NOTE ***Internal Medicine***

Seasonal Changes of Blood Composition in Captive Bottlenose Dolphins

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ABSTRACT. To determine how blood values in bottlenose dolphins changed during the year, 504 blood samples were taken from 9 dolphins from 1991 to 1999 and clinical blood examinations were undertaken monthly including 3 hematological and 19 serum chemistry tests. In creatinine, significant seasonal changes were found among three groups of adult males, adult females and juveniles, and the average values in summer were 15–38% higher than those in winter. In two out of three groups the average total cholesterol value were h ighest in winter, and the lowest of all groups were in summer. In two other groups the peaks of average FFA value were recorded in sum mer, and the lows were in winter.

KEY WORDS: bottlenose dolphin, seasonal change, serum chemistry.

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The physiology of free-living marine mammals adapts to seasonal environmental changes. It is known that bottlenose dolphins migrate seasonally, and the migration is probably a response to temperature changes [16, 21]. Several physiological parameters, for example, the thickness of blubber [25], the body mass [21] and the rectal body temperature [23], are known to have seasonality even in captive dolphins.

Seasonal changes in hematology have been recorded in birds [1, 5, 9, 17, 20, 27], bears [8], deer [14], bats [12] and fish [4]. However, there have been few reports of seasonal changes of hematological values for marine mammals [26]. It is important to know how blood values change seasonally.

The blood samples taken from wild dolphins may be affected by several factors, including their handling stress and their diet. We previously reported how diet affected the blood composition of this species [24]. Therefore, it is necessary to examine the case of bottlenose dolphins in captivity. The information presented should help to establish a hematological base line of seasonal changes for bottlenose dolphins. It is important to be aware of seasonal changes for evaluating long-term physiological variations, for example, during pregnancy. Therefore, this report examines how blood values change seasonally in captive bottlenose dolphins, *Tursiops truncatus*, from the results of clinical blood examinations at Enoshima Aquarium from 1991 to 1999.

Hematological data were compiled from the routine health records of 9 bottlenose dolphins (Table 1) which were divided into three groups: adult males (two dolphins, aged over 12 years), adult females (two dolphins, aged over 12 years), and juveniles (one male and four females, aged under 6 years) at Enoshima Aquarium, Kanagawa, Japan. This age classification was not changed throughout the study period. Three of these dolphins were born at this facility. The other six dolphins originated in waters around Japan. When they were brought into captivity, we estimated their ages from body length and body mass [22]. Clinical data over a 9-year period (1991 to 1999) for adult males and females, and a 5-year period (1995 to 1999) for juveniles were used, and a total of 504 blood samples were taken. Only blood samples from dolphins judged to be normal by behavior, appearance, or food consumption were used.

The tank is outdoors with a total seawater volume of 5000 m^3 (45 m × 25 m, oval-shaped, with a depth of 3.5 to 5.5 m). During the study period from 1991 to 1999, the average monthly water temperature varied between 12.4 ± 0.6°C in February to 27.1 ± 1.5°C in August, and the average monthly air temperature ranged from 8.8 ± 2.3°C in January to 27.9 ± 2.3°C in August. Both of these measurements were taken daily at 15:00.

The body mass was measured during husbandry on a digital bar scale (DIGI Compo, Teraoka, Tokyo, Japan, 0.5 kg accuracy) every two weeks for one adult male and four juvenile females (Table 1). The other four animals' body mass could not be measured.

Monthly blood samples were taken from the fluke by venipuncture, using a plastic disposable syringe with a (21-gauge) butterfly needle, and clinically examined [11]. All blood samples (8–10 m*l*) were taken between 09:00–10:00, before feeding. This meant that the dolphins had not eaten

Table 1. Animals used in this study

Animal[a)]	Birth date[b)]	Arrival date	Body length[c)]
AM[d)]	1979 Feb 22	–	–
AM	–	1988 May 11	304 cm
AF	–	1974 Nov 13	285 cm
AF	–	1978 Nov 11	290 cm
JM	1993 May 24	–	–
JF[d)]	1994 Dec 23	–	–
JF[d)]	–	1996 Oct 17	232 cm
JF[d)]	–	1996 Oct 17	236 cm
JF[d)]	–	1996 Oct 17	226 cm

a) AM=Adult male; AF=Adult female; JM=Juvenile male; JF=Juvenile female. b) Animals were born at Enoshima Aquarium. c) Body length was measured at arrival time. d) Body mass of these animals was measured during husbandry.

for 16–17 hr after the last feed. Blood was placed in EDTA-2K tubes, clot tubes and 3.8% sodium citrate tubes. All samples were refrigerated at 4°C and transported to the biochemical laboratory (Showa Medical Science, Machida, Tokyo, Japan). Blood was centrifuged to separate the serum at 3000 rpm for 10 min, 6 hr after collection.

