Nutritional Status In Captive Bottlenose Dolphins (*Tursiops truncatus*)

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Nutritional status of captive dolphins (*Tursiops truncatus*) was examined using biochemical analysis. Voluntary blood samples, obtained from four healthy captive bottlenose dolphins at Brookfield Zoo, were analyzed for vitamin D metabolites [25(OH)D and $1,25(OH)_2D$], lipids (total cholesterol, triacylglycerides, HDL-cholesterol, and LDL-cholesterol), and fatty acids. Fish fed to these dolphins were analyzed for dry matter, crude protein, fat, energy, vitamin D, cholesterol, and fatty acids. Plasma values for 25(OH)D were higher than those published for a variety of cetacean species, but similar to published bottlenose dolphin data. Total cholesterol was lower than that reported for captive beluga, but similar to wild beluga. Palmitic, oleic, eicosapentaenoic and docosahexaenoic acids were high in both dolphin plasma and the fish sampled. Vitamin D in herring was similar to that found in other species of fish and was reflected in the high circulating levels of 25(OH)D. Capelin was found to be considerably lower in vitamin D than other species of fish. Total cholesterol in fish samples was higher than that of fillets of other fish species.

Key words: marine mammals; cetaceans; vitamin status; lipids; cholesterol

INTRODUCTION

Determinations of circulating levels of vitamins, lipids and fatty acids can provide a base for examining nutritional status of captive cetaceans (Crissey et al., 1999). Limited information exists regarding circulating concentrations of these nutrients in cetaceans. This study examines vitamin D metabolites, lipids and fatty acids in captive bottlenose dolphins and relates those values to the fish they consumed.

MATERIALS AND METHODS

Blood

Voluntary blood samples were collected from two male (age: 17 and 19 yr) and two female (age: 18 and 7 yr) bottlenose dolphins at Brookfield Zoo. Both females were gestating at the time blood samples were obtained. Animals were housed in their usual exhibit and fed their usual fish diet with supplemental thiamin, vitamin E and a multivitamin. Venous blood was drawn by animal management staff into tubes containing either heparin or EDTA and chemically analyzed. Plasma was separated by centrifugation and frozen for less than two months at -80ºC until thawed for analysis. Samples from all four animals were analyzed for fatty acids. Samples for vitamin D metabolites and lipids were not obtained from the 7-yr old female.

Samples were analyzed for the vitamin D metabolites 25(OH)D and $1,25(OH)_2D$ at Boston University School of Medicine (Boston, MA). 25(OH)D was extracted from 0.05 ml plasma with 100% ethanol, followed by a protein-binding assay using rat serum vitamin D- binding protein which has a high affinity for 25(OH)D and using a high specific activity $[^3H]25(OH)D_3$ as a tracer [Chen et al., 1990b]. To determine $1,25(OH)_2D$, 1.0 ml of plasma was extracted with acetonitrile. The extracts were then applied to C-18-OH reversed-phase cartridge to separate $1,25(OH)_2D$ from 25(OH)D and tri-hydroxylated vitamin D metabolites. The quantitation of $1,25(OH)_2D$ was accomplished by a radio-receptor binding assay using calf thymus receptor and high specific activity $[^3H]1,25(OH)_2D_3$ [Chen et al., 1990a].

Total cholesterol [Allain et al., 1974; Beckman-Coulter, 1997] triacylglycerides, high density lipoprotein-cholesterol (HDL-cholesterol) and low density lipoprotein-cholesterol (LDL-cholesterol) [Beckman-Coulter, 1997] concentrations were measured at Loyola University Medical Center (Maywood, IL) using the Synchron Delta CX7 Analyzer (Beckman-Coulter, Inc., Brea, CA).

Fatty acid analyses were conducted at the Kennedy Krieger Institute (Baltimore, MD). Fatty acids were determined by capillary gas chromatography of fatty acid methyl esters as described by Moser et al. [1999].

Diet

Proximate analysis of whole capelin (*Mallotus villosus*) and herring (*Clupea* spp.) was conducted at the Brookfield Zoo Nutrition Laboratory. Fish were pureed with liquid nitrogen until they formed a fine powder, then dried at 60°C using a forced draft oven. Crude protein was determined using the Kjeldahl method x 6.25 [AOAC, 1995]. Crude fat was determined by Soxhlet ether extraction and energy by bomb calorimetry (Parr Instruments Moline, IL). Vitamin D, cholesterol and fatty acid analyses of fish were performed by Covance Laboratories (Madison, WI). Vitamin D was determined by HPLC using AOAC method 45.1.22, modified [AOAC, 1995]. Total cholesterol was analyzed

according to AOAC method 994.10 [1995]. Fatty acids were determined by gas chromatography according to the American Oil Chemists Society [1997].

