homeostasis on overall animal health.

Key Words: vitamin α- and γ-tocopherols, retinol, retinyl palmitate, ascorbic acid, bottlenose dolphin, *Tursiops truncatus*, reference values

Introduction

Baseline levels of vitamins in blood have not been described for most marine mammal species. The majority of studies published on vitamins has been on tissue levels, had small sample sizes, and were mainly performed on pinnipeds (St. Aubin & Geraci, 1980; Mazzaro et al., 1995, 2003; Kakela et al., 1997; Simms & Ross, 2000). Previous studies also focused on marine mammals in managed care, which may not represent wild populations given their more restricted diet. For bottlenose dolphins (*Tursiops truncatus*), one study reported vitamin levels in managed-care dolphins (Kasamatsu et al., 2009), and another study compared managed-care with wild individuals (Crissey & Wells, 1999). Potential changes in vitamin levels secondary to age, sex, reproductive status, health, and possible regional dietary differences have not been thoroughly investigated in cetaceans and are important factors in determining baseline values.

It is improbable that vitamins A and E deficiency occurs in free-ranging marine mammals under normal circumstances as both vitamins are found in abundance in the marine diet (Worthy, 2001), although stress, disease, and contaminant exposure can alter vitamin concentrations. Vitamin E has a low potential for toxicity, and levels much higher than required to prevent deficiency appear to be beneficial in combating a variety of oxidative stress disorders (Combs, 2008). Vitamin E (tocopherols and tocotrienols) is a major contributor in the antioxidant defense system (Combs, 2008). It acts as a free radical scavenger, reducing free radicals produced as a result of normal biological functions and secondary to xenobiotic metabolism, thus limiting lipid peroxidation (Slim et al., 1999; Combs, 2008). Outside of adipose tissue, vitamin E is found primarily in cell membranes and plays an important role in the maintenance of cell membrane stabilization in virtually all cells in the body (Slim et al., 1999; Combs, 2008).

Vitamin A (retinol and retinyl esters) plays a role in many physiologic functions, including normal health of vision and skin, growth and fetal development, reproduction, and immune functions. Two forms of dietary vitamin A are available: (1) preformed vitamin A (retinol and its esterified form, retinyl ester) and (2) provitamin A carotenoids. Preformed vitamin A is found in foods from animal sources such as fish and meat. In marine mammals, retinyl esters are likely the most common dietary source of the vitamin. The esters are hydrolyzed to retinol in the intestines and converted back to retinyl esters for storage in target tissues (Simms & Ross, 2000); thus, it is important to measure both of these compounds. Several aspects of vitamin A metabolism tend to protect against hypervitaminosis, including hepatic storage of vitamin A which tends to mitigate against the development of intoxication due to intakes in excess of physiological needs (Combs, 2008). In the free-ranging animal, excessive vitamin A levels are not likely. It also appears to be involved in the cytochrome P-450 system and provides protection against the effects of several chemical carcinogens (Combs, 2008). The highest concentration of retinoids in cetaceans occurs in the liver followed by the blubber (Tornero et al., 2005; Desforges et al., 2013). Vitamin A levels in blood are subject to tight homeostatic regulation, and less variation is expected in blood levels compared to liver and blubber concentrations (Borrell et al., 2002). Therefore, it has been suggested that vitamin A blood levels are not reliable indicators of retinoid disruption (Tornero et al., 2005). Vitamin A has been investigated as a biomarker for persistent organochlorine contaminants (POCs) in marine mammals (Nyman et al., 2003; Debier et al., 2004, 2005; Tornero et al., 2005; Rosa et al., 2007; Vanden Berghe et al., 2010; Desforges et al., 2013) as POCs and their metabolites affect uptake, transport, storage, and elimination (Novák et al., 2008). Although liver contains the highest concentration of vitamin A, the invasive nature of obtaining liver biopsies precludes the use of liver vitamin A as a biomarker in marine mammals. Circulating concentrations of retinol represent the least invasive measurement of vitamin A.