Complete blood counts (white blood cell, red blood cell and platelet) were performed using a SYSMEX NE 7000 ™ automated hematology analyzer. Serum chemical analyses (total protein, albumin/globulin ratio, aspartate amino transferase, alanine amino transferase, lactate dehydrogenase, alkalinephosphatase, creatine kinase, amylase, blood urea nitrogen, creatinine, triglycerides, total cholesterol, free fatty acid, total bilirubin, sodium, chloride, iron and lipase) were performed using a HITACHI 7450 automatic analyzer, and fibrinogen was measured using a SYSMEX CA 5000™ automated coagulation analyzer. The allowable limit of error for fibrinogen is: <10%, and for the other parameters is: <5%. Within 12 hr of collection, all samples were examined. The methods of analysis have been previously reported [24].

Fish used for feeding were caught in Japanese waters during the previous year (December to February), and were quick-frozen and packaged in materials impervious to air and moisture at a temperature below –20°C. The species of fish food were *Scomber japonicus*, *Cololabis saira*, and *Trachurus japonicus*. Approximately 50–60% of the average diet was *Scomber japonicus*, and the remaining percentage was a combination of the other species. The composition of the dolphin's diet was almost the same throughout the study. They were fed 4 to 8 times per day between 09:00 and 17:30. Adult males consumed approximately 10 to 13 kg in a day, adult females from 13 to 14 kg, and juveniles around 9 kg.

To determine seasonal changes in blood composition, 504 samples were analyzed over the four seasons: spring (March to May), summer (June to August), autumn (September to November), and winter (December to February). The results were processed and analyzed using one-way analysis of variance (ANOVA). All results are presented as means ± SD. Values of $p<0.05$ were designated as significant.

Creatinine was shown to have seasonal variations in three groups (Table 2). Total cholesterol and free fatty acids changed similarly in two out of the three groups.

Creatinine (CRE): CRE values ranged from 0.8 to 2.5 mg/d*l*. In all groups, the averages in summer were the highest, and in winter the lowest. The average CRE values in summer were 15–38% higher than in winter. The annual average CRE values were highest in adult males, followed by adult females and lowest in juveniles. The levels for adult females were 92.2% that of adult males and the levels for juveniles were 73.8% that of adult males.

Total cholesterol (T-Cho): T-Cho values ranged from 96 to 348 mg/d*l*. In adult males and juveniles the peak for the average T-Cho value was recorded in winter, and in adult females the highest level was observed in spring. The lowest values in all groups were in summer. The levels in adult females were higher than those in juveniles, except in autumn. The levels in adult males were consistently the lowest of the three groups throughout the year.

Free fatty acids (FFA): FFA values ranged from 0.10 to 1.53 mEq/*l*. In adult males and adult females the peak for the average FFA value was recorded in summer, and the lows were in winter. However, in adult females, the average FFA values for winter and autumn were the same. In juveniles, the average peak of the FFA values was recorded in spring, and the lows were in autumn. The magnitude of variation in juveniles was the smallest and seasonal changes were not shown as clearly as in the other two groups.

Relationship of body mass to CRE: In one adult male dolphin, the average monthly body mass for 9 years ranged from 290.7 to 327.9 kg (Fig. 1). The peak for body mass was recorded in May, and the lowest body mass was in October. The average body mass value in May for the nine years was 12.8% higher than in October. The average CRE values ranged from 1.38 to 1.94 mg/d*l*. The peak for CRE occurred in July and the lowest recording was in February. The average CRE value in July was 40.6% higher than in February.