RESULTS AND DISCUSSION

Blood

Vitamin D metabolite, lipid and fatty acid concentrations in bottlenose dolphin samples are listed in Table 1. There was little variation among dolphins in the concentrations of vitamin D metabolites. Plasma concentrations of 25(OH)D in Brookfield Zoo dolphins were similar to those published for wild bottlenose dolphins [Keiver et al., 1988]. Values were higher than published values for wild beluga whales (*Delphinapterus leucas*), wild pilot whales (*Globicephala malaena*), and wild white-sided dolphins (*Lagenorhynchus acutus*) [Keiver et al., 1988]. Concentrations of $1,25(OH)_2D$ have not been reported in cetaceans. Total cholesterol and HDL cholesterol showed less individual variation than did triacylglycerides and LDL cholesterol. Total cholesterol was lower than previously noted for captive beluga but within the ranges obtained for wild beluga [Cook et al., 1990]. No data published for other cetaceans were found. Monounsaturated and polyunsaturated fatty acid percentages in dolphin plasma were similar at 36% and 38%, respectively, with saturated fatty acids accounting for roughly 20% of the total. Fatty acid composition of dolphin plasma was similar to that previously published for captive bottlenose dolphins [Nelson, 1973] with oleic, eicosapentaenoic and palmitic as the three highest fatty acids for both groups. Omega 3- and omega 9-fatty acids made up more than 50% of the fatty acids measured.

Diet

Composition of fish is known to vary with species, gender, age, season and the location where they are caught [Bernard and Allen, 1997]. Nutrient composition of the capelin and herring fed to Brookfield Zoo dolphins on a dry matter basis (DMB) is presented in Table 2. Protein content was similar between the fish types, however fat content was higher in herring. Vitamin D content of the herring was higher than that for capelin. Fish liver oils are known to be a good source of vitamin D, yet few studies have examined vitamin D content of whole fish. Published values for Baltic herring fillets with skin had higher levels of vitamin D than the herring sampled in this report (3828 IU/100g and 1257 IU/100g, respectively DMB). Fresh water perch fillets without skin had lower levels of vitamin D (326-1527 IU/100g DMB) than the Baltic herring with an upper range similar to the sampled herring [Mattila, 1997].

Total cholesterol in capelin was higher than the herring. Lie et al. [1994] examined the fatty acid and cholesterol content of a variety of fish species, but analyzed fillets, not whole fish. Cholesterol of the sampled fish was twice that of the fish fillets examined by Lie, which was to be expected.

Myristic, palmitic, palmitoleic, oleic, eicosenoic, eicosapentaenoic and docosahexaenoic acids were the highest in both herring and capelin. Eicosanoic was highest in capelin while palmitic was highest in herring. Overall, herring had higher levels of both saturated and polyunsaturated fatty acids. Palmitic and oleic acids were high in many fish species examined by Lie et al. [1994], as it was in the sampled fish. However, herring and capelin were considerably higher in eicosenoic acid compared to most species reported.

Plasma concentrations of vitamin D metabolites, lipids and fatty acids in captive bottlenose dolphins appear to be reflective of the fish diet consumed. The supplemental multivitamins contributed to the vitamin D concentrations in plasma, but were consumed at such low levels that the impact should not have been substantial. Diet was noted as the primary factor for difference found in the fatty acid composition of the liver of freshwater and marine ringed seals [Katela and Hyvarinen, 1998].

REFERENCES

Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. 1974. Enzymatic determination of total serum cholesterol. Clin Chem 20:470-5.

[AOAC] Association of Analytical Chemists. 1995. Official Methods of Analysis of AOAC International. 16th edition. Arlington, VA:AOAC International.

[AOCS] American Oil Chemists Society. 1997. Ce 1-62 fatty acid composition by gas chromatography. *In*: Official Methods and Recommended Practices of the AOCS, 5th ed. American Oil Chemists' Society, Champaign, IL.

Beckman-Coulter, Inc. 1997. Synchron Delta CX7 Technical Procedure Manual. Brea, CA.

Bernard J, Allen ME. 1997. Feeding piscivorous animals: nutritional aspects of fish as food. In: Baer DJ, Crissey SD, Ullrey DE, editors. Nutrition Advisory Group Handbook Fact Sheet 5. Silver Spring, MD: American Zoo and Aquarium Association.

Chen TC, Turner AK, Holick MF. 1990a. A method for the determination of the circulating concentration of 1,25-hydroxyvitamin-D. J Nutr Biochem 1:320-7.

Chen TC, Turner AK, Holick MF. 1990b. Methods for determination of the circulating concentration of 25-hydroxyvitamin-D. J Nutr Biochem 1:315-9.

Cook RA, Stoskopf MK, Dierenfeld ES. 1990. Circulating levels of vitamin E, cholesterol, and selected minerals in captive and wild beluga whales *(Delphinapterus leucas*). J Zoo Wildl Med 21:65-9.