Many contaminants can disrupt vitamin A physiology as shown both in laboratory investigations (Zile, 1992; Kakela et al., 2003) and in field studies (Rolland, 2000; Simms et al., 2000; Desforges et al., 2013). While decreases in circulating retinol levels have been observed in some studies with experimental animals and wildlife, including marine mammals, others have shown increases or no relationship which may reflect differences in species-specific responses as well as study design (Simms & Ross, 2000). Effects of polychlorinated biphenyls (PCBs) on vitamin E levels in birds and mammals have been documented in several species (Katayama et al., 1991; Halouzka et al., 1994; Nyman et al., 2003). Exposure to PCBs cause increases in serum vitamin E levels possibly due to increased intestinal absorption and increased mobilization of vitamin E stores (Katayama et al., 1991). *In vitro*, vitamin E blocks the oxidative stress and endothelial barrier dysfunction induced by PCBs (Slim et al., 1999). Therefore, vitamin E has been proposed as a biomarker for contaminant exposure. Unfortunately, the lack of knowledge on baseline levels of vitamins E and A in marine mammals and the potential effects of variables on circulating levels remain an impediment to using these vitamins as biomarkers (Simms et al., 2000).

Vitamin C is an essential water-soluble nutrient with important actions on host defense mechanisms and immune homeostasis and, therefore, is considered the most important physiological antioxidant (Pavlovic & Sarac, 2011). Numerous studies have demonstrated that vitamin C stimulates the immune system and decreases the risk of pathophysiologic consequences. Vitamin C contributes to the maintenance of the redox integrity of cells and thereby protects them against reactive oxygen species generated during the respiratory burst in the inflammatory response. Vitamin C concentrations decline rapidly in plasma and leukocytes during infections and stress responses. Supplementation was found to improve components of the human immune system such as antimicrobial and natural killer cell activities, lymphocyte proliferation, chemotaxis, and delayed-type hypersensitivity (Wintergerst et al., 2006). Vitamin C and zinc play important roles in immune function and in the modulation of host resistance to infectious agents, reducing the risk, severity, and duration of infectious diseases (Wintergerst et al., 2006). Most animals synthesize ascorbic acid and do not require dietary intake. Animals that do not synthesize ascorbic acid include humans and other primates, guinea pigs, invertebrates, most fish, and a few bird species (Combs, 2008). Bottlenose dolphins, pygmy sperm whales (*Kogia breviceps*), pilot whales (*Globicephala*), and false killer whales (*Pseudorca crassidens*) are thought to be incapable of synthesizing vitamin C (Moore, 1980). However, vitamin C concentrations in emaciated stranded cetaceans which had not been feeding for some time did not differ from healthy animals, suggesting that ascorbic acid levels may be independent of dietary intake (St. Aubin & Geraci, 1980). The highest concentration of ascorbate in bottlenose dolphins was found in the adrenal glands, followed by the epidermis, liver, and pancreas (St. Aubin & Geraci, 1980).

The purpose of this study was to (1) describe plasma levels of circulating vitamins A, E, and C in two free-ranging bottlenose dolphin

populations; (2) compare levels by sex, age, and geographic location; and (3) develop reference intervals for these estuarine populations.

Methods

Sample Collection

Samples were collected during dolphin capture-release health assessments conducted at two study sites: Charleston (CHS), South Carolina, and the Indian River Lagoon (IRL), Florida (Figure 1), in 2003 and 2004. The CHS site (32° 46' 35" N, 79° 55' 51" W) included the Charleston Harbor; portions of the main channels and creeks of the Ashley, Cooper, and Wando Rivers; and the Stono River Estuary. For the IRL site, assessments were conducted near Titusville (28° 36' 43" N, 80° 48' 27" W) and Stuart (27° 11' 51" N, 80° 15' 10" W), Florida, and included portions of the Mosquito Lagoon, Indian River, Banana River, north and south forks of the St. Lucie River, and Sebastian Inlet. Dolphins in these two estuarine locations have high site fidelity as indicated by long-term