Table 2. Values of CRE (mg/d*l*), T-Cho (mg/d*l*) and FFA (mEq/*l*) in four seasons and annual average values in two captive adult male, two adult female and five juvenile bottlenose dolphins

Test	Group[a)]	Winter[b)]	Spring[c)]	Summer[d)]	Autumn[e)]	*p*	Annual
CRE	AM	1.42±0.11 (42)[f)]	1.72±0.23 (39)[f)]	1.97±0.25 (36)[f)]	1.65±0.18 (34)[f)]	<0.001	1.68±0.28 (151)[f)]
	AF	1.45±0.16 (32)[f)]	1.49±0.15 (17)[f)]	1.69±0.19 (12)[f)]	1.68±0.15 (18)[f)]	<0.001	1.55±0.19 (79)[f)]
	JU	1.15±0.20 (84)[f)]	1.23±0.21 (87)[f)]	1.33±0.23 (46)[f)]	1.30±0.24 (57)[f)]	<0.001	1.24±0.23 (274)[f)]
T-Cho	AM	177.5±40.1	168.7±33.7	140.3±26.1	141.2±32.3	<0.001	158.2±37.3
	AF	231.2±21.0	244.9±27.9	193.4±14.1	193.7±21.6	<0.001	217.4±28.6
	JU	219.1±39.3	203.9±33.1	180.1±29.3	196.7±36.3	<0.001	203.1±37.5
FFA	AM	0.363±0.151	0.430±0.189	0.610±0.191	0.453±0.190	<0.001	0.458±0.200
	AF	0.583±0.155	0.650±0.185	0.789±0.309	0.583±0.255	0.036	0.629±0.224
	JU	0.483±0.144	0.592±0.179	0.547±0.151	0.479±0.142	<0.001	0.528±0.164

Results shown as means ± SD. a) AM=Adult males; AF=Adult females; JU= Juveniles, b) December to February, c) March to May, d) June to August, e) September to November, f) Number of samples. There were significant differences ($p<0.05$; ANOVA one-way) in all four seasons.

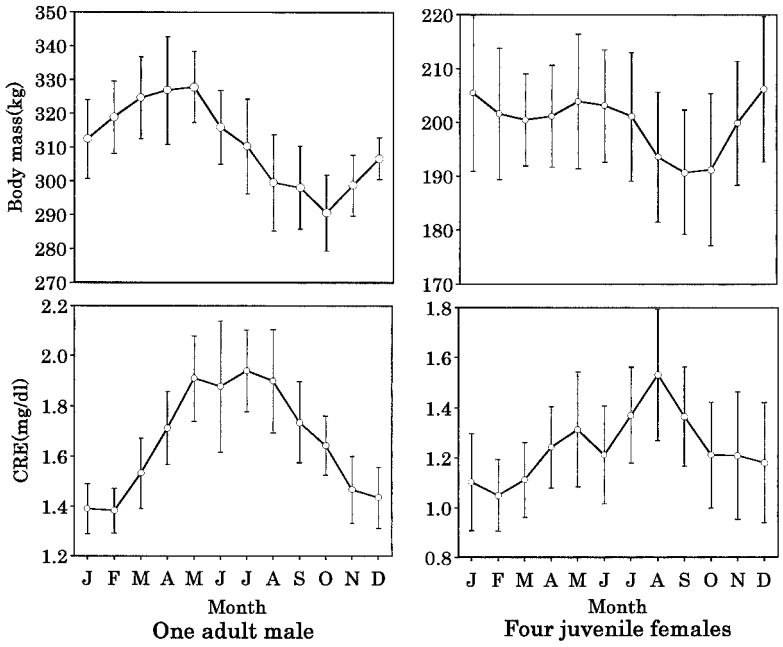

Fig. 1. Changes of the average monthly body mass value and the average monthly creatinine value in one adult male and four juvenile female bottlenose dolphins. These data cover a 9-year period for adult male and a 5-year period for juveniles, and this study focused on the changes of body mass and CRE in each month excluding the factors of year. Each graph represents the mean and SD (vertical bar).

In four juvenile female dolphins, the average monthly body mass for 5 years ranged from 190.8 to 206.3 kg. There were two peaks in December and May, and the highest level was recorded in December. The lowest was in September. The average body mass value in December was 8.1% higher than in September. The average CRE values ranged from 1.05 to 1.53 mg/d*l*. The peak for CRE occurred in August and the lowest recording was in February. The average CRE value in August was 45.7% higher than in February.

In captive dolphins, the body mass changes seasonally [21]. The present study showed that the same results were obtained for one male adult and four juvenile female dolphins (Fig. 1). Ross and Cockcroft [21] reported that an increase in a captive dolphin's body mass was probably due to an increase in its blubber mass. In captive bottlenose dolphins, the blubber thickness was thickest in January at San Diego, and in December at Hawaii. The thickness of the insulating layer of dolphins changes with acclimation temperature [25]. At Enoshima Aquarium, the blubber thickness may also change seasonally. If water temperature is related to blubber thickness inversely, the body mass for winter would probably be heaviest in the year. However, in the present study, the peaks for body mass in adult male and juvenile dolphins were recorded in May. This suggests the possibility that some other tissue in addition to the blubber mass might also change seasonally.

