NOTE *Internal Medicine*

Hyperlipemia of Captive Bottlenose Dolphins during Pregnancy

Fumio TERASAWA[1)] and Masakazu KITAMURA[1)]

[1)]*Enoshima Aquarium, 2–19–1 Katase-kaigan, Fujisawa, Kanagawa 251–0035 Japan*

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ABSTRACT. In this study values for total cholesterol and triglycerides were measured in 110 blood samples taken from 360 days pre-partum to 90 days post-partum in ten parturitions of six bottlenose dolphins, and in 75 blood samples when the dolphins were not pregnant as a control group. The average total cholesterol values in the second, third and fourth stages and in the puerperium were significantly higher than the average value of the control group by 11.0%, 30.2%, 19.3% and 13.4% respectively. The average triglycerides values for the third and fourth stages and in the puerperium were also significantly higher than those in the control group by 59.7%, 84.3%, and 42.1% respectively.

KEY WORDS: bottlenose dolphin, changes in pregnancy, hyperlipemia.

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In humans [12, 14], normal pregnancy leads to an increase in plasma lipids. Both plasma cholesterol and triglyceride concentrations increase markedly during pregnancy. Some studies have suggested that the rise in plasma triglycerides might enhance the availability of essential and non-essential triglyceride fatty acids for placental transfer to the fetus, and that the cholesterol rise might increase the supply of cholesterol needed for placental progesterone synthesis and transplacental cholesterol transfer to the fetus [10]. However, its physiologic significance is not yet completely understood [6].

Pregnant harbor seals have a higher level of triglycerides than non-pregnant seals [11]. However, there are no reports in the literature of a longitudinal study for total serum lipids in pregnancy and during the puerperium for marine mammals. Measurement of lipid concentrations during pregnancy periods would relate not only to health management for the mother and the fetus, for example, but also the prevention of toxemia. Currently, toxemia in cetaceans has not been observed in Japanese aquariums, but the possibility of suffering from this disease exists even in cetaceans. Additionally, toxemia is occasionally associated with hyperlipemia in humans [12].

This study describes longitudinal changes in the concentration of total cholesterol and triglycerides in captive bottlenose dolphins during pregnancy and in the puerperium, based on our previous papers, dolphin dietary factors [21] and seasonal changes [22]. The bottlenose dolphin's gestation period is 12 months, plus or minus 10–14 days [17]. In the present study, from 360 days pre-partum to 90 days post-partum, total serum cholesterol and triglyceride levels were measured for ten parturitions of six bottlenose dolphins by clinical blood examinations at Enoshima Aquarium from 1992 to 2002.

Six female bottlenose dolphins and ten parturitions were used for the analysis (Table 1). All dolphins originated in waters around Japan. The time of parturition for each animal kept at Enoshima Aquarium ranged from 2 to 23 years (average 10 years) from the time of entry into the facility. All parturitions occurred normally with the infant dolphins surviving. However, in five out of the ten parturitions, these infants died before 90 days post partum (Table 1).

The tank is outdoors with a total sea water 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 1992 to 2002, the average monthly water temperature varied between 12.5 ± 0.6°C in February to 27.3 ± 1.4°C in August, and the average monthly air temperature ranged from 8.7 ± 2.4°C in January to 27.9 ± 2.2°C in August. Both of these measurements were taken daily at 15:00.

One hundred and ten blood samples (8–10 m*l*) were taken from the fluke, using a plastic disposable syringe with a (21-gauge) butterfly needle, and were clinically examined [9]. Additionally, 75 blood samples were also taken as a control group from these six dolphins when they were not pregnant. All samples were obtained between 09:00–10:00, before feeding. These timings indicate that the dolphins had been fasting for 16–17 hr since the previous feed [21]. Blood was placed in clot tubes. All samples were refrigerated at 4°C and transported to a biochemical laboratory (Showa Medical Science, Machida, Tokyo, Japan). Blood was centrifuged to separate the serum at 3,000 rpm for 10 min, 6 hr after collection. Total cholesterol and triglyceride values

Table 1. Animals and parturitions used in this study

ID Number	Arrival date	Parturition Date
EAM.161	1972/11/13	1992/05/11[a)]
EAM.187	1978/11/11	1993/05/24
EAM.227	1988/05/11	1994/12/23
EAM.161	–	1995/07/29[a)]
EAM.227	–	1998/06/13
EAM.237	1994/12/23	2000/06/07
EAM.249	1998/01/29	2000/07/23[a)]
EAM.242	1996/10/21	2001/06/18[a)]
EAM.249	–	2001/08/01[a)]
EAM.242	–	2002/07/15

EAM.161, 227, 242 and 249 had two parturitions in the present study.
a) Infants died before 90 days post- partum.

were measured using a HITACHI 7450 automatic analyzer.