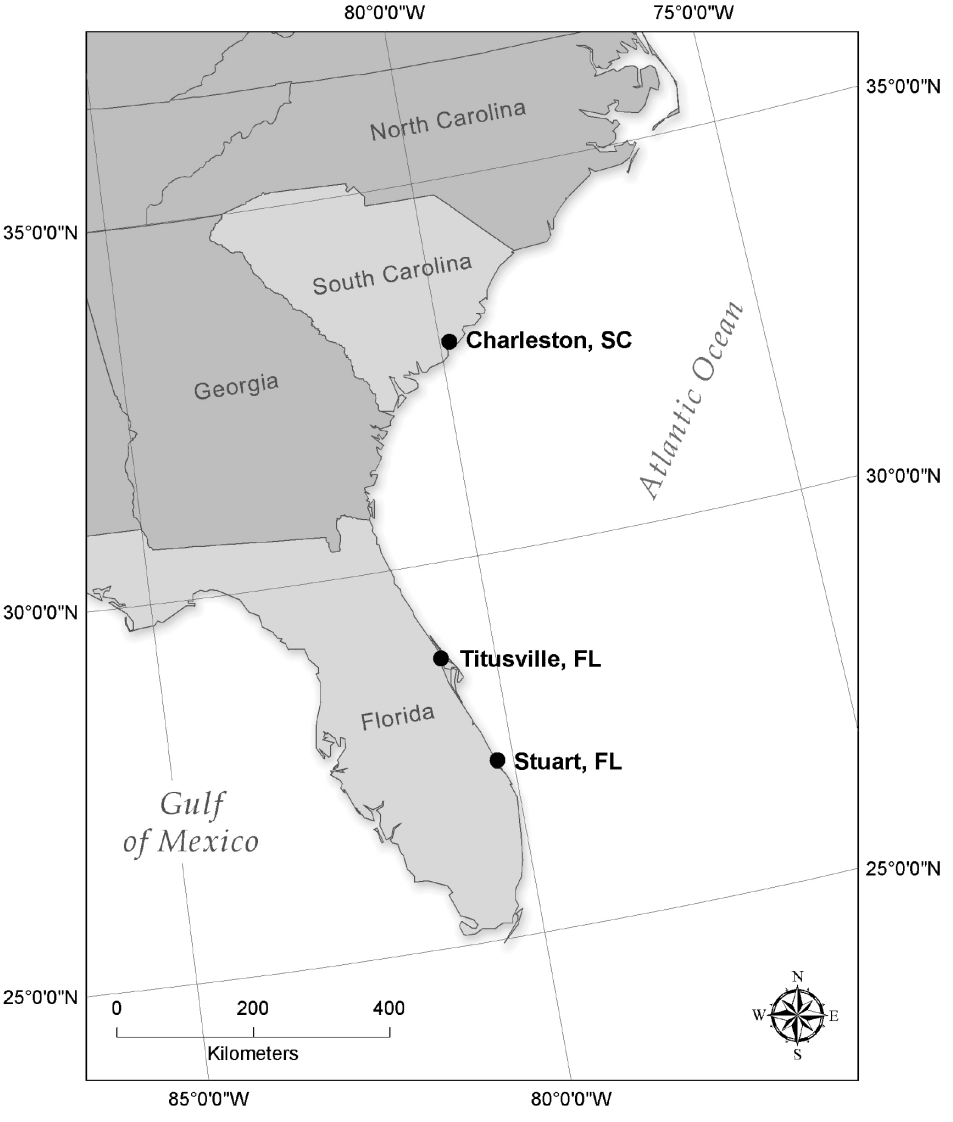

Figure 1. Study sites in estuarine areas of Charleston, South Carolina, and the Indian River Lagoon, Florida (between Titusville and Stuart, Florida) along the U.S. southeast Atlantic coast

photo-identification data (Zolman, 2002; Mazzoil et al., 2008; Speakman et al., 2010).

Techniques used for the capture, sampling, and release of dolphins have been described previously by Fair et al. (2006). Briefly, dolphins were followed by boat and encircled with a large mesh seine net. They were encircled in shallow water, and experienced marine mammal personnel and veterinarians were deployed around the net circumference for handling and restraint. Once restrained, blood samples were collected from the periarterial venous rete in the flukes using a 19-gauge needle and 1.9-cm butterfly catheter with a vacutainer™ attachment (Becton-Dickinson, Franklin Lakes, NJ, USA). The dolphin was then moved to a boat designed for conducting clinical evaluation and additional tissue collection as previously described (Fair et al., 2006). Following the clinical evaluation, all dolphins were returned to the water and released.