Crissey SD, Maslanka MT, Ullrey DE. 1999. Assessment of nutritional status of captive and free-ranging animals. In: Baer DJ, Kirk-Baer C, editors. Nutrition Advisory Group Handbook Fact Sheet 8. Silver Spring, MD: American Zoo and Aquarium Association.

Kakela R, Hyvarinen H. 1998. Composition of pilyunsaturated fatty acids in the liver of freshwater and marine ringed seals (*Phoca hispida* spp) differs largely due to the diet of the seals. Comp Biochem Physiol 120B:231-7.

Keiver KM, Ronald K, Draper HH. 1988. Plasma levels of vitamin D and some metabolites in marine mammals. Can J Zool 66:1297-1300.

Lie O, Lied E, Maage A, Njaa LR, Sandnes K. 1994. Nutrient content in fish and shellfish. Fisk Dir Skr Ser Ernaering 6:83-105.

Mattila P, Piironen V, Haapala R, Hirvi T, Uusi-Rauva E. 1997. Possible factors responsible for the high variation in the cholecalciferol contents of fish. J Agric Food Chem 45:3891-6.

Moser AB, Jones DS, Raymond GV, Moser HW. 1999. Plasma and red blood cell fatty acids in peroxisomal disorders. Neurochem Res 24:187-97.

Nelson GJ. 1973. The lipid composition of the blood of marine mammals-III. The fatty acid composition of plasma and erythrocytes of Atlantic bottlenose dolphin, *Tursiops truncatus*. Comp Biochem Physiol 46B:257-68.

TABLE 1. Circulating concentrations of vitamin D metabolites, lipids and selected fatty acids in the plasma total lipids of captive bottlenose dolphins[1]

Vitamin D metabolites and lipids *N* = 3		Mass percentage of fatty acids *n* = 4	
Vitamin D metabolites		**Saturated fatty acids**	%
25(OH)D, ng/ml	210 ± 12.9	Myristic 14:0	1.9±0.09
1,25 $(OH)_2$ D, pg/ml	135 ± 13.1	Palmitic 16:0	11.3±0.36
		Stearic 18:0	6.0±0.34
Lipids		Arachidic 20:0	1.2±0.07
Total cholesterol, mg/dL	136 ± 6.3	**Monounsaturated fatty acids**	
Triacylglycerides, mg/dL	61±13.4	Oleic 18:1ω9	20.9±0.60
HDL-cholesterol, mg/dL	101±2.9	Gondoic 20:1ω9	2.1±0.11
LDL-cholesterol, mg/dL	25±5.4	22:1ω11	1.9±0.07
		Nervonic 24:1ω9	2.9±0.19
		Palmitoleic 16:1ω7	5.9±0.39
		Vaccenic 18:1ω7	2.6±0.17
Fatty acid totals, %		**Polyunsaturated fatty acids**	
Total saturated fat	21.63 ± 0.78	Linoleic 18:2ω6	1.5±0.03
Total ω9	28.7 ± 0.28	Arachidonic 20:4ω6	3.6±0.16
Total ω7	9.4± 0.58	Alpha linolenic 18:3ω3	2.2±.010
Totalω6	6.2 ±0.09	Eicosapentaenoic 20:5ω3	15.3±0.62
Totalω3	32.0±0.57	22:5ω3	3.4±0.31
Total fatty acids (ug/ml)	1419± 363.6	Docosahexaenoic 22:6ω3	10.9±0.45
		Palmitelaidic 16:1T	1.1±0.05

[1]Fatty acids that exceeded 1% were listed.

TABLE 2. Selected nutrient composition of fish fed to Brookfield Zoo dolphins on a dry matter basis[1]

Nutrient	Capelin	Herring
Dry Matter, %	19.14	25.79
Crude Protein, %	69.93	62.79
Crude Fat, %	12.12	22.21
Energy, kcals/g	5.46	6.88
Vitamin D, IU/100g	99	1257
Cholesterol, mg/100g	893	477
Saturated fatty acids, g/100g		
Capric 10:0	.104	.04
Myristic 14:0	1.25	1.75
Pentadecanoic 15:0	<.05	.08
Palmitic 16:0	1.88	2.76
Stearic 18:0	.26	.31
Monounsaturated fatty acids, g/100g		
Palmitoleic 16:1	1.78	1.13
Oleic 18:1	1.62	2.37
Eicosenoic 20:1	3.29	2.68
Polyunsaturated fatty acids, g/100g		
Linoleic 18:2	.26	.27
Linolenic 18:3	<.05	.16
Octadecatetraenoic 18:4	.10	<.04
Eicosapentaenoic 20:5	.94	1.20
Docosapentaenoic 22:5	.157	.16
Docosahexaenoic 22:6	1.20	1.75
Saturated fat, g/100g	3.24	4.85
Polyunsaturated fat, g/100g	2.45	3.76

[1]Fatty acids that exceeded 0.05 g/100g were listed.