During the pregnancy, the average diets for the ten cases were 12.1 kg in the first stage (360 to 271 days pre-partum), 13.0 kg in the second stage (270 to 181 days pre- partum), 13.3 kg in the third stage (180 to 91 days pre-partum) and 13.2 kg in the fourth stage (90 to 1 day pre-partum). In the puerperium (0 to 90 days post-partum), the average diet for the five cases whose infants survived until 90 days post-partum was 18.8 kg. The species of fish and the composition of the diet have been previously reported [22].

To determine the changes in total cholesterol and triglyceride values during pregnancy and in the puerperium, blood data were analyzed over the four stages before parturition and the puerperium. The results were processed and analyzed using two-way analysis of variance (ANOVA), and the Dunnet test. All results are presented as means ± SD. Values of $p<0.05$ were considered as significant.

Total cholesterol (T-Cho): T-Cho values ranged from 114 to 364 mg/d*l*, 360 days pre-partum to 90 days post-partum. In the control group, T-Cho values ranged from 127 to 388 mg/d*l*. The average T-Cho values in the control group and in the first stage were at almost the same level. No statistical differences were found between them. The average T-Cho values elevated from the second stage, and peaked at the third (Table 2). Then the values began to decline from the fourth stage until puerperium. The average T-Cho values for the first, second, third and fourth stages were higher than the values for the control group by 2.3%, 11.0%, 30.2%, and 19.3% respectively. The average T-Cho values for the puerperium were still 13.4% higher than those in the non-pregnant control group. Additionally, there were significant differences in these four periods; the second, third and fourth stages, and in the puerperium.

The average levels during the same season for the third stage and for non-pregnant dolphins were compared using two-way ANOVA (Table 3). The results showed that a significant difference was found in the effect of pregnancy, however there were no statistical differences for seasonal variation, or their interaction.

Triglycerides (TG): TG values ranged from 8 to 69 mg/d*l*, 360 days pre-partum to 90 days post-partum. In the control group, TG values ranged from 10 to 28 mg/d*l*. As the pregnancy progressed, the average of the TG values increased continuously, peaking in the fourth stage (Table 2). At the fourth stage the average of the TG values was nearly double that of the control group. T-Cho values subsequently dropped after the parturitions, and the average TG value of the puerperium still remained between the levels of the second and third stages. The average TG values for the first, second, third and fourth stages and in the puerperium were higher than those in the non-pregnant control group by 14.5%, 19.5%, 59.7%, 84.3%, and 42.1% respectively. There were significant differences between the third and fourth stages and the puerperium compared to the control group.

In humans, the increase in T-Cho and TG during pregnancy and in the puerperium has been reported by many investigators [12, 14]. Similar findings are reported for the dog [5] and the guinea pig [8]. In the rhesus monkey [13] and the baboon [24], the opposite situation occurs: the serum cholesterol decreases during pregnancy and increases post-partum. This study demonstrated an increase in T-Cho and TG during bottlenose dolphins' pregnancies in comparison to the values of a non-pregnant control group.

The hyperlipidemia of human pregnancy is characterized by a two-fold to four-fold rise in plasma TG and a 10% to 50% rise in plasma cholesterol at term [14]. In this study, a 1.8-fold rise at the fourth stage in average TG values and a

Table 2. Average values for total cholesterol (mg/d*l*) and triglycerides (mg/d*l*) during pregnancy and in the puerperium for six captive dolphins (ten parturitions), and for the same six dolphins when non-pregnant

Items (n)[f]	Non-pregnant Dolphins (75)	Stages of Pregnancy FIS[a] (15)	SS[b] (29)	TS[c] (34)	FOS[d] (23)	PU[e] (9)
T-Cho	203.2 ± 60.5	207.9 ± 56.4	225.6 ± 56.4*	264.6 ± 49.3*	242.4 ± 39.9*	230.4 ± 43.8*
TG	15.9 ± 4.3	18.2 ± 4.4	19.0 ± 6.4	25.4 ± 15.7*	29.3 ± 15.6*	22.6 ± 15.8*

Results shown as means ± SD. a) The first stage (360 to 271 days pre-partum), b) the second stage (270 to 181 days pre-partum), c) the third stage (180 to 91 day pre- partum), d) the fourth stage (90 to 1 day pre-partum), and e) the puerperium (after parturition to 90 days post-partum). f) Number of samples. Significant increase compared to non-pregnant dolphins (*; $p<0.05$, a Dunnet test).

Table 3. Comparison of average total cholesterol values between third stage of pregnancy and non-pregnant dolphins in winter and spring

Season	Third Stage of Pregnancy	Non-pregnant Dolphins	ANOVA Pregnancy (A)	Season (B)	AxB
Winter[a]	260.6 ± 50.1(24)	233.2 ± 60.5(25)	< 0.05	NS[c]	NS[c]
Spring[b]	274.4 ± 48.3(10)	208.1 ± 72.5(22)			

Results shown as mean ± SD. a) December to February, b) March to May. Probabilities calculated using two-way analysis of variance (ANOVA). c) Not Significant.