Whole blood was collected in 10-ml sodium heparin vacutainers (Benton Dickinson) and immediately centrifuged at 3,500 rpm for 15 min. The plasma was transferred to amber transport tubes and frozen on dry ice. Blood samples were collected from a total of 151 individuals: 72 from CHS in August of 2003 and 2004 and 79 from IRL in June of 2003 and 2004. Age was determined by examining the postnatal dentine layers of an extracted tooth (Hohn et al., 1989). Adult males were defined as individuals 10 y of age or older, and adult females were defined as individuals 7 y of age or older (Mead & Potter, 1990). All animal capture and sampling protocols were conducted under National Marine Fisheries Permit No. 998-1678-00 and approved by the Harbor Branch Oceanographic Institution (HBOI) Institutional Animal Care and Use Committee (IACUC).

Analyses of Vitamins in Plasma

Freshly obtained plasma samples collected from dolphins in 2003 and 2004 were shipped on dry ice overnight and analyzed by the Associated Regional and University Pathologists (ARUP; Salt Lake City, UT, USA). Analysis of retinol, retinyl palmitate, α-tocopherol, γ-tocopherol, and retinyl acetate (internal standard) was performed using an extraction method with hexane followed by detection on a High-Performance Liquid Chromatography (HPLC) system (Sowell et al., 1994). The analytes were detected by their ultraviolet absorbance at 296 nm (tocopherols) and 325 nm (retinol, retinyl palmitate, and retinyl acetate). The measurement range for each analyte was as follows: retinol, 0.06 to 5.0 mg/L; retinyl palmitate, 0.01 to 5.0 mg/L; α-tocopherol, 0.5 to 180 mg/L; and γ-tocopherol, 0.05 to 20 mg/L. Intra-assay precision (% CV) was as follows: 6.5% for retinol, 8.1% for retinyl palmitate, 6.3% for α-tocopherol, and 6.8% for γ-tocopherol. For vitamin C analysis, plasma was extracted using trichloroacetic acid to obtain a protein-free filtrate followed by dehydroascorbic acid oxidation in the presence of acid-washed charcoal. Dehydroascorbic acid reacts with 2,4-dinitrophenylhydrazine to form the 2,4-dinitrophenylosazone, and sulfuric acid produces a reddish complex, which is measured by spectrophotometry at 515 nm (Burtis & Ashwood, 1999). The measurement range was 0.1 to 5.0 mg/dL. Intra-assay precision (% CV) for samples with mean values of 0.5 mg/dL was 13.6%, and it was 7.6% for mean values 2.9 mg/dL.

Statistical Analysis

Mean, standard deviation, and percentiles were calculated for each site and compared using a *t* test. Variables were assessed for normality and log transformed to meet test assumptions when appropriate. Adult females were defined as 7 y and older, adult males were defined as 10 y and older, while juveniles were categorized as less than these ages. Age and sex were stratified by sampling site and compared using an analysis of variance (ANOVA) across categories. We also compared mean vitamin E concentrations between pregnant and nonpregnant female dolphins from both sites using a *t* test. Eleven of the 56 females were pregnant as determined by ultrasound examination. Statistical significance was set at $p < 0.05$, and all analysis was done using *SPSS for Windows 2014*, Version 22 (IBM Corp., Armonk, NY, USA).

Results

Site-specific mean vitamin plasma concentrations for the two dolphin populations are provided in Table 1. Significant differences were found between CHS and IRL dolphins in mean levels of α-tocopherol, retinyl palmitate, and ascorbic acid with higher levels in CHS dolphins. No significant differences were observed in concentrations of γ-tocopherol and retinol between the two dolphin populations. The 50th, 75th, and 90th percentiles for α-tocopherol, γ-tocopherol, retinol, retinol palmitate, and ascorbic acid are also shown in Table 1 for dolphins from both sites.

Mean vitamin concentrations were compared by age class (adults and juveniles; see Table 2). There were no statistically significant differences in vitamin concentrations between adults and juveniles in either population. Female dolphins had higher α-tocopherol mean levels than males at both sites (Table 3). The difference was statistically significant in the CHS dolphins but not in IRL dolphins. There was no statistically significant difference in the concentration of any vitamin between pregnant ($n = 11$) and nonpregnant ($n = 45$) females.