In terrestrial mammals, CRE is a non-protein, nitrogenous substance formed during the muscle metabolism of creatine and phosphocreatine. The quantities of CRE excreted are closely related to the body muscle mass and the metabolic rate of the animal [7]. In humans, the level of CRE is directly proportional to the individual's muscle mass, and the quantity of CRE in an adult man is highest, followed by an adult woman and lowest in a child [10]. In the present study, the annual averages of CRE were also related to the muscle mass, and were highest in adult males, followed by adult females and were lowest in juveniles.

In one adult bottlenose dolphin, the peaks for body mass and CRE occurred at different times, and there were approximately two or three months delay between them (Fig. 1). In four juvenile dolphins, a similar relationship between body mass and CRE was shown. This study focused on the monthly changes of body mass and CRE. In a minke whale, *Balaenoptera acutorostrata*, of the Antarctic, the muscle mass in March was larger than in December with the results being measured from December to March [13]. The present study, therefore, suggests the possibility that the changes in CRE levels were in response to the seasonal muscle volume changes. It is recommended that in further studies, a non-invasive ultrasound be used throughout the year in order to determine muscle mass in relation to CRE.

Our measurements for T-Cho are comparable to values reported for bottlenose dolphins in the literature [2, 3, 19]. Ridgway *et al.* [19] reported that levels of cholesterol in females were higher than in males. The same result was also observed in this study. Furthermore, the juvenile values

were consistent for the levels of females and males. While the food intake of adult males was higher than that of juveniles, the levels of T-Cho in adult males were consistently lower than those of juveniles (Table 2).

In sparrows, free cholesterol was elevated during premigratory and migratory periods, minimal during summer breeding periods, and reduced during periods of body molting [5]. In both immature and adult pelicans, cholesterol levels were elevated in winter through spring [27], while in reindeer, total and HDL-bound cholesterol were increased in summer [14]. In the present study also, T-Cho levels of bottlenose dolphins changed, increasing for adult male and juvenile groups in winter, and decreasing for all groups in summer.

Seasonal variations in the lipid and fatty acid composition of frigate mackerel are known [18]. However, the same stored frozen fish were used during this study. In a study involving bottlenose dolphins, the T-Cho value at 4 hr after feeding was not changed, compared to before feeding [24]. Asper *et al.* [2] reported that the level of cholesterol in dolphins is influenced by diet. In humans, dietary cholesterol takes several days to equilibrate with cholesterol in the plasma and several weeks to equilibrate with cholesterol in the tissues [15]. It is likely, therefore, that the level of T-Cho is affected by the volume of food intake fed to dolphins several weeks, or more, prior.

In the present study, the food intake of adult male dolphins fed in winter was approximately 20–30% more than the food intake in summer, and the averages for T-Cho in winter were 26.5% higher than in summer. In reindeer, total and HDL-bound cholesterol coincided with the seasonal changes in food intake [14]. In this study, although the food intake for two adult female and five juvenile dolphins was the same volume throughout the study, seasonal changes in T-Cho were observed. In laboratory animals, seasonal variations in cholesterol were shown in spite of constant diet [6]. This suggests that seasonal variations may not only be due to nutritional factors.

Stable diet and limited exercise both contribute to serum cholesterol and albumin elevations in captives [27]. Also, cholesterol levels were higher in captive dolphins than in wild dolphins [2]. In the present study, the quantity of exercise for dolphins was not compared for each season.

Levels of FFA appear to be the best indicator of the rate of mobilization of deposited fat. In bottlenose dolphins, the blubber thickness was thinnest during warm seasons and thickest during cooler winter months [25]. As the level of FFA is inversely related to the blubber thickness, it is most likely that variations of FFA are released or deposited to the blubber seasonally.

FFA arises in the plasma as a result of lipolysis of triacylglycerol in adipose tissue or as a result of the action of lipoprotein lipase during uptake of plasma triacyglycerols into tissue [15]. The present study did not measure lipoprotein lipase. Further research will be needed to measure FFA and lipoprotein lipase, and blubber thickness.

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