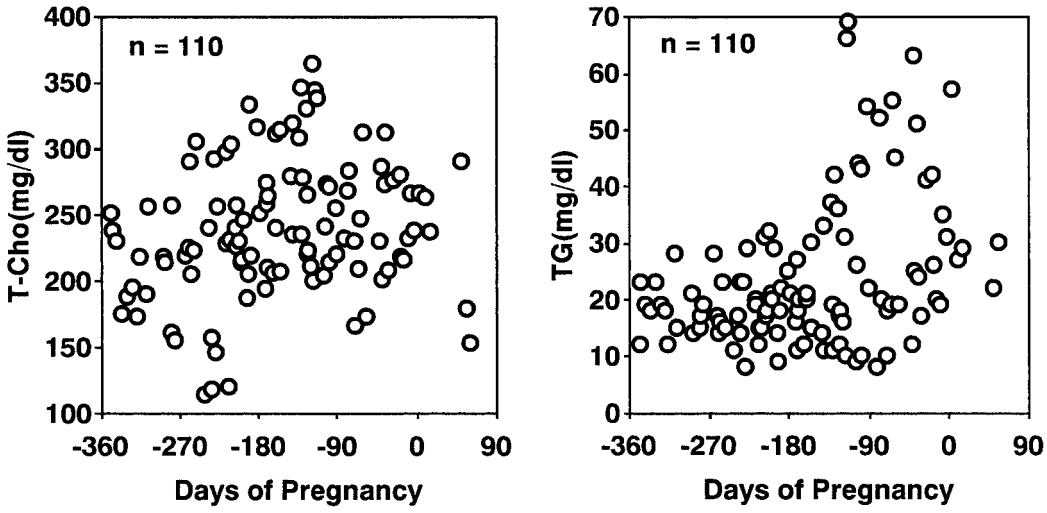

Fig.1. The distributions of total cholesterol and triglyceride values from 360 days pre-partum to 90 days post-partum for ten parturitions of six bottlenose dolphins.

30% rise at the third stage in average T-Cho values were observed. These values are thought to be a result of the similarity.

Previously reported values for the proportion of cholesterol in the fetus at term derived from maternal sources were 15–20% in the rat [2], 22% in the guinea pig [3] and 42% in the rhesus monkey [13]. There might be a positive correlation between body size and maternally derived cholesterol. This suggests the possibility that the rate of maternally derived cholesterol in bottlenose dolphins is higher than in those animals previously reported [2, 3, 13].

In most orders of mammals, there is a definite relationship between the duration of the gestation period and the size of the young at birth since the rate of fetal growth is the same for those orders of mammals [19]. Many studies [12, 14] have demonstrated elevated lipid concentrations at the end of human pregnancy. The present results also showed that the highest levels for TG were in the fourth stage and the highest levels for T-Cho were in the third stage. The pattern for the period at which the peak for T-Cho occurs in dolphins is different from the pattern that occurs in humans.

In nine out of the ten cases, parturitions were observed from May to August. All data in the third stage were taken from December to April (Table 3). In our previous report [22], a tendency showed for the level of T-Cho to be higher in winter and lower in summer. In this study, the timing in which blood was taken corresponding to the third stage was from winter to spring, and that for the fourth stage was from spring to summer. The average T-Cho value in the third stage was higher than at any other time during pregnancy or puerperium, and this level was also higher than those reported in the literature [1, 15, 22]. In the third stage, the level in spring was higher than that of winter and also the control group. It suggests that T-Cho values increase as pregnancy progresses without seasonal variations, and the possibility that the species patterns in bottlenose dolphins might be responsible for the peak level of T-Cho in the third trimester of pregnancy and the puerperium.

Currently, in cetacean reproduction, monitors for progesterone levels in sera [16, 23] and ultrasound examination [4] are being used for the determination of pregnancy, and rectal temperature [20] is used to determine time of parturition. In 1992 and 2002 for all Japanese aquariums, the number of normal parturitions for bottlenose dolphins was four and nine respectively [7]. However, the numbers for abortion and stillbirth were six in 1992 and seven in 2002 (unpublished data). Although the normal parturition rate has improved, the numbers of abortions and stillbirths has not changed for ten years. However, there have been few reports of physiological studies during pregnancy and in the puerperium for bottlenose dolphins. In a human's late stage of pregnancy, positive correlations were found between the level of maternal circulating TG and the birth weight of the infant [18]. This study suggests the possibility that levels of total cholesterol and triglycerides might be one indicator for the health management of the mother and the fetus during the pregnancy.

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