Table 1. Reference values for plasma vitamins by percentiles in bottlenose dolphins (*Tursiops truncatus*) from the Indian River Lagoon (IRL), Florida, and from Charleston, South Carolina (CHS)

	Indian River Lagoon (n = 79)				Charleston (n = 72)				
Vitamin	Mean (SD)	50th	75th	90th	Mean (SD)	50th	75th	90th	p value
α-tocopherol (ug/ml)	10.20 (2.69)	10.08	12.11	13.42	12.46 (3.36)	12.00	14.35	16.78	< 0.01*
γ-tocopherol (ug/ml)	0.08 (0.10)	0.10	0.10	0.20	0.07 (0.09)	0.04	0.10	0.20	0.20
Retinol (ng/ml)	0.05 (0.02)	0.05	0.07	0.07	0.05 (0.05)	0.04	0.05	0.06	0.78
Retinol palmitate (ng/ml)	0.02 (0.02)	0.01	0.03	0.05	0.04 (0.12)	0.01	0.05	0.06	< 0.01*
Vitamin C (mg/dL)	0.59 (0.21)	0.60	0.70	0.80	0.75 (0.23)	0.60	0.70	0.80	< 0.01*

* Statistically significant

Table 2. Plasma vitamin concentrations for IRL and CHS dolphins by age class mean (± SD). Adult males were ≥ 10 y; adult females were ≥ 7 y.

	Indian River Lagoon (n = 68)			Charleston (n = 61)		
Vitamin	Juvenile (n = 21)	Adult (n = 47)	p value	Juvenile (n = 19)	Adult (n = 42)	p value
γ-tocopherol (ug/ml)	0.11 (0.13)	0.08 (0.08)	0.22	0.08 (0.08)	0.06 (0.09)	0.50
Retinol (ng/ml)	0.05 (0.02)	0.05 (0.02)	0.50	0.04 (0.01)	0.05 (0.06)	0.11
Retinyl palmitate (mg/ml)	0.02 (0.03)	0.01 (0.02)	0.63	0.02 (0.03)	0.05 (0.15)	0.83
Vitamin C (mg/dl)	0.56 (0.15)	0.60 (0.21)	0.42	0.79 (0.27)	0.74 (0.21)	0.47

Table 3. Plasma vitamin concentrations for IRL and CHS dolphins by sex mean (+ SD)

	Indian River Lagoon (n = 79)			Charleston (n = 72)		
Vitamin	Male (n = 48)	Female (n = 31)	p value	Male (n = 47)	Female (n = 25)	p value
α-tocopherol (ug/ml)	9.82 (2.64)	10.78 (2.70)	0.12	11.90 (2.88)	13.50 (3.98)	0.05
γ- tocopherol (ug/ml)	0.07 (0.08)	0.10 (0.12)	0.22	0.06 (0.08)	0.10 (0.09)	0.22
Retinol (ng/ml)	0.05 (0.06)	0.04 (0.02)	0.88	0.05 (0.02)	0.04 (0.02)	0.38
Retinyl palmitate (mg/ml)	0.02 (0.03)	0.02 (0.03)	0.21	0.04 (0.02)	0.02 (0.03)	0.14
Vitamin C (mg/dl)	0.57 (0.16)	0.62 (0.27)	0.62	0.75 (0.22)	0.75 (0.25)	0.97

* Statistically significant

Discussion

Overall, dolphins inhabiting the CHS estuarine waters had higher levels of α-tocopherol, retinyl palmitate, and ascorbic acid compared to those in the IRL. These site-specific differences may reflect the nutritional differences and/or environmental stressors that they are exposed to such as contaminants and pathogens. Although dietary differences are possible, this explanation is unlikely for vitamin E due to the abundance of vitamin E in the marine diet (Worthy, 2001). An inconsistent correlation between vitamin E and the contaminant load has been shown in several studies of marine mammals. One study found a positive correlation between serum vitamin E levels and both PCB and DDT levels in Baltic ringed (*Phoca hispida*) and grey (*Halichoerus grypus*) seals (Nyman et al., 2003), supporting the role of vitamin E in oxygen free radical scavenging due to contaminant levels. No correlation was found between circulating vitamin E levels and PCB or DDT contaminant concentrations in juvenile California sea lions (*Zalophus californianus*) (Debier et al., 2005). No relationship

was found between serum, liver, and blubber vitamin E levels and serum and blubber POC concentrations in the bowhead whale (*Balaena mysticetus*) (Rosa et al., 2007). However, bowhead whales generally have a low POC burden compared to other mysticetes. CHS dolphins have higher concentrations of POCs than IRL dolphins, including PCBs, pesticides such as DDT, perfluorinated compounds, and polybrominated diphenyl ethers (Fair et al., 2010). It is possible that the higher vitamin E levels are related to the higher contaminant loads in the CHS population. The relationships between contaminant load and circulating vitamin levels will be evaluated in a future study.

The plasma vitamin E concentrations reported herein are comparable to those in other published studies, although the literature for wild dolphins is very limited. CHS dolphins had a mean α-tocopherol concentration of 12.46 μg/ml, which is very similar to the mean level found in Sarasota dolphins (12.5 μg/ml; Crissey & Wells, 1999). IRL dolphins had a lower mean level (10.20 μg/ml) than either the South Carolina or Sarasota dolphin populations. Similar laboratories were used for each of the vitamin analyses from the CHS and IRL dolphins; however, it should be noted that other studies used different laboratories and, thus, the data may not be directly comparable. The values for vitamin E in wild dolphins tend to be lower than those reported for managed-care dolphins at three different facilities with mean values of 16.4 μg/ml, 13.0 μg/ml (Crissey & Wells, 1999), and 20.61 μg/ml (Kasamatsu et al., 2009). Many facilities supplement their captive dolphins with a multivitamin containing vitamin E as a common component. Thus, the differences in vitamin E concentrations between wild and managed-care dolphins could be due to their supplemented diet.

Vitamin E stores within adipose tissue are very slowly metabolized, and levels can be virtually normal even in animals showing clinical signs of deficiency. However, vitamin stores outside of adipose tissue, such as in plasma and liver, are rapidly depleted in states of vitamin E deficiency (Combs, 2008). It has been suggested that vitamin E may be mobilized from adipose tissue only when lipolysis is induced under certain metabolic conditions (Combs, 2008). In pinnipeds, pregnancy and lactation have been found to affect vitamin E levels (Schweigert et al., 2002). Vitamin E levels are much higher in colostrum than later in lactation in harp (*Pagophilus groenlandicus*) and hooded (*Cystophora cristata*) seal milk (Debier et al., 1999). This supports a lack of transplacental movement of vitamin E as well as the importance of vitamin E in the nutrition of the neonate. Human maternal vitamin E levels increase during pregnancy, while fetal levels remain low (Combs, 2008), further supporting a lack of transplacental transfer of vitamin E. Levels of α-tocopherol were significantly higher in female dolphins compared to males in CHS, with a similar trend apparent in IRL dolphins. Higher levels of vitamin E in female compared to male dolphins in our study may in part be due to reproductive status and pregnancy. However, when we compared mean values between pregnant and nonpregnant females from both sites, we found no significant differences and, thus, we do not believe that pregnancy is impacting the results in this small sample.

Our study found no differences in concentrations of γ-tocopherol between dolphins at the two sites (CHS, 0.07 μg/ml; IRL, 0.08 μg/ml). In another study, levels of γ-tocopherol were nondetectable in wild dolphins and managed-care dolphins at one facility, although at another facility, dolphins had concentrations of 0.72 μg/ml (Crissey & Wells, 1999). The concentrations in our dolphins were approximately ten times lower than those reported for the above managed-care dolphins. Often, vitamin E supplements contain a mixture of tocopherols such as γ-tocopherol in addition to α-tocopherol; this may explain the higher γ-tocopherol levels reported in the one dolphin facility. Vitamin E occurs in eight structurally related forms, with α-tocopherol as the major form of vitamin E found in animals and humans (Jiang et al., 2001), although γ-tocopherol is the most abundant form of vitamin E in the U.S. diet. Animal studies have shown that the bioavailability and bioactivity of γ-tocopherol are lower than the alpha-form. Thus, γ-tocopherol was considered less important since it was not maintained at the same concentrations as α-tocopherol. Recent evidence has shown γ-tocopherol having superior properties compared to α-tocopherol with regard to detoxifying electrophiles and anti-inflammatory activity, which may contribute significantly to health in ways that are just beginning to be recognized (Jiang et al., 2001). Therefore, it would be useful to measure both forms of tocopherols in marine mammals to gain further insight into their role in health and disease.

The retinol levels found in our study populations were similar to levels found in bottlenose dolphins along the Gulf coast of Sarasota, Florida (Crissey & Wells, 1999). Retinyl palmitate, a retinol ester, was not measured in the serum of the Sarasota dolphins (Crissey & Wells, 1999), while low concentrations of 0.05 ng/ml were detected in both CHS and IRL dolphins. There have been limited studies on comparisons in vitamin A levels between wild and captive dolphins. One study found higher levels in captive dolphins from one facility but not another (Crissey & Wells, 1999). It has been suggested that the presence of circulating retinyl esters may be an adaptive mechanism to handle high levels of

vitamin A in carnivorous diets (Schweigert et al., 1991). The presence of retinyl palmitate in our study populations suggests the presence of abundant vitamin A in the diet. Vitamin A represents an essential nutrient for all mammals and maintains intact epithelial tissues as a physical barrier to infection as well as maintaining both innate and acquired immune function (Mora et al., 2008).

No statistically significant differences were observed in retinol concentrations between dolphins from CHS and IRL or across sex and age groups. To better understand vitamin A physiology in the harbor seal (*Phoca vitulina*), Mos & Ross (2002) quantified vitamin A in plasma, liver, blubber, and skin and found positive correlations between vitamin levels across tissues, supporting its use in biomarker studies. They found no sex-related differences in vitamin A concentrations for any of the tissues (i.e., liver, blubber, skin, and plasma). However, they reported site-related differences in concentrations of plasma and blubber retinol between the harbor seal populations from British Columbia, Canada, and Washington State, USA (Mos et al., 2007). The study also found that concentrations of both circulatory vitamin A (retinol) in plasma and stored vitamin A in blubber were negatively associated with blubber PCB concentrations.

Vitamin C levels were significantly higher in CHS dolphins than in IRL dolphins. Vitamin C is an important physiological antioxidant shown to regenerate other antioxidants within the body, including α-tocopherol, and plays an important role in immune function (Jacob et al., 2002). Vitamin C concentrations in the plasma and leukocytes rapidly decline during infections and stress (Wintergerst et al., 2006). Some viral infections are often associated with oxidative stress, and vitamin C promotes detoxification and neutralization of reactive oxygen species associated with infection (Wintergerst et al., 2006). While the basis of the lower levels in the IRL dolphins is unknown, potential increased microbial and pathogen exposure of IRL dolphins compared to the CHS population may be suspect. Thus, the differences in vitamin C levels between dolphins at the two study sites may reflect disparity in environmental stressors such as contaminants and/or pathogens.

Reference intervals are critical prerequisites for interpreting health information in all species, including humans. This study contains the first report of reference intervals (50th, 75th, and 90th percentiles) for vitamins measured in these two dolphin populations. These vitamin reference values determined for bottlenose dolphin populations in CHS and the IRL represent a necessary first step for biomarker investigations. In humans, sociodemographic variations in serum concentrations of α-tocopherol and γ-tocopherol have been reported among U.S. adults (Ford et al., 2006). Such differences may also be relevant to wildlife populations such as dolphins as reported herein. The implications that vitamin concentrations may have for adequacy of diet and growth patterns in wild cetaceans are still to be determined. Reference intervals should ideally be determined using blood samples from a large cohort of healthy subjects (Sasse, 2003). Sampling dolphins over a 2-y period in our study provided a robust sample size of 151 individuals from both sites. The large sample size is valuable in establishing reference values; however, it should be noted that these data represent a single point in time and may not adequately reflect baseline values which can be affected by seasonal changes or other variables. Since vitamins play an important role in growth, development, and immune function, small differences in vitamin levels may be important to the overall health of an animal and may reflect their nutritional status as well as stressors in their environment. Development of vitamin reference values for marine mammal populations should aid in future studies investigating contaminant or other disruption of vitamin homeostasis